

RESEARCH

Open Access



# Nutritional properties of wheat flour supplemented with modified tacca (*Tacca involuocrata*) flour for production of healthy biscuits

Emmanuel Omotayo Ojewumi, Olufunmilayo Sade Omoba and Olugbenga Olufemi Awolu 

## Abstract

**Background:** Tacca flour obtained from tacca tubers, an underutilized crop rich in carbohydrate and phytochemicals, was subjected to physical, chemical and enzymatic modifications which were supplemented into wheat flour for the production of healthy wheat–tacca flour biscuits. While the proximate composition and the antioxidants properties of the native and modified samples were carried out in order to produce modified samples with the best antioxidant properties for subsequent preparation of biscuits. The haematological, in vitro antioxidative and lipid peroxidative potentials of the biscuit samples were evaluated.

**Results:** The results show that while native tacca flour had the best proximate composition, the flour sample from enzymatic modification had the best antioxidants properties. The biscuit produced from wheat–tacca flour at varying compositions of tacca flour ranging from 5 to 20% incorporations (TEB5%, TEB10%, TEB15 and TEB20%) showed that all the samples substituted with modified tacca flour had better haematological properties, in vitro antioxidative properties and lipid peroxidative properties compared to the 100% wheat biscuit. Specifically, the sample TEB20% (20% tacca flour incorporation) had the best nutritional qualities. The toxicological studies showed that the samples with tacca flour incorporation are better than 100% wheat flour biscuit and basal diet.

**Conclusion:** Tacca flour would successfully supplement wheat flour in the production of nutritionally rich and toxicologically safe biscuit with over 70% overall sensory acceptability.

**Keywords:** Antioxidants, Biscuits, Enzymatic modifications, Flours, Haematological properties, Tacca

## Background

The current trend of health challenges which resulted from life style or hereditary has elicited research into development of functional foods derived from food crops (Awolu et al. 2015; Omoba et al. 2020). Baked foods from flours are one of the most consumed, and hence, the focus is to manipulate wheat-based baked foods in order to enrich them in dietary fibre, protein, ash content and bioactive compounds

(Awolu et al. 2015, 2017). These specially formulated foods called nutraceuticals are gaining attentions worldwide. (Awolu et al. 2017). Wheat-based baked products, though widely consumed, have been found to be deficient in several nutrients in addition to development of celiac diseases for consumers that are gluten intolerant (Franco et al. 2020).

Tacca is a plant in Nigeria that grow naturally under the shade of thick forest and sometimes found growing in heap of decayed organic matter (Adefegha et al. 2014; Autsavak- itipong et al. 2015). However, tacca flour and starch usage are limited due to the absence of gluten. Nevertheless, its low fat and sodium contents make it desirable and suitable

\*Correspondence: [ooawolu@futa.edu.ng](mailto:ooawolu@futa.edu.ng)

Department of Food Science and Technology, Federal University of Technology Akure, Akure, Nigeria

raw material for food formulations (biscuits and bread) for patient with gluten allergies and hypertension. Hence, the need to improve the functionality through modification would greatly enhance its utilization as functional ingredients for domestic and industrial uses.

Composite flours formulation may or may not include wheat flours in addition to some cereals, legumes and tubers flours (Omoba et al. 2020; Awolu 2017). Several baked products have been developed from these composite flours. Advantages of baked products from the composite flours are their nutritional adequacy and presence of bioactive compounds from the gluten-free raw materials.

Tubers with nutraceutical potentials abound worldwide. Some of the tubers that are widely available in Nigeria are *Tacca spp.*, yam, potato and cassava. *Tacca* is naturally grown in Nigeria and in some Pacific Island Nations (Ukpabi et al. 2009). Important compounds isolated from *Tacca* widely studied include steroidal saponins, steroidal glycosides (Arifanti et al. 2020), taccalonolides (*Plantaginea*, *leontopetaloides* and *integrifolia*) (Adebiyi et al. 2011; Nwokocha 2017). Taccalonolides have been reported with antitumor activities (Peng et al. 2010).

Since fresh *tacca* tubers are highly perishable due to its high moisture contents, it is processed into flour in order to enhance its shelf life (Nduoyang et al. 2015). *Tacca* tuber made into flour might be a potential replacement for wheat flour in the production of baked food products.

Flour modification is meant to enhance the functional, physicochemical and rheological characteristics of native flour (Olorunfemi et al. 2021; Awolu et al. 2020). Modifications have actually improved several flour qualities especially when used with gluten-free flours. Modifications can be physical, chemical and enzymatic (Olorunfemi et al. 2021; Awolu and Olofinlae 2016). While starch modifications are very common, modifications of flours are recently being given considerations (Olorunfemi et al. 2021).

This study was carried out to determine the effects of various modification methods (physical, acetylated and enzymatic) on the functional, physicochemical and antioxidant properties of the composited wheat–*tacca* biscuits in order to arrive at the biscuit sample with the best nutraceutical potential.

## Methods

### Collection and authentication of materials

*Tacca* tuber used in this study was obtained from Eruwa in Oyo state and authenticated at the Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo State, Nigeria.

### Purchase of experimental albino rats

A total of 35 Wistar rats of about 12 weeks old, weighing between 120 and 150 g each, were obtained from Animal

House Biochemistry Department, Federal University of Technology, Akure, Ondo State, for this experiment.

### Preparation of *tacca* flour

*Tacca* tubers were washed, peeled and cut into about 2 cm slices and dried in an oven at 40 °C for 8 h. The dried *tacca* chips were pulverized into flour and stored in sealed plastic container stored at 4 °C for subsequent use (Nduoyang et al. 2015).

### Preparation of *tacca* acetylated flour

Acetylation of *Tacca* flours was carried out according to the modified method of Awolu et al. 2020. About 2 g of *tacca* flour was mixed with about 5 ml distilled water. Exactly 1 g of sodium hydroxide pellets was added and mixed thoroughly with 5 ml acetic anhydride and kept at 40 °C for 1 h. After about 3 to 4 times washings with water and alcohol, the sample (with pH 7.0) was further dried at 40 °C to 8% moisture.

### Preparation of enzyme modified flour

Glucoamylase (from *Rhizopus* mould, 21,100 units/g solid) at (5%) (20 U mg<sup>-1</sup> flour) was added to 1 g of the flour sample in acetate buffer (pH 4.6, 6 ml). The reaction mixture was incubated at 60 °C for 90 min. The incubated samples were centrifuged, and the sediment after centrifugation was collected, washed with alcohol about 3 to 4 times. Samples were later dried and stored at 12 °C for further analysis. (Kaur et al. 2012).

### Physically modified flour

*Tacca* flour was modified by the method of heat treatment. Flour sample was heated at the temperature of 100 °C for about 14 h and cooled and kept in zip lock polybag for future use (Awolu and Olofinlae 2016).

### Proximate composition of *tacca* flour and wheat–*tacca* biscuits

The proximate composition was carried out using official Methods of Analysis by Association of Analytical Chemists (AOAC 2010).

### Determination of antioxidant properties of samples

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activities of the *tacca* flour were determined using the method described by Marinova and Batchvarov (2011) with slight modification. The hydroxyl radical scavenging assay was based on a method described by Girgih et al. (2013). The chelating activity of the extract on Fe<sup>2+</sup> as well as the scavenging activity of sample against 2, 2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) was determined according to the

method described (Yadav et al. 2020). The total phenol content (TPC) was determined by Folin–Ciocalteu assay (Sánchez-Rangel et al. 2013).

Sample with best proximate and antioxidants properties was used in the production of biscuits, which was used for the preparation of the experimental diet.

#### Animals

A total of thirty-five male Wistar albino (*Rattus norvegicus*) rats (120–150 g) were acquired and kept in the Laboratory Animal House of the Biochemistry Department, Federal University of Technology, Akure, Nigeria. The animals were randomly apportioned into 6 groups, of 7 rats per group. Rats were housed individually in metabolic cages under laboratory conditions of temperature ( $23 \pm 2$  °C) and relative humidity ( $45 \pm 5$ %) and fed ad libitum with the control diet for 14 days of acclimatization prior to the experiment. For the 28 days experimental period, 100 g of feed was administered to rats per group per day.

#### Collection of blood samples

At the end of 28 days, the animals were weighed and killed by suffocation with diethyl ether after an overnight fasting. Plasma was collected by cardiac puncture, stored in the heparinized bottle and frozen at  $-20$  °C for biochemical assay. The experimental protocol was approved by the Institutional Ethics Approval Committee for Laboratory Animals (FUTA/SAAT/2020/016).

#### Haematological and biochemical assay

The haematological indices, specifically, pack cell volume (PCV), haemoglobin concentration (Hbc), white blood cells (WBC), red blood cells (RBC), neutrophil (NEU) and lymphocytes (LYM) were determined using methods described by Bain et al. (2016). Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentrated (MCHC) were calculated. Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphate (ALP) were determined using the method outlined by Lab test Diagnostica S.A. (Lagoa Santa, Minas Gerais, Brazil).

#### In vivo enzymatic antioxidant

Superoxide dismutase (SOD) activity of the tissue homogenates, activity of catalase in the tissue homogenates and thiobarbituric acid-reactive substances (TBARS) measurements were carried out according to the methods adopted by Alvariz et al. (2019). Glutathione peroxidase (GPx), glutathione S-transferase (GST) and reduced glutathione (GSH) activities in brain homogenate were determined using the method of Rizzeti et al. (2013).

#### Diet

The processed diets used for the animal study are as follows:

- Group A—5% tacca flour + 95% wheat flour.
- Group B—10% tacca flour + 90% wheat flour.
- Group C—15% tacca flour + 85% wheat flour.
- Group D—20% tacca flour + 80% wheat flour.
- Group E—100% wheat flour.

#### Production of wheat–tacca biscuit

Wheat–tacca biscuit was produced according to the modified method of Olagunju et al. (2020). The proportion of ingredients used consisted of composite flour (200 g), margarine (0.3%), salt (0.75%) and baking powder (1.5%). All dry ingredients (flour, salt and baking powder) were weighed and mixed in a bowl to obtain an even mixture after which margarine was added and mixed followed by the egg and the batter was kneaded and rolled out. It was then cut (5-mm diameter), arranged on a baking tray and baked for 10 min at 180 °C in a preheated oven. Baked biscuits were cooled at room temperature and packaged in polyethylene bags for further analysis.

#### Sensory evaluation of biscuit

The sensory evaluation of biscuit samples was carried by fifty untrained panellists in order to determine the consumer acceptance and preferences. The colour, aroma, taste, texture and overall acceptability were evaluated using a nine-point Hedonic scale where 1 represents “extremely dislike” and 9 “extremely like”, respectively. Means and standard errors of the mean (SEM) of replicate scores were determined and subjected to analysis of variance (ANOVA) using the statistical package for social statistics (SPSS version 16.0) as well as means using the Duncan’s new multiple range test.

#### Statistical analysis

Data were analysed by analysis of variance (ANOVA) using SPSS version 16.0 software (SPSS Inc., Chicago, IL, USA). All analyses were carried out in triplicate. The results were presented as mean  $\pm$  standard deviation (SD) of 3 determinations. The means were separated using Tukey’s test. Level of significance was set at  $P < 0.05$  (Omoba et al. 2020).

#### Results

##### Proximate composition (g/100 g) of modified Tacca flour

The proximate composition of the native and modified tacca flour is presented in Table 1. The moisture content of all the samples ranged from 7.34 to 10.14%; total ash from 1.19 to 2.59%; crude protein from 5.56 to 14.08%; crude fibre from 1.23 to 2.91%; and carbohydrate content

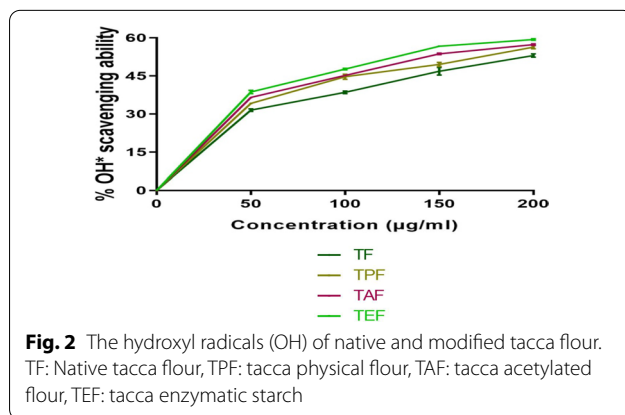
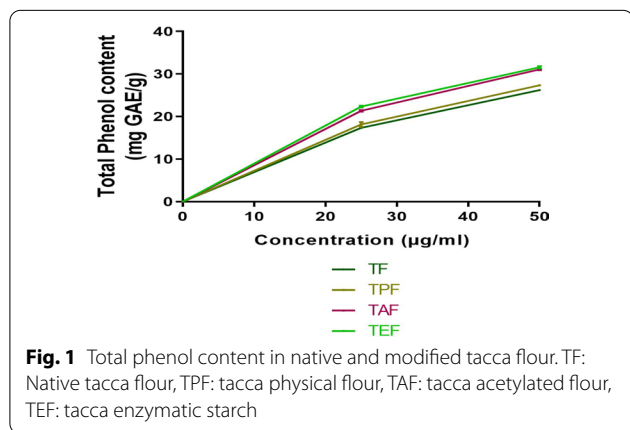
**Table 1** Proximate composition and energy of tacca native and modified flour (dry weight basis)

Samples	Moisture (g/100 g)	Total ash (g/100 g)	Crude protein (g/100 g)	Crude fat (g/100 g)	Crude fibre (g/100 g)	Carbohydrate (g/100 g)	Energy (Kcal/100 g)
TF	8.46 ± 0.02 <sup>a</sup>	2.59 ± 0.01 <sup>a</sup>	5.56 ± 0.06 <sup>d</sup>	3.38 ± 0.01 <sup>a</sup>	2.91 ± 0.01 <sup>a</sup>	82.10 ± 0.14 <sup>a</sup>	381.06 ± 3.13 <sup>a</sup>
TPF	7.34 ± 0.01 <sup>d</sup>	1.91 ± 0.01 <sup>b</sup>	11.38 ± 0.01 <sup>c</sup>	2.83 ± 0.05 <sup>b</sup>	2.45 ± 0.01 <sup>b</sup>	74.09 ± 0.70 <sup>b</sup>	367.35 ± 3.01 <sup>b</sup>
TAF	10.12 ± 0.01 <sup>c</sup>	1.22 ± 0.01 <sup>c</sup>	12.80 ± 0.01 <sup>b</sup>	2.69 ± 0.01 <sup>c</sup>	2.24 ± 0.03 <sup>c</sup>	68.93 ± 0.01 <sup>c</sup>	351.13 ± 1.90 <sup>c</sup>
TEF	12.87 ± 0.01 <sup>b</sup>	1.19 ± 0.02 <sup>c</sup>	14.08 ± 0.05 <sup>a</sup>	1.77 ± 0.01 <sup>d</sup>	1.23 ± 0.01 <sup>d</sup>	68.86 ± 0.01 <sup>d</sup>	347.69 ± 2.04 <sup>d</sup>
*Ref	< 10.00	> 3.00	> 14.00	< 10–25	> 5	64.00	400–425

Means (± SEM) with different alphabetical superscripts in the same column are significantly different at  $P < 0.05$

TF Native tacca flour, TPF Tacca physical flour, TAF Tacca acetylated flour, and TEF Tacca enzymatic starch

\*Ref - stands for standard values of the components



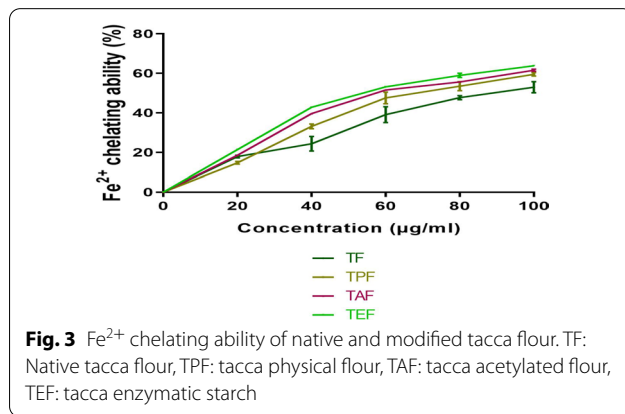
from 68.86 to 82.10%. The energy values ranged from 347.69 to 381.06 kcal/ 100 g.

**Antioxidant activities of tacca (native and modified flour)**

The antioxidant activities of Tacca (native and modified flour) samples are shown in Figs. 1, 2, 3, 4, and 5. The hydroxyl radical (\*OH) ranged from 42.46% in TF–50.58% in TEF; ABTS\* radical scavenging activities (42.46% in TF–50.58% in TEF), Fe<sup>2+</sup> chelation (42.46% in TF–50.58% in TEF), DPPH\* radical scavenging activities (22.50 mmol TEAC/ g in TF–59.88 mmol TEAC/ g in TEF).

**Haematological parameters of animal fed with wheat–tacca biscuits**

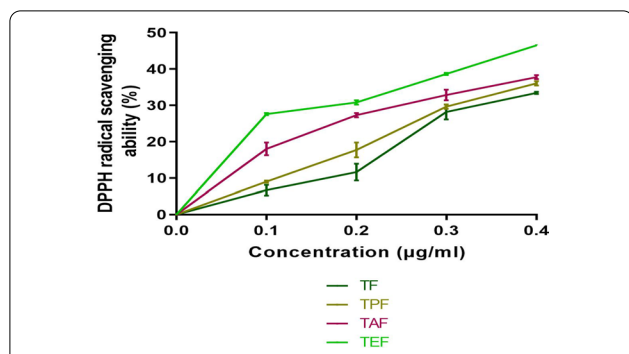
The results of the haematological studies of the basal diet, 100% wheat biscuit and the biscuit produced from composite flours consisting enzymatic modified flours and wheat flours (TEB) are presented in Table 2. The higher the tacca flour incorporation in the composite flour, the



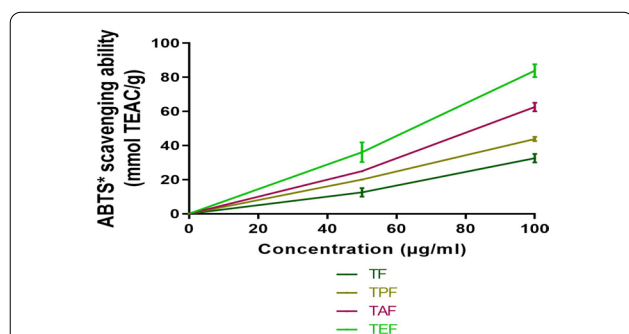
higher the haematological values (TEB20% > TEB15 > TEB10% > TEB5% %).

**Effects of the biscuit samples on liver functions**

The results of the effect of alanine aminotransferase (ALT), alkaline phosphate (ALP) and aspartate aminotransferase (AST) on the enzymatic modified tacca–wheat biscuit in albino Wistar rats of the liver are shown in Figs. 6, 7 and 8. The properties are cytosolic enzymes found in the liver and heart, and they are biomarkers for liver functionality.



**Fig. 4** DPPH radical scavenging ability of native and modified tacca flour. TF: Native tacca flour, TPF: tacca physical flour, TAF: tacca acetylated flour, TEF: tacca enzymatic starch



**Fig. 5** ABTS\* scavenging ability of native and modified tacca flour. TF: Native tacca flour, TPF: tacca physical flour, TAF: tacca acetylated flour, TEF: tacca enzymatic starch

**Effects of enzymatic tacca–wheat biscuit on kidney function**

The effect of enzymatic tacca–wheat biscuit on kidney functions as defined by the creatinine and uric acid levels is shown in Figs. 9 and 10, respectively.

**Effects of enzymatic tacca–wheat biscuit on in vivo enzymatic antioxidant**

The results of the effect of the samples on the *in vivo* antioxidant enzymes, namely catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione S-transferase (GST) and reduced glutathione (GSH), are shown in Figs. 11, 12, 13, 14 and 15.

**Lipid peroxidation activities of the sample biscuits**

Effect of enzymatic tacca–wheat biscuit on albino Wistar rats TBARS level is shown in Fig. 16.

**Sensory attributes of enzymatic wheat–tacca biscuit**

Biscuits samples produced with 5%, 10%, 15% and 20% of wheat–tacca flour are shown in Fig. 17, while the sensory attributes of the tacca–wheat biscuit are presented in Table 3.

**Discussion**

The results of the proximate composition of the native and modified tacca flour as presented in Table 1 indicated that the moisture content of all the samples ranged from 7.34 to 12.87%, which is similar to previous work of Onabanjo and Ighere (2014) who reported moisture content

**Table 2** Haematological parameter of rats fed with tacca enzymatic biscuit

Parameters	Basal	100% Wheat	TEB 5%	TEB 10%	TEB 15%	TEB 20%
PVC (%)	17.00 ± 0.52 <sup>c</sup>	20.02 ± 0.13 <sup>b</sup>	21.04 ± 0.16 <sup>ab</sup>	22.05 ± 0.32 <sup>a</sup>	22.10 ± 0.90 <sup>a</sup>	22.20 ± 0.34 <sup>a</sup>
Hb (g/dl)	8.57 ± 0.22 <sup>b</sup>	9.10 ± 0.31 <sup>b</sup>	9.70 ± 0.09 <sup>ab</sup>	9.50 ± 0.10 <sup>a</sup>	9.50 ± 0.30 <sup>a</sup>	9.60 ± 0.50 <sup>a</sup>
WBC (× 10 <sup>3</sup> mm <sup>-3</sup> )	6.45 ± 0.31 <sup>d</sup>	6.70 ± 0.41 <sup>d</sup>	8.40 ± 0.32 <sup>b</sup>	9.90 ± 0.43 <sup>a</sup>	7.70 ± 0.31 <sup>c</sup>	9.50 ± 0.30 <sup>a</sup>
RBC (× 10 <sup>3</sup> mm <sup>-3</sup> )	3.55 ± 0.01 <sup>d</sup>	4.50 ± 0.02 <sup>c</sup>	5.00 ± 0.11 <sup>b</sup>	5.30 ± 0.17 <sup>a</sup>	5.45 ± 0.55 <sup>a</sup>	5.45 ± 0.51 <sup>a</sup>
MCHC (g/dl)	26.40 ± 0.30 <sup>d</sup>	33.30 ± 0.11 <sup>b</sup>	33.30 ± 0.21 <sup>b</sup>	34.20 ± 0.45 <sup>a</sup>	31.10 ± 0.16 <sup>c</sup>	33.10 ± 0.13 <sup>b</sup>
MCH (pg)	19.20 ± 0.40 <sup>d</sup>	29.80 ± 0.20 <sup>c</sup>	30.00 ± 0.40 <sup>c</sup>	38.40 ± 0.50 <sup>a</sup>	33.90 ± 0.20 <sup>b</sup>	34.30 ± 0.10 <sup>b</sup>
MCV (fl)	17.20 ± 0.11 <sup>d</sup>	89.30 ± 0.11 <sup>c</sup>	90.00 ± 0.20 <sup>b</sup>	98.50 ± 0.30 <sup>a</sup>	91.00 ± 0.50 <sup>b</sup>	89.90 ± 0.50 <sup>c</sup>
Neutrophils (%)	14.00 ± 0.03 <sup>f</sup>	20.00 ± 0.21 <sup>e</sup>	32.00 ± 0.41 <sup>d</sup>	41.20 ± 0.48 <sup>c</sup>	45.0 ± 0.22 <sup>b</sup>	52.00 ± 0.41 <sup>a</sup>
Lymphocytes	60.00 ± 0.20 <sup>e</sup>	60.00 ± 0.47 <sup>e</sup>	65.00 ± 0.46 <sup>d</sup>	70.40 ± 0.15 <sup>c</sup>	75.00 ± 0.40 <sup>b</sup>	77.00 ± 1.25 <sup>a</sup>
Monocytes (%)	0.00 ± 0.00 <sup>d</sup>	1.00 ± 0.00 <sup>c</sup>	2.00 ± 0.00 <sup>b</sup>	3.20 ± 0.00 <sup>a</sup>	2.00 ± 0.00 <sup>b</sup>	3.00 ± 0.00 <sup>a</sup>
Eosinophils (%)	0.00 ± 0.00 <sup>b</sup>	0.00 ± 0.00 <sup>b</sup>	0.00 ± 0.00 <sup>b</sup>	1.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>b</sup>	0.00 ± 0.00 <sup>b</sup>
Basophils (%)	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>

Means (± SEM) with different alphabetical superscripts in the same row are significantly different at *P* < 0.05

100% Wheat: Rats fed with 100% wheat flour biscuit

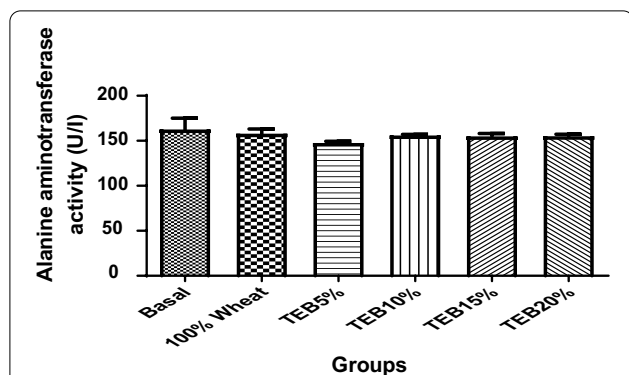
TEB5%: Rats fed with 5% enzymatic tacca flour + 95% wheat flour biscuit

TEB10%: Rats fed with 10% enzymatic tacca flour + 90% wheat flour biscuit

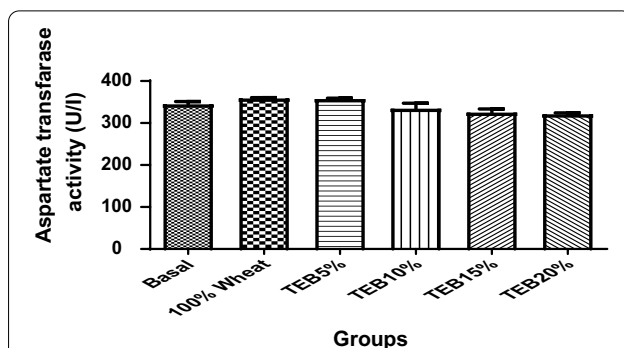
TEB15%: Rats fed with 15% enzymatic tacca flour + 85% wheat flour biscuit

TEB20%: Rats fed with 20% enzymatic tacca flour + 80% wheat flour biscuit

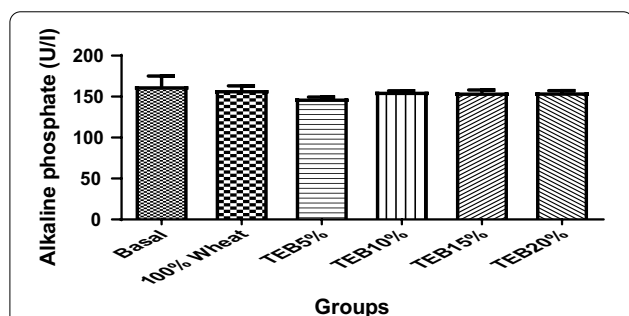




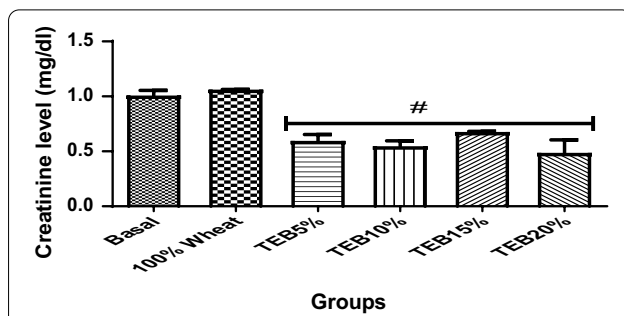
**Fig. 6** Effect of enzymatic tacca–wheat biscuit on albino Wistar rats by alanine aminotransferase. 100% Wheat: Rats fed with 100% wheat flour biscuit, TEB5%: Rats fed with 5% enzymatic tacca flour + 95% wheat flour biscuit, TEB10%: Rats fed with 10% enzymatic tacca flour + 90% wheat flour biscuit, TEB15%: Rats fed with 15% enzymatic tacca flour + 85% wheat flour biscuit, TEB20%: Rats fed with 20% enzymatic tacca flour + 80% wheat flour biscuit



**Fig. 8** Effect of enzymatic tacca–wheat biscuit on albino Wistar rats by aspartate transferase (AST). 100% Wheat: Rats fed with 100% wheat flour biscuit, TEB5%: Rats fed with 5% enzymatic tacca flour + 95% wheat flour biscuit, TEB10%: Rats fed with 10% enzymatic tacca flour + 90% wheat flour biscuit, TEB15%: Rats fed with 15% enzymatic tacca flour + 85% wheat flour biscuit, TEB20%: Rats fed with 20% enzymatic tacca flour + 80% wheat flour biscuit



**Fig. 7** Effect of enzymatic tacca–wheat biscuit on albino Wistar rats by alkaline phosphate (ALP). 100% Wheat: Rats fed with 100% wheat flour biscuit, TEB5%: Rats fed with 5% enzymatic tacca flour + 95% wheat flour biscuit, TEB10%: Rats fed with 10% enzymatic tacca flour + 90% wheat flour biscuit, TEB15%: Rats fed with 15% enzymatic tacca flour + 85% wheat flour biscuit, TEB20%: Rats fed with 20% enzymatic tacca flour + 80% wheat flour biscuit

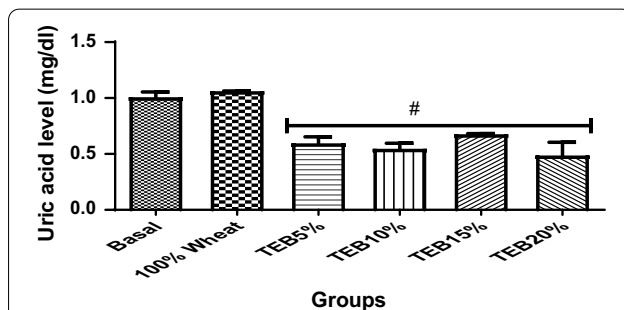


**Fig. 9** Effect of enzymatic tacca–wheat biscuit on albino Wistar rats by creatinine. Basal: Rats fed with animal chow. 100% Wheat: Rats fed with 100% wheat flour biscuit, TEB5%: Rats fed with 5% enzymatic tacca flour + 95% wheat flour biscuit, TEB10%: Rats fed with 10% enzymatic tacca flour + 90% wheat flour biscuit, TEB15%: Rats fed with 15% enzymatic tacca flour + 85% wheat flour biscuit, TEB20%: Rats fed with 20% enzymatic tacca flour + 80% wheat flour biscuit

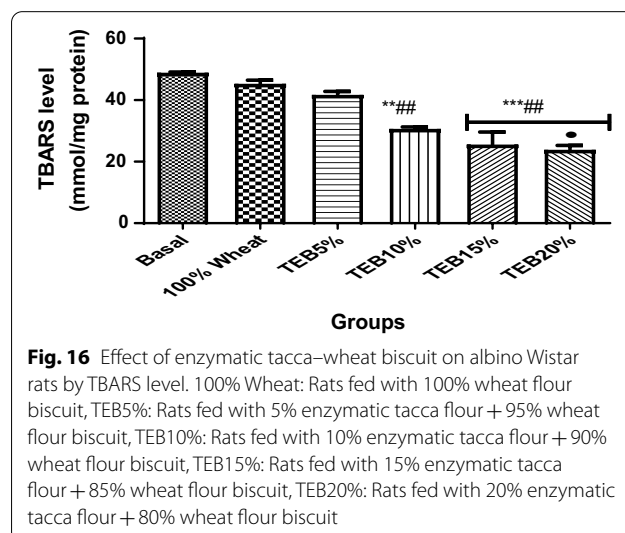
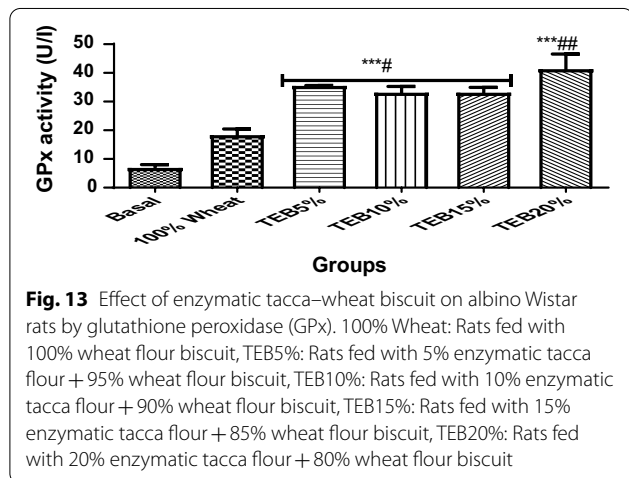
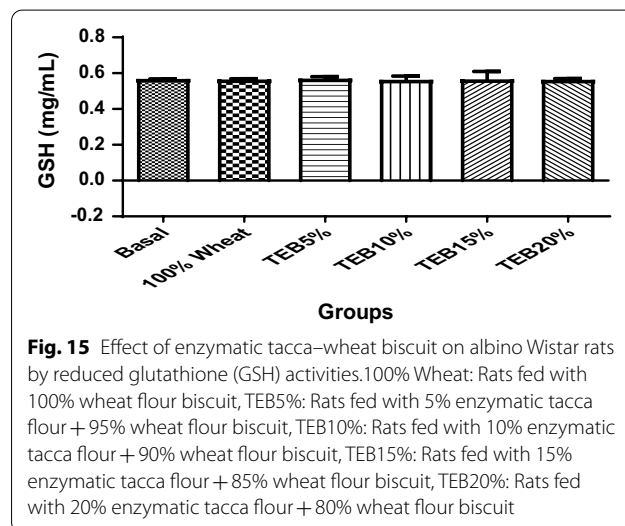
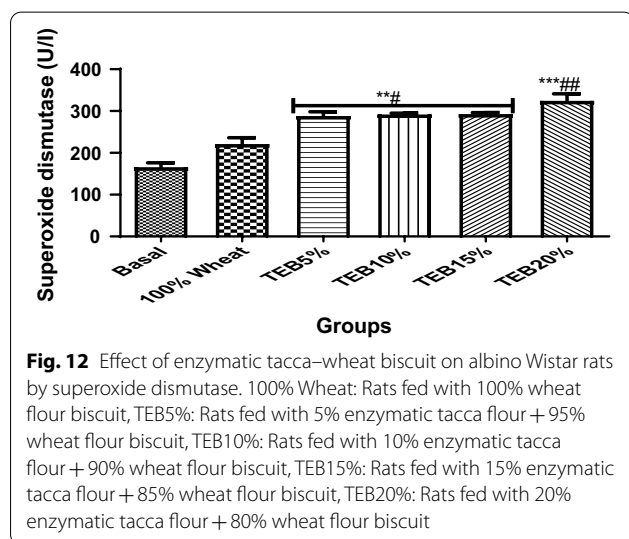
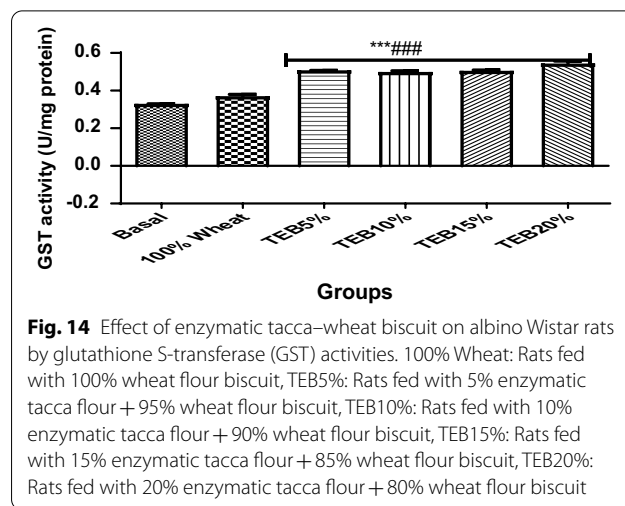
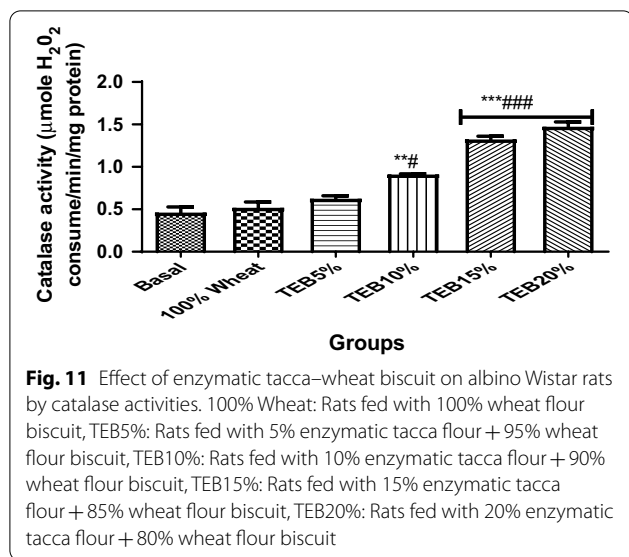
of wheat–sweet potato flour biscuits to be between 9.34 and 12.71%. The slight differences reported in the moisture content might not be unconnected to the processing conditions like modification, washing and drying (Nduoyang et al. 2015). According to DeMan et al. (1999), flours with moisture content less than 14% can resist microbial growth and hence enhance storage stability.

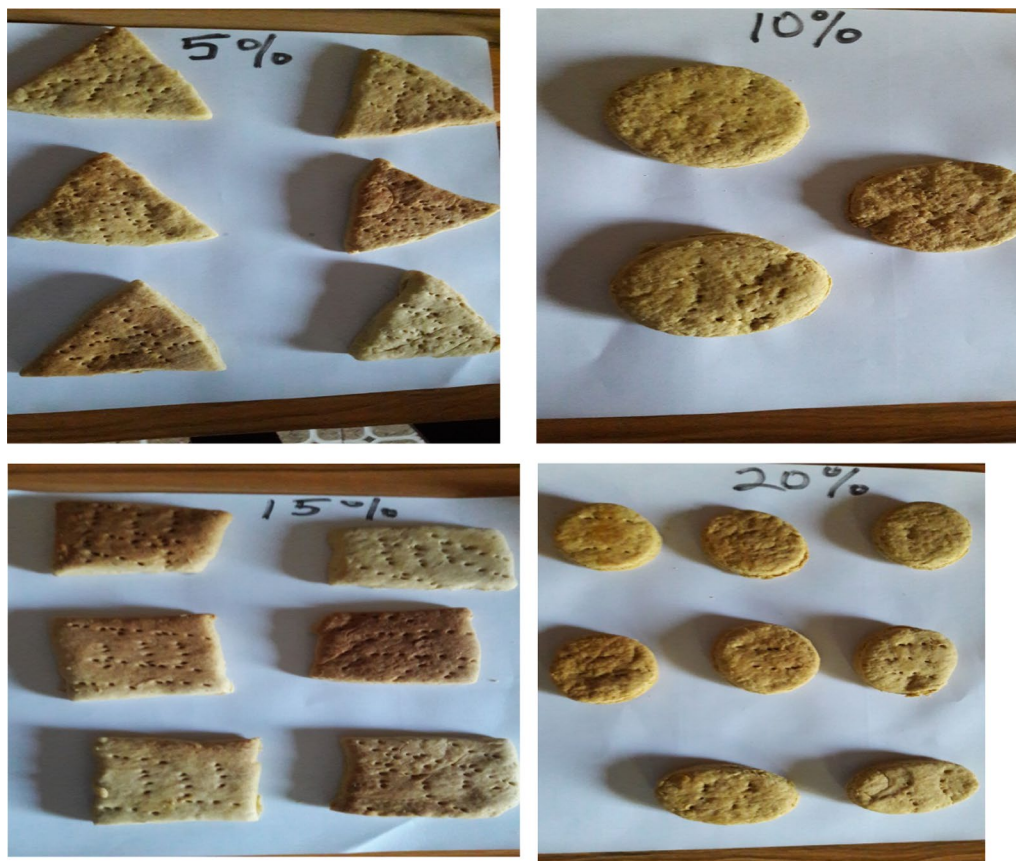
The ash contents of the native sample were significantly reduced by modifications. The values were, however, higher than 0.45% reported for wheat flour (Peng et al. 2010). Enzymatically modified flour recorded the least ash content.

The crude protein for native, physically, acetylated and enzymatically modified tacca flour was 5.56, 11.38, 12.80



**Fig. 10** Effects of enzymatic tacca–wheat biscuit on albino Wistar rats by uric acid. Basal: Rats fed with animal chow. 100% Wheat: Rats fed with 100% wheat flour biscuit, TEB5%: Rats fed with 5% enzymatic tacca flour + 95% wheat flour biscuit, TEB10%: Rats fed with 10% enzymatic tacca flour + 90% wheat flour biscuit, TEB15%: Rats fed with 15% enzymatic tacca flour + 85% wheat flour biscuit, TEB20%: Rats fed with 20% enzymatic tacca flour + 80% wheat flour biscuit





**Fig. 17** Biscuits samples produced with 5%, 10%, 15% and 20% of wheat–tacca flour, respectively. TEB (5%): Rats fed with 5% enzymatic tacca flour + 95% wheat flour biscuit, TEB (10%): Rats fed with 10% enzymatic tacca flour + 90% wheat flour biscuit, TEB (15%): Rats fed with 15% enzymatic tacca flour + 85% wheat flour biscuit, TEB (20%): Rats fed with 20% enzymatic tacca flour + 80% wheat flour biscuit

**Table 3** Sensory attributes of tacca–wheat biscuit

Sample	Appearance	Aroma	Texture	Crispiness	Taste	Overall acceptability
100%Wheat	7.24 ± 1.33 <sup>a</sup>	6.40 ± 1.63 <sup>b</sup>	6.40 ± 1.43 <sup>b</sup>	6.90 ± 0.12 <sup>a</sup>	7.30 ± 1.96 <sup>a</sup>	7.20 ± 0.89 <sup>a</sup>
TEB5%	6.14 ± 1.04 <sup>c</sup>	6.60 ± 1.53 <sup>a</sup>	6.70 ± 1.53 <sup>a</sup>	6.70 ± 0.42 <sup>b</sup>	7.22 ± 0.13 <sup>b</sup>	7.11 ± 0.22 <sup>b</sup>
TEB10%	6.19 ± 1.00 <sup>b</sup>	6.50 ± 1.03 <sup>b</sup>	6.50 ± 1.07 <sup>c</sup>	6.90 ± 0.53 <sup>b</sup>	7.18 ± 1.32 <sup>b</sup>	7.10 ± 0.61 <sup>b</sup>
TEB15%	6.14 ± 1.06 <sup>c</sup>	6.45 ± 1.11 <sup>b</sup>	6.40 ± 1.56 <sup>d</sup>	6.79 ± 0.42 <sup>b</sup>	7.22 ± 1.36 <sup>b</sup>	7.14 ± 0.77 <sup>b</sup>
TEB20%	6.21 ± 1.39 <sup>b</sup>	6.49 ± 1.95 <sup>b</sup>	6.50 ± 1.69 <sup>d</sup>	6.75 ± 0.16 <sup>b</sup>	7.20 ± 1.17 <sup>b</sup>	7.15 ± 0.89 <sup>b</sup>

Means with different alphabetical superscripts in the same column are significantly different at  $P < 0.05$

100% Wheat: 100% wheat flour biscuit

TEB5%: 5% enzymatic tacca flour + 95% wheat flour biscuit

TEB10%: 10% enzymatic tacca flour + 90% wheat flour biscuit

TEB15%: 15% enzymatic tacca flour + 85% wheat flour biscuit

TEB20%: 20% enzymatic tacca flour + 80% wheat flour biscuit

and 14.08%, respectively. Crude protein values in the native flour were significantly lower than 15.1% reported for wheat flour (Ade-Omowaye et al. 2008). However, crude protein in modified flour sample was significantly higher ( $p > 0.005$ ) than that of native samples especially

in enzymatically modified sample which had over 200% increase. Processing increases the activity of glucoamylase on the cell wall matrix, thereby setting loose some bound protein molecules (Alrahmany and Tsopmo 2012), which could lead to the increased protein contents



observed in the modified samples. Also, modification techniques involving the use of glucoamylase might lead to the breaking down of carbohydrates which led to an increase in the percentage of protein content.

The crude fat and fibre contents of the native samples were significantly reduced by modifications; physically modified samples, however, retained crude fibre and fat contents most, while enzymatic modified samples had the least contents. The values of crude fat in native flour were higher than 1.3% reported for wheat flour according to Ade-Omowaye et al. (2008).

The antioxidant activities of Tacca (native and modified flour) samples shown in Figs. 1, 2, 3, 4 and 5 indicated that the hydroxyl radical ( $\cdot\text{OH}$ ) ranged from 42.46% in TF–50.58% in TEF; ABTS\* radical scavenging activities (42.46% in TF–50.58% in TEF),  $\text{Fe}^{2+}$  chelation (42.46% in TF–50.58% in TEF), DPPH\* radical scavenging activities (22.50 mmol TEAC/ g in TF–59.88 mmol TEAC/ g in TEF). It was observed that the antioxidant activities ( $\text{OH}^*$ , ABTS\*,  $\text{Fe}^{2+}$  chelation, DPPH\* and total phenol) obtained from the present study were significantly ( $p > 0.05$ ) higher in the modified samples (enzymatic > acetylated > physical) than native samples, respectively. The total phenol in native and modified tacca flour ranges from 21.78% in TF to 26.96% in TEF, which is about 5.18% increase in antioxidant activities.

Antioxidants are molecules that slow down the oxidation of other molecules and function as protective agents against oxygen free radicals, thereby preventing some types of cell damage (Krishnaiah et al. 2011). Oxidation is a chemical reaction that generates free radicals, leading to chain reactions that may damage and affect cells. Free radicals cause “oxidative stress”, leading to cell damages (Ademosun et al. 2020). Oxidative stress is involved in the onset of many diseases including cancer, arteriosclerosis, cardiovascular diseases, diabetes, Alzheimer’s disease, Parkinson’s disease and eye diseases such as cataracts and age-related macular degeneration (Oboh and Rocha 2007). However, antioxidant functions by scavenging the free radicals thereby prevent oxidative stress and cell damage and also lead to reduced risk of cardiovascular diseases (Ademosun et al. 2020; Aruoma 1998).

Plant food materials are reported to be rich in total phenol and flavonoid content, also leading to high antioxidant activities in some plant materials (Ademosun et al. 2020; Awika and Rooney 2004).

The haematological studies of the basal diet, 100% wheat biscuit and the biscuit produced from composite flours consisting of enzymatic modified flours and wheat flours (TEB) are presented in Table 2. The results revealed that the composite flours biscuits had better haematological values than the 100% wheat biscuit and the basal diet. We noticed an increase in the

haematological values with increase in the incorporation of tacca flour in the composite flour (TEB20% > TEB15 > TEB10% > TEB5%). This increase might be due to the impact of modification on the tacca flour leading to an increased antioxidant activities as well as the level of mineral particularly iron present in the enzymatically modified tacca flour. The literature has it that iron is beneficial to blood formation (Abbaspour et al. 2014, Camaschella 2015).

However, the result obtained showed that the value obtained in TEB 15% and TEB 20% was significantly ( $p < 0.05$ ) lower than values obtained in other experimental diet and there is a significant ( $p > 0.05$ ) different between (TEB 15% and TEB 20%), TEB 10% and other experimental diets. This implies that TEB 15% and TEB 20% consumption in rats reduced the rate of free oxygen production and hence reduced the rate of cell damage and occurrence of cardiovascular diseases.

The high concentration of PCV and RBC observed in rats fed TEB 20% compared to other experimental animal group further indicates the nutritional qualities of the food samples. Scientific study has proven that diets containing poor protein would usually result in poor production of haemoglobin and poor immunity (Roberts et al. 2000). However, the values obtained from the present study suggest that the formulated diet will promote adequate blood production in the body as well as more immunity against germs and infections (Dewan 2007). Hence, TEB 20% contains better haematological parameter compared with other experimental samples.

Alanine aminotransferase (ALT), alkaline phosphate (ALP) and aspartate aminotransferase (AST) are cytosolic enzymes found in the liver and heart, and they are biomarkers for liver functionality. An increase in serum AST, ALT or ALP activity is an indicator of liver and kidney destruction. The ALT, ALP and ASP values of the TEB samples were not higher than 100% wheat biscuit (Figs. 6, 7 and 8). The essence of this comparison was to ensure that consumption of the tacca-based samples is safe for human consumption. In other words, the result implies that the tacca-based biscuit would have acceptable liver functionality with no side effects on the liver.

The effect of enzymatic tacca–wheat biscuit on kidney functions as defined by the creatinine and uric acid levels is shown in Figs. 9 and 10, respectively. Creatinine is an endogenous metabolite and substrate for multiple transporters expressed in the proximal tubule cells of the kidney (Scotcher et al. 2020). It is a key indicator of renal function (Arroyo and Pepper 2020). The results obtained from enzyme activities of creatinine and uric acid showed that animal fed with 100% wheat flour biscuit and basal diets had significantly higher values of creatinine and uric acid than TEB samples. The

reduced values of the TEB biscuit samples indicated that they had higher kidney protection, better than 100% wheat biscuit.

The effect of the samples on the *in vivo* antioxidant enzymes, namely catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione S-transferase (GST) and reduced glutathione (GSH), is shown in Figs. 11, 12, 13, 14 and 15. Antioxidant enzymes (CAT, SOD, GPx, GST and GSH) are present in the tissue (heart, kidney and liver) of living subject and inhibit free radicals production by minimizing reactive oxygen species (ROS) and/or scavenging radical, chelating and metal breaking chain reactions (Yadav et al. 2020). Glutathione is a small tri-peptide protein synthesized in the liver. It is a potent antioxidant with high redox potential, and it also serves as a co-factor for antioxidant enzymes (Valko et al. 2007). The results showed that increasing quantities of tacca flour in the samples resulted in increased catalase activities. The same trend was observed in SOD, GPx, GST and GSH (Figs. 12, 13, 14 and 15). In fact, highest values of the antioxidants activities were observed in TEB 20% (20% tacca flour incorporation). Tacca flour therefore showed a great antioxidant potential which would be beneficial to consumers of tacca flour biscuits. This high antioxidant activities of biscuits with tacca flour will be an added advantage over wheat flour biscuits.

Thiobarbituric acid-reactive substances (TBARS) are formed as a by-product of lipid peroxidation. Lipid peroxidation is the oxidative degradation of lipids (Trevisan et al. 2001) and results in cell damage (Muller et al. 2007; Weber et al. 2013). Lipid peroxidation proceeds by a free radical chain reaction mechanism, which must be terminated fast enough in order to prevent damage to the cell membrane, which consists mainly of lipids (Trevisan et al. 2001). The TBARS of the biscuit samples are shown in Fig. 16. Samples with higher tacca flour incorporation had the least TBARS, hence least lipid peroxidation. This result therefore shows that biscuits with higher tacca flour incorporation will not pose negative lipid peroxidation effects which could lead to mutagenic and carcinogenic activities.

The tacca-incorporated biscuits are shown in Fig. 17, while the sensory attributes of enzymatic wheat–tacca biscuit (TEB) and the control (100% wheat) biscuit samples are presented in Table 3. The sensory parameters used in the sensory evaluations were appearance, aroma, texture, crispiness, taste and overall acceptability. It was observed that the control sample (100% wheat) has the highest rating by panellist in terms of overall acceptability. However, TEB5% had the best aroma and texture. There were no significance differences in the overall acceptability of all the TEB samples. Quantitatively and probably qualitatively, the acceptability of TEB samples was above 70%, which is very close to 72% obtained for wheat flour biscuit (Table 3).

## Conclusions

Tacca flour could serve as a veritable substitute of wheat flour in the production of non-gluten baked products. In addition, biscuits produced with wheat–tacca flour were rich in bioactive compounds with acceptable haematological properties. The toxicological analysis result implies that incorporation of tacca flour into wheat flour up to 20% was very safe for consumption. Enzymatic modification of the incorporated tacca flours considerably improved the antioxidants properties of the biscuits. The biscuits produced from wheat-modified tacca flour composite flour has better *in vivo*, *in vitro* antioxidant properties and better haematological attributes in albino rats fed with it when compared with that of 100% wheat biscuits and hence could be said to exhibit nutraceutical potentials.

## Abbreviations

TF: Native tacca flour; TPF: Tacca physical flour; TAF: Tacca acetylated flour; TEF: Tacca enzymatic starch; OH: Hydroxyl radicals; DPPH: 2,2-Diphenyl-1-picrylhydrazyl; ABTS: 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); PVC: Packed cell volume; Hb: Haemoglobin; WBC: White blood cell; RBC: Red blood cell; MCH: Mean corpuscular haemoglobin; MCHC: Low mean corpuscular haemoglobin concentration.

## Author contributions

EOO carried out the laboratory analyses, interpreted the data, carried out statistical analyses and wrote the manuscript draft. OSO conceived the research, monitored research execution, interpreted the data and corrected the drafts. OOA co-conceived the research, monitored the research execution, jointly wrote the manuscript, interpreted the data and corrected the drafts. All authors have read and approved the manuscript.

## Funding

No funding was received for this research work.

## Availability of data and materials

Data are available on request.

## Declarations

### Ethical approval and consent to participate

The study protocol was approved by the Ethical Committee School of Agriculture and Agricultural Technology, Federal University of Technology, Akure, Nigeria (FUTA/SAAT/2020/016). Verbal consent to participate was obtained from the sensory panellist who participate in sensory evaluation.

### Consent for publication

Not applicable.

### Competing interests

No competing interest within authors.

Received: 15 December 2021 Accepted: 25 June 2022

Published online: 07 July 2022

## References

- Abbaspour N, Hurrell R, Kelishadi R (2014) Review on iron and its importance for human health. *J Res Med Sci* 19(2):164–174 (PMCID3999603)
- Adebiji AB, Omojola MO, Orishadipe A, Afolayan M, Olalekan D (2011) Tacca starch citrate - a potential pharmaceutical excipient. *Arch Appl Sci Res* 3(6):114–121

- Adefegha SA, Obogh G, Adefegha OM, Boligon AA, Athayde ML (2014) Antihyperglycemic, hypolipidemic, hepatoprotective and antioxidative effects of dietary clove (*Syzygium aromaticum*) bud powder in a high-fat diet/streptozotocin-induced diabetes rat model. *J Sci Food Agric* 94:2726–2737. <https://doi.org/10.1002/jsfa.6617>
- Ademosun AO, Akuine KC, Oyeleye SI, Obogh G (2020) Effect of bitter leaf (*Vernonia amygdalina*) aqueous extract on pancreatic  $\alpha$ -amylase and intestinal  $\alpha$ -glucosidase repressive potentials of Acarbose. *Biokemistri* 32(2):157–163
- Ade-Omowaye B, Akinwande B, Bolarinwa I, Adebisi A (2008) Evaluation of tigernut (*Cyperus asculentus*) wheat composite flour and bread. *Afr J Food Sci* 2(1):87–91
- Alrahmany R, Tsopmo A (2012) Role of carbohydrases on the release of reducing sugar, total phenolics and on antioxidants properties of oat bran. *Food Chem* 132(1):413–418. <https://doi.org/10.1016/j.foodchem.2011.11.014>
- Alvariz RM, Moreira IT, Cury GK, Vargas CR, Barschak AG (2019) In vitro effect of globotriaosylceramide on electron transport chain complexes and redox parameters. *An Acad Bras Ciênc* 91(02)
- AOAC (2010) Official methods of analysis of association of official analytical chemists. 18th Edition, Washington, DC
- Ariefanti L, Sukardiman S, Indriyanti N, Widayati R (2020) Anticancer property of *Orthosiphon stamineus* Benth. Extracts in different solvent systems against T47D human breast cancer cell lines. *Fabard J Pharm Sci* 45(3):187–194
- Aruoma OI (1998) Free radicals, oxidative stress, and antioxidants in human health and disease. *J Am Oil Chem Soc* 75(2):199–212
- Arroyo EN, Pepper M (2020) B cells are sufficient to prime the dominant CD4+ Tfh response to Plasmodium infection. *J Exp Med*. <https://doi.org/10.1084/jem.20190849>
- Autsavakitpong T, Khonsung P, Panthong A, Chiranthan N, Kunanusorn P, Nuntasae N, Jaipetch T, Bunteang S, Reutrakul V (2015) Preliminary evaluation of the analgesic and anti-inflammatory effects of *Tacca integrifolia* in rodents. *Int J Appl Res Nat Prod* 8:19–25
- Awika JM, Rooney LW (2004) Sorghum phytochemicals and their potential impact on human health. *Phytochemistry* 65(9):1199–1221. <https://doi.org/10.1016/j.phytochem.2004.04.001>
- Awolu OO (2017) Optimization of the functional characteristics, pasting and rheological properties of pearl millet-based composite flour. *Heliyon* 3(2):e00240. <https://doi.org/10.1016/j.heliyon.2017.e00240>
- Awolu OO, Olofinlae SJ (2016) Physico-chemical, functional and pasting properties of native and chemically modified water yam (*Dioscorea alata*) starch and production of water yam starch-based yoghurt. *Starch-Stärke* 68(7–8):719–726. <https://doi.org/10.1002/star.201500302>
- Awolu OO, Oluwaferanmi PM, Fafowora OI, Oseyemi GF (2015) Optimization of the extrusion process for the production of ready-to-eat snack from rice, cassava and kersting's groundnut composite flours. *LWT* 64(1):18–24. <https://doi.org/10.1016/j.lwt.2015.05.025>
- Awolu OO, Odoro JW, Adeloye JB, Lawal OM (2020) Physicochemical evaluation and Fourier transform infrared spectroscopy characterization of quality protein maize starch subjected to different modifications. *J Food Sci* 85:3052–3060. <https://doi.org/10.1111/1750-3841.15391>
- Awolu OO, Oyeibanji OV, Sodipo MA (2017). Optimization of proximate composition and functional properties of composite flours consisting wheat, cocoyam (*Colocasia esculenta*) and bambara groundnut (*Vigna subterranea*). *Int Food Res J*. <http://www.ifrj.upm.edu.my>
- Bain BJ, Bates I, Laffan MA, Lewis SM (2016) Dacie and Lewis practical haematology: expert consult: online and print. Elsevier Health Sciences
- Camaschella C (2015) Iron-deficiency anemia. *N Engl J Med* 372(19):1832–1843. <https://doi.org/10.1056/NEJMra1401038>
- DeMan JM, Finley JW, Hurst WJ, Lee CY (1999) Principles of food chemistry, vol 478. Aspen Publishers, Gaithersburg, p 446
- Dewan M (2007) A prospective study of physical activity and its role in management and prevention of diabetes. *J Exerc Sci Fit* 3(2):111–119
- Franco VA, Garcia LGC, Silva FAD (2020) Addition of hydrocolloids in gluten-free bread and replacement of rice flour for sweet potato flour. *Food Sci Technol* 40:88–96. <https://doi.org/10.1590/fst.05919>
- Girgih AT, Udenigwe CC, Hasan FM, Gill TA, Aluko RE (2013) Antioxidant properties of Salmon (*Salmon salar*) protein hydrolyzate and peptide fractions isolated by reverse-phase HPLC. *Food Res Int* 53:315–322
- Kaur B, Ariffin F, Bhat R, Karim AA (2012) Progress in starch modification in the last decade. *Food Hydrocoll* 26:398–404. <https://doi.org/10.1016/j.foodhyd.2011.02.016>
- Krishnaiah D, Sarbatly R, Nithyanandam R (2011) A review of the antioxidant potential of medicinal plant species. *Food Bioprod Process* 89(3):217–233. <https://doi.org/10.1016/j.fbp.2010.04.008>
- Marinova G, Batchvarov V (2011) Evaluation of the methods for determination of the free radical scavenging activity by DPPH. *Bulgarian J Agr Sci* 17(1):11–24
- Muller F, Lustgerten MS, Jang Y, Richardson A, Van Remmesh H (2007) Trends in Oxidative ageing theories. *Free Radic Biol Med* 43(4):477–503. <https://doi.org/10.1016/j.freeradbiomed.2007.03.034>
- Nduoyang CJ, Njintang NY, Facho B, Scher J, Mbofung CM (2015) Effect of processing method on the antinutrient content of *Tacca leontopetaloides* (L.) Kuntze Flour. *Br J Appl Sci Technol*. 5(3):258
- Nwokocha L (2017) Chemical composition and rheological properties of white and yellow *Tacca involucreta* flours. *Niger J Sci* 51(1):57–63
- Obogh G, Rocha JBT (2007) Distribution and antioxidant activity of polyphenols in ripe and unripe tree pepper (*Capsicum pubescens*). *J Food Biochem* 31(4):456–473. <https://doi.org/10.1111/j.1745-4514.2007.00123.x>
- Olagunju AI, Omoba OS, Enujiugha VN, Wiens RA, Gough KM, Aluko RE (2020) Influence of acetylation on physicochemical and morphological characteristics of pigeon pea starch. *Food Hydrocoll* 100:105424. <https://doi.org/10.1016/j.foodhyd.2019.105424>
- Olorunfemi MA, Awolu OO, Enujiugha VN (2021) Evaluation of the chemical, antinutritional and antioxidant properties of composite flour comprising native and modified acha (*digitaria exilis* stapf) flour supplemented with mango kernel seed and soy cake flours. *Food Sci Technol Int* 28:40–49
- Omoba OS, Oyewole GO, Oloniyo RO (2020) Chemical composition and antioxidant properties of orange fleshed sweet potato leaves and consumer acceptability in vegetable soup. *Prev Nutr Food Sci* 25(3):293
- Onabanjo OO, Ighere DA (2014) Nutritional, functional and sensory properties of biscuit produced from wheat-sweet potato composite. *J Food Technol* 1(2):111–121. <https://doi.org/10.18488/journal.58/2014.1.2/58.2.111.121>
- Peng J, Jackson EM, Babinski DJ, Risinger AL, Helms G, Frantz DE, Mooberry SL (2010) Evelynin, a cytotoxic benzoquinone-type retro-dihydrochalcone from *Tacca chantrieri*. *J Nat Prod* 73(9):1590–1592. <https://doi.org/10.1021/np100350>
- Roberts DL, Cooper RJ, Petit LJ (2000) Flock characteristics of ant-following birds in premontane moist forest and coffee agroecosystems. *Ecol Appl* 10(5):1414–1425. [https://doi.org/10.1890/1051-0761\(2000\)010\[1414:FCOAFBJ2.0.CO;2](https://doi.org/10.1890/1051-0761(2000)010[1414:FCOAFBJ2.0.CO;2)
- Sánchez-Rangel JC, Benavides J, Heredia JB, Cisneros-Zevallos L, Jacobo-Velázquez DA (2013) The Folin-Ciocalteu assay revisited: improvement of its specificity for total phenolic content determination. *Anal Methods* 5(21):5990–5999
- Scotcher D, Arya V, Yang X, Zhao P, Zhang L, Huang SM, Galetin A (2020) Mechanistic models as framework for understanding biomarker disposition: prediction of creatinine-drug interactions. *CPT Pharmacomet Syst Pharmacol* 9(5):282–293. <https://doi.org/10.1002/psp4.12508>
- Trevisan M, Browne R, Ram M, Multi P, Freudenheim J, Carosella AM, Armstrong D (2001) Correlates of markers of oxidative status in the general population. *Am J Epidemiol* 154(4):348–356. <https://doi.org/10.1093/aje/154.4.348>
- Ukpabi U, Ukenye E, Olojede A (2009) Raw material potentials of Nigerian wild polynesian arrowroot (*Tacca leontopetaloides*) tubers and starch. *J Food Technol* 7(4):135–138
- Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J (2007) Free radicals and antioxidants in normal physiological functions and human disease. *J Biochem Cell Biol* 39(1):44–84. <https://doi.org/10.1016/j.biocel.2006.07.001>
- Weber C, Hametner C, Tuchscherer A, Losand B, Kanitz E, Otten W, Kanitz W (2013) Hepatic gene expression involved in glucose and lipid metabolism in transition cows: effects of fat mobilization during early lactation in relation to milk performance and metabolic changes. *J Dairy Sci* 96(9):5670–5681. <https://doi.org/10.3168/jds.2012-6277>
- Yadav N, Pal A, Sihag S, CR N (2020) Antioxidant activity profiling of acetonetic extract of *Jamun* (L.) seeds in different models. *Open Food Sci J* 12(1)

## Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.