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Bioactive composition and anti-hyperglycemic properties of biofortified yellow maize-based gluten-free cookies

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The use of gluten-free ingredients in the baking of cookies is increasing due to gluten-associated disorder and other nutritional-related non-communicable diseases, and consumer's pursuit for health-promoting foods. In this study, gluten-free cookies (GFC) were made from biofortified yellow maize supplemented with sodium carboxyl methylcellulose (SCMC), a reference hydrocolloid, and *Brachystegia eurycoma* flour (BEF) at four different proportions (2.5, 5, 7.5, and 10%), respectively. The GFC were then assessed for their bioactive components (flavonoids, tannins, and saponins), carotenoids, starch-hydrolyzing enzymes (alpha-amylase and alphasglucosidase), and glycemic index. Cookies made from commercial wheat flour (100% CWF) served as control. The total flavonoid contents of the 100% BYF cookies (144.48 ± 0.18 mg/g) and saponins (1.04 ± 0.00 mg/g) were significantly ($p < 0.05$) higher than the 100% CWF cookies (93.84 ± 0.36 mg/g; 1.02 ± 0.00 mg/g). The total tannin contents of the cookies ranged from 3.12 ± 0.00 mg/g (100% CWF) to 4.87 ± 0.00 mg/g (2.5%BEF-97.5% BYF). The carotenoid profiles indicated the presence of lutein (range from 0.62 to 1.63 µg/g), β-carotene (range from 3.81 to 6.06 µg/g), β-cryptoxanthin (range from 0.19 to 1.75 µg/g) and zeaxanthin (range from 0.83 to 2.48 µg/g) in the cookies. Further, the GF cookies significantly ($p < 0.05$) inhibited the alpha-amylase (IC₅₀ value ranged from 1.42 ± 0.01 µg/mL (100% BYF) to 2.63 ± 0.01 µg/mL (100% CWF)) and alpha-glucosidase with IC₅₀ value range from 1.12 ± 0.01 µg/mL (2.5%SCMC-97.5% BYF) to 6.72 ± 0.01 (7.5%BEF-92.5% BYF). The GF cookies showed a low glycemic index (GI) value (GI < 50). The biofortified yellow maize-based GFC have significant amounts of bioactive compounds and anti-hyperglycemic properties, and low GI. Hence, they can serve as functional baked food with potential health benefits.

KEYWORDS

biofortified yellow maize, carotenoid, bioactive compound, gluten-free cookies, *Brachystegia eurycoma* flour

1 Introduction

Cookies are among the bakery products consumed as a snack by human beings (Susman et al., 2021). It is characterized by low water content which contributes to its lengthy shelf life due to the absence of microbiological deterioration (Dhankhar, 2013). Cookies are usually made from refined wheat flour, which contains gluten that gives its dough a remarkable viscoelastic



FIGURE 1
Plant materials. (A) Biofortified yellow maize grain, (B) *Brachystegia eurycoma* seed.

properties (Elif and Duygu, 2020; Irondi et al., 2023; Imam et al., 2024). However, there has been an increase in the baking of cookies using gluten-free (GF) ingredients owing to gluten-associated disorders like the increasing prevalence of celiac disease, consumers' pursuit for health-promoting foods as well as other nutrition-related non-communicable diseases (Irondi et al., 2023; Imam et al., 2024). Pseudocereals, tubers, seeds, legumes, nuts, fruits, and cereals constitute a few of these elements (Jnawali et al., 2016; Bashir et al., 2020; Xu et al., 2020; Irondi et al., 2023; Imam et al., 2024). Previous investigation has suggested that GF products can provide medical advantages including anti-diabetic, anti-cancer, anti-hypertensive, and hypo-cholesterolemia characteristics (Manzoor, et al., 2020; Irondi et al., 2023; Imam et al., 2024). These have been related to their chemical composition, particularly the presence of bioactive substances (Fouad and Rehab, 2013; Sethiya et al., 2014). Typical GF cookie ingredients including flour, rice starch, and corn starch have been linked to possible worries among those with metabolic issues (such as diabetes and obesity). Risks include a high glycemic index after ingesting owing to their starch content (Elif and Duygu, 2020). When making GF cookies, ingredients that might mitigate these risks and have superior health benefits might be a good substitute (Di Cairano et al., 2018).

Maize (*Zea mays* L.), is an arable crop cultivated predominantly for its grains, which are crucial for both human and animal nutrition. It is the third-popular grain cultivated worldwide following rice and wheat (Yousaf et al., 2020) and serves as an excellent source of minerals, iron, vitamin B, carbohydrates, and protein (Siwela et al., 2020). Biofortified yellow maize (provitamin A biofortified maize) is a major dietary source of bioactive substances like carotenoids, vitamin C, polyphenolic compounds, and anthocyanins (Alamu et al., 2018; Beta and Hwang, 2018; Elemosho et al., 2020). These bioactive chemicals may provide anti-obesity, antidiabetic, and antioxidant effects on health (Žilić et al., 2012; Irondi et al., 2019a). Moreover, *Brachystegia eurycoma* (BE) is a naturally occurring hydrocolloid source that is underutilized and can imitate the viscoelastic properties of gluten (Irondi et al., 2021a; Irondi et al., 2022). Additionally, it provides

nutrients including protein, calories, carbs, minerals, and vitamins (Nwosu, 2012) as well as antioxidants and important phytochemicals such as flavonoids, saponins, phenolic compounds, tannins that render health benefits (Irondi et al., 2015; Irondi et al., 2021a). The health advantages of gluten-free cookies can be increased by baking them with biofortified yellow maize and *Brachystegia eurycoma*. Therefore, the aim of this study is to evaluate the bioactive content and anti-hyperglycemic qualities of biofortified gluten-free cookies made from biofortified yellow maize and *Brachystegia eurycoma*.

2 Materials and methods

2.1 Materials

The materials used in this study were biofortified yellow maize (BYM) grains and *B. eurycoma* (BE) seed (Figure 1), commercial wheat flour (CWF) (*Triticum aestivum*), and sodium carboxyl methylcellulose (SCMC) (reference hydrocolloids). These materials were purchased from a neighborhood market in Ilorin, Kwara State. The chemicals and reagents used in this study were of analytical grade.

2.2 Methods

2.2.1 Preparation of flour samples used for cookie production

BE seeds and BYM grains were ground into distinct flours (Figure 2). The biofortified yellow maize flour (BYF) blends used to make BYF-based cookies were formulated by adding SCMC and BE flour (BEF) to BYF at different proportions (2.5, 5, 7.5, and 10%), respectively. CWF was used to produce the control cookies.

2.2.2 Baking of cookies

The cookies were baked by employing Okaka and Isieh's (1990) method. Using a Kenwood Chef, the 200 g flour sample was mixed

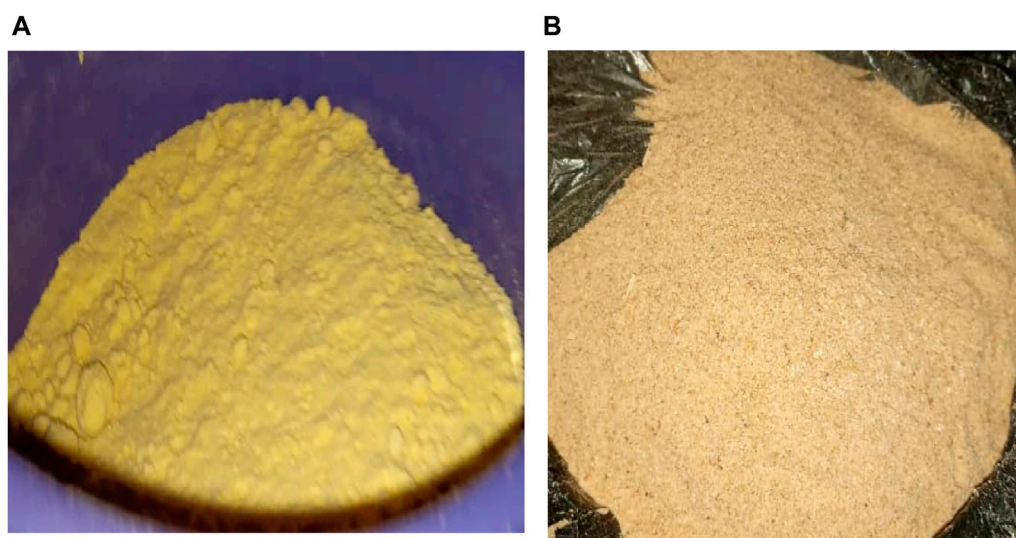


FIGURE 2
Flour samples. (A) Biofortified yellow maize flour, (B) *Brachystegia eurycoma* flour.



FIGURE 3
Cookie samples.

with 100 g margarine until a light and fluffy consistency was obtained. When the speed mark reached the 6 marks on the indicator, 60 g of whole egg, 0.1 g of baking powder, 20 g of powdered milk, and 1 g of salt were combined with the flour sample to create a batter or stiff paste. Next, the batter was rolled to thickness (0.2–0.3 cm) using a floured board and rolling pin. After shaping the rolled batter into forms, it was placed on a tray coated with oil and cooked for 20 min at 150°C. The cookies (as seen in Figure 3) were removed and placed in cellophane bags for further analysis.

2.2.3 Preparation of methanolic extract

The methanolic extract of the cookies flour was prepared using the procedure of Chan et al., 2007. Methanol (10 mL) was added to a capped 50 mL centrifuge tube containing 1 g of the cookie samples. After a few minutes of vigorous shaking at ambient temperature, the mixture was left overnight. It was then filtered to obtain the

supernatant (methanolic extract) and was stored at -4°C until analysis.

2.2.4 Quantitative determination of bioactive compounds of the cookies

The following standard techniques were followed to quantitatively quantify the bioactive chemicals present in the samples.

2.2.4.1 Determination of total flavonoids content

The aluminum chloride method as reported by Kale et al. (2010) was used to quantify the total flavonoid contents. Methanolic extract (0.5 mL) was dispensed into a test tube, followed by 1.5 mL of methanol, 0.1 mL of aluminum chloride (10%), 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water. The reaction mixture was shaken, incubated at room temperature for 30 min and absorbance was then read at 514 nm. The total flavonoid content was expressed as quercetin equivalent (QE) in mg/g material.

2.2.4.2 Determination of total tannin contents

The tannin content of the sample was measured using the technique described by Amorim et al. (2008). To sum up, 1 mL of 35% sodium carbonate solution, 7.5 mL of distilled water, 0.5 mL of Folin-Denis reagent, and 0.1 mL of supernatant were combined and then further diluted to 10 mL using distilled water. A UV/Visible spectrophotometer was then used to measure the mixture's absorbance (760 nm) after shaking it and letting it sit at room temperature for 30 min. The tannic acid equivalent was used to express the amount of tannin in mg/g of material.

2.2.4.3 Determination of total saponins

The total saponins content of the extract was determined by the method described by Makkar et al. (2007). An aliquot (0.25 mL) of the supernatant was pipette into a test tube after which 0.25 mL vanillin reagent (8% vanillin in ethanol) and 72% aqueous H_2SO_4

(2.5 mL) were added. The reaction mixture in the tubes was heated in a water bath (60 °C) for 10 min. The tubes were cooled in ice for 4 min and allowed to acclimatize to room temperature. Thereafter, the absorbance was read in a UV/Visible spectrophotometer at 544 nm. Diosgenin was used as a standard, and the result obtained was expressed as mg diosgenin equivalent per gram.

2.2.5 Quantification of carotenoid contents of the cookies

The carotenoid content of the cookie sample was quantified using the procedure outlined by [Howe and Tanumihardjo \(2006\)](#). In a nutshell, 0.6 g of the sample and 6 mL of ethanol (with 0.1% butylated hydroxyl toluene) were combined to extract carotenoids from the flours. The mixture spent 5 minutes in an 85°C water bath. The interfering oil in the mixture was then saponified for 5 minutes at 85°C in a water bath using potassium hydroxide (80% w/v). After combining the suspension with a vortex, it was put back into the water bath for an additional 5 minutes. It was put into an ice-cold bath right away, and 3 mL of deionized water were added. Three times in a row, the mixture's carotenoid contents were extracted using 3 mL of n-hexane and centrifuged at 1,000 rpm for 10 s. A 50 mL concentrator tube was filled with the upper layer of the mixture. The mixed hexane fraction was centrifuged for 10 s at 1,000 rpm, vortexed three times, and then cleaned with deionized water. Using a Turbo Vap (LIV) Concentrator with nitrogen gas for 25 min, the n-hexane fraction was dried down. Then, an aliquot (100 µL) was injected into the HPLC system to quantify the carotenoids after the dried extract was reconstituted with 1 mL of methanol/dichloromethane, 50:50 v/v.

The HPLC system (Waters Corporation, Milford, MA, USA) included an auto-sampler (Waters 717), a photodiode array detector (Waters 2996), a binary HPLC pump (Waters 626), a guard column, and a C30 YMC carotenoid column (4.6 × 250 mm, 3 µM). Empower one software (Waters Corporation) was used to run the system. The mobile phase was made up of solvent B, which contained 100% methyl tertiary-butyl ether, and solvent A, which included methanol-water (92:8 v/v) with 10 mmol/L ammonium acetate. Gradient elution was carried out in the following circumstances, with a flow rate of 1 mL/min: There were four linear gradients in total—29 min from 83% to 59% A, 6 min from 59% to 30% A, 1 min at 30% A, 4 min from 30% to 83% A, and 4 min at 83%. Chromatograms were generated at 450 nm, and their carotenoids were identified and quantified using the external standards approach. This involved comparing the absorption spectrum and co-elution with standard carotenoids, as well as utilizing the calibration curve from pure standards.

2.2.6 *In vitro* starch -digesting enzymes (alpha-amylase and alpha-glucosidase) inhibitory assays of the cookies

2.2.6.1 *In vitro* alpha-glucosidase inhibition assay

Alpha-glucosidase inhibitory activity was conducted following the method reported by [Kim et al. \(2005\)](#) using α-glucosidase (EC 3.2.1.20) and para-nitrophenyl glucopyranoside (PNPG) as the substrate. An aliquot of α-glucosidase (Five units) was incubated with cookies methanol extracts (20 µg/mL) for 15 min. Next, 3 mM PNPG was dissolved in 20 mM phosphate buffer (pH 6.9) as a

substrate to initiate the hydrolytic reaction. The hydrolytic reaction was allowed to proceed for 20 min at 37°C, after which it was terminated by adding 0.1 M Na₂CO₃ (2 mL). The absorbance of the yellow *p*-nitrophenol released from PNPG hydrolysis was read at 400 nm. The percentage (%) inhibition of α-glucosidase was calculated as follows:

$$\% \text{ Inhibition} = \left(\frac{\text{Absorbance } 400_{\text{Reference}} - \text{Absorbance } 400_{\text{Sample}}}{\text{Absorbance } 400_{\text{Reference}}} \right) \times 100$$

2.2.6.2 *In Vitro* alpha-amylase inhibition assay

The method described by [Kwon et al. \(2008\)](#) was used to conduct alpha-amylase inhibition assay. Porcine pancreas α-amylase (EC 3.2.1.1) and soluble starch were used in this assay as enzyme and substrate respectively. Appropriate dilutions of methanol extract of cookies samples (500 µL) and 0.02 M sodium phosphate buffer (500 µL) (pH 6.9 with 0.006 M NaCl) containing 0.5 mg/mL α-amylase solution were incubated (37°C) for 10 min. Afterward, 1% starch solution (500 µL) in 0.02 M sodium phosphate buffer was added. Next, the reaction mixture was incubated at 37°C for 15 min, after which 1.0 mL of DNSA color reagent (1% 3, 5-dinitro salicylic acid, and 12% sodium potassium tartrate in 0.4 M NaOH) was added to terminate the reaction. Next, the reaction mixture was incubated for 5 min in a boiling water bath, cooled to room temperature, and diluted with 10 mL of distilled water. The absorbance was read at 540 nm. The percentage (%) inhibition of α-amylase will be calculated as follows:

$$\% \text{ Inhibition} = \left(\frac{\text{Absorbance } 540_{\text{Reference}} - \text{Absorbance } 540_{\text{Sample}}}{\text{Absorbance } 540_{\text{Reference}}} \right) \times 100$$

2.3 Evaluation of the glycemic index of cookies

The study on the glycemic index (GI) was approved by the Kwara State Ministry of Health, Ilorin with approval number MOH/KS/EU/777/493. Sixty (6) healthy, non-diabetic human subjects (30 males and 30 females) between 28 and 50 years were recruited for the clinical trials upon obtaining their information consent. Measurement and calculation of GI were based on the method described by [FAO/WHO \(1998\)](#). Subjects used had no known diseases and were not on any medications which could influence the results of the study. They were strictly informed to abstain from cigarette smoking or alcohol and caffeine-containing drinks within the period of the study. All subjects fasted overnight (7.30 p.m.–7.30 a.m.) before the morning testing and were instructed not to engage in strenuous physical activities before testing days. Data on height, weight, and age were obtained for each subject before the test.

2.4 Data analysis

Results of triplicate experiments were expressed as mean ± standard deviation (SD). Analysis of variance (ANOVA) was

TABLE 1 Bioactive compounds of the cookies samples.

Samples	Total flavonoids (mg/g)	Tannins (mg/g)	Total saponins (mg/g)
100% CWF	93.84 ± 0.36 ^d	3.12 ± 0.00 ^a	1.02 ± 0.00 ^a
100%BYF	144.48 ± 0.18 ^b	3.70 ± 0.02 ^e	1.04 ± 0.00 ^f
2.5%BEF-97.5% BYF	121.15 ± 0.18 ^g	4.87 ± 0.01 ^j	1.05 ± 0.00 ^g
5%BEF-95% BYF	80.00 ± 0.36 ^c	4.50 ± 0.01 ⁱ	1.03 ± 0.00 ^d
7.5%BEF-92.5% BYF	106.53 ± 0.18 ^f	4.04 ± 0.01 ^f	1.04 ± 0.00 ^f
10%BEF-90%BYF	59.35 ± 0.54 ^a	4.41 ± 0.00 ^h	1.04 ± 0.00 ^c
2.5%SCMC-97.5% BYF	59.48 ± 0.36 ^a	3.36 ± 0.00 ^b	1.03 ± 0.00 ^d
5%SCMC-95% BYF	95.76 ± 0.18 ^e	3.41 ± 0.00 ^c	1.02 ± 0.00 ^b
7.5%SCMC-92.5%BYF	58.71 ± 0.36 ^a	3.44 ± 0.00 ^d	1.03 ± 0.00 ^c
10%SCMC-90% BYF	71.92 ± 0.54 ^b	4.08 ± 0.00 ^g	1.03 ± 0.00 ^c

Data are the mean values ± standard deviation (SD) of independent triplicate determinations. Data with different lowercase superscript letters along the same column vary significantly ($p < 0.05$). CWF, commercial wheat flour; BYF, biofortified yellow maize flour; BEF, *brachystegia eurycoma* flour; SCMC, sodium carboxymethyl cellulose.

carried out on the result data and Duncan's Multiple Range Test was performed to compare the means of the data at a 95% confidence level using SPSS software (21st version). Graph pad prism eight was used in plotting the graphs.

3 Result and discussion

3.1 Bioactive compounds of the cookies

The result of the bioactive compounds determination is presented in Table 1. From the result, 100% BYF had the highest value of total flavonoid content (144.48 ± 0.18 mg/g) which is significantly higher ($p < 0.05$) than that of the 100% CWF cookies (93.84 ± 0.36 mg/g). The lowest flavonoid content was found in 7.5%SCMC-92.5%BYF cookies (58.71 ± 0.36 mg/g) and is significantly comparable ($p > 0.05$) with 10%BEF-90%BYF (59.35 ± 0.54 mg/g) and 2.5% SCMC-97.5% BYF (59.48 ± 0.36 mg/g) cookies. Further, the total tannin contents of the cookies ranged from 3.12 ± 0.00 mg/g (100% CWF) to 4.87 ± 0.00 mg/g (2.5%BEF-97.5% BYF). The tannins contents of 100%BYF (3.70 ± 0.02 mg/g) were significantly ($p < 0.05$) higher than the 100% CWF (3.12 ± 0.00 mg/g). A similar trend has previously been reported for yellow maize and wheat flour by Irondi et al. (2021b). The total saponin level of 100% CWF cookies (1.02 ± 0.00 mg/g) was significantly ($p < 0.05$) lower than 100% BYF (1.04 ± 0.00 mg/g) cookies and the BEF-BYF cookies. The bioactive compounds (flavonoids, and tannins) found in the study are renowned for their positive impact on health. According to reports, the presence of these substances in gluten-free products may offer anti-obesity, anti-hypertensive, anti-diabetic, and antioxidant advantages (Bai et al., 2017; Irondi et al., 2018; Irondi et al., 2021a; Avila-Roman et al., 2021; Irondi et al., 2021b; Irondi et al., 2022) have reported that besides their health advantages, they may additionally prevent oxidative storage, prolong food shelf-life, and conserve food's nutritional value.

3.2 Carotenoid contents of the cookies

The carotenoid profile of the cookies is shown in Table 2. Based on HPLC fingerprinting, the results indicated that all cookie samples had significant ($p < 0.05$) amounts of carotenoid. Specifically, lutein (range from 0.62 to 1.63 µg/g), β-carotene (range from 3.81 to 6.06 µg/g), β-cryptoxanthin (range from 0.19 to 1.75 µg/g), and zeaxanthin (range from 0.83 to 2.48 µg/g) were among the principal carotenoid. The cookies with 2.5%BEF-97.5% BYF had the lowest retinol activity (0.76 µg/g), whereas the cookies with 5% SCMC-95% BYF had the highest value (1.14 µg/g). The presence of carotenoids in the cookies suggests that the BYF-based cookies may have health benefits. Carotenoids have anti-oxidant and pro-vitamin A properties (Seifried et al., 2007; Elemosho et al., 2020); they guard against cancer, including prostate cancer (Agarwal and Rao, 2000; Elemosho et al., 2020); they prevent chronic diseases (Gammone et al., 2017) and type 2 diabetes (Sugiura et al., 2015), insulin resistance (Ni et al., 2015), and hyperglycemia associated with diabetic conditions (Elemosho et al., 2020).

3.3 Starch-digesting enzymes (alpha-amylase and alpha-glucosidase) inhibitory activity of the cookies

The alpha-amylase and alpha-glucosidase inhibitory activities of the cookies were presented in Table 3 as the concentrations of extracts of the cookies that caused 50% inhibition (IC₅₀). The cookies of 100% BYF with IC₅₀ values of 1.42 ± 0.01 µg/mL (alpha-amylase and alpha-glucosidase respectively) showed better inhibition against alpha-amylase and alpha-glucosidase than 100% CWF cookies (alpha-amylase, 2.63 ± 0.01 and alpha-glucosidase, 2.29 ± 0.02 µg/mL). A lower IC₅₀ value shows stronger inhibitory activity against an enzyme (Irons et al., 2021b; Irondi et al., 2022). Further, the IC₅₀ value ranged from 1.42 ± 0.01 µg/mL (100% BYF) to 2.63 ± 0.01 µg/mL (100% CWF) for alpha-amylase and 1.12 ± 0.01 µg/mL (2.5%SCMC-97.5% BYF) to 6.72 ± 0.01 (7.5%BEF-92.5% BYF) for alpha-glucosidase. Among the composite cookies,

TABLE 2 Carotenoid profile of cookies sample.

Samples	TC (µg/g)	LUT (µg/g)	ZEA (µg/g)	β-Cryp (µg/g)	α-Caro (µg/g)	13-Cis-β (µg/g)	trans-β (µg/g)	9 Cis-β (µg/g)	TotalXanthophyl (µg/g)	Total βC (µg/g)	PVA (µg/g)	Retinol activity (µg/g)
100% CWF	9.13 ^c	1.63 ^c	0.83 ^a	0.19 ^a	0.19 ^a	1.41 ^b	3.99 ^c	0.65 ^b	2.46 ^a	6.06 ^d	6.25 ^b	1.04 ^b
100%BYF	8.91 ^d	0.76 ^a	1.51 ^b	1.16 ^b	0.52 ^b	1.10 ^a	2.70 ^a	0.50 ^a	2.27 ^a	4.30 ^b	5.14 ^a	0.86 ^a
2.5%BEF-97.5% BYF	7.73 ^c	0.62 ^a	1.40 ^b	1.02 ^b	0.47 ^b	0.83 ^a	2.44 ^a	0.54 ^a	2.02 ^a	3.81 ^a	4.55 ^a	0.76 ^a
5%BEF-95% BYF	3.74 ^a	0.88 ^b	2.10 ^c	1.63 ^c	0.61 ^c	1.06 ^a	3.15 ^b	0.75 ^c	2.97 ^b	4.96 ^b	6.08 ^b	1.01 ^b
7.5%BEF-92.5% BYF	6.68 ^b	0.96 ^b	2.23 ^c	1.61 ^c	0.58 ^c	1.30 ^b	3.15 ^b	0.72 ^c	3.18 ^b	5.18 ^c	6.27 ^b	1.04 ^b
10%BEF-90%BYF	9.38 ^c	0.93 ^b	2.31 ^c	1.54 ^c	0.59 ^c	1.33 ^b	3.16 ^b	0.68 ^b	3.24 ^b	5.17 ^c	6.24 ^b	1.04 ^b
2.5%SCMC-97.5% BYF	9.32 ^c	0.72 ^a	2.23 ^c	1.56 ^c	0.58 ^c	1.23 ^b	3.19 ^b	0.73 ^c	2.95 ^b	5.14 ^c	6.22 ^b	1.04 ^b
5%SCMC-95% BYF	9.20 ^c	1.07 ^b	2.48 ^c	1.75 ^c	0.61 ^c	1.37 ^b	3.50 ^c	0.79 ^c	3.55 ^c	5.65 ^c	6.83 ^c	1.14 ^b
7.5%SCMC-92.5%BYF	9.04 ^d	0.98 ^b	2.24 ^c	1.65 ^c	0.62 ^c	1.39 ^b	3.25 ^b	0.78 ^c	3.22 ^b	5.42 ^c	6.55 ^c	1.09 ^b
10%SCMC-90% BYF	8.73 ^d	0.87 ^b	2.07 ^c	1.49 ^c	0.62 ^c	1.34 ^b	3.03 ^a	0.74 ^c	2.93 ^b	5.11 ^c	6.17 ^b	1.03 ^b

Data are the mean values of independent triplicate determinations. Data with different lowercase superscript letters along the same column vary significantly ($p < 0.05$). CWF, commercial wheat flour; BYF, biofortified yellow maize flour; BEF, *brachystegia eurycoma* flour; SCMC, sodium carboxymethyl cellulose.

TABLE 3 Starch -digesting enzymes (alpha-amylase and alpha-glucosidase) inhibitory activity of the cookies.

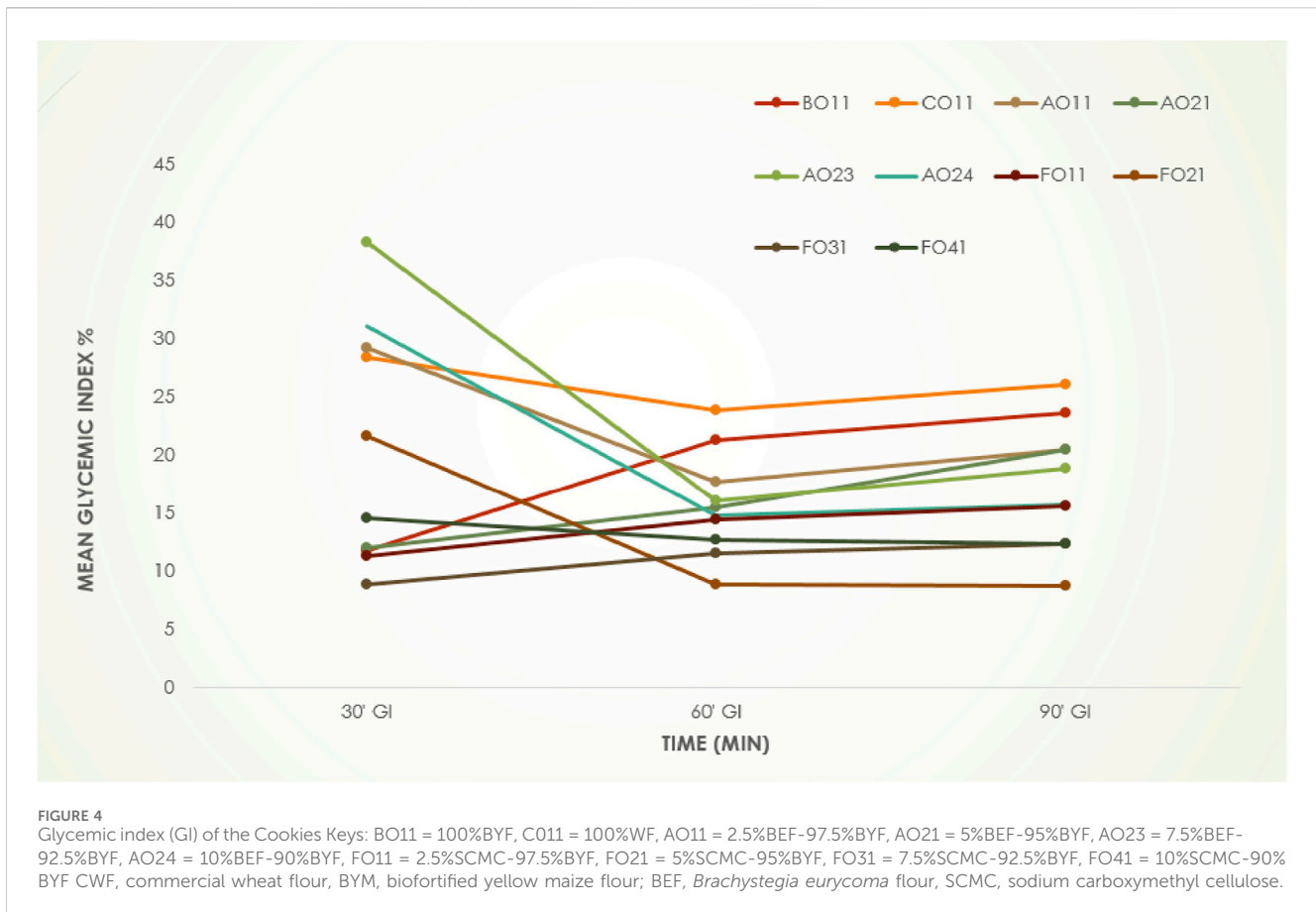
Samples	Alpha-amylaseIC ₅₀ (µg/mL)	Alpha-glucosidaseIC ₅₀ (µg/mL)
100% CWF	2.63 ± 0.01 ^g	2.29 ± 0.02 ^b
100%BYF	1.42 ± 0.01 ^f	1.42 ± 0.01 ^c
2.5%BEF-97.5% BYF	2.97 ± 0.02 ^{cd}	1.34 ± 0.02 ^g
5%BEF-95% BYF	2.23 ± 0.01 ^{ed}	2.51 ± 0.01 ^f
7.5%BEF-92.5% BYF	3.52 ± 0.03 ^{de}	6.72 ± 0.01 ^h
10%BEF-90%BYF	1.94 ± 0.00 ^b	1.94 ± 0.04 ^d
2.5%SCMC-97.5% BYF	1.96 ± 0.00 ^b	1.12 ± 0.01 ^a
5%SCMC-95% BYF	2.17 ± 0.02 ^c	2.15 ± 0.03 ^d
7.5%SCMC-92.5%BYF	1.77 ± 0.00 ^c	1.33 ± 0.01 ^{cd}
10%SCMC-90% BYF	1.91 ± 0.00 ^d	2.51 ± 0.01 ^f

Data are the mean values ± standard deviation (SD) of independent triplicate determinations. Data with different lowercase superscript letters along the same column vary significantly ($p < 0.05$). CWF, commercial wheat flour; BYF, biofortified yellow maize flour; BEF, *brachystegia eurycoma* flour; SCMC, sodium carboxymethyl cellulose.

7.5%SCMC-92.5%BYF cookies (1.77 ± 0.00 µg/mL) have better inhibition against alpha-amylase while 2.5%SCMC-97.5% BYF (1.12 ± 0.01 µg/mL) has better inhibition against alpha-glucosidase.

The digestion of dietary carbohydrates, especially starch, is facilitated by both alpha-amylase and alpha-glucosidase (Kareem et al., 2022). Alpha-amylase catalyzes the first stage of the starch hydrolysis process, resulting in the production of maltose, dextrin, and maltotriose. After alpha-glucosidase hydrolyzes these products,

absorbable monosaccharides (such as fructose and glucose) are produced (Irondi et al., 2019b; Kareem et al., 2022). As per Butterworth et al. (2022), the inhibition of α-amylase and α-glucosidase enzymes is strongly linked to a reduction in blood glucose response. This implies that inhibiting these enzymes can be a therapeutic method for managing Type-2 Diabetes by reducing postprandial hyperglycemia (Irondi et al., 2019b). Many anti-diabetes therapies (natural items, medications, and functional



foods) work by inhibiting α -amylase and α -glucosidase and a meal that has this ability also has anti-hyperglycemic properties (Irondi et al., 2021b). The result therefore showed that the cookie samples had significant ($p < 0.05$) anti-hyperglycemic qualities and could be used as functional foods to treat obesity and type 2 diabetes.

3.4 Glycemic index of the cookies

Figure 4 shows the glycemic index (GI) of all the cookies at 0, 30, 60, and 90 min intervals. There was a significant ($p < 0.05$) increase in the blood glucose concentration within 30 min of cookies consumption. A peak level of blood glucose concentration was observed at 60 min. The 7.5%SCMC-92.5%BYF cookie (FO31) has the lowest GI mean value at 30 min time whereas, 7.5%BEF-92.5%BYF cookie (AO23) has the highest. The GI mean of 7.5%BEF-92.5%BYF cookies (AO23) significantly decreased ($p < 0.05$) after 30 min of consumption. According to Zhu (2019), GI values are determined by how quickly carbohydrates are metabolized. Those with a slow pace of digestion are classified as low-GI ($GI < 55$), while those with a high rate of digestion ($GI > 70$) are classified as high-GI. All of the cookies fall into the low GI category because their average GI varied between 10 to 45. High GI diets, such as those high in carbohydrates, are connected to several health challenges, including metabolic disorders and non-communicable diseases

like hyperlipidemia, heart problems, obesity, cardiovascular diseases (CVDs), and diabetes (Zhu and Li, 2019; Gorenand et al., 2023). Since low-GI diets were not associated with these illnesses, it is possible that they can be used in place of high-GI foods to help manage these health issues (Butterworth et al., 2022; Haini et al., 2022). Therefore, the cookie samples with low GI values can be regarded as high-quality baked goods.

4 Conclusion

A Biofortified yellow maize-based gluten-free cookie was produced from biofortified yellow maize flour (BYF). Additionally, the biofortified yellow maize flour (BYF) was combined with *Brachystegia eurycoma* flour (BEF) and sodium carboxyl methylcellulose (SCMC), a reference hydrocolloid, in four different amounts (2.5, 5, 7.5, and 10%), respectively. The resulting blends were then used to manufacture gluten-free cookies. Compared to conventional wheat flour cookies (100% CWF), the BYF (100%) and BYF-BEF cookies have a lower glycemic index, greater bioactive components and carotenoid concentrations, and superior starch-hydrolyzing enzyme inhibition. This implied that eating 100% BYF and BYF-BEF cookies may be beneficial to one's health and that they may be used as a baked good to manage diseases like diabetes, heart disease, hyperlipidemia, hyperglycemia, obesity, and celiac disease: disorders linked to nutrition.

Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by the Kwara State Ministry of Health, Ilorin with approval number MOH/KS/EU/777/493. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

KA: Writing—original draft, Formal Analysis, Investigation. YI: Writing—original draft, Writing—review and editing. EI: Conceptualization, Supervision, Writing—review and editing. EA: Supervision, Writing—review and editing.

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