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Research Article
Innovative Nutritious Biscuits Limit Aflatoxin Contamination

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Abstract

Background and Objective: Incorporation of food byproducts in biscuit could increase the safety, nutritional and enhance dough properties. These byproducts were wheat bran (WB), goldenberry fruit (GBF) and goldenberry peel (GBP) contains active ingredients. **Materials and Methods:** Wheat flour (WF) was partially replaced in biscuit dough. Antioxidant activity, chemical composition and baking quality were evaluated. Anti-aflatoxigenic and antifungal activities of WB, GBF and GBP have estimated also aflatoxin reduction was evaluated. **Results:** The results were showed biscuit acceptable sensories. The GBF and GBP exhibited the highest antioxidant and phenolic content explaining its antimicrobial behaviour. The addition of WB, GBF or GBP to fungal media inhibited the growth, however, using 20% GBF in *Aspergillus flavus* media showed the greatest aflatoxin reduction. The biscuit-specific volume was more pronounced when GBF and GBP were included in the formulation. No great differences were seen for colour, baking quality or texture of biscuit mixes. **Conclusion:** This novel safe biscuit appears a safer alternative to traditional biscuits.

Key words: Aflatoxins, antifungal activity, *Aspergillus flavus*, baking quality, goldenberry, safe biscuit

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The main byproduct of wheat milling is wheat bran (WB), which composes 10-15% of the kernel weight[1]. The bran is considered a unique source of bioactive compounds that are low cost and directly available from breadstuff milling. The physiological effects of WB are categorized into nutritional, mechanical and antioxidant effects[2]. Recently, food byproducts, as a source of fibre have been found to possess health and safety benefits related to their application as mycotoxin reducers[3]. Blending bran into biscuit or cake formulations results in less undesirable changes than blending bran into bread does, either in terms of the dough or product characteristics[4]. A previous study found that biscuits manufactured with fine bran developed a more compact structure, lower surface area and internal defects[5].

Physalis peruviana L. is known as goldenberry (GB) in many countries and is an annual plant that is grown commercially worldwide. The GB is a common fruit known for its organoleptic, nutritional and health characteristics. Although GB is generally sold as a fresh product, it is also incorporated into sauces, syrups and marmalades[6]. Additionally, it may be dehydrated similar to raisins for use in baked goods, cocktails, snacks and breakfast cereal.

In many food products, mycotoxins pose a recognized risk and can be a source of economic loss. This hazard is elevated because these toxins cause health problems for humans and animals. Cereals and their products are incorporated as a base material in food products such as baked goods and biscuits and have suitable characteristics for fungal growth and toxin production. Active components from either plants or food byproducts have shown beneficial effects on reducing mycotoxins in food and food products[7]. This reduction could be related to physical (binding) or chemical reactions with byproduct materials[8,9]. Antioxidant and pro-oxidant balance improve metabolic pathways, maintains development and reduces stress conditions in cells[10,11]. Dietary antioxidants such as vitamin E, selenium and carotenoids could regulate this balance, but other nutritional stressors have a negative impact on this balance[12,13]. Mycotoxins are assumed to be some of the most important food-borne stress factors[14].

The WB and GBP are food byproducts that contain bioactive molecules. In recent years, no detailed studies have been published on the anti-aflatoxigenic effect of GB. Additionally, GB application for food safety improvements needs to be evaluated. Therefore, the authors aimed to produce new biscuits that can prevent mycotoxin contamination and that possess an extended shelf life because of the delayed fungal contamination. This aim was achieved through partial substitution of the flour in dough with WB, GBF or GBP. These substitutes improved the quality and safety of the produced biscuits. These results will aid the development of safe and nutritious infant products.

MATERIALS AND METHODS

Materials

Raw materials: Wheat flour (WF, 72% extract) and WB were obtained from the north Cairo Flour Mill Company, Egypt. Goldenberry fruit (GBF) and peel (GBP) were obtained from the Agriculture Research Farm at the Agriculture Research Institute, Giza, Egypt. All materials were dried in a Binder FDL 115 hot air oven and the dried materials were ground and then sieved (80 mesh) to collect fine dust. All materials were determined to be free of toxigenic fungi and aflatoxins.

Bacterial strains: The strains used in the tests to determine antimicrobial properties and minimum inhibitory concentrations (MICs) were *Enterococcus faecium* ATCC 19434, *Staphylococcus aureus* NCTC 10788, *Salmonella typhi* ATCC 14028 and *Escherichia coli* ATCC 11229. These strains were purchased from the Standards Development Organization (SDO), LGC Standards, Sesto San Giovanni, MI Italy.

Fungal strains: The strains of toxin-producing fungi used in the tests to determine antifungal properties and minimum fungicidal concentrations (MFCs) were *Aspergillus flavus* ITEM 698, *Aspergillus niger* ITEM 3856, *Fusarium solani* ITEM 250 and *Penicillium notatum* ATCC 10106. These strains were purchased from the SDO, LGC Standards, Sesto San Giovanni, MI Italy.

Methods

Preparation of flour mixtures: The WF (72% extract) was well blended with WB and GBF to produce individual mixtures containing 0, 5, 10, 15 and 20% replacement levels and 1, 3, 5 and 7% GBP replacement, these mixtures were then used for biscuit manufacturing. Fresh GBF was dried in a vacuum oven (J.P. Selecta 4001489, Vaciotem-T, Spain) at 30°C for 48 h, ground using a hummer mill and sieved using a sieve 40 mesh to produce a powder. The GBP was dried under the same conditions, then ground to a powder and sieved (60 mesh). All samples were stored in airtight containers and stored at 3-4 °C until use.

Evaluation of the chemical properties of prepared biscuits: Biscuit composition was determined according to the AACC official methods of analysis[15] and the determined parameters were carbohydrates, protein, moisture, ash, bre and fat.

Determination of the total phenolic content (TPC): The TPC was determined according to the Folin-Ciocalteu procedure[16] by means of a calibration curve prepared with gallic acid and expressed as mg GAE g^{-1} sample and the results are expressed as the Means±SD of triplicate measurements.

Determination of the antioxidant capacity

Determination of the DPPH radical-scavenging activity: The free radical-scavenging capacity of applied materials was determined using the stable DPPH*, according to Hwang and Thi[17]. The absorbance was measured at 517 nm against a blank. The percentage inhibition of the free DPPH radical was calculated by the following equation:

$$\text{Inhibition } (\%) = \left[\frac{\left(A_{\text{control}} - A_{\text{sample}}\right)}{A_{\text{control}}}\right] \times 100$$

where, $A_{control}$ is the absorbance of the control reaction and A_{sample} is the absorbance of the test compound.

Determination of the ABTS radical-scavenging activity: The ABTS* values of WB, GBF and GBP were measured at 734 nm using a spectrophotometer according to Hwang and Thi[17]. A standard curve was prepared using Trolox and the results are expressed as mM Trolox equivalents (TE) g^{-1} sample.

Ferric reducing antioxidant power (FRAP) assay: The FRAP values of WB, GBF and GBP were determined according to Hwang and Thi[17]. The reaction product was measured at 593 nm, a standard curve was prepared and the results are expressed as mM TE g^{-1} sample.

Determination of the raw fibre fraction: An FIWE raw fibre extractor (VELP) unit, (VELP Co., Italy) was used to determine the hemicellulose, cellulose, neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) contents according to the method of Tosh and Yada[18].

Preparation and evaluation of baking and sensory qualities of biscuits: Biscuits were prepared by mixing WF (72%) with 35 g sucrose, 28 g shortening, 30 g egg, 1.5 g baking powder, 0.9 g salt and 1 g vanilla, then, a suitable amount of water was added according to the AACC method[15]. The dough was baked at 200°C for 15 min. Baking quality and organoleptic (sensory) characteristics of biscuits were evaluated according to Chauhan *et al.*[19]. The biscuit formulations were subjected to sensory analysis by 20 panellists.

Colour analysis of biscuits: The colour parameters of the raw materials and prepared biscuits were evaluated using a HunterLab LabScan XE spectrophotometer (Reston, VA) calibrated with a white HunterLab colour standard tile (LX No. 16379, x = 77.26, y = 81.94 and z = 88.14, L* = 92.43, a* = -0.88, b* = 0.21).

Texture analysis of biscuits: Texture profile analysis (TPA) was performed using a Brookfield texture metre (model CT3-10 kg, USA) equipped with a cylinder probe (TA.AACC36) and calculations were performed by texture metre software. The TPA parameters of control samples and the supplemented biscuits were evaluated using double compression tests[20]. The biscuit radius and depth were 29 and 8 mm, respectively and the biscuits were compressed by 20% twice to produce a two-bite texture profile curve. Texture analyses were performed 6 h after baking. The trigger load and test speeds were 9.00 N·g and 2.5 mm sec^{-1}, respectively.

Antimicrobial activity

Determination of the MIC: The raw materials were prepared using iso-propanol:water (1:1) according to the method of Abdel-Razek *et al.*[7]. The MIC was determined as described by Mostafa *et al.*[21]. Tetracycline, as an antibiotic standard was used as a reference material because of its antimicrobial properties.

Determination of the MFC: The MFC of the raw materials was evaluated according to the method described in Abdel-Razek *et al.*[22]. Hypha growth inhibition was used to determine the antifungal activity against fungal strains, as described by Dellavalle *et al.*[23]. Nystatin was utilized as a standard antifungal material for comparison of the MFCs.

Preparation of aflatoxin standards: Standards of aflatoxins B and G from Sigma-Aldrich were received as dry films or crystals. Methanol:acetonitrile (9:1) was added as a solvent and the concentration in ng mL^{-1} was calculated.

Determination of aflatoxins using HPLC: An agilent 1100 HPLC instrument was utilized for aflatoxins detection. One hundred microliters of the samples were injected into the HPLC column (ZORBAX Eclipse XDB-C18, 4.6×150 mm, 3.5 µm) which was heated to 40°C. The mobile phase was acetonitrile:methanol:water (1:3:6, v/v). The flow rate was adjusted by 1 mL min^{-1}. For the fluorescence detection of aflatoxins, the excitation wavelength was 254 nm and the emission wavelength was 366 nm. Aflatoxins were determined (as ng aflatoxin mL^{-1} of media).

Evaluation of the impacts of WB, GBF and GBP on aflatoxin production in media: The WB, GBF and GBP reduction effect on aflatoxin production was tested in inoculated YES media by *A. flavus* ITEM 698 (produces aflatoxin B_1, aflatoxin B_2, aflatoxin G_1 and aflatoxin G_2). The WB, GBF and GBP at four concentrations each were added to inoculated flasks, while a control flask contained only the fungi. The reduction in aflatoxin production was calculated by comparing the growth rates.

Statistical evaluation: The obtained results were evaluated statistically via one-way analysis of variance (ANOVA) using SPSS 16.0 as reported by Salama *et al.*[24].

RESULTS

Gross chemical composition of the raw materials: Table 1 shows that the protein content of the raw materials ranged from 6.10-10.82%. The GBP featured high contents of fibre and protein and low fat and ash contents, but a higher fibre content was recorded for GBF. The carbohydrate content was very similar in WB and GBF but was lower in GBP. Moreover, the ash contents in WB and GBP were similar. The contents of cellulose, lignin, hemicellulose, NDF, which is considered non-dissolved fibre, ADF and ADL in WF, WB, GBF and GBP are presented in Table 1. Hemicellulose was the most abundant raw fibre fraction (i.e., cellulose and lignin) in all raw materials (WF, WB, GBF and GBP). The same trend was observed for NDF, ADF and ADL in the WF, WB, GBF and GBP. However, the highest hemicellulose level was found in WB, followed by GBP and GBF.

TPC and antioxidant activity (AA) of the raw materials: The TPCs of WF, WB, GBF and GBP were determined. Additionally, the AA of all samples were tested based on their ability to scavenge DPPH, ABTS and FRAP radicals. As shown in Fig. 1, GBP was characterized by a high TPC, followed by GBF and then WB. Additionally, the AA (ABTS, DPPH and FRAP) was highest for GBP followed by GBF and then by WB and WF.

Colour attributes of the raw materials and biscuit mixes: The colour of the raw materials was evaluated using a Hunter colorimeter (unpublished data). The WB, GBF and GBP were darker than WF. The lightness (L*) ranged from 70.64-76.40, the redness (a*) from 6.13-7.08 and the yellowness (b*) from 19.71-36.63. This result could be linked to the hull and germ contents of WB, which caused the bran and peel to be darker (lower L*) than the WF. As the ratios of WB, GBF and GBP increased, biscuit lightness (L*) and yellowness (b*) decreased, but redness (a*) increased. All biscuit formulations had noticeably darker crusts than the control sample (100% WF).

Baking quality of biscuits: Table 2 shows the effects of WB, GBF and GBP addition on the baking quality of biscuits. Biscuit volume was increased by WB, GBF and GBP addition, while the diameter and spread ratio were slightly increased by the additions. The increase in specific biscuit volume was more pronounced when WB, GBF and GBP were added at levels of 5, 10, 15 and 20%, consequently.

Table 1: Chemical composition and fibre fraction of the raw materials used to prepare biscuits

Composition	Wheat flour (WF)	Wheat bran (WB)	Goldenberry fruit powder (GBF)	Goldenberry peel powder (GBP)
Moisture	13.55±1.12	9.31±0.85	14.07±0.67	10.06±0.59
Protein	10.82±0.66	8.50±0.45	9.64±0.33	6.10±0.29
Fat	1.08±0.03	2.83±0.06	0.19±0.001	0.82±0.02
Fibre	1.29±0.04	6.29±0.11	16.03±0.39	37.15±0.52
Ash	0.56±0.001	14.58±0.26	5.17±0.10	14.34±0.32
Carbohydrate	72.70±0.76	58.49±0.60	54.90±0.48	31.53±0.36
NDF	1.73±0.03	55.73±0.39	41.23±0.32	39.14±0.29
ADF	0.01±0.0	39.54±0.17	22.68±0.19	27.46±0.32
ADL	ND	18.38±0.11	11.18±0.13	9.02±0.15
Lignin	ND	8.53±0.17	4.87±0.10	5.13±0.09
Cellulose	0.01±0.0	16.19±0.10	11.48±0.08	11.67±0.12
Hemicellulose	1.72±0.08	20.81±0.22	18.55±0.17	19.49±0.15

NDF: Neutral detergent fibre, ADF: Acid detergent fibre, ADL: Acid detergent lignin. Values in the table are the means±SDs

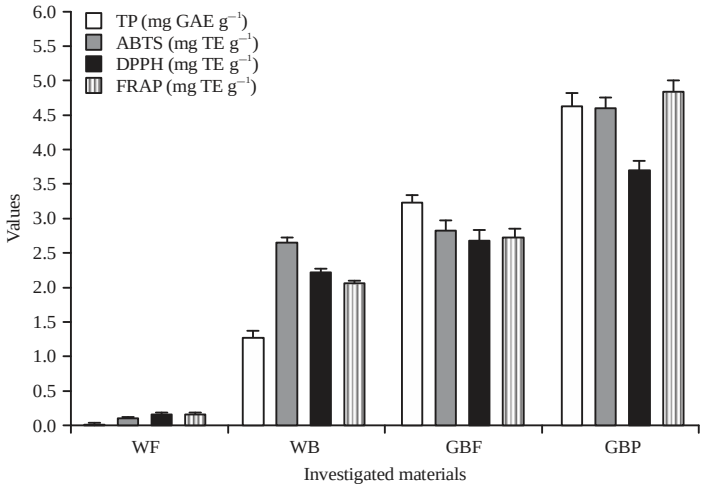

Fig. 1: Total phenolic content and antioxidant activity of the raw materials used in biscuit manufacturing
WF: Wheat flour, WB: Wheat bran, GBF: Goldenberry fruit powder, GBP: Goldenberry peel powder, GAE: Gallic acid equivalents, TE: Trolox equivalents

Table 2: Baking quality of biscuit formulations

Samples	Weight (g)	Volume (cm^3)	Specific volume (v\w)	Diameter (cm)	Thickness (cm)	Spread ratio
(1) Control	10.95^{a}	18.70^{c}	1.71^{d}	5.98^{b}	0.61^{a}	9.80^{d}
(2) 5% WB	11.10^{a}	19.00^{c}	1.71^{d}	6.00^{b}	0.58^{a}	10.34^{c}
(3) 10% WB	11.26^{a}	19.50^{b}	1.73^{d}	6.00^{b}	0.56^{a}	10.71^{b}
(4) 15% WB	11.28^{a}	19.80^{b}	1.76^{d}	6.20^{a,b}	0.57^{a}	10.88^{b}
(5) 20% WB	11.01^{a}	19.50^{b}	1.77^{d}	6.50^{a}	0.59^{a}	11.02^{a}
(6) 5% GBF	10.89^{a}	19.30^{b}	1.77^{d}	5.92^{b,c}	0.58^{a}	10.21^{c}
(7) 10% GBF	10.46^{b}	19.00^{c}	1.82^{c}	6.13^{b}	0.57^{a}	10.75^{b}
(8) 15% GBF	8.98^{d}	21.50^{a}	2.39^{a}	6.10^{b}	0.55^{a}	11.09^{a}
(9) 20% GBF	10.56^{a,b}	19.50^{b}	1.85^{c}	6.31^{a}	0.57^{a}	11.07^{a}
(10) 1% GBP	10.33^{b}	19.72^{b}	1.91^{c}	6.28^{a}	0.55^{a}	11.42^{a}
(11) 3% GBP	9.85^{c}	18.95^{c}	1.92^{c}	5.82^{c}	0.57^{a}	10.21^{c}
(12) 5% GBP	9.95^{b}	20.30^{c}	2.04^{b,c}	5.91^{c}	0.55^{a}	10.75^{b}
(13) 7% GBP	9.90^{c}	21.40^{a}	2.16^{b}	5.98^{b}	0.55^{a}	10.87^{b}
LSD at 0.05	0.625	1.066	0.115	0.271	0.051	0.499

*WB: Wheat bran, GBF: Goldenberry fruit powder, GBP: Goldenberry peel powder. Values with different superscript letters are different at $p \leq 0.05$

Sensory evaluation: Mixing WB, GBF and GBP with WF in the biscuit formula resulted in very small decreases in the biscuit sensory scores (Table 3). Increasing the proportions of WB, GBF and GBP in biscuits slightly decreased the sensory scores for colour, texture, odour, taste, appearance and overall acceptability.

Texture parameters of biscuits: The biscuit formulations exhibited hardness (N) values ranging from 10027-10247 N. Increased biscuit hardness is related to a low moisture content. As the ratio of WB, GBF and GBP (5, 10, 15 and 20%) to flour increased, the hardness (N) increased (Table 3).

Approximate biscuit composition: The approximate compositions of biscuits prepared with different proportions of WB, GBF and GBP were estimated (Table 4). The biscuit composed of 100% WF contained 0.33% ash, 6.46% crude protein, 17.37% fat, 0.77% fibre and 75.07% carbohydrate. The ranges of protein, fat, ash, fibre and carbohydrate were 6.38-6.15, 17.27-17.49, 0.47-2.00, 0.92-2.53 and 72.99-74.55% in all biscuits mixes.

MICs of WB, GBF and GBP for bacterial and toxigenic fungal strains: As shown in Table 5, WB possessed the lowest antimicrobial activity followed by GBF. The GBP had the highest antimicrobial activity after tetracycline (standard reference antibiotic). The TPC, flavonoid content and antioxidant capacity were considered the main antimicrobial agents in the raw material and these values are shown in Fig. 1. On the other hand, the GBF showed the highest antifungal activity followed by GBP, but the lowest antifungal activity was recorded for WB as the byproduct ingredient with weak effect.

Table 3: Sensory evaluation and texture profile analysis of biscuit mixes

	Sensory evaluation						Texture profile parameters			
Samples	Colour (10)	Texture (10)	Odour (10)	Taste (10)	Appearance (10)	Overall acceptability (10)	Hard-ness (N)	Deformation at hardness (mm)	Deformation at hardness (%)	Hardness work (mJ)
(1) Control	8.54^{a}	8.21^{a}	7.46^{b}	8.10^{a}	8.44^{a}	8.63^{a}	10053	17.98	276.60	0.20
(2) 5% WB	7.82^{b}	7.46^{b}	7.39^{b}	7.64^{b}	7.37^{b}	7.49^{b}	10116	11.95	183.80	0.20
(3) 10% WB	7.57^{b}	7.00^{b}	8.54^{a}	7.12^{b}	7.00^{b}	7.13^{b}	10247	0.66	10.20	34.80
(4) 15% WB	7.09^{c}	6.38^{c}	8.29^{a}	6.56^{c}	6.95^{b}	7.12^{b}	10242	10.73	165.10	599.70
(5) 20% WB	8.63^{a}	8.53^{a}	8.56^{a}	8.65^{a}	8.83^{a}	8.39^{a}	10056	2.23	34.30	136.70
(6) 5% GBF	8.45^{a}	8.19^{a}	7.39^{b}	8.30^{a}	8.47^{a}	8.67^{a}	10053	3.84	59.10	200.30
(7) 10% GBF	7.55^{b}	7.21^{b}	8.71a	7.21^{b}	7.25^{b}	7.54^{b}	10027	5.85	90.00	280.50
(8) 15% GBF	6.13^{d}	5.56^{d}	8.62^{a}	5.50^{d}	5.80^{d}	6.42^{c}	10093	5.53	85.10	340.10
(9) 20% GBF	8.72^{a}	8.74^{a}	8.36^{a}	8.48^{a}	8.56^{a}	8.67^{a}	10028	12.60	193.80	275.10
(10) 1% GBP	8.63^{a}	8.54^{a}	8.32^{a}	8.33^{a}	8.45^{a}	8.54^{a}	10051	13.18	202.80	490.80
(11) 3% GBP	7.21^{b,c}	7.19^{b}	8.41^{a}	7.30^{b}	7.46^{b}	7.82^{a,b}	10090	9.75	150.00	451.60
(12) 5% GBP	8.81^{a}	8.65^{a}	8.64^{a}	6.53^{c}	8.90^{a}	8.71^{a}	10060	15.74	242.20	940.50
(13) 7% GBP	6.05^{d}	5.50^{d}	7.40^{b}	5.55^{d}	5.85^{d}	6.41^{c}	10070	15.02	231.10	817.10
LSD at 0.05	0.539	0.572	0.493	0.652	0.667	0.693				

WB: Wheat bran, GBF: Goldenberry fruit powder, GBP: Goldenberry peel powder. Deformation at hardness (mm), deformation at hardness (%), hardness work (mJ) and fracturability with 1% load sensitivity (N) of biscuit. Values with different superscript letters are different at $p \leq 0.05$

Table 4: Chemical composition of biscuit samples

Composition	Ash	Protein	Fat	Fibre	Carbohydrate
(1) Control	0.33^{h}	6.46^{a}	17.37^{a}	0.77^{k}	75.07^{a}
(2) 5% WB	0.75^{e}	6.38^{a}	17.40^{a}	0.92^{j}	74.55^{a}
(3) 10% WB	1.17^{c}	6.31^{a}	17.43^{a}	1.07^{i}	74.02^{a}
(4) 15% WB	1.58^{b}	6.23^{a}	17.46^{a}	1.21^{h}	73.52^{a}
(5) 20% WB	2.00^{a}	6.15^{b}	17.49^{a}	1.36^{g}	73.00^{a}
(6) 5% GBF	0.47^{g}	6.43^{a}	17.34^{a}	1.21^{h}	74.55^{a}
(7) 10% GBF	0.61^{f}	6.39^{a}	17.32^{a}	1.65^{e}	74.03^{a}
(8) 15% GBF	0.91^{d}	6.26^{a}	17.33^{a}	2.27^{b}	73.23^{a}
(9) 20% GBF	0.75^{e}	6.36^{a}	17.29^{a}	2.09^{c}	73.51^{a}
(10) 1% GBP	0.89^{d}	6.32^{a}	17.27^{a}	2.53^{a}	72.99^{a}
(11) 3% GBP	0.42^{g}	6.43^{a}	17.36^{a}	0.98^{j}	74.81^{a}
(12) 5% GBP	0.58^{f}	6.37^{a}	17.35^{a}	1.41^{f}	74.29^{a}
(13) 7% GBP	0.75^{e}	6.31^{a}	17.34^{a}	1.84^{d}	75.07^{a}
LSD at 0.05	0.052	0.304	1.237	0.072	2.985

WB: Wheat bran, GBF: Goldenberry fruit powder, GBP: Goldenberry peel powder. Values calculated on a dry weight basis, values with different superscript letters are different at $p \leq 0.05$

Table 5: Minimum fungicidal and inhibitory concentrations of wheat bran and goldenberry (fruit and peel) against toxigenic and pathogenic micro-organisms

	Minimum fungicidal concentration (MFC)				Minimum inhibitory concentration (MIC)			
Parameters	*Aspergillus flavus* ITEM 698	*Aspergillus niger* ITEM 3856	*Fusarium solani* ITEM 250	*Penicillium notatum* ITEM 10106	*Enterococcus faecium* ATCC 19434	*Staph. aureus* NCTC 10788	*Salmonella typhi* ATCC 14028	*Escherichia coli* ATCC 11229
Goldenberry fruit (mg mL^{-1})	150	170	150	150	100	120	120	120
Goldenberry peels (mg mL^{-1})	260	300	260	250	90	110	110	90
Wheat bran (mg mL^{-1})	770	720	700	730	460	480	460	460
Nystatin (µg mL^{-1})	26	26	26	26	-	-	-	-
Tetracycline (µg mL^{-1})	-	-	-	-	16	32	32	16

Nystatin was used as a standard antifungal compound against toxigenic fungi. Tetracycline used as a standard antibiotic compound against pathogenic bacteria

Inhibition ratio for WB, GBF and GBP on fungal growth of toxigenic fungal strains: The inhibition of mycotoxigenic fungi by the three materials (WB, GBF and GBP) were tested using *A. flavus* ITEM 698, *Aspergillus ochraceus* NRRL 419 and *P. notatum* ATCC 10106, all of which are mycotoxin producers. The addition of 10 or 20% GBF to media containing *P. notatum* ATCC 10106 resulted in the best inhibition ratio. For *A. ochraceus* ATCC 10106 20% GBP resulted in a good reduction in fungal growth. Moreover, 20% GBF resulted in the greatest inhibition. Therefore, 20% GBF is the most effective fungal inhibitor. These results may support the addition of GBF to food products to increase their shelf life (Fig. 2).

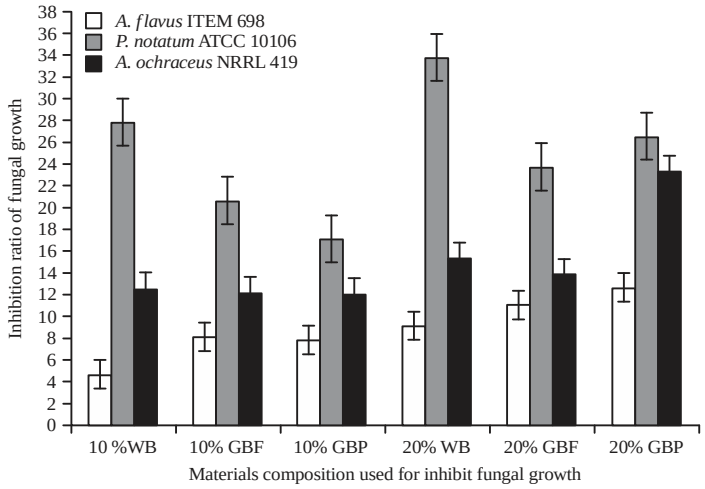

Fig. 2: Inhibition of 3 strains of toxigenic fungi by different materials (powder form)
WF: Wheat flour, WB: Wheat bran, GBF: Goldenberry fruit powder, GBP: Goldenberry peel powder

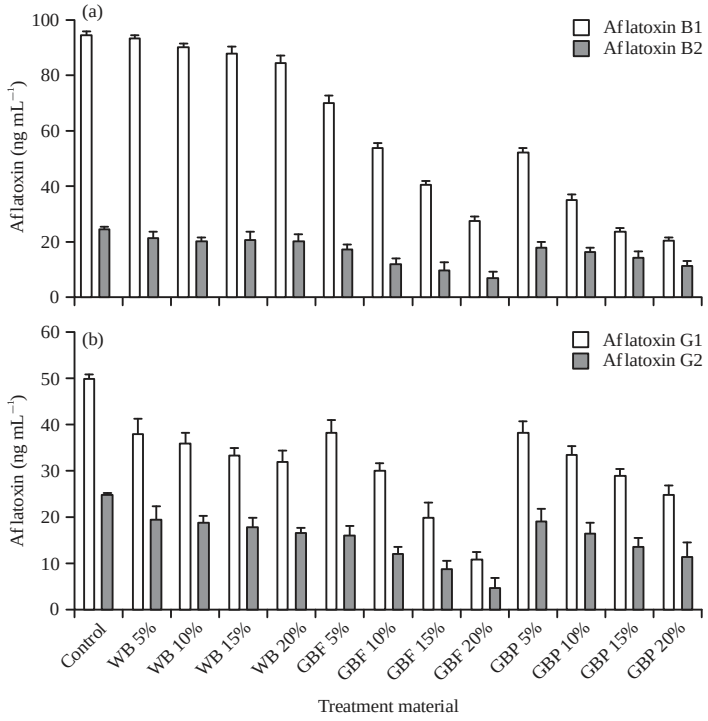

Fig. 3: Aflatoxin reduction by wheat bran and goldenberry (fruit and peel)
WF: Wheat flour, WB: Wheat bran, GBF: Goldenberry fruit powder, GBP: Goldenberry peel powder, (%): Composition of each material used

Reduction in aflatoxin concentration by WB, GBF and GBP: The results displayed in Fig. 3 showed that GB was the most effective at reducing the amount of aflatoxins in the YES media, the toxin reduction increased with the GB concentration. The reduction was greatest at a 20% concentration. The efficiency of the materials at aflatoxin reduction followed an order of GBF powder>GBP powder>WB fine dust.

DISCUSSION

The WB contained more cellulose, NDF and ADF than all the other raw materials. These results agree with those reported by Krishnan *et al.*[25], among the cell wall components, lignin was reported to have maximum interaction with other dietary components, leading to decreased bioavailability[26]. The highest lignin level was found in WB followed by GBP and

GBF. These components could play a significant role in aflatoxin degradation[27,28]. The TPC and AA are related to the ability of these materials to resist the oxidative stress caused by the presence of mycotoxins. The degradation of mycotoxins, particularly aflatoxins was reported to be affected by the oxidative stress caused by other ingredients in food products[14]. Colour is an important sensory characteristic that directly affects product preference, however, the darkness increased as a result of the presence of peel and bran in any product[29,30].

Nevertheless, increasing the level of WB, GBF and GBP showed a little decrease of biscuits baking quality. This finding was similar to the results of a study conducted by El Shebini *et al.*[31]. The approximate WF biscuit compositions reported closed to the results obtained in previous studies[32,33], while, The compositions of WB, GBF and GBP biscuits were similar to those found in other studies[34,35]. Biscuit hardness is perceptible to consumers and may correlate with the expansion and cell structure of the product, independent of the feed moisture content[32,36,37].

According to the chemical compositions of the by-product materials, GBF contained high levels of fibre and low levels of fat and ash. The GBF fibre content was in between those of WB and GBP and the lignin content of GBF was closely associated with the ability of the GBF biscuits to reduce the aflatoxin concentration. In this respect, Prosky *et al.*[26] referred to lignin's ability to interact with dietary components, which could occur for aflatoxin in contaminated diets. Moreover, the TPCs of GBF and GBP were high and the phenolic compounds with AA that was evaluated using three assays (DPPH, ABTS and FRAP), form a barrier of pro-oxidants that prevents aflatoxin contamination[9]. The inhibition related to the TPC and antioxidant potency was recorded using the raw materials either in media or in biscuit dough. The resultant biscuits with different additives (GBF, GBP and WB) in different proportions (5, 10, 15 and 20% replacement of flour) only slightly different in their rheological parameters and not all replacements resulted in rheological changes.

To evaluate the inhibition effect of byproduct ingredients, it was examined against toxigenic fungi strains included *A. flavus* ITEM 698, which is one of the most common toxigenic fungi used for food health and safety studies[38]. The MIC and MFC of GBF and GBP reflect an inhibition of pathogenic bacteria and toxigenic fungi, which improves biscuit safety and extends shelf life. The highest aflatoxin reduction was recorded for 20% GBF, which also showed a small inhibition effect on the producer strain *A. flavus*, in this respect, the combination of the TPC and AA could explain the antimicrobial and antifungal properties of WB, GBF and GBP on the toxigenic strain[7] *A. flavus* ITEM 698, which is expected to have the same effects as aflatoxins in biscuits. Moreover, antimicrobial activity of goldenberry reported in agreement with the results of Erturk *et al.*[39], who recorded the antimicrobial activity of Turkish GB fruits, seeds, roots and leaves to determine the antioxidant and antimicrobial activity of this plant. Additionally, these authors evaluated the TPC, flavonoid content and volatile compound contents. The TPC was high in seeds, followed by fruits and then leaves. The same order was observed for the total flavonoid content. The AA of flowers was highest, followed by that of the roots. These results agree with those of Shehata *et al.*[9], who utilized a bioactive film loaded with agricultural byproducts to reduce the risk of mycotoxin exposure. Roidaki *et al.*[40] were studied the antioxidant and antifungal activity of some food plants and reported the antifungal activity of some fruits, including GBF. Of the 16 studied plants, GB exhibited the highest antifungal activity, particularly against *Aspergillus parasiticus*. Commonly, plant antifungal properties is linked to the antioxidant capacity and phenolic content. The antifungal properties of this plant may be due to the presence of diverse phytoconstituents.

CONCLUSION

The GB is a fruit with numerous health benefits and is a source of bioactive molecules. This study compared dried GBF powder with byproducts (WB and GBP) to improve biscuit safety. The novel GB biscuits looked like the traditional biscuits in colour and sensory characteristics but had excellent baking, textural and rheological properties. The GBF exhibited the best antimicrobial and antifungal properties and showed acceptable inhibition of toxigenic fungi and mycotoxin production. On the basis of these results, it is recommended that these materials be used as food preservatives and food additives to increase the safety and shelf life of food products.

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SIGNIFICANCE STATEMENT

The WB, GBF and GBP as sources of bioactive components, combined with WF in biscuit formulations led to the production of a safe and nutritious product. By replacing WF with GBF and GBP in biscuit formulations, the fibre and mineral contents of biscuits were increased. Additionally, 20% GBF in the biscuit formulation resulted in the best antifungal activity with highly efficient aflatoxin reduction. Thus, this research could lead to a novel safe biscuit derived from agricultural byproducts, which is important for paediatric nutrition.

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