IMPACT OF FERMENTATION ON THE BIOCHEMICAL PROPERTIES, ANTINUTRITIONAL COMPOUNDS AND ANTIOXIDANT ACTIVITIES OF GELATINIZED FLOURS FROM TWO FONIO VARIETIES

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Abstract
The impact of fermentation on the biochemical properties, antinutritional compounds and antioxidant activities of gelatinized flours from white and brown fonio grains were investigated. The gelatinized flour was fermented at different periods (2, 4, 8. 16 and 24 h) using *Saccharomyces cerevisiae*. Flour samples with moderate acidity were produced following gelatinization, and combined fermentation and gelatinization. Gelatinization significantly ($p \leq 0.05$) reduced the level of phytic acid, tannin and saponin content in both varieties compared to the raw flour. In a time dependent manner, fermentation led to substantial reduction ($p \leq 0.05$) in antinutritional compounds, and improved phytochemical constituents (total phenolic content and total flavonoids content) and antioxidant activities of the gelatinized fonio flours. This study demonstrated that combined fermentation with *Saccharomyces cerevisiae* and gelatinization could be utilized to produce fonio flour with low residual antinutrients and higher antioxidant activities.

Keywords: White and brown fonio grains, Fermentation, Gelatinization, Antinutritional Compounds,
Antioxidant capacity

1.0 Introduction

Cereal grains form the major portion of the diet of the global population. Wholegrain cereals provide an affordable and accessible nutritional foundation (Erenstein *et al*., 2022). White fonio (*Digitaria exilis*) and brown fonio (*Digitaria iburua*) are the two commonly cultivated fonio varieties in West Africa (Agu *et al*., 2020), are attracting research attention due to their low gluten content and glycemic index and rich phytochemicals, encouraging their use in functional foods formulation (Adams and Yakubu, 2020). Fonio grains are rich in macro- and micronutrients as well as phytochemicals for human nutrition, with chemical compositions similar to other cereals and pseudo-cereals (Zhu, 2020). On the other hand, antinutritional compounds such as phytate present in fonio grains significantly reduce bioavailability of nutrients (Zhu, 2020), which limits its use in food system. Therefore, processing is a prerequisite for the consumption of whole grains.

Several traditional and novel food processing methods have been employed to reduce the level of antinutritional compounds and improve the nutritional quality of cereals including whole grains. Research has established that biotechnological approach combining gelatinization and fermentation caused significant reduction in the concentration of antinutritional compounds, improved the nutritional, antioxidant and technological properties of grains for the development of functional foods (De Pasquale *et al*., 2020). Accordingly, fermentation involves biochemical changes that occur in the macromolecules of the food materials by the action of microorganism-derived enzymes, thus, modifying the functionality of the derived product. In this study, the effect of fermentation on the biochemical properties, antinutritional factors and antioxidant activities of two fonio varieties (white fonio, *Digitaria exilis* and brown fonio, *Digitaria iburua*) were evaluated.

2.0 Materials and Methods

2.1 Materials

The white and brown fonio grains were purchased from an Agro seed Company in Minna Central Market, Minna, Nigeria. Chemicals and reagents were procured from a reputable Agrochemical industry in Minna and Abuja

2.2 Sample preparation

Two fonio varieties (white and brown varieties) were prepared the same way. Cleaned fonio grains were washed in clean tap water, drained, and dried in air draft-oven (Gallenkamp, Cheshire, UK) at 50 ^oC 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 flour .

For the gelatinized flour, fonio grains from the two varieties were separately 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 ^oC), drained and the soaked seeds was steamed at 100 °C for 20 min. Afterwards, the steamed grains were oven dried at 50 ^oC 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 fonio flour.

The fermented-gelatinized flour samples were prepared as follows: 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 °C in a fermentation cabinet (National MEG Company, Lincoln, USA) at different periods (0, 2, 4, 8, 16 and 24 h). The fermented 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-gelatinized flour.

2.3 Determination of pH and total titratable acidity

The pH of the fonio flours were determined by homogenizing 10 g of respective flours with 90 mL distilled water and determination using a calibrated pH meter (PHS-25, Techmel, USA). Afterwards, the filtered slurry of the sample was used to determine the titratable acidity (%), which was titrated against 0.05 M NaOH solution with phenolphthalein as an indicator (Azeez *et al*., 2022).

2.3 Determination of antinutritional factors

Phytic acid and tannin content in the samples were assayed as reported by Azeez *et al*. (2022) while total saponin was assayed by a method reported by Lai *et al*. (2013). The phytic acid, tannin and saponin content results were reported as mg/100 g, CE/mg and mg/ 100g, respectively, on dry weight basis.

2.4 Determination of phytochemical constituents and antioxidant properties

The samples extracts (methanolic extract) of the flour samples were assayed for the determination of total phenolic content (TPC) and antioxidant activities (DPPH and ABTS radical scavenging activities) according to a method reported by Chinma *et al.* (2014). Total phenolic content was determined following by the Folin–Ciocalteau reagent method (Chinma *et al.*, 2014), presented as mg GAE/g. The TFC was assayed following a standard procedure (Sant'Anna *et al.*, 2005) and presented as mg CE/g. The DPPH (1,1-diphenyl-2-picryl-hydrazil) radical scavenging activity and ABTS (2,2'-Azino-bis-3-ethylbenzthiazoline-6-sulfonic acid) were profiled as reported by Chinma *et al.* (2014) and Sant'Anna *et al.*, 2016), respectively.

2.5 Statistical analysis

Data obtained in this study were subjected to analysis of variance using a statistical software (SPSS 20, BM, Armonk, NY, USA). Tukey's test was also employed to determine significant differences at 5% probability level among means.

3. Results and Discussion

3.1 pH and TTA of raw, gelatinized, and gelatinized-fermented fonio flours

A drop in pH was recorded in the gelatinized, and gelatinized-fermented samples compared to the raw samples from white and brown fonio grains (Table 1). In a time dependent manner, fermentation led to significant reduction in pH with a corresponding increase in total titratable acidity in gelatinized fonio samples compared to the control. Such decrease in pH values and corresponding increase in TTA, may partly be attributed to hydrolysis of starch into organic acids by microorganisms. The moderate pH range recorded in treated fonio samples is within the optimum pH of some food enzymes (such as α-amylase, protease, phytase, and β-glucanase), and such pH could make them activated and improve the quality attributes of food products (Ilowefah *et al.*, 2017). Moreover, higher total titratable acidity of the fermented-gelatinized white and brown fonio flours may inhibit the proliferation of undesirable microorganisms that may cause poor fermentation of the fonio grains. This may influence the functionality of the flour. Therefore, such information should be established in future studies.

3. *2 Antinutritional composition of raw, gelatinized, and gelatinized-fermented fonio flours*

The antinutritional composition of raw, gelatinized, and fermented-gelatinized fonio flour samples are presented in Table 1. The level of antinutritional compounds (phytic acid, tannin and saponin) were significantly ($p \leq 0.05$) reduced in gelatinized fonio flours from both varieties compared to the raw flour, probably due to hydrothermal effect that caused degradation of the antinutritional compounds. In addition, the soaking of the grains in water probably facilitated the leaching-out of the antinutritional compounds in water. In a time dependent manner, fermentation caused an enormous reduction ($p \leq 0.05$) (in the level of antinutrient compounds probably due to increased activities of microorganisms and or enzymes that caused hydrolysis of ANFs; thus, in accordance with the report where significant reduction in ANFs were recorded following lactic acid fermentation of legume flours (De Pasquale *et al.*, 2020). The level of residual antinutrients obtained in this study following gelatinization, and combined gelatinization and fermentation were much lower than the concentration recommended as safe level for human consumption (Farinde *et al.*, 2018).

3.3 Antioxidant activities of raw, gelatinized, and gelatinized-fermented fonio flours

Total phenolic content and total flavonoids content of the raw flour from brown variety was significantly higher than the white fonio. Table 1 shows that the TPC and TFC of the gelatinized samples were significantly higher than the raw flour. Compared to the raw flour from both varieties, fermentation caused significant increase in TPC and TFC of the flours probably due to the increased activities of microorganism/microbial enzymes that caused the release of phenolics which also accounted for increase total flavonoids content. The increased TPC and TFC probably caused significant increase in the antioxidant properties of gelatinized, and combined fermented and gelatinized fonio flours from both varieties. The fermented-gelatinized flour had high antioxidant activities followed by the gelatinized flour, and the control. In addition, the brown fonio samples had higher antioxidant activities than the white fonio.

Conclusions

This study revealed that the moderate acidity of the gelatinized, and fermented-gelatinized fonio flours indicated that the flours could be incorporated in food formulations. The study established that fermentation could be sufficient to produce gelatinized fonio flours with low residual antinutritional compounds and higher antioxidant activities. Further studies are needed to provide insight on the impact of fermentation on the nutritional, functional and structural properties of gelatinized flours from white and brown fonio grains for specific food application.

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Table 1. Effect of fermentation on the biochemical properties, antinutritional compounds and antioxidant activities of gelatinized flours from two fonio varieties (white and brown)

Parameters	FT	pH	TTA (mL)	Phytic (mg / 100 g)	Tannin (mg CE/g)	Saponin (mg/100 g)	TPC (mg GAE/g)	TFC (mg CE/g)	DPPH (µmol TE/g)	ABTS (µmol TE/g)
White fonio	raw	6.05±0.01^{a}	0.50±0.00^{e}	520.13±1.42^{b}	0.27±0.02^{a}	6.44±0.04^{c}	1.45±0.01^{h}	5.37 ± 0.02^{k}	12.60±0.01^{i}	21.19±0.02^{k}
	0 (GF)	5.89±0.01^{b}	0.67±0.01^{d}	474.05±1.17^{d}	0.18±0.01^{b}	5.98±0.02^{d}	1.63±0.01^{g}	5.50±0.01^{j}	12.97±0.01^{h}	21.70±0.06^{j}
	2	5.64±0.00^{c}	0.89±0.01^{c}	423.07±1.29^{f}	0.15±0.01^{b}	5.61± 0.03^{e}	1.70±0.01^{g}	5.65±0.01^{i}	13.24±0.01^{g}	21.99±0.03^{i}
	4	5.57±0.01^{c}	1.07±0.01^{b}	380.56±1.10^{h}	0.13±0.01^{b}	5.39±0.01^{f}	1.84±0.02^{f}	5.97±0.02^{g}	13.48±0.03^{f}	22.26±0.05^{h}
	8	5.54±0.00^{c}	1.17±0.01^{ab}	326.93±1.25^{j}	0.10±0.01^{bc}	4.85±0.01^{g}	1.97±0.01^{e}	6.13±0.01^{f}	13.70±0.03^{e}	22.64±0.02^{g}
	16	5.49±0.00^{c}	1.20±0.01^{ab}	257.45 ±1.36^{l}	0.07±0.01^{c}	4.40±0.03^{k}	2.15±0.01^{d}	6.60 ±0.01^{e}	13.95±0.02^{d}	22.88±0.07^{f}
	24	5.46±0.01^{c}	1.18±0.01^{ab}	203.60±1.14^{o}	0.07±0.01^{c}	3.81±0.01^{j}	2.33±0.01^{c}	7.05±0.01^{c}	14.28±0.03^{c}	23.50±0.04^{d}
Brown fonio	raw	6.11±0.00^{a}	0.52± 0.02^{e}	552.40±1.23^{a}	0.31±0.01^{a}	6.92±0.05^{a}	1.67±0.01^{g}	5.83±0.00^{h}	13.21±0.01^{g}	22.35±0.01^{g}
	0 (GF)	5.94±0.01^{b}	0.70±0.01^{d}	496.52±1.21^{c}	0.20±0.01^{b}	6.64±0.02^{b}	1.90±0.03^{f}	6.24±0.01^{f}	13.50±0.00^{f}	22.83±0.03^{f}
	2	5.63±0.01^{c}	0.98±0.01^{c}	463.17±1.17^{e}	0.18±0.01^{b}	6.30±0.01^{c}	2.03±0.01^{e}	6.57±0.01^{e}	13.66±0.01^{e}	23.20±0.06^{e}
	4	5.60±0.01^{c}	1.09±0.00^{b}	397.84±1.19^{g}	0.16±0.00^{b}	5.92±0.01^{d}	2.17±0.01^{d}	6.79±0.01^{d}	13.98±0.01^{d}	23.61±0.02^{d}
	8	5.58±0.01^{c}	1.19±0.01^{ab}	341.13±1.12^{i}	0.14±0.00^{b}	5.54±0.03^{e}	2.28±0.02^{c}	6.96±0.02^{c}	14.23±0.03^{c}	23.96±0.05^{c}
	16	5.53±0.01^{c}	1.24±0.01^{a}	285.62±1.15^{k}	0.11±0.01^{b}	4.77±0.01^{g}	2.45±0.01^{b}	7.27±0.01^{b}	14.49±0.03^{b}	24.17±0.01^{b}
	24	5.48±0.01^{c}	1.27±0.01^{a}	239.25±1.04^{m}	0.10±0.01^{b}	4.14±0.01^{i}	2.59±0.00^{a}	7.64±0.04^{a}	14.85±0.03^{a}	24.43±0.04^{a}

Data presented as means ± standard deviation from triplicate analysis. Means with no common letters within a column are significantly different at ($p \leq 0.05$).
DPPH: 2,2-diphenyl-1-picrylhydrazyl; ABTS= 2,2'-Azino-bis-3-ethylbenzthiazoline-6-sulfonic acid, FT= Fermentation time; GF = Gelatinized flour