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# *In vitro* digestibilities, predicted glycemic index and sensory evaluation of biscuits produced from composite flours of wheat and processed tiger nut.

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# Abstract

This study aimed to evaluate the *In vitro* digestibilities, predicted glycemic index and sensory evaluation of biscuits produced from composite flours of wheat and processed tiger nut. The tiger nut was sorted and processed by air-drying, blanching, fermentation and dehydration. The processed samples were oven dried at a temperature of 60°C for 17 hours before milling into fine flour to blend with wheat flour (Wf) at the ratio of 20:80. Biscuits were produced from blends of wheat flour and processed tiger nut namely: wheat-tiger nut air dried (W-TNA), wheat- tiger nut blanched (W-TNB), wheat- tiger nut fermented (W-TNF) and wheat- tiger nut dehydrated (W-TND). The results of *In-vitro* protein digestibility of the composite biscuit showed W-TNF biscuit to have the highest digestibility (52.53%) and W-TND as the least (39.44%) when compared to wheat flour (48.18%). Highest starch digestibilities were recorded for composite biscuits from  $W_f$  (99.45±2.32%) and W- TNF (89.99±1.67%). The highest hydrolysis index (HI) of 138.85%, and 113.79% were observed in W-TNF and W-TND respectively; which also resulted in higher predicted glycemic index (PGI) of 115.93% (W-TNF) and 102.17% (W-TND). The sensory evaluation revealed that the composite biscuits were generally accepted although there were reductions in aroma, texture and taste of the composite biscuits when compared with the control (Wf)

Keywords: In vitro digestibilities; Predicted glycemic index; Sensory evaluation; Biscuit; Wheat; Processed tiger nut.

# 1. Introduction

Wheat flour used for the production of biscuits is insufficient in many regions of the world resulting in importation of the flour by regions with limited supplies [1]. Wheat production in Nigeria is limited due to climatic conditions and wheat is imported to meet local flour needs for baking. As a result of this, huge amount of foreign exchange is spent annually for wheat importation [2]. Efforts have been made to promote the use of composite flour in which locally grown crops with high protein values with the best processing methods replaces a portion of wheat flour thereby decreasing the demand for imported wheat [3]. Soft wheat flour is the suitable flour for biscuit making. This is due to its content of gliadin (a prolamin) and glutamine (glutelin) which undergoes hydration in the presence of water, salt and sugar. This protein forms a visco-elastic matrix known as gluten, which is responsible for the rising nature of dough or permit substantial increase in the volume of baked product of dough and its gas retention capability [4]. There is therefore, a compelling need to develop an adequate substitute for wheat flour. This substitute should be readily available, cheap and capable of replacing wheat in functionality.

Tiger nut (*Cyperus esculenta*) is of the Cyperacea family and of the same genus as the papyrus plant[5-6]. It is an edible perennial grass-likeC4 plant of the sedge family[7]. Common names of the plant are earth almond, yellow nut grass,

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ground almond, chufa, bambara groundnut and edible galingale[8-9]. The production and distribution of tiger nut in Nigeria is seasonal; this fluctuates with season of abundance and scarcity which leads to the inability to explore the nutritional content and and its medicinal value[10]. Tiger nut is high in protein that plays important role in human nutrition. A detailed study shows that it makes an excellent source of supplementing proteins in the diet [11]. Tiger nuts have long been recognized to contain almost twice the quantity of starch as potato or sweet potato tubers. Its tuber is a good source of energy [12]. It has high vitamin and mineral contents like iron, phosphorous and calcium. It also has a high content of crude fibre and high level of sulphur containing amino acids which are limited in cereals. These compositions give tiger nut its high nutritive and health value [13]. Its tubers can be eaten raw, roasted with sugar, soaked in water or processed into starch and flour [14]. It can be processed into a milky beverage called "Horchata de Chufa" in Spain or "Atadwe" milk in Ghana [15]. In Nigeria, tiger nut is well grown and available in semi-dried form in Nigerian markets where it is sold locally and consumed uncooked [16]. Tiger nut can be used as composite flour for baking because of its nutritional value. However, processing techniques such as blanching, fermentation and dehydration are means of improving its nutritional value. Composite flour has the added advantage of improving the nutrient value of biscuits and other bakery products especially when cereals are blended with legumes or tubers. Osuntogun [17] suggested the ratio of 50:50 (wheat and non-wheat) flour mixture for biscuit and other bakery products.

Biscuit is an important edible confectionary crisp product of wheat which is deficient in protein and could be enhanced by supplementing the wheat flour with more nutritional pods and other leguminous crops [18]. Biscuit and other baked food products are important items belonging to the class of food that are sold in ready to serve form. All biscuits are expected to be nutritious, contributing valuable quantities of iron, calcium, protein, calorie, fibre and some of the Bvitamins to our diet and daily food requirement. Composite flour has the added advantages of improving the nutrient value of biscuits and other bakery products especially when cereals are blended with legumes. This research however, is aimed at improving the quality of biscuit based products by the addition of processed earth-almond as supplement to wheat flour.

# 2. Material and methods

The wheat, earth-almond (tigernut) and other ingredients used for the production of biscuits were purchased from Bori camp market in Obio/Akpor Local Government Area of Rivers State; Nigeria. All chemical reagents used in this study were of analytical grade.

# 2.1. Processing of earth-almond

Dried tiger nut tubers were carefully selected to remove dust particles and shared into four sets. The first set was further air-dried (TNA) for four days and blended using laboratory miller. The second, third and fourth sets were soaked in water for four days to rehydrate. After which the following processing methods were applied; blanching at 80°C for 10 minutes (TNB), soaking in water to ferment for 4 days (TNF) and dehydrated (TND) by oven drying again after rehydration respectively. The 2nd – 4th sets were then oven dried at a temperature of 60°C for 17 hours before milling into flour. They were all sieved through100 mesh size and the flour was stored in a sealed container at 4°C until ready for use.

# 2.2. Equipment used

Equipment used include: UV-Visible spectrophotometer, electronic weighing balance, laboratory oven, incubator, centrifuge, electrothermal water bath, heating mantle and kjeldahl apparatus.

### 2.3. Chemical / reagents

Sulphuric acid, hydrochloric acid, distilled water, sodium hydroxide, anthrone reagent, standard glucose and indicator.

### 2.4. Preparation of flour blends

Wheat flour (Wf) and the various processed tigernut samples which included air dried (TNA), blanched (TNB), fermented (TNF) and dehydrated (TND) were used in formulating blends for biscuits making in the proportion of 80:20 respectively. A kenwood mixer was used for mixing samples at speed 6 for 5 minutes to achieve uniform mixing. After which the *in-vitro* digestibilities, glycemic index and sensory evaluation of the biscuits made from processed wheat-earth-almond composite flour were determined.

Sample	Blend ratio
W-TNA	80:20
W-TNB	80:20
W-TNF	80:20
W-TND	80:20
Wf	100

## **Table 1** Composite flour compositions of wheat and procecced earth-almond

 $\label{eq:WTNA} Wheat \mbox{-tiger nut air dried; W-TNB} = \mbox{Wheat-tiger nut blanched; W-TNF} = \mbox{Wheat-tiger nut fermented} \\ W-TND = \mbox{Wheat-tiger nut dehydrated; } W_f = \mbox{Wheat flour} \end{cases}$ 

## 2.5. Production of wheat-earth-almond biscuits

The method described by Arisa *et al.*, [19] was used in the production of wheat-earth-almond biscuit. Sugar (75 g) was added to 125 g of margarine in a Kenwood mixer and mixed at medium speed until fluffy. Whole egg and milk powder were added during mixing and then mixing continued for about 30 min. Composite flour (wheat flour + processed earth-almond), baking powder and flavor were slowly added to the mixture; and kneaded to form dough. It was then rolled on a flat rolling board sprinkled with flour to a uniform thickness of about 0.4 cm, circular cookies of 5.8-6.0 cm were cut, placed in oiled baking trays and baked in the oven at 160 °C for 15 mins.

## 2.6. Determination of In vitro protein digestibility of wheat-earth-almond composite biscuit

The *In-vitro* protein digestibility of the composite biscuits and the control (wheat flour) were determined using the method of Saunders *et al.*, [20] and modified by Monsour and Yusuf [21]. A known weight of each sample containing 16 mg nitrogen was taken and digested with 1mg of pepsin (Cat. No. P6887, Sigma Chemicals Ltd, USA) in 15 ml of 0.1 MHCl at 37 °C for 3 h, the pepsin hydrolysed solutions were neutralized with 0.5 M NaOH and incubated with 6.0 mg of pancreatin (Sigma Chemicals Ltd, Cat. No. P 1750) in 7.5 ml of 0.2 M phosphate buffer at pH 7.6 for 18 h. The reaction was terminated by adding 22.5 ml of 10% TCA. The TCA soluble fraction was assayed for nitrogen using the micro Kjeldahl method [22]. A blank sample was also determined; protein digestibility was calculated using the formula:

Protein digestibility % = <u>Nitrogen in supernatant - Nitrogen in blank x 100</u>

### Nitrogen in blank

# 2.7. Determination of In vitro starch digestibility of wheat-earth-almond composite biscuits

The total starch in the control (wheat flour) biscuit and composite (W-TNA, W-TNB, W-TNF, W-TND) flour biscuits developed was determined enzymatically according to the modified method of Goni *et al.*, [23]. The samples were ground to pass through a 0.5mm sieve. Subsequently, 25mg of each sample was dissolved in 6ml of 2M KOH and shaken vigorously for 30 mins at roomtemperature. A stock solution of  $\alpha$ -amylase was prepared by mixing 20 mg of porcine pancreatic  $\alpha$  -amylase with 50ml of 0.2M phosphate buffer (pH 6.9). One milliliter (1ml) of the  $\alpha$ - amylase solution was added to the sample suspension and incubated at 37°C for 45 mins in a shaking water bath. After incubation, 1ml of 3 – 5 dinitrosalicyclic acid was immediately added and the mixture was heated for 5 mins to inactivate the enzymes. The solutions after cooling were centrifuged for 10 mins at 3000xg, colour absorption of glucose concentration in the supernatant was determined using a spectrophotometer (Cecil 1021, U.K) at a wavelength of 450nm. A standard glucose graph (Y = 18.719x and R2 = 0.9445) of 0.02 mg/l to 0.1mg/l was also prepared and the glucose concentrations of each sample obtained. The results were converted to starch by multiplying the percentage glucose concentration by a factor 0.9.

# 2.8. In-vitro starch digestion rate and prediction of glycemic index (PGI)

The starch digestion rate for the biscuits produced was expressed as the percentage of total starch hydrolysed over time intervals of 30, 60, 90 and 120minutes of incubation. The Hydrolysis Index (HI) was derived from the ratio between the areas under the hydrolysis curve of the various products developed and the reference sample (glucose). From the hydrolysis index obtained, the predicted glycemic index (PGI) was calculated using the equation established by Goni *et al.*, [23].

$$PGI = 39.7 + 0.548$$
HI

$$HI = \frac{AUC \ of \ product}{AUC \ of \ glucose} \times 100$$

Where, AUC = Area under the curve

#### 2.9. Sensory evaluation of wheat-earth-almond biscuits

Sensory evaluation of wheat-earth-almond biscuits were carried out after baking using the method described by Giami and Barber [24]. The sensory attributes included colour, texture, taste and general acceptability were evaluated using a 9 – point hedonic scale with 1 representing the least score (dislike extremely) and 9, the highest score (like extremely) as described by Iwe [25].

#### 2.10. Statistical analysis

Results were expressed as mean values and standard deviation of duplicate determinations. The obtained data were analysed using a one way Analysis of Variance (ANOVA) using Statistical Package for Social Science (SPSS) version 20.0 software 2011 (Soft Inc. Tulsa, USA) to test the level of significance (p<0.05). Duncan New Multiple range Test was used to separate the means where significant differences existed.

### 3. Results

*In vitro* starch hydrolysis rates of biscuits produced from wheat flour and processed tiger nut are shown in Table 2. The hydrolysis of biscuits produced from blends of Wheat flour and processed tiger nut varied with digestion time. Starch digestibility at various time (0-180 minutes) for composite biscuits produced from W-TNA, W-TNB, W-TNF, W-TND and W<sub>f</sub> are within the ranges of; 18.68-59.61%, 15.48-77.72%, 22.14-89.99%, 52.11-74.12% and 63.72-99.45% respectively. Highest starch digestibilities were recorded for biscuits from W<sub>f</sub> (99.45%) and W-TNF (89.99%).

Table 2Percentage (%) starch hy	ydrolysis of biscuits produced fro	om wheat flour and processed earth-almond
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Time(min)	W-TNA	W-TNB	W-TNF	W-TND	W <sub>f</sub>
0	$34.47 \pm 0.04^{a}$	31.73±0.00ª	89.99±0.12°	52.11±0.04 <sup>b</sup>	63.72±0.33 <sup>b</sup>
30	$51.57 \pm 0.00^{b}$	$33.57 \pm 0.02^{a}$	$66.51 \pm 0.02^{bc}$	74.12±0.06 <sup>c</sup>	$99.45 \pm 0.24^{d}$
60	59.61±0.14 <sup>b</sup>	77.72±0.08 <sup>b</sup>	$48.38 \pm 0.23^{a}$	62.60±0.34 <sup>b</sup>	70.11±0.23 <sup>b</sup>
90	$18.68 \pm 0.12^{a}$	$15.48 \pm 0.04^{a}$	39.92±0.19 <sup>b</sup>	70.92±0.87°	77.67±0.29°
120	$28.89 \pm 0.04^{a}$	$40.55 \pm 0.07^{b}$	60.21±0.31 <sup>c</sup>	64.94±0.91 <sup>c</sup>	$81.90 \pm 0.34^{d}$
150	45.86±0.56 <sup>b</sup>	$24.89 \pm 0.84^{a}$	$22.14 \pm 0.15^{a}$	71.42±0.75°	83.34±0.97°
180	$40.10 \pm 0.87^{b}$	20.48±0.00 <sup>a</sup>	$38.93 \pm 0.05^{b}$	53.28±0.94 <sup>c</sup>	79.43±0.33 <sup>d</sup>

Values are means  $\pm$  standard deviation of duplicate samples.Mean values bearing different superscript in the same row differ significantly (p<0.05). \*W-TNA=Wheat -tiger nut air dried,W-TNB = Wheat- tiger nut blanched, W-TNF =Wheat-tiger nut fermented, W-TND =Wheat-tiger nut dehydrated, W<sub>f</sub> = Wheat flour

The Area Under Curve (AUC), Hydrolysis Index (HI) and Predicted Glycemic Index (PGI) are shown in Table 3. The highest hydrolysis index (HI) of 138.85% was observed in W-TNF and 113.79% in W-TND which resulted in higher predicted glycemic index (PGI) of 115.93% for W-TNF and 102.17% for W-TND. The values were significantly different from that obtained for  $W_f$  with HI of 44.67%, PGI 64.22% and W-TNA with HI value of 48.00% and PGI of 66.05%.

Table 3 Calculated hydrolysis index and predicted glycemic index

Samples	AUC	HI	GI
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W-TNA	55.78±4.25 <sup>d</sup>	48.00±3.65 <sup>d</sup>	66.05±2.01 <sup>d</sup>
W-TNB	94.63±2.66 <sup>c</sup>	81.44±2.29°	84.41±1.26 <sup>c</sup>
W-TNF	161.35±4.38ª	138.85±3.77ª	115.93±2.07ª
W-TND	132.23±4.35 <sup>b</sup>	113.79±3.74 <sup>b</sup>	$102.17 \pm 2.05^{b}$
W <sub>f</sub>	$51.91 \pm 0.68$ d	$44.67 \pm 0.55^{d}$	$64.22 \pm 0.30^{d}$

Values are means  $\pm$  standard deviation of duplicate samples. Mean values bearing different superscript in the same column differ significantly (p<0.05).

AUC= area under the curve; HI= hydrolysis index; GI= glycaemic index

*In vitro* protein digestibilities are presented in Figure 1.The protein digestibilities for biscuits from W-TNF (52.53%) and Wf (48.18 %) were significantly (p<0.05) higher than the biscuits from W-TND (39.44 %); but not significantly higher than biscuits from W-TNB (48.16 %) and W-TNA (43.77 %).

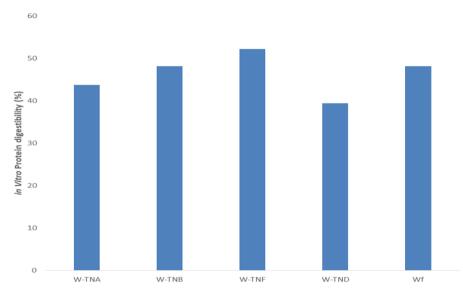


Figure 1 In vitro protein digestibility of composite biscuits from wheat flour and processed tiger nut flours

Mean score for sensory evaluation of biscuit given in Table 4 revealed significant differences ( $p \le 0.05$ ) between treatments for sensory attributes like taste, colour and texture. Sensory rating of biscuit for colour shows that sample W-TND was rated highest (7.90), followed by sample Wf (7.85) and sample W-TNB (7.70) while sample W-TNA was rated lowest (6.12).

Sample	Colour	Taste	Texture	Aroma	Acceptability
W-TNA	6.12± 0.22 a	4.82± 0.18 a	4.61± 0.22 a	5.33± 0.23a	4.45±0.01 a
W-TNB	7.70± 0.09 b	7.34± 0.19 c	6.48± 0.18 c	6.84± 0.18 b	4.86±0.00 a
W-TNF	6.62± 0.13 a	5.83± 0.23 b	5.62± 0.18 b	6.49± 0.22 b	4.34±0.04 a
W-TND	7.90± 0.10 b	5.76± 0.24 b	6.07± 0.20 c	6.84± 0.17 b	4.21±0.03 a
Wf	7.85± 0.13 b	7.42± 0.17 c	6.91± 0.22 c	7.06± 0.22 b	4.89±0.03 a

Table 4 Sensory evaluation of biscuit produced form wheat and processed tiger nut

### 4. Discussion

Starch digestibility involves the disruption of their complex cellular structure. The variation in digestion time may be attributed to the fact that the heat treatment applied in some cases may not be sufficient to fully gelatinize starch granules thus making it resistant for  $\alpha$ -amylase digestion. The digestibilities observed with the composite biscuits agree

with the report of Kiin-Kabari and Giami, [26]. Starch digestibility for wheat flour biscuit was higher than the composite biscuits. Increase in total starch hydrolysed inbiscuit from frementation blend (W-TNF) could be attributed to the fact that fermentation activates starch hydrolyzing enzymes such as alpha-amylase and maltase which degrade starch into maltodextrins and simple sugars respectively [10, 13].

Glycemic index gives an idea about how fast the body converts carbohydrate in a food into glucose. High glycemic index reported in this study agrees with the report of Kiin-Kabari and Giami, [26]. The lowest HI and PGI were recorded for wheat flour. The result showed that the lower HI subsequently led to the reduced predicted glycemic index (PGI). Wheat flour has been reported to have glycemic index of 54 [26]. This value differs slightly from what the present study reported. This difference may be attributed to factors such as processing methods, product compositions such as proteins [27] and fat [28]. According to Foster-Powell *et al.*, [29] glycemic index of foods are classified as follows: low (GI<55), medium (GI 56-69) and high (GI>70). This may be that wheat contains carbohydrates whose bulk may consist of non-starch polysaccharides with a low GI. Studies have shown that a low GI diet not only improves certain metabolic consequence of insulin resistance but also reduces insulin resistances [30]. In addition to improvement in glucose and lipid metabolism, there are indications of improvements in fibrinolytic activity suggesting beneficial roles in the management of diabetes and cardiovascular diseases [31]. Although, predicted glycemic index for white bread had been reported to be 94.61% [32] which compares with the present study. The highest HI with a corresponding GI was observed in the fermented composite biscuit. Fermentation has dual effect on glycemic index (GI). Some studies have reported increased [33] while others have reported decreased [34] GI after consumption of fermented foods. The low GI of fermented food has been attributed to the short-chain organic acids produced during fermentation such as lactic acid, acetic acid, and propionic acid [35]. While the increase was likely due to ease of digestion and absorption of glucose; which is as a result of degradation of fiber by microorganisms during fermentation. However, there is still gap on scientific knowledge on the increase in GI due to fermented cereal and legumes products.

*In vitro* protein digestibility has the potential to give useful measures of *in vivo* protein and amino acid digestibility for humans. In this study, *In vitro* protein digestibility is high with fermented product because during fermentation there is an increase in the release of proteolytic enzymes that can not only degrade the antinutrient which complex with proteins but also brake down complex proteins thereby liberating more peptides and amino acids [36]. The protein digestibility level recorded in this study was lower to the digestibility reported by Kiin-Kabari and Giami [26]. Possible reason for the low digestibility recorded for wheat flour biscuit may be due to the interraction of various components during formulation.

The result for sensory evaluation shows that wheat flour sample was most preferred by the panelist in terms of taste, aroma, acceptability and texture; while W-TND was preferred in terms of colour. Processing of tiger nut did not affect the sensory evaluation of the composite biscuits. This may be attributed to the ratio of the wheat (80%) and the various processed earth-almond (20%) flours. Colour is a very important parameter in judging properly baked biscuits that not only reflect the suitable raw material used for the preparation but also provides information about the formation and quality of the product [37]. Darker colour was observed with composite flour biscuits compared to the control. The change in colour may be due to the non-enzymatic reaction between reducing sugar molecules and additional protein in wheat flour and earth-almond flour respectively. The observed darker colour of biscuits produced from composite flours has been earlier reported by Neha and Ramesh [38]. Evaluation of taste showed that W-TNA had the least value (4.82). The decline in taste could be ascribed to the effect of non-enzymatic browning reactions resulting from the reaction between amino acids and free sugars causing slight bitterness during baking [39].

In terms of aroma, composite flour biscuits were rated lower compared to the control. A different observation was noted by supplementing wheat flour with mustard protein concentrate and cotton seed flour in the production of cookies [40]. It is important to note that modification in production process and slight change in the physical, chemical properties of ingredients in blended foods may also affect the sensorial quality.

# 5. Conclusion

Results from this research have shown that when processed tiger nut was used to substitute with wheat flour in the production of biscuits, digestibilities improved although improvement was dependent on processing. HI and PGI of W-TNA were low and alike to Wf values; this is an indication that W-TNA can serve as food source for obesity and diabetic patients. Biscuit prepared fom W-TNB, compared favourably in physical characteristics with the control (Wf). Sensory attributes of the biscuits were not adversely affected since there was general acceptability of all the biscuits.

## **Compliance with ethical standards**

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### Disclosure of conflict of interest

All authors declare that they have no conflict of interest.

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