ANTIOXIDANT PROPERTIES OF FERMENTED BLACK TURTLE BEANS AND PEARL MILLET FLOURS

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ABSTRACT

The impact of short-term fermentation on the phytochemical and antioxidant properties of black turtle beans and pearl millets flours were investigated. Black turtle beans and pearl millet grains were fermented at different periods (24, 48 and 72 h) and processed into flour. In a time-dependent manner, fermentation increased ($p \leq 0.05$) total phenolic content, total flavonoid content and antioxidant properties (ABTS, DPPH and FRAP). The 72-h fermented grains contained higher content of phytochemicals and antioxidant properties; thus, short-term fermentation could serve as a natural means to improve the health properties of black turtle beans and pearl millets flour as a novel flour in food product development.

Keywords: Antioxidants, Antinutritional factors, Fermentation, Black turtle beans and Pearl millet.

5.0 Introduction

In recent years, there has been a high demand for foods rich in antioxidants, not only due to their cost-effectiveness but also for their numerous health benefits, including anti-aging properties, cancer prevention, cardiovascular protection, and anti-inflammatory effects (Ghosh & Sil, 2022). Crops like black turtle beans and pearl millet are excellent sources of plant-based protein, dietary fiber, and antioxidants (Kumar et al., 2023).

Black turtle beans (*Phaseolus vulgaris*), referred to as Akidi in Eastern Nigeria, are recognized for their excellent nutritional value, particularly in providing protein, dietary fiber, antioxidants, and essential vitamins and minerals like magnesium, calcium, and potassium. These nutrients contribute to lowering cholesterol, managing diabetes, aiding weight loss, and reducing the risks of colon cancer and cardiovascular diseases (Odo, 2010; Johnston *et al.,* 2015). Several studies have indicated that legumes may serve as excellent dietary sources of natural antioxidants for disease prevention and health promotion Madhujith *et al.,* 2004; Oomah *et al.,* 2005; Xu and Chang, 2007)

Pearl millet (*Pennisetum typhoideum*) is a versatile cereal cultivated for food and forages as compared to the major cultivated cereal crops which provides numerous health benefits, including weight management, diabetes control, bone strength (Kumar *et al.,* 2020). Its high content of dietary fiber, gluten-free nature, and unique protein and fat composition are additional qualities that make this cereal a well-balanced food option, potentially aiding in the management of various disorders (Vila-Real *et al.,* 2017). Like other cereals, it contains a high amount of antinutritional components such as phytic acid, phenolic compounds and condensed tannins which are known to reduce mineral bioavailability (Baye *et al.,* 2015).

Processing treatments such as fermentation is a long-standing economical traditional method of processing plant foods that significantly reduce antinutritional factors, improve the nutritional value, antioxidant, techno-functional properties, and modify the structural properties of legumes and cereals (Chinma *et al.,* 2024).

There is limited information available in literature on the impact of fermentation on the antioxidant properties of black turtle beans and pearl millet flours. This study was therefore aimed to determine the impact of fermentation on the phytochemical and antioxidant properties of black turtle beans and pearl millets flours. Our findings will contribute to increasing their utilization in the food industry, as well as increasing the

2.0 Materials and Methods

2.1 Source of materials

Black turtle beans (dark purple) and pearl millets grains (grayish brown variety) were procured from Central Market, Minna, Nigeria. All reagents used for the study are of analytical grade.

2.2 Preparation of raw, and fermented Black turtle beans and pearl millet flours

The cleaned Black turtle bean (BTB) was soaked with tap water (boiled and allowed to cool in a cleaned container with a lid) for 24 h at room temperature. The soaked BTB were dehulled with an attrition mill and dried in an oven at 40°C until constant weight was achieved. The dried BTB were milled and passed through a 100 µm sieve to produce the native BTB flour. Likewise, cleaned pearl millet grains were soaked and dried in a hot air oven. The dried grains were milled using a hammer mill and passed through a 100 µm sieve to produce the pearl millet flour (PMF). The flours were divided into two parts; one part was packed into a polyethylene bag kept in airtight container and stored at 4°C prior to analyses, while the other part was used for the fermentation process. The fermentation was done according to the modified method of Ilowefah *et al.* (2017) and Azeez *et al.* (2022). The BTB and pearl millet flours were separately dispersed in 65 mL of tap water. The beakers were covered with aluminum foil, and the samples were then allowed to ferment using an incubator at 40°C for 24, 48 and 72 h. The fermented BTB and pearl millet flour batters were oven dried at 40°C until constant moisture was achieved. The dried samples were milled and sieved to produce fermented Black turtle bean (FBTB 24, 48 and 72h) and pearl millet flour (PMF 24, 48 and 72 h), respectively.

2.3 Determination of phytochemicals and antioxidant activities

A methanolic extract was prepared from the samples based on a standard procedure (Chinma *et al*. 2014). A 0.20 g of sample was mixed with 4 mL 80% methanol. Thereafter, the mixture was centrifuged (for 20 min at 4000 × g). Afterwards, the supernatant was transferred into test tubes, evaporated (under nitrogen stream) and stored at 4 ^{o}C. The extraction of the samples was carried out in triplicates. The methanolic extracts were used in profiling the phytochemical and antioxidant properties of the samples. TPC (presented in mg GAE/g) was assayed based on Folin Ciocalteau reagent method detailed by Chinma *et al*. (2014). The ABTS [free radical scavenging -azino-bis (3-ethyl-benzthiazoline-6-sulfonic acid)] was determined as explained by Chinma *et al*. (2022) and presented as mg TE/g (dry basis). The DPPH (1,1-diphenyl-2- picryl-hydrazil) radical scavenging activity (presented as mg AAE/g) and ferric reducing antioxidant power (FRAP, mg TE/g) were assayed using a standard method (Chinma *et al*., 2014).

2.4 Statistical analysis

All analyses were measured in triplicates and data obtained subjected to analysis of variance using SPSS 22 (IBM, Armonk, USA). Differences among the means of the measured parameters were separated by Tukey's test at 5% probability.

3.0 Results and Discussion

Table 1 shows the phytochemical and antioxidant properties of raw and fermented black turtle bean and pearl millet flours. The total phenolic content (TPC) and total flavonoid (TFC) of the raw flours from both grains were significantly lower than the fermented grains (Table 1). Compared to the raw flour from both grains, an increase in fermentation time 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 antioxidant activities (evaluated in terms of DPPH, FRAP and ABTS radical scavenging activities) of the fermented flours from both grains were significantly higher than the raw flour samples; thus, suggesting that the fermented flours demonstrated higher potential as functional ingredients compared to the raw flour. The increased content of TPC and TFC; suggests that a strong correlation has been established between phenolic compounds and antioxidant activities (Xu *et al*., 2019).

4.0 Conclusion

This study showed that short-time fermentation of black turtle beans and pearl millet could serve as a natural means and effective strategy for enhancing their phytochemicals and antioxidant activities. The 72 hour fermented grains contained higher phytochemicals and antioxidant properties; thus, the utilization of the bioprocessed flours in food formulation could be promising in the development of health promoting foods. Further studies are ongoing in our laboratory on the impact of fermentation on the techno-functional and structural properties of black turtle bean and pearl millet flours.

Table 1. Effect of fermentation on the antioxidant properties of black turtle bean and pearl millet flours

Parameter	Fermentation time	TPC (mg GAE/g)	TFC (mg CE/g)	DPPH (mgAAE/g)	FRAP (mg TE/g)	ABTS (µmTE/g)
Black turle beans	Raw flour	2.11 ± 0.05^{d}	3.35 ± 0.01^{d}	2.42 ± 0.01^{d}	2.51 ± 0.01^{d}	18.24 ± 0.17^{d}
	24 h	2.74 ± 0.06^{c}	3.60 ± 0.01^{c}	2.66 ± 0.01^{c}	3.10 ± 0.01^{c}	19.39 ± 0.12^{c}
	48 h	3.30 ± 0.11^{b}	3.84 ± 0.00^{b}	3.14 ± 0.01^{b}	3.55 ± 0.01^{b}	20.42 ± 0.19^{b}
	72 h	3.97 ± 0.13^{a}	4.02 ± 0.01^{a}	3.39 ± 0.01^{a}	4.17 ± 0.01^{a}	21.66 ± 0.15^{a}
Pearl millet	Raw flour	1.29 ± 0.01^{d}	2.11 ± 0.01^{d}	1.12 ± 0.01^{d}	1.67 ± 0.01^{d}	13.10 ± 0.13^{d}
	24 h	1.41 ± 0.02^{c}	2.33 ± 0.01^{c}	1.56 ± 0.02^{c}	1.80 ± 0.02^{c}	13.75 ± 0.11^{c}
	48 h	1.94 ± 0.01^{b}	2.70 ± 0.01^{b}	1.92 ± 0.01^{b}	2.11 ± 0.01^{b}	14.20 ± 0.15^{b}
	72 h	2.06 ± 0.02^{a}	2.95 ± 0.03^{a}	2.15 ± 0.01^{a}	2.28 ± 0.02^{a}	14.67 ± 0.11^{a}

Mean ± standard deviation from triplicate analysis. Means with no common letters within a column is significantly, different at ($p\leq0.05$) for a particular grain. TPC = Total phenolic content, TFC = Total flavonoid content, DPPH: 2,2-diphenyl-1-picrylhydrazyl; ABTS= 2,2′-Azino-bis-3-ethylbenzthiazoline-6-sulfonic acid, FFRAP-Ferric reducing antioxidant power; ABTS [free radical scavenging -azino-bis(3-ethyl-benzthiazoline-6-sulfonic acid)

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