

Nutritional, antioxidant and α -amylase inhibitory properties of cracker biscuits from high quality cassava and sour sop (*Annona muricata*) seed flours.

Abstract

This study evaluated the nutritional, antioxidant and inhibitory effect on α -amylase enzyme linked to type-2 diabetes of cracker biscuits made from seven blends of high quality cassava-wheat flour and sour sop seed flours. The composite mixture of high quality cassava (HQC), whole wheat flour (WHT)) and Sour sop seeds flour (SSs) in the proportion (w/w) of 100:0:0, 100:0:0, 85:10:5, 80:10:10, 75:10:15, 70:10:20 and 65:10:25 were prepared respectively to produce functional cracker biscuits. The proximate analysis of the cracker biscuits showed that the protein, ash fat and fiber contents ranged from 2.72-5.60 %, 4.03-9.70 %, 1.24-18.99 % and 0.43-0.95 % while the carbohydrate ranged from 58.72-87.05 % respectively. Magnesium and sodium content of the cracker biscuits increased with increasing soursop seeds flour. L*, a* and b* color of the samples ranged from 30.44-50.24, 2.55-3.78 and 8.0. The ferric reducing antioxidant power ranged from 1.45-6.97 % while the total flavonoid and phenolic contents ranged from 1.64-8.52 mgQuercetin/g and 3.32-35.99 mgGAE/g and the DPPH ranged from 29.51-66.26 %. The formulated cracker biscuits especially (65HQ10WH25SSs) have high α -amylase inhibition with value of 79.49 % and significantly inhibited the key digestive enzymes (α -amylase) in dose-dependent manner when compared with the control samples and the standard (arcarbose). Also, the formulated cracker biscuits compared favorably with the control samples which exhibited highest acceptability among the panelist. Conclusively, the crackers biscuits in particular, (65HQ10WH25SSs) could serve as a functional food in the management of hyperglycemia and prevention of associated degenerative diseases among children and adults.

Keywords: cassava, wheat, diabetes, sour sop, nutritional, α -amylase.

Introduction

Diabetes mellitus has been described as a metabolic disorder with a significant high morbidity and mortality rate. This degenerative disease is caused either by deficiency in insulin secretion or degradation of secreted insulin (Cao *et al.*, 2012). During the development of diabetes, the cells of the body cannot metabolize sugar properly due to deficient action of insulin on target tissues resulting from insensitivity or lack of insulin (a peptide hormone that regulates blood glucose). The inability of insulin to metabolize sugar occurs when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces. This triggers the body to break down its own fat, protein, and glycogen to produce sugar, leading to the presence of high sugar levels in the blood with excess by-products called ketones being produced by the liver (Buowari, 2013; Folorunso and Oguntibeju, 2013).

Diabetes is distinguished by chronic hyperglycemia with disturbances in the macromolecules' metabolism as a result of impairments in insulin secretion, insulin action, or both. Diabetes causes long-term damage, dysfunction, and failure of various organ systems (heart, blood vessels, eyes, kidneys, and nerves) leading to disability and premature death (Bahijri *et al.*, 2016). Several symptoms such as thirst, polyuria, blurring of vision, and weight loss also accompany diabetes (Sperling *et al.*, 2014). The α -glucosidase inhibitors, such as acarbose and miglitol, impede certain enzymes responsible for the breakdown of carbohydrates in the small intestine. This class of hypoglycemic agents acts mostly by reducing the absorption rate of carbohydrates in the body. Acarbose equally reversibly inhibits both pancreatic α -amylase and α -glucosidase enzymes by binding to the carbohydrate-binding region and by interfering with their hydrolysis into monosaccharides, which leads to a slower absorption together with a reduction in postprandial blood sugar levels (Salehi *et al.*, 2018; Sharifi-Rad *et al.*, 2018). However, some of these drugs exhibit side effects such as abdominal distension, bloating, flatulence, and diarrhea.

There are multiple number of plant products available that are assisting in controlling the progression of diabetes mellitus (Abo *et al.*, 2008). The anti-diabetic potential of these plants are often attributed to the functional ingredients in them such as the dietary fiber and antioxidant compounds which possess the ability to inhibit the enzymes associated with carbohydrate metabolism which in turn eventually helps to modulate glucose and insulin response in human body system (Onwulata *et al.*, 2010; Trinidad *et al.*, 2010).

Soursop (*Annona muricata*) belongs to the family *Annonaceae* and a native of Tropical North and South America (Fasakin *et al.*, 2008). They are either irregular, ovoid or heart shaped fruits which is 15-30 cm long and a width of 10-30 cm. It has a thick skin which is dark green with sparse curved spines [10]. The fruit mesocarp resembles white cotton and possesses a stinging and sweet-sour taste containing many dark seeds (Iombor *et al.*, 2014). Sour sop is often consumed as a dessert fruit or utilized by food industries for the production of beverages, ice cream, wine, candy and syrup (Okigbo, 2008). The utilization of sour sop flour for the production of bread has also been reported by Zabidi and Yunus (2014). The effect of sour sop flour inclusion to wheat flour for bread production have also been investigated and reported that the flour from sour sop has quality attributes which could be utilized for the production of bread and other baked goods thereby diversifying the utilization of sour sop and improving the quality of food products produced from it (Iombor and Banjo, 2018). Sour sop flour could be utilized in enhancing the nutritional content (especially dietary fibre, minerals and protein contents) of various food products (Zabidi and Yunus, 2014). It has been reported that sour sop possesses some therapeutic properties such as antioxidant and anticancer. They further added that the sour sop fruits can be explored as a viable source of natural antioxidants for the production of functional foods (Akomolafe and Ajayi, 2015). However, there is dearth of information on the utilization of the seeds of sour sop in the production of cracker biscuits, thus the study aimed to produce cracker biscuits from the blends of high quality cassava and soursop seeds flours and determine the nutritional, antioxidant and antidiabetic effect of the cracker biscuits on key enzyme (α -amylase) linked to type-2 diabetes.

Materials and Methods

Source of raw materials

Sour sop fruit was obtained locally from fruit market at Sango area Saki Oyo State, Nigeria. Refined wheat flour and other ingredients such as margarine, eggs, sugar, salt, milk and vegetable oil were purchased from Agbeni market Ibadan, Oyo State Nigeria. High quality cassava was gotten from Pсалtry international company limited, Ado awaye, Iseyin local government, Oyo state.. Chemicals used for all analysis were of analytical grade.

Methods

Processing of sour sop seeds flour

The seeds of sour sop were removed from the fruits, washed and air-dried at room temperature (28 ± 2.00 °C) for two weeks. The seed coat of the seeds were removed by manual cracking and thereafter the dehulled seeds were pulverized using an electric blender (Holt Star, Model BE 768-2, John Holt Co. UK). The finely powdered samples were stored at room temperature in air-tight polythene bag until needed for analyses.

Product formulation

The composite high quality cassava flour and sour sop pulp flour were formulated in percentages as shown in Table 1

Production of crackers

High quality cassava flour and sour sop pulp flour were mixed with vanilla, table salt and cold water to form the dough. The dough was transferred to a rolling parchment paper and covered with plastic wrap. A rolling pin was used to flatten the dough to a thin layer of desired thickness. The plastic wrap was removed and the dough cut into rectangular shapes by simply drawing lines on it with a sharp knife. The cut pieces of dough were carefully placed in a baking tray that had been previously lightly greased with cooking oil, lined with parchment paper and baked at 120°C for 30 min. The crackers were allowed to cool at room temperature for 10 min, inverted and baked again at 100°C until crispy. The crackers were allowed to cool at room temperature, packaged and stored for further analysis.

Table 1. The product formulation for high quality cassava flour and sour sop seed composite flours

Samples	High quality cassava	Wheat	Sour sop seed
100HQ	100	-	-
100WH	-	100	-
85HQ10WH5SS	85	10	05
80HQ10WH10SS	80	10	10
75HQ10WH15SS	75	10	15
70HQ10WH20SS	70	10	20
65HQ10WH25SSS	65	10	25

HQ- High quality cassava flour

WH- Wheat flour

SS- Sour sop seed flour

Determination of proximate composition

The proximate compositions of the samples were determined using AOAC (2012) method. Moisture content was obtained by drying in the oven until a constant weight was obtained at 105 °C. The crude fat was extracted with petroleum ether using the Soxhlet method and the ash was determined from the weight of residue obtained after heating (incinerating) the sample at 525 °C for 4 h. The crude protein was obtained using the micro-Kjeldahl method while 6.25 was taken as the nitrogen conversion factor. The carbohydrate content was obtained by difference by subtracting the percentage contents of moisture, ash, fat, fibre and protein from 100.

Determination of mineral composition

Evaluation of the mineral constituents (magnesium, phosphorus and sodium) of the biscuits were done according to standard methods of the AOAC (2012).

Determination of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability

The DPPH free radical scavenging ability of the extract was determined as previously reported (Agunbiade *et al.*, 2022). Briefly, 1 ml of the extract was mixed with 1 ml of the 0.4 mM methanolic solution of the DPPH. The mixture was left in the dark for 30 min before measuring the absorbance at 517 nm with (Healicom 721S China) spectrophotometer. The control consisted of methanol instead of the sample and the radical scavenging ability of the sample was calculated as

$$\% \text{ DPPH} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

Determination of ferric reducing antioxidant power (FRAP)

The reducing property of the extract was determined as earlier reported (Aderinola, 2018). Briefly, 0.25 ml of the extract was mixed with 0.25 ml of 200 mM of sodium phosphate buffer (pH 6.6) and 0.25 ml of 1 % potassium ferricyanide. The mixture was incubated at 50 °C for 20 min, thereafter 0.25 ml of 10 % trichloroacetic acid was added and centrifuged at 2000 rpm for 10 min, 1 ml of the supernatant was mixed with 1 ml of distilled water and 0.1% of FeCl₃ and the absorbance was measured at 700 nm with Healicom 721S China) spectrophotometer. Ascorbic acid (0.01 mg/ml) was used as the standard.

Determination of total phenolic contents

The total phenol contents of the sample were determined using earlier method (Singleton *et. al.*, 1999). 0.2 ml of the extract was mixed with 0.5 ml of 10 % Folin Ciocalteau's reagent and 2 ml of 7.5 % Sodium carbonate. The mixture was incubated for 40 min at 45 °C, and the absorbance was taken at 700 nm in the spectrophotometer (Healicom 721S China), gallic acid served as the standard phenol. The total phenolic contents of the sample were expressed as mg gallic acid equivalent.

Determination of total flavonoid contents

The total flavonoid content was determined as previously reported (Ademosun *et al.*, 2021). Briefly, about 200 μ L of the extract was added to 300 μ l of 5 % sodium nitrate. After 5 min, 600 μ l of 10 % Aluminium chloride was added. Also, 2 ml of 1 M sodium hydroxide was added to the mixture after 6 min, followed by the addition of 2.1 ml of distilled water. Absorbance was read at 415 nm with Healicom 721S (China) UV-spectrophotometer against the reagent blank. The flavonoid content was expressed as mg quercetin equivalent.

Determination of color properties

The color (L^* , a^* , and b^*) was determined with the aid of a Minolta CR-400 colorimeter, according to the CIE system. L^* represents lightness or darkness, with values between 0 (completely black) and 100 (totally white); a^* presents values between -80 and +100, where the extremes correspond, respectively, to green and red; and b^* may vary from -50 to +70, with intensity from blue to yellow. The readings were made at three distinct points in each sample

Sensory evaluation

Crackers samples were organoleptic evaluated for its sensory characteristics. Crackers sample was served on white, odorless and disposable plates and water was provided for rinsing between samples for fifty panelists. Samples were scored for color, taste, flavor, texture, appearance and overall acceptability. Control crackers were used to compare with tested samples for sensory test.

Statistical analysis

The results were expressed as the mean \pm standard deviation (SD). Data obtained from the analysis was subjected to one-way analysis of variances, all means were separated using Duncan Multiple Range Test (DMRT) at 5% probability level ($p > 0.05$) using Minitab Statistical Software (Minitab Inc., State College, PA, USA).

Result and Discussions

Proximate composition of cracker biscuits made from blends of high quality cassava and sour sop seed flours.

Table 2 presents the proximate composition of cracker biscuits made from blends of high quality cassava and sour sop seeds flour. The moisture content of the cracker biscuits ranged between 4.28 and 6.95 % with the lowest and highest values observed in (100WHT) and 100HQC) respectively. The protein content of the cracker biscuits ranged between 2.32 and 5.60 % where the lowest value were observed in (100WHT) and the highest value observed in (65HQ10WH25Ss). The percentage ash content of the samples ranged between 4.03 and 9.70 % for (100WHT) and (65HQ10WH25Ss) while the fat content of the cracker biscuits ranged between 1.24 and 18.99 % where the lowest value was observed in (100WHT) and the highest value observed in (65HQ10WH25Ss) respectively. The fiber content ranged between 0.43 and 1.02 % where the lowest value was observed in (85HQ10WH5Ss) and the highest value observed in (100WHT). The carbohydrate content ranged between 58.72 to 87.05 % for (65HQ10WH25Ss) as the lowest and (100WHT) as highest value respectively. The high moisture content of the enriched crackers as compared to the control crackers may be due to the incorporation of sour sop seeds. The moisture level of any food is an indicator of its water activity and is used to assess its vulnerability to microbial infection. The moisture content of biscuits should not surpass 14% as reported by Rebellato *et al.*, (2015). Hence the moisture content of the cracker biscuits formulated in this study was well within an appropriate range for an optimum shelf life. The moisture content observed in this study compared favorably with the value of 5.04-5.60 % for enriched cracker biscuits made from wheat and cashew kernel seeds flour blends as reported by Anim *et al.*, (2023). This finding is similar to 4.52–10.62 % reported for biscuits supplemented with onion residue (Jiang *et al.*, 2020). Generally, this study revealed that the protein content of the cracker biscuits increased with an increment in the addition of the sour sop seed flour that is as the concentration of sour sop seed flour increased, the protein content of the crackers biscuits increased compared with the control samples. Oludunmila and Adetimehin (2016) reported 2.44-3.04 % protein content of biscuits made from the combination of unripe plantain-wheat and watermelon seeds flours. Ifediba and Egbuna (2019) who also fortified biscuit by blending wheat flour with moringa leaf powder and the results showed that

addition of moringa leaf powder increased protein content of the biscuits from 11.14 to 11.47 %. The result revealed that the protein content of the fortified crackers biscuit in this present study would be of better quality as compared with 100 % wheat and 100 % high quality cassava flour cracker biscuits. The increase in the protein content of the cracker biscuits in this study may be due to the high percentage of protein content reported to be found in sour sop seeds (Solis-Fuentes 2020). The ash content of the samples ranged between 4.03 to 9.70 % for (100WHT) and (65HQ10WH25Ss). It was observed that cracker biscuits enriched with 25 % sour sop seed flour has the highest ash content while the control sample has the lowest ash content. The ash content reported in this study was higher than the values of 1.33-3.51 % reported for cracker biscuits obtained from wheat and acha seeds flour (Olagunju *et al.*, 2018). Low value of ash content (0.94-0.99 %) was also observed for crackers biscuits obtained from wheat-african yam bean enriched with tigernut seeds which was significantly ($p < 0.05$) lower to the ash content in this study as reported by (Dada *et al.*, 2023). Increase in the ash content on substitution sour sop seeds is expected as seeds of soup sop are high in ash content and the leaves are rich in mineral elements such as sodium, magnesium, and calcium and phosphorous (Emilike and Akusu, 2017). The percentage ash of a sample gives an idea on the inorganic content of the samples from where the mineral content could be obtained. Samples with high ash contents is expected to have high concentration of various mineral elements, which are expected to speed up metabolic processes, improve growth and development. The fat content of the cracker biscuits in this study compared favorably with the fat content of cracker biscuits made from wheat flour enriched with cashew seeds with the values of 12.45-13.72 % as reported by Ujong *et al.*, (2023). The elevation in fat content of the crackers biscuit was mostly due to the incorporation of undefatted soursap seeds flour. Odunlade *et al.*, (2017) also reported a significant content in the fat content of wheat bread (1.27–2.00 %) upon supplementation with *Telfairia occidentalis*, *Amaranthus viridis* and *Solanum macrocarpon*. The presence of fat influences the shelf life of foods (Addisu *et al.*, 2019). A high fat content may hasten deterioration by encouraging rancidity, which may give rise to unpleasant flavors and odors. Despite the observed increase, the fat content remained below 19 %, which is the minimum limit for fat in bakery food products (Adissu *et al.*, 2019). The fiber content of the developed cracker biscuits in the study was significantly ($p < 0.05$) lower than the values of 1.30-7.20 % obtained for crackers made from wheat flour enriched with cashew seeds and 2.36-7.30 % for cracker biscuits made from wheat-african yam bean enriched

with tigernut seeds (Ujong *et al.*, 2023; Dada *et al.*, 2023). However, with the low fiber content observed in the study, there was an increased in the fiber content of the crackers as the concentration of sour sop seed flour increased, thus, consuming foods crackers made from high quality cassava flour enriched with sour sop seed flour may lower the risk of cardiovascular disease, certain cancers, and type 2 diabetes, as well as enhance the regulation of physiological functions of the body system (Sharma *et al.*, 2016). The decrease in the carbohydrate content of the cracker biscuits as the supplementation with sour sop seeds increased agrees with the report of Olubukola *et al.*, (2017) who also reported a decreased in the carbohydrate content of chin-chin enriched with Ugu seeds flour. Ifediba and Egbuna (2019) equally showed a significant decrease in carbohydrate from 49.38-47.80 % for biscuit made from wheat and moringa seed flour blends.

Table 2. Proximate composition (%) of cracker biscuits made from blends of high quality cassava and sour sop seed flours.

Sample	Moisture	Protein	Ash	Fat	Fibre	Carbohydrate
100HQC	6.95±0.17 ^a	2.72±0.27 ^f	5.38±0.25 ^c	1.24±0.69 ^c	0.74±0.03 ^b	82.97±0.83 ^b
100WHT	4.28±0.16 ^g	2.32±0.04 ^g	4.03±0.18 ^a	1.30±0.41 ^b	1.02±0.01 ^a	87.05±0.68 ^a
85HQ10WH5Ss	5.95±0.13 ^f	3.68±0.00 ^e	7.97±0.08 ^f	15.19±0.04 ^a	0.43±0.02 ^d	67.21±0.09 ^c
80HQ10WH10Ss	4.58±0.50 ^e	4.29±0.09 ^d	8.94±0.06 ^f	17.11±0.02 ^d	0.53±0.02 ^d	64.65±0.52 ^d
75HQ10WH15Ss	5.64±0.32 ^e	4.81±0.05 ^c	8.98±0.06 ^e	18.57±0.45 ^b	0.63±0.03 ^e	61.37±0.71 ^e
70HQ10WH20Ss	6.25±0.20 ^b	4.90±0.00 ^b	9.02±0.02 ^b	18.88±0.19 ^e	0.72±0.00 ^d	60.23±0.35 ^f
65HQ10WH25Ss	6.04±0.15 ^c	5.60±0.04 ^a	9.70±0.13 ^d	18.99±0.19 ^c	0.95±0.00 ^c	58.72±0.16 ^g

Mean values are of triplicate determinations. Mean values within a column with different superscripts are significantly different at ($p < 0.05$).

Mineral composition (mg/100g) of cracker biscuits made from blends of high quality cassava and sour sop seed flours.

Table 3 shows the mineral composition of the crackers biscuits made from high quality cassava flour enriched with sour sop seed flour. Magnesium content of the samples ranged between 0.04 and 8.07 mg/100 g with the control samples having the lowest values and (65HQ10WH25Ss) having the highest value. The phosphorus content ranged between 0.00 to 10.01 mg /100g) while the sodium content ranged between 3.70 to 9.55 mg/100 g for 100 WHT and (65HQ10WH25Ss) respectively. The sour sap seeds flour enriched crackers have better magnesium, phosphorus and sodium contents than the crackers biscuits without the addition of sour sop seeds flour and this is in agreement with the work of Ndife *et al.*, (2013). The high mineral contents observed in the developed cracker biscuits confirms previous research by Emelike *et al.*, (2015). Thus the flour blends can serve as functional ingredient in the formulation of other snack and confectionaries products. It is have been reported that vegetables and plant seeds are good sources of minerals which are usually in short supply in daily diets (Ajayi, 2015).

Table 3. Mineral composition (mg/100 g) of cracker biscuits made from blends of high quality cassava and sour sop seed flours.

Sample	Magnesium	Phosphorus	Sodium
100HQ	0.04±0.00 ^f	0.00±0.00 ^g	4.11±0.01 ^f
100WH	0.04±0.01 ^f	0.02±0.00 ^f	3.70±0.00 ^g
85HQ10WH5Ss	3.05±0.01 ^e	6.00±0.00 ^e	5.27±0.00 ^e
80HQ10WH10Ss	5.05±0.00 ^d	7.00±0.00 ^d	6.45±0.00 ^d
75HQ10WH15Ss	6.06±0.01 ^c	8.00±0.00 ^c	8.70±0.00 ^c
70HQ10WH20Ss	7.07±0.00 ^b	9.01±0.00 ^b	8.84±0.00 ^b
65HQ10WH25Ss	8.07±0.01 ^a	10.01±0.00 ^a	9.55±0.00 ^a

Mean values are of triplicate determinations. Mean values within a column with different superscripts are significantly different at (p <0.05).

Color characteristic of cracker biscuits made from blends of high quality cassava and sour sop seed flours.

L^* value ranged from 30.44 to 50.24 for (80HQ10WH10Ss) and (100WHT) and a^* ranged from 2.55 to 3.78 for (65HQ10WH25Ss) and (80HQ10WH10Ss) respectively as shown in Table 4. The b^* value ranged from 8.04 to 15.79 for (65HQ10WH25Ss) and (100WHT). ΔL and ΔB values of the developed cracker biscuits ranged from 13.78 to 21.51 and 6.37 to 14.11 for (70HQ10WH20Ss) and (100HQC) and (65HQ10WH25Ss) and (100WHT) while ΔE value ranged from 14.70 to 35.27 for (80HQ10WH10Ss) and (100WHT). The (100WHT) showed more lightness than the developed cracker biscuits while the (80HQ10WH10Ss) showed more redness than the control samples. Sample (100WHT) showed more yellowness than the developed cracker biscuits. There is a distinct color difference from control samples and the developed biscuits. The reduction of lightness tones observed across the formulated cracker biscuits could be attributed to enhanced synthesis of Maillard reaction between reducing sugars and amino acids caused by the addition of sour sap seeds flour and the development of melanoidins which led to non-enzymatic browning (Jiang *et al.*, 2020). Baking temperature and time could also affect the colour (Pereira *et al.*, 2013). The reduced redness intensity could also be due to the destruction of anthocyanin by some circumstances such as pH, heat, light, and temperature; anthocyanin is responsible for the redness (Sukasih and Musada 2018). The overall difference in colour (ΔE) is a parameter used to show the degree of color variation between control and stored samples (Pathare *et al.*, 2013; Patras *et al.*, 2011). Adekunle *et al.*, (2010) stated that variances in color can be analytically categorized as small differences ($1.5 < \Delta E$) distinct ($1.5 < \Delta E < 3$), and very distinct ($\Delta E > 3$). Assessment of browning index is important in food industries for effective grouping and rating practices for market conditions to be met. This arises from the oxidation (both enzymatic and non-enzymatic) of phenolic compounds present in the commodities (Pathare *et al.*, 2013). Color is an essential feature since it influences the cravings of consumers. It is a key quality criterion used as a procedure check during food processing such as roasting and baking (Pereira *et al.*, 2013). Color helps to direct the market performance of a product. The use of food grade colors may not be out of place to make them more appealing to consumers. The sensory attributes of foods usually influence the consumers' desire and approval of products that ultimately determine the success or failure of any food product.

UNDER PEER REVIEW

Table 4. Color characteristic of cracker biscuits made from blends of high quality cassava and sour sop seeds flour.

Sample	L*	a*	b*	ΔL^*	ΔA^*	ΔB^*	ΔE^*
100HQC	39.32±0.29 ^b	3.73±0.04 ^a	11.80±0.13 ^b	21.51±0.29 ^b	0.16±0.03 ^a	10.12±0.13 ^b	23.59±0.35 ^b
100WHT	50.24±0.62 ^a	3.12±0.20 ^{bc}	15.79±0.21 ^a	32.31±0.62 ^a	-0.65±0.01 ^d	14.11±0.21 ^a	35.27±0.49 ^a
85HQ10WH5Ss	35.33±0.23 ^c	3.23±0.06 ^b	11.12±0.12 ^c	17.40±0.23 ^c	-0.34±0.06 ^b	9.43±0.12 ^c	19.80±0.25 ^c
80HQ10WH10Ss	30.44±0.07 ^f	3.78±0.01 ^a	9.39±0.05 ^d	12.52±0.08 ^f	0.20±0.02 ^a	7.71±0.04 ^d	14.70±0.09 ^e
75HQ10WH15Ss	33.06±0.05 ^d	3.06±0.01 ^{bc}	9.19±0.02 ^d	15.11±0.07 ^d	-0.51±0.01 ^c	7.50±0.02 ^d	16.87±0.07 ^d
70HQ10WH20Ss	31.71±0.60 ^e	2.91±0.08 ^c	8.62±0.22 ^e	13.78±0.60 ^e	-0.66±0.08 ^d	6.93±0.22 ^e	15.44±0.63 ^e
65HQ10WH25Ss	34.41±0.11 ^c	2.55±0.01 ^d	8.04±0.04 ^f	16.48±0.11 ^c	-1.03±0.01 ^e	6.37±0.05 ^f	17.70±0.12 ^d

Mean values are of triplicate determinations. Mean values within a column with different superscripts are significantly different at ($p < 0.05$).

Antioxidant properties of cracker biscuits made from blends of high quality cassava and sour sop seeds flour.

The ferric reducing antioxidant potential (FRAP) content ranged between 1.45 to 6.97 % with the control sample (100HQC) having the lower value while (65HQ10WH25Ss) have the highest value. Total flavonoid and total phenolic contents of the developed cracker biscuits as shown in Table 5 followed dose-dependent patterns. At 0.05 mg/ml concentration of the biscuit extracts, the total phenol content ranged between 1.64 to 8.52 mg Quercetin/g for (100HQC) and (65HQ10WH25Ss) and 3.63 to 35.99 mgGAE/mg for (100WHT) and (65HQ10WH25Ss) respectively. The DPPH radical scavenging activity of the cracker biscuit ranged between 29.51 to 66.26 % with (100HQC) having the lowest value and (65HQ10WH25Ss) having the highest value. It was shown from the study that the total flavonoid and total phenolic, FRAP and DPPH contents of the cracker biscuits increased as the concentration of the sour sop seeds flour increased. The total flavonoid and total phenolic content of the developed cracker biscuits was significantly ($p < 0.05$) higher than the value of 0.02-0.09 mg Quercetin/g and 2.75-6.75 mgGAE/g for shallot-enriched plantain biscuits reported by (Adeyemo *et al.*, 2022). However, the value was lower than the value of 48.66 to 95.05 mg GAE/g and 37.45 to 58.33 mg Quercetin/g for cracker biscuits made from wheat-african yam bean flour enriched with tigernut seed flour as reported by (Dada *et al.*, 2023). The increase in total phenol and total flavonoid contents of developed biscuits as the concentration of sour sop seeds increased could be ascribed to the high amount of polyphenols present in the seeds. Sour sop seeds has been reported to contain high amount of phytochemicals such as quercetin, gallic and kaempferol (Nguyen *et al.*, 2020; Reshi *et al.*, 2012). However, Adefegha *et al.*, (2014) reported that phenolic compounds are powerful antioxidants that can protect the body against free radicals. Also, it was reported that flavonoids are active against many diseases such as cardiovascular diseases, cancer, and other age-related diseases (Masek *et al.*, 2017). Polyphenolic compounds have been widely documented to exhibit outstanding antioxidant potentials (Aladesanmi *et al.*, 2020; Liu *et al.*, 2019). This explains the reason for the higher level of free radicals (FRAP and DPPH) scavenging abilities of crackers biscuits made from high quality cassava enriched with sour sop seeds flour than the control samples. An increase in sour sap seeds flour supplementation in the cracker biscuits exhibited higher scavenging and reducing abilities of these free radicals. These results agreed with the discoveries of Jiang *et al.*, (2020) where increased total phenol, total

flavonoid contents, and DPPH radical scavenging ability of biscuits was reported due to augmentation of biscuits with onion residue. The information on the impact of nutritional polyphenols on human health is constantly growing which consolidates their protective role against many human diseases (D'Angelo 2020; Krzysztoforska *et al.*, 2019). They are an essential group of secondary metabolites generated as an adaptive reaction to biological stress situations (Ashraf *et al.*, 2018). Polyphenols are capable of scavenging a wide range of reactive oxygen species via various mechanisms such as suppression of reactive oxygen species formation by inhibiting the enzymes involved in their production and upregulation/protection of antioxidants. Therefore, the antioxidant abilities of the developed cracker biscuits could be credited to the polyphenols present in the sour sop seeds which has been reported to contain various polyphenols (Fasihzadeh *et al.*, 2016; Sittisart *et al.*, 2017; Soininen *et al.*, 2012; Sun *et al.*, 2019).

Table 5. Antioxidant activity of cracker biscuits made from blends of high quality cassava and sour sop seed flours.

Sample	FRAP (%)	TFC (mgQuercetin/g)	TPC (mgGAE/g)	DPPH (%)	TAC (%)
100HQC	1.45±0.06 ^{de}	1.64±0.00 ^d	3.63±0.04 ^e	29.51±0.33 ^d	0.23±0.00 ^d
100WHT	1.59±0.04 ^e	1.67±0.00 ^{bc}	3.32±0.04 ^c	30.57±0.16 ^d	0.39±0.00 ^c
85HQ10WH5Ss	2.76±0.01 ^c	4.42±0.39 ^{ab}	14.49±0.02 ^f	43.04±0.13 ^c	0.43±0.00 ^f
80HQ10WH10Ss	3.65±0.02 ^a	5.97±0.09 ^a	17.21±0.01 ^b	51.07±0.18 ^b	0.77±0.03 ^a
75HQ10WH15Ss	4.65±0.00 ^d	6.64±0.03 ^{ab}	20.04±0.08 ^g	59.72±0.05 ^f	1.98±0.00 ^g
70HQ10WH20Ss	5.72±0.02 ^d	7.42±0.17 ^{ab}	26.79±0.01 ^c	62.22±0.09 ^g	2.63±0.00 ^c
65HQ10WH25Ss	6.97±0.02 ^b	8.52±0.69 ^{ab}	35.99±0.01 ^e	66.26±0.22 ^a	3.59±0.01 ^d

Mean values are of triplicate determinations. Mean values within a column with different superscripts are significantly different at ($p < 0.05$).

α -amylase inhibitory activity of cracker biscuits extracts made from blends of high quality cassava and sour sop seeds flour.

The percentage α - amylase inhibition of cracker biscuits made from blends of high quality cassava and soursop seeds flour is shown in Table 6. Percentage α - amylase inhibition increased with increase in supplementation of cassava flour with sour sop seeds. For instance, samples (65HQ10WH25Ss) exhibited higher inhibitory activity of 79.49 % while (100HQC) had the least inhibitory activity of 5.73 %. However, all the developed cracker biscuits exhibited an optimum α -amylase inhibitory, although samples (70HQ10WH20Ss) and (65HQ10WH25Ss) compared favorably with the standard drug known as acarbose which exhibited inhibitory activity of 33.525 %. The observed inhibitory effect of the Sour sop seeds extracts on α -amylase activity in vitro suggests the possible mechanism by which Sour sop seeds exert their antidiabetic effect and may be part of the underlying basis for their folkloric use in the management/treatment of diabetes. Several studies have revealed that α -amylase and α -glucosidase activity have a great influence on blood glucose level and their inhibition could significantly reduce the postprandial increase of blood glucose (Nair *et al.*, 2013). It has also been established that reducing postprandial hyperglycemia is an important strategy towards Type-2 diabetes management (Banu *et al.*, 2015). From this study, the incorporation of sour sop seed flour into the high quality cassava flour to make cracker biscuits exhibited the highest total phenol and flavonoid contents hence, the highest α -amylase inhibitory effects. This is consistent with earlier studies where α -amylase and α glucosidase inhibitory effect of plant foods are attributed to their phenolic constituents (Saravanan and Parimelazhagan, 2014; Adefegha and Oboh, 2012; Ademiluyi *et al.*, 2015). One of the risk factors in type-2 diabetes mellitus and its cardiovascular complication (hypertension) is oxidative stress. Oxidative stress has been reported to play a vital role in the etiology and development of type-2 diabetes and hypertension. Free radicals induced oxidative damage of pancreatic β -cells has been implicated in impaired insulin production/function, a major risk factor of diabetes development (Jiménez *et al.*, 2014). Also, oxidative damage to endothelia cell of the blood vessel could compromise the elasticity of the vessel resulting in hypertension or some other cardiovascular complications (Schiffrin, 2010). Thus combating oxidative stress could be a practical way to ensure holistic management of type 2 diabetes and hypertension. Conclusively, the developed cracker biscuits has the potential of combating the oxidative stress, hence in the management of Type-2 diabetes.

Table 6. α -amylase inhibitory activity of cracker biscuits extracts made from blends of high quality cassava and soup sop seeds flour.

Sample	% inhibition	IC ₅₀ (μ g/m)
100HQC	5.73 \pm 0.36 ^f	191.08 \pm 1.81 ^d
100 WHT	12.33 \pm 0.93 ^d	507.27 \pm 7.62 ^b
85HQ10WH5Ss	17.67 \pm 0.71 ^f	2385.90 \pm 27.45 ^a
80HQ10WH10Ss	20.17 \pm 0.39 ^c	348.47 \pm 3.27 ^c
75HQ10WH15Ss	27.43 \pm 2.04 ^c	177.81 \pm 1.92 ^d
70HQ10WH20Ss	46.49 \pm 0.02 ^b	138.78 \pm 2.46 ^e
65HQ10WH25Ss	79.49 \pm 0.56 ^a	9.32 \pm 0.36 ^f

Mean values are of triplicate determinations. Mean values within a column with different superscripts are significantly different at (p <0.05).

Note: concentration of the standard (Acarbose)- 100 μ g/ml

% inhibition of the standard at IC₅₀ (Acarbose)- 33.525 %

IC₅₀ of the standard (Acarbose)- 57.65%

Sensory properties of cracker biscuits made from blends of high quality cassava and sour sop seeds flour.

The sensory characteristics of cracker biscuits made from blends of high quality cassava and soursop seeds flour is shown in Table 7. The mean scores for the color and crunchiness of the samples ranged from 4.20-7.40 and 2.08-6.67 for (65HQ10WH25Ss) and (100WHT) respectively while the mean scores for the taste and aroma ranged from 5.20-6.90 and 4.10-6.47 for (65HQ10WH25Ss) and (100WHT). The mean score for the overall acceptability of the samples ranged from 4.00-6.99 for (65HQ10WH25Ss) and (100WHT) respectively. According to Nwatum *et al.*, (2020), the baking conditions (temperature and time variables), the state of the biscuit constituents, such as fiber, starch, protein (gluten), whether damaged or undamaged and

the amounts of absorbed water during dough mixing contribute to the outcome of its overall acceptability

Table 7. Sensory characteristics of cracker biscuits extracts made from blends of high quality cassava and sour sop seeds flour

Sample	Color	Crunchiness	Taste	Aroma	Overall acceptability
100HQC	6.83±0.36ab	6.47±0.29a	6.53±0.33ab	5.87±0.29ab	6.93±0.19a
100WH	7.40±0.23a	6.67±0.38a	6.90±0.29a	6.47±0.28a	6.99±0.33a
85HQ10WH5Ss	6.33±0.33b	4.43±0.31a	5.93±0.38ab	5.80±0.35b	6.40±0.31ab
80HQ10WH10Ss	6.10±0.24bc	3.63±0.37a	5.67±0.43b	5.80±0.42b	6.03±0.37ab
75HQ10WH5Ss	6.00±0.39bc	2.67±0.42a	5.67±0.33b	5.00±0.39b	5.33±0.32ab
70HQ10WH20Ss	5.17±0.38bc	2.23±0.31a	5.63±0.36b	4.27±0.40ab	4.67±0.35b
65HQ10WH25Ss	4.20±0.49c	2.08±0.43aa	5.20±0.40ab	4.10±0.44b	4.00±0.36ab

Mean values are of triplicate determinations. Mean values within a column with different superscripts are significantly different at (p <0.05)

Conclusion

Nutritional, antioxidant and α -amylase inhibitory properties of cracker biscuits from high quality cassava and sour sop (*Annona muricata*) seed flours was evaluated. The formulated cracker biscuits exhibited an increase in the protein, fat, ash and fiber contents as the concentration of sour sop seeds increased. An improved mineral content was also observed more importantly magnesium, phosphorus and sodium with an increasing amount of sour sop. The cracker biscuits demonstrated a significant amount of total phenolic content, total flavonoid content and DPPH as the concentration of sour sop increased more importantly (65HQ10WH25SS). A high α -amylase inhibitory activity responsible for the breakdown and absorption of carbohydrate was observed among the samples as the concentration of sour sop increased, however, (65HQ10WH25SS) showed a very high α -amylase inhibitory activity compared to other samples and the control

The developed cracker biscuits was generally acceptable in terms of taste, crunchiness and aroma compared to the control samples. It can therefore be concluded the formulated cracker biscuits may be considered as functional foods for dual purposes of preventing protein energy malnutrition among children and management of degenerative disease such as Type-2 diabetes.

References

- Abo K. A., Fred-Jaiyesimi A. A & Jaiyesimi A. E. (2008). Ethnobotanical studies of medicinal plants used in the management of diabetes mellitus in South Western Nigeria. *J. Ethnopharmacol.* 115(1): 67-71
- Adefegha, S.A & Oboh, G. (2012). "Inhibition of key enzymes linked to type 2 diabetes and sodium nitroprusside-induced lipid per oxidation in rat pancreas by water extractable phytochemicals from some tropical spices," *Pharmaceutical Biology* 50 (7). 857-65
- Adefegha, S.A., Oboh, G., Adefegha, O.M., Boligon, M.L & Athayde, M.L (2014). "Antihyperglycemic, hypolipidemic, hepato protective and antioxidative effects of dietary clove (*Syzygium aromaticum*) bud powder in a high-fat diet/streptozotocin induced diabetes rat model," *Journal of the Science of Food and Agriculture* 94 (13). 2726-2737
- Ademosun, A.O., Odanye, O.S & Oboh G (2021) Orange peel flavored unripe plantain noodles with low glycemic index improved antioxidant status and reduced blood glucose levels in diabetic rats. *Food Measure* 15:3742–3751
- Aderinola, T.A., Aluko, R.M., Fagbemi, T.N., Enujiugha, V.N 1 & Alashi, A.M (2018). In vitro antihypertensive and antioxidative properties of trypsin derived *Moringa oleifera* seed globulin hydrolyzate and its membrane fractions. *Food Sci Nutr* 7(1): 132–138.
- Addisu, A., Shimelis, A & Biniyam, T (2019). Shelf-life prediction of edible cotton, peanut and soybean seed oils using an empirical model based on standard quality tests. *Cogent Food Agric.* 5 (1).
- Adekunte, A.O., Tiwari, B.K., Cullen, P.J., Scannell, A.G.M & O'donnell, C.P (2010) Effect of sonication on colour, ascorbic acid and yeast inactivation in tomato juice. *Food Chem* 122(3):500–507

- Ademiluyi, A.O., Oboh, G & Aragbaiye, F.P (2015). "Antioxidant properties and in vitro α -amylase and α -glucosidase inhibitory properties of phenolics constituents from different varieties of *Corchorus* spp.," *Journal of Taibah University Medical Sciences* 10 (3). 278-287.
- Adeyemo, A.E., Omoba, O.S., Olagunju, A.I & Josiah, S.S (2022). Chemical composition, in vitro antioxidant properties, and phenolic profile of shallot (*Allium ascalonicum* L.)-enriched plantain biscuit. *Bulletin of the National Research Centre* 46:85
- AOAC. Association for Official, Analytical Chemist. Official Methods for Analysis (2012). nineteenth ed., 2012. Washington DC.
- Ajayi, A (2015). Mineral contents of *Ocimum gratissimum* leaves (African basil), *Int. J. Sci. Res.* 6 (10). 1572-1573
- Akomolafe, S & O. Ajayi, (2015). "A comparative study on antioxidant properties, proximate and mineral compositions of the peel and pulp of ripe *Annona muricata* (L.) fruit," *International Food Research Journal* 22: 2381-2388
- Aladesanmi, O.O., Akinmoladun, A.C., Josiah, S.S., Olaleye, M.T & Akindahunsi, A.A (2020) Modulatory activities of polyphenols on crude acetylene induced cardiac and hepatic dysfunctions in a rat model. *Drug Chem Toxicol.* <https://doi.org/10.1080/01480545.2020.1853766>
- Ashraf M.A, Iqbal M, Rasheed R, Hussain I, Riaz M, Arif M.S (2018). Environmental stress and secondary metabolites in plants: an overview. In: Ahmad P, Ahanger MA, Singh VP, tripathi DK, Alam P, Alyemini MN (eds) *Plant metabolites and regulation under environmental stress*, Academic Press. 153-167.
- Bahijri, S.M., Jambi H.A., Al Raddadi R.M., Ferns G & Tuomilehto J (2016). The prevalence of diabetes and prediabetes in the adult population of Jeddah, Saudi Arabia-A community-based survey. *PLoS ONE*
- Banu ,S Nabir, N.R & N.C.Manjunathet, N.C (2015). "Reduction of post-prandial hyperglycemia by mulberry tea in type-2 diabetes patients," *Saudi Journal of Biological Sciences* 22 (1): 32-36.
- Buowari O (2013). *Diabetes Mellitus—Insights and Perspectives*. InTechOpen; Rijeka, Croatia: Chapter 8: Diabetes mellitus in developing countries and case series.
- Cao, A., Tang, Y & Liu, Y. (2012). Novel fluorescent biosensor for α -glucosidase inhibitor screening based on cationic conjugated polymers. *ACS Appl. Mater. Interfaces.* 4 (8): 3773-3778.

- Dada, M.A., Bello, F.A., Omobulejo, F.O & Olukunle, F.E (2023). Nutritional quality and physicochemical properties of biscuit from composite flour of wheat, African yam bean and tigernut. *Heliyon* 9 (11): 1-32
- D'Angelo, S (2020). Current evidence on the effect of dietary polyphenols intake on brain health. *Curr Nutr Food Sci* 16(8):1170–1182
- Emelike, N.J.T., Akusu, M.O & Ujong, A.E (2017). Antioxidant and physicochemical properties of oils extracted from cashew (*Anacardium occidentale*) kernels, *Int. J. Food Sci. Nutr.* 2 (6) 122–128.
- Emelike, N.J.T., Barber, L.I & Ebere, C.O (2015). Proximate, mineral and functional properties of defatted and undefatted cashew (*Anacardium occidentale* Linn.) kernel flour, *Eur. J. Food Sci. Technol.* 3 (4). 11-19.
- Fasakin, A., Fehintola, E., Obijole, O & Oseni, O (2008). "Compositional analyses of the seed of sour sop (*Annona muricata* L) as a potential animal feed supplement," *Scientific Research and Essays* 3 : 521-523.
- Fasihzadeh, S., Lorigooini, Z & Jivad, N (2016). Chemical constituents of *Allium stipitatum* regel (*Persian shallot*) essential oil. *Der Pharm Lett* 8(1):175-180.
- Folorunso O & Oguntibeju O. (2013). *Diabetes Mellitus—Insights and Perspectives.* InTechOpen; Rijeka, Croatia: Chapter 5: The role of nutrition in the management of diabetes mellitus.
- Ifediba, D.I & Egbuna, H.I (2019). Proximate composition and organoleptic properties of whole wheat biscuit fortified with Moringa (*Moringa oleifera*) Leaf powder, *Int. J. Sci. Res. Publ.* 9 (11) (2019) 637–642.
- Iombor, T.T & Banjo, A.U. (2018). "Evaluation of the potential of soursop (*Annona muricata*) flour in bread production," *Confluence Journal of Pure and Applied Sciences* 2:19-26.
- Iombor, T.T., Olaitan, I.N & Ede, R.A (2014). "Proximate composition, antinutrient content and functional properties of soursop flour as influenced by oven and freeze drying methods," *Current Research in Nutrition and Food Science Journal* 2. 106-110.
- Iombor, T.T & Banjo, A.U (2018). "Evaluation of the potential of soursop (*Annona muricata*) flour in bread production," *Confluence Journal of Pure and Applied Sciences* 2: 19-26.

- Jiang, G., Wu, Z., Ameer, K & Song, C. (2020). Physicochemical, antioxidant, micro structural, and sensory characteristics of biscuits as affected by addition of onion residue. *J Food Meas Charact* 57(12):4551-4561
- Jiménez, V.M., Gruschwitz, M., Schweiggert, R.M., Carle, R & Esquivel, P (2014) “Identification of phenolic compounds in soursop (*Annona muricata*) pulp by high-performance liquid chromatography with diode array and electrospray ionization massspectrometric detection,” *Food Research International* 65. 42-46
- Krzysztoforska, K., Mirowska-Guzel, D & Widy-Tyszkiewicz E (2019). Pharmacological effects of protocatechuic acid and its therapeutic potential in neurodegenerative diseases: Review on the basis of in vitro and in vivo studies in rodents and humans. *Nutr Neurosci* 22(2):72–82.
- Liu, K., Luo, M & Wei, S (2019). The bioprotective effects of polyphenols on metabolic syndrome against oxidative stress: evidences and perspectives. *Oxid Med Cell Longev.* <https://doi.org/10.1155/2019/6713194>
- Masek, A., Chrzescijanska, E., Latos, M., Zaborski, M & Podsedek, A (2017). Antioxidant and antiradical properties of green tea extract compounds *Int. J. Electrochem. Sci.* 12 (7). 6600-6610.
- Nair, S.S., Kavrekar, V and Mishra, A (2013) “In vitro studies on alpha amylase and α glucosidase inhibitory activities of selected plant extracts,” *European Journal of Experimental Biology* 3 (1): 128–132.
- Ndife, J., Obiegbuna, J & Ajayi, S (2013). Comparative evaluation of the nutritional and sensory quality of major commercial whole-wheat breads in Nigerian market, *Adv. J. Food Sci. Technol.* 5. 1600-1605
- Nguyen, M.T., Nguyen, V.T & Minh, L.V (2020). “Determination of the phytochemical screening, total polyphenols, flavonoids content, and antioxidant activity of sour sop leaves (*Annona muricata* Linn)” *IOP Conference Series. Materials Science and Engineering* 736 (6).
- Nwatum, I.A., Ukeyima, M.T & Eke, M.O (2020). Production and quality evaluation of cookies from wheat defatted peanut and avocado composite flour. *Asian Food Sci. J* 15 (4). 1-12.
- Odunlade, T.V., Famuwagum, A.A., Taiwo, K.A., Gbadamosi, S.O., Oyedele, D.J & Adebooye, O.C. (2017). Chemical composition and quality characteristics of wheat bread supplemented with leafy vegetable powders, *Hindawi J. Food Qual.* 3. 1-7.

- Onwulata, C. I., Thomas, A. E., Cooke, P. H., Phillips, J. G., Carvalho, C., Ascheri, J & Tomasula, P. M. (2010). Glycemic Potential of Extruded Barley, Cassava, Corn, and Quinoa Enriched With Whey Proteins and Cashew Pulp. *Int. J. Food Prop.* 13, 338–359.
- Okigbo, R.N (2008). "Mycoflora and production of wine from fruits of soursop (*Annona Muricata* L.)," *International Journal of Wine Research* 1: 1-9.
- Olagunju, O.F., Ezekiel, O.O., Ogunshe, A.O., Oyeyinka, S.A & Ijabadeniyi, O.A (2018) Effects of fermentation on proximate composition, mineral profile and antinutrients of tamarind (*Tamarindus indica* L.) seed in the production of daddawa-type condiment. *Lebensmittel-Wissenschaft & Technologie*, 90:455–459
- Oludumila, O.R & Adetimehin, A.M (2016). Quality characteristics of cookies produced from composite flours of unripe plantain, wheat and water melon seeds. *Indian J Nutr* 2(2):2326-2395.
- Olubukola, A., Olasunkanmi, G., Kehinde, T., Oyedele, D.J & Adebooye, C. (2017). Proximate, mineral, sensory evaluations and shelf stability of chinchin enriched with Ugu and Indian spinach vegetables, *Int. J. Biochem. Res. Rev.* 18 (4). 1-14.
- Patras, A., Brunton, N.P., Tiwari, B.K & Butler F (2011) Stability and degradation kinetics of bioactive compounds and colour in strawberry jam during storage. *Food Bioproc Tech* 4(7):1245–1252
- Pereira D, Correia P.M, Guine R.P (2013) Analysis of the physical-chemical and sensorial properties of maria type cookies. *Acta Chim Slovaca* 6:269-280
- Pathare, P.B., Opara, U.L & Al-Said, F.A.J (2013) Colour measurement and analysis in fresh and processed foods: a review. *Food Bioproc Tech* 6(1):36–60.
- Reshi, M., Kaul, R.K., Bhat, A & Kaur, P (2012) “The role of antioxidants in human health,” *International Journal of Medical Sciences.* 6 (1). 14-22
- Rebellato, A.P., Pacheco, B.C., Prado, J.P & Pallone, J.A.L (2015). Iron in fortified biscuits: a simple method for its quantification, bioaccessibility study and physicochemical quality. *Food Research International* 77 (3). 385-391.
- Saravanan, S & Parimelazhagan, T (2014). “In vitro antioxidant, antimicrobial and anti-diabetic properties of polyphenols of *Passiflora ligularis* Juss, fruit pulp,” *Food Science and Human Wellness*, 3 (2). 56-64

- Sharma, S.K., Bansal, S., Mangal, M., Dixit, A.K., Gupta, R.K & Mangal, A.K. (2016). Utilization of food processing by-products as dietary, functional, and novel fiber: a review, *Crit. Rev. Food Sci. Nutr.* 56 (10). 1647-1661
- Salehi B., Kumar N.V.A., Şener B., Sharifi-Rad M., Kılıç M., Mahady G.B., Vlaisavljevic S., Iriti M., Kobarfard F & Setzer W.N (2018). Medicinal plants used in the treatment of human immunodeficiency virus. *Int. J. Mol. Sci* 19:1459
- Schiffrin, E.L (2010). “Antioxidants in hypertension and cardiovascular disease,” *Molecular Interventions* 10 (6). 354-362
- Sukasih, E & Musada, D (2018) Physico-chemical characteristics of shallot new superior varieties (NSV) from Indonesia. In *IOP Conference Series: Earth and Environmental Science*, IOP Publishing, 102, p 012037
- Singleton, V.L., Orthofer, R & R. M. Lamuela-Raventos, R.M (1999) “Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-ciocalteu reagent,” *Methods in Enzymology* 299:152–178.
- Sittisart P., Yossan, S and Prasertsan, P (2017). Antifungal property of chili, shallot and garlic extracts against pathogenic fungi, *Phomopsis* spp, isolated from infected leaves of Para rubber (*Hevea brasiliensis* Muell. Arg.). *Agric Nat Resour* 51:485-491
- Soininen, T.H., Jukarainen, N., Julkunen-Tiitto, R., Karjalainen, R & Vepsäläinen, J.J (2012) The combined use of constrained total-line-shape 1H NMR and LC–MS/MS for quantitative analysis of bioactive components in yellow onion. *J Food Compost Anal* 25:208-214.
- Sun W, Shahrajabian, M.H & Cheng, Q (2019.) The insight and survey on medicinal properties and nutritive components of shallot. *J Med Plant Res* 13:452–457
- Sperling M., Tamborlane M., Batteling T., Weinzimer S., Phillip M (2014). *Diabetes mellitus*. 4th ed. Elsevier; Amsterdam, The Netherlands: Pediatric endocrinology.
- Sharifi-Rad M., Salehi B., Sharifi-Rad J., Setzer W.N & Iriti, M. (2018). *Pulicaria vulgaris* Gaertn. essential oil: An alternative or complementary treatment for leishmaniasis. *Cell. Mol. Biol* 64:18-21.
- Trinidad, T. P., Mallillin, A. C., Loyola, A. S., Sagum, R. S & Encabo, R. R. (2010). The potential health benefits of legumes as a good source of dietary fibre. *Br. J. Nutr.* 103 (4):569-74.

Ujong A.E., Emelike N.J .T., Woka F.I & Francis Otekeiwebia J.N.R (2023). Formulation of fiber enriched crackers biscuit: Effect on nutritional composition, physical and sensory properties. *Heliyon* 9. 1-9

Zabidi, M.A & Yunus , A.M. (2014). "Effect on physicochemical and sensory attributes of bread substituted with different levels of matured soursop (*Annona muricata*) flour," *International Journal of Nutrition and Food Engineering* 8: 740-744.

UNDER PEER REVIEW