EFFECT OF FERMENTATION ON THE ANTINUTRITIONAL COMPOUNDSAND ANTIOXIDANT ACTIVITIESOFGELATINIZED BAMBARA GROUNDNUT FLOUR

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Abstract

This study investigated the effect of fermentation on the antinutritional compounds and antioxidant activities of gelatinized Bambara groundnut flour (GBGF). The GBGF was fermented at different periods (2, 4, 8. 16 and 24 h) using *Saccharomyces cerevisiae*. Gelatinization significantly ($p \leq 0.05$) reduced the level of phytic acid, oxalate, tannin, trypsin inhibitors and raffinose by 27.18%, 18.35%, 22.79%, 18.24% and 18.89%, respectivelywhile phytochemical constituents (total phenolic content, TPC and total flavonoid content, TFC) and antioxidant activities (DPPH, FRAP and ABTS radical scavenging activity) were significantly ($p \leq 0.05$) improvedcompared to raw Bambara groundnut flour.Fermentationfurther decreased phytic acid (32.50-62.70%), oxalate(21.27-60.39%), tannin (31.94-79.84%), trypsin inhibitors (21.07-83.65%) and raffinose (31.50-70.80%). However, fermentation further improved the TPC (5.70-6.89 mg GAE/g), TFC (4.17-4.96 mg CE/g), DPPH (56.50-72.48%), FRAP (3.24-3.92 µmol TE/g) and ABTS(2.19-2.88 µmol TE/g) of the gelatinize Bambara groundnutflours. These results revealed that combined gelatinization and fermentation with *Saccharomyces cerevisiae*could serve as an effective biotechnological approach for substantial reduction of antinutritional compounds and improvement of antioxidant potential of Bambara groundnut flour.

Keywords: Bambara groundnut, Fermentation, Gelatinization, Antinutritional Compounds, Antioxidant potential

1.0 Introduction

The demand for high nutritional value-added food products has increase over the last few years throughout the world partly due to human population growth and consumer health awareness (Alrosan*et al.*, 2021). Bambara groundnut (BGN) is an underutilized, drought-tolerant African legume next to groundnut and cowpea (Chinma*et al.*, 2021). BGN contains high content of protein (17.4-30.7%) and lysine, rich in dietary fiber, micronutrients and resistant starch (Chinma*et al.*, 2021). BGN, like other legumes,contains a wide range of phytochemicals and bioactive constituents and its consumption is associated with numerous health benefits including the prevention of chronic and non-communicable diseases (Adebiyi *et al.*, 2019).

However, the utilization of BGN in the food system is limited by the presence of antinutritional factors (ANFs) which limit nutrient digestibility and have adverse toxicological effects in the human system. Several thermal processing and bioprocessing methods have been employed to reduce the concentration of antinutrient compounds in cereals and legumes. Aside degradation of antinutrient compounds, heat treatment methods (such as autoclaving, cooking, autoclaving, roasting, extrusion, infrared, microwaving, among others) cause a total or partial starch gelatinization,enhanced emulsifying, foaming, and water retention capacity and thickening; thus, improving technological suitability of legumes flours to be used as ingredients in baked goods, snacks and pasta. Research has also established that bioprocessing techniques such as fermentation with baker's yeast (*Saccharomyces cerevisiae*) has been employed to improve the functionality of grains (Chinma*et al.*, 2020). Furthermore, the use of biotechnological approach, combining thermal treatment ("gelatinization") and fermentation has been employed to reduce antinutritional compounds, improve nutritional composition and functional properties of legume flours (De Pasquale *et al.*, 2020). Nevertheless, the effect of fermentation with baker's yeast (*Saccharomyces cerevisiae*) on the antinutritional compounds of gelatinized Bambara groundnut flour has not been investigated. It can be hypothesized that fermentation with *Saccharomyces cerevisiae* may modify the level of antinutrient compounds, phytochemical constituents, and antioxidant activities in gelatinized BGN flour for improved nutrition. The objective of this study was to evaluate the effect of fermentation time (2, 4, 8, 16, and 24 h) on the antinutrient composition and antioxidant properties of gelatinized Bambara groundnut flour.

2.0 Materials and Methods

2.1 Materials

Bambara groundnut(brown variety) seedswas purchased from an Agro seed Company based in Minna Central Market, Minna, Nigeria. Chemicals and reagents were procured from a reputable Agrochemical industry in Minna and Abuja.

2.2 Sample preparation

Cleaned BGN seeds were washed in clean tap water, drained, and dried in air draft-oven (Gallenkamp, Cheshire, UK) at 50 °C for 24 h. The dried BGN grains were milled (Globe P 44, Diamond Tools Co. Ltd. Henan, China) and sieved (mesh size of 100 µm) to produce raw Bambara groundnut flour (RBGF).

For the gelatinized flour, Bambara groundnut seeds were washed in cold tap water and thereafter soaked in cold tap water (1: 5 w/v) for about 4 h at room temperature (28 ± 1 °C), drained and the soaked seeds was steamed at 100 °C for 20 min. Afterwards, the steamed grains were oven dried at 50 °C for 24 h and milled in a hammer mill (Globe P 44, Diamond Tools Co. Ltd. Henan, China) and sieved using a mesh size of 100 µm to obtain gelatinized Bambara groundnut flour (GBGF).

For the fermented-gelatinized flour, 1 g of baker's yeast was dissolved in 100 mL of distilled water. The mixture was then added to 100 g of the flour to act as starter. The batter was fermented at 37 °Cin a fermentation cabinet (National MEG Company, Lincoln, USA) at different periods(2, 4, 8, 16 and 24 h). Thefermented BGN batter was dried in an oven at 50 °C for 24 h and the dried flour was blended and sieved (to pass through 100 µm screen) to produce fermented-gelatinizedBambara groundnut flour (GFBGF).

2.3 Determination of antinutritional factors

Phytic acid and tannin content were determined as previously reported by Chinma*et al.* (2021). Trypsin inhibitor activity (TIA) was measured using a trypsin substrate (0.04 g/L of BAPA, Nα-benzoyl-L-arginine 4- nitroanilide hydrochloride) following a standard procedure (Chinma*et al.*, 2021).Oxalate content was assayed according to the method described by Adebiyi *et al.* (2019).Raffinose content was determined using the Raffinose/D -Galactose Assay Kit (K-RAFGA, Megazyme International, Ireland).

2.4 Determination of phytochemical constituents and antioxidant properties
The methanolic extract from BGN flour samples for the profiling of total phenolic content (TPC) and antioxidant properties was prepared according to a standard procedure (Chinma*et al.*, 2014).The TPC was determined following the Folin–Ciocalteau reagent method previously described by Chinma*et al.* (2014) and expressed as mg GAE/100 g. The total flavonoids content (TFC) was determined according to the method of Bao *et al.* (2005) and presented as mg CE/g. The DPPH (1,1-diphenyl-2-picryl-hydrazil) radical scavenging activity and ferric reducing antioxidant power (FRAP) were determined as described by Chinma*et al.* (2014) while ABTS (2,2'-Azino-bis-3-ethylbenzthiazoline-6-sulfonic acid)was determined according to a standard procedure (Sant'Anna*et al.*, 2016).

2.5 Statistical analysis
Data obtained were subjected to analysis of variance using a statistical software (SPSS 20, BM, Armonk, NY, USA)while Tukey's test was used to determine significant differences (5% probability) among means.

3.Results and Discussion
3.1 Antinutritional composition of raw, gelatinized, and gelatinized-fermented BGN flours
The antinutritional composition of raw, gelatinized and gelatinized-fermented Bambara groundnut flour samples are presented in Table 1. The raw BGN flour contained 5.63 mg/100 g phytic acid, 7.90 mg/100g oxalate, 6.45 mg/100g tannin, 3.18 TIU/mg and 1.27 mg/100 g raffinose; which is in close to the values (except raffinose) reported for raw BGN by other researchers (Adebiyi *et al.*, 2019; Chinma*et al.*, 2021). Compared to raw BGN, the concentration of antinutrient compounds (phytic acid, oxalate, tannin and raffinose) were reduced significantly in gelatinized BGN flours probably due to leaching-out of the antinutrients during soaking or boiling to aid gelatinization or due to thermal gradient that occurred during the first step of the gelatinization process which induced the activation of endogenous enzymes that degraded antinutrients including raffinose (De Pasquale *et al.*, 2020). It was also observed that higher reductions in antinutritional factors (ANFs) were recorded following fermentation of gelatinized BGN flourscompared to raw BGN, partly due to increased activity of lactic acid bacteria in reducing ANFs following fermentation.

3.2 Antioxidant potential of raw, gelatinized, and gelatinized-fermented BGN flours
The TPC and TFC of the raw BGN samples were 5.43 GAE mg/100 g and3.67mg CE/g, respectively, which is in close range with the values previously reported for raw BGN (Adebiyi *et al.* ,2019). The TPC and TFC of the gelatinized samples were significantly higher than the control(Table 2) probably due torelease of conjugated phenolic compounds upon disruption of complex structures during hydrothermal treatment (Nickel *et al.*, 2016). It was also observed that higher reductions of phytochemical constituents were recorded in fermented-gelatinized BGN samples; which also increased ($p \leq 0.05$) with fermentation time (2-24 h). The observed increase may be attributed to the release of bound components by microbial enzymes during

fermentation as well as formation of Maillard reaction products with phenol and reductone-type structures caused by partial destruction of cellular structure during thermal treatment (Zou *et al.*, 2018). Consequently, higher antioxidant activities were recorded in fermented-gelatinized BGF samples followed by gelatinized BGF samples and raw BGF; which corroborates that high phytochemical constituents are associated with high antioxidant activities(evaluated in terms of DPPH, FRAP and ABTS in this study). From this perspective, the antioxidant activities of the samples were in the order: FGBGF > GBGF > RBGF. These results contradicts the findings of De Pasquale*et al.* (2020) whoreported higher antioxidant activities in raw legume (red lentil, yellow lentil, white beans, black beans, and chickpea) flours than lactic acid fermented-gelatinized, and gelatinizesamples; thus, ascribed to the effect of thermal treatment that caused significant reduction of the total phenols due to polymerization.

Conclusions

This study demonstrated that gelatinization, and combined fermentation and gelatinization caused significant reduction in antinutrient compounds while phytochemical constituents and antioxidant activities of Bambara groundnut flours were improved.Consequently, the low level of residual antinutrients in BGN after gelatinization, and combined gelatinization and fermentation is expected to improve the nutritional properties of the flour for food fortification. However, research is on-going in our laboratory on the impact of fermentation on the nutritional composition and techno-functional properties of gelatinized Bambara flours for food product development.

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Table 1. Effect of fermentation time on the antinutritional properties of gelatinized Bambara groundnut flour

Parameters	Raw flour	Gelatinized flour	2-hFGF	4-hFGF	8-h F GF	16-hFGF	24-hFGF
Phytic acid (mg/ 100g)	5.63±0.02^a	4.10±0.01^b	3.80 ± 0.01^c	3.06±0.01^d	2.77 ± 0.02^e	2.42± 0.01^f	2.05± 0.01^g
Phytic acid reduction (%)	-	27.18	32.50	27.88	33.04	46.36	62.70
Oxalate	7.90±0.02^a	6.45±0.02^b	6.22^b ±0.01^c	5.40±0.01^d	4.91± 0.01^e	4.45± 0.01^f	3.13±0.03^g
Oxalate reduction (%)	-	18.35	21.27	31.64	37.85	43.67	60.39
Tannin (g/kg)	6.45±0.01^a	4.98± 0.02^b	4.39±0.01^c	4.05±0.01^d	3.80±0.03^e	2.52± 0.02^f	1.30±0.02^g
Tannin reduction (%)	-	22.79	31.94	37.21	41.09	60.93	79.84
TIA/TIU/ mg)	3.18±0.03^a	2.60±0.01^b	2.51 ± 0.02^c	2.23 ±0.03^d	1.74±0.02^e	1.06±0.01^f	0.52±0.01^g
TIA reduction (%)		18.24	21.07	29.85	45.28	66.67	83.65
Raffinose (g/100 g)	1.27± 0.01^a	1.03± 0.01^b	0.87±0.00^c	0.75^d ±0.01^d	0.62±0.01^e	0.50±0.01^f	0.38±0.01^g
Raffinose reduction (%)	-	18.89	31.50	40.94	51.18	60.62	70.08

Data presented as means ± standard deviation from triplicate analysis. Means with no common letters within a row are significantly different at ($p \leq 0.05$).
TIA = Trypsin inhibitor activity; 2-hFGF = 2-h fermented and gelatinized flour, 4-hFGF = 4-h fermented and gelatinized flour, 8-hFGF = 8-h fermented and gelatinized flour, 16-hFGF = 16-h fermented and gelatinized flour and 24-hFGF = 24-h fermented and gelatinized flour

Table 2. Effect of fermentation time on the antioxidant properties of gelatinized Bambara groundnut flour

Parameters	Raw flour	Gelatinized flour	2-hFGF	4-hFGF	8-h F GF	16-hFGF	24-hFGF
Total phenolic content (mg GAE/ g)	5.43±0.03^g	5.70±0.02^f	5.98 ± 0.01^e	6.24±0.02^d	6.47 ± 0.03^c	6.65± 0.01^b	6.89± 0.02^a
Total flavonoid content (mg CE/g)	3.67±0.01^g	3.89±0.01^f	4.17 ± 0.02^e	4.39±0.01^d	4.53 ± 0.00^c	4.70± 0.01^b	4.96 ± 0.03^a
DPPH (%)	44.51±0.19^g	50.22±0.14^f	56.50 ± 0.16^e	57.06±0.11^d	59.15 ± 0.16^c	65.29± 0.14^b	72.48 ±0.20^a
FRAP (µmol TE/g)	2.84±0.01^g	3.06±0.02^f	3.24± 0.02^e	3.38±0.01^d	3.57± 0.01^c	3.74± 0.01^b	3.92± 0.01^a
ABTS (µmol TE/g)	1.65±0.02^f	1.93±0.01^e	2.19± 0.01^d	2.25±0.01^d	2.49 ± 0.00^c	2.65^a ± 0.01^b	2.88 ± 0.01^a

Data presented as means ± standard deviation from triplicate analysis. Means with no common letters within a row are significantly different at ($p \leq 0.05$).
DPPH: 2,2-diphenyl-1-picrylhydrazyl; FRAP: Ferric reducing anti-oxidant power and ABTS= 2,2'-Azino-bis-3-ethylbenzthiazoline-6-sulfonic acid.

2-hFGF = 2-h fermented and gelatinized flour, 4-hFGF = 4-h fermented and gelatinized flour, 8-hFGF = 8-h fermented and gelatinized flour, 16-hFGF = 16-h fermented and gelatinized flour and 24-hFGF = 24-h fermented and gelatinized flour