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 ofhigh qualitycassava-wheat flour and soursop 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 (65HQ10WH25Ss) 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, (65HQ10WH25Ss) 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, soursop, 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 (*Annonamuricata*) 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 stingy and sweet-sour taste containing many dark seeds (Iombor*et al*., 2014). Soursop 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 soursop flour for the production of bread has also been reported by Zabidi and Yunus (2014). The effect of soursop flour inclusion to wheat flour for bread production have also been investigated and reported that the flour from soursop has quality attributes which could be utilized for the production of bread and other baked goods thereby diversifying the utilization of soursop and improving the quality of food products produced from it(Iombor and Banjo, 2018). Soursop 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 have been reported that soursop possesses some therapeutic properties such as antioxidant and anticancer. They further added that the soursop 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 soursop in the production of cracker biscuits, thus the study aimed to produced 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

Soursop 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 fromPsaltry international company limited, Ado awaye, Iseyin local government, Oyo state.. Chemicals used for all analysis were of analytical grade.

Methods

Processing of soursop seeds flour

The seeds of soursopwere removed from the fruits, washed and air-dried at room temperature (28 ± 2.00 ℃) 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 soursop pulp flour were formulated in percentages as shown in Table 1

Production of crackers

High quality cassava flour and soursop 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 soursop 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 ℃. 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 ℃ 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

$$\textbf{\% DPPH} = \frac{Acontrol\ - Asample}{Acontrol} x\ 100$$

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 ℃ 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 $FeCl_3$ 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 ℃, and the absorbance was taken at 700 nm in the spectrophotometer (Healicom 721S China), garlic 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 µL of the extract was added to 300 µl of 5 % sodium nitrate. After 5 min, 600 µl 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 ±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 ofcracker 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 soursop 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 soursop seed flour that is as the concentration of soursop 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 (Solıs-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 % soursop 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 that 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 soursop seeds is expected as seeds of soupsop 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 undefattedsoursap 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 that 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 soursop 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 soursop seeds increased agrees with the reportof Olubukola *et al*., (2017) who also reported a decreased in the carbohydrate content ofchin-chin enriched with Ugu seeds flour. Ifediba and Egbuna (2019)equallyshowed a significant decrease in carbohydrate from 49.38-47.80 % forbiscuit 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 qualitycassava 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
100HQC	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 cassavaand soursop 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). Adekunte*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.

Table 4. Color characteristic of cracker biscuits made from blends of high quality cassava and soursop 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 cassavaand 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 sopseeds has been reported to contain high amount of phytochemicals such as quercetin, gallic and kaempferol (Nguyen *et al*., 2020; Reshi *et al*., 2012).However, Adefeghа*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 poly phenols 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 sopseed 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 soursop 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 Soursop seeds extracts on α-amylase activity in vitro suggeststhepossiblemechanismbywhichSoursop 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 soursop 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 (Jim´enez*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 soupsop seeds flour.

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

% inhibition of the standard at IC$_{50}$ (Acarbose)- 33.525 %

IC$_{50}$ of the standard (Acarbose)- 57.65%

Sensory properties of cracker biscuits made from blends of high quality cassava and soursop 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 mixingcontribute to the outcome of its overall acceptability

Table 7. Sensory characteristics of cracker biscuits extracts made from blends of high qualitycassava and soursop 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.

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