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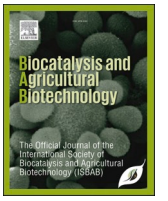

Impact of probiotics co-culture fermentation process on functional properties of sobia fortified with barley flour

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ABSTRACT

Traditional Egyptian beverages are widely consumed across the Middle East and North Africa. This study aimed to enhance the nutritional and functional properties of these beverages and pudding in powder formulations with replacing the main ingredients by barley flour (BF). In addition, BF-fortified sobia fermented with monoculture and co-cultured lactic acid bacteria was investigated to produce a synbiotic beverage. Compared to control, the sahlab and sobia fortified with 30 % BF and the mahalabia fortified with 50 % BF had higher nutritional values and were the most palatable in all sensory aspects. Furthermore, the BF was a good source of antioxidant-active polyphenols and vitamin B which would have a positive effect on the nutritional value of the drinks as well as the consumer's health. Aflatoxin B1 was reduced and product contamination was lower in BF-fortified products after storage. Three fortified samples gain more bioavailability via fermentation, particularly a BF-fortified sobia formulation that used central composite design to provide ideal growth conditions for *Lactobacillus plantarum* and *Bifidobacterium lactis*. With a 3.0 % inoculum level from each bacterium, the fermented drink had the highest viable counts (12.6 Log CFU/mL). Fermented sobia-fortified with BF had higher levels of flavonoids, antioxidants, and antibacterial properties than unfermented product. Moreover, HPLC analysis demonstrated that the organic acids, polyphenols, and amino acids were successfully changed by co-fermentation. In conclusion, the products fortified with BF can be utilized to formulate unique synbiotic foods rich in bioactive components, as well as to formulate healthful and functional foods and serve as food carriers for probiotics.

1. Introduction

Due to its high nutritional value, bioactive qualities, and affordability, barley (*Hordeum vulgare* L.) flour has drawn a lot of interest for use in the production of healthy functional foods (Raj et al., 2023). Barley ranks fourth globally in terms of cereal crop production (Haas et al., 2019). Barley's nutritional benefits are ascribed to its elevated levels of essential amino acids, protein, minerals, vitamins, and phytochemicals, along with its useful carbohydrates, particularly β-glucan (Huang et al., 2020). Its flour is rich in linoleic acid, resistant starch, polysaccharides, β-glucan, and bioactive compounds such as tocols, phenolic acids, flavonoids, and anthocyanins (Geng et al., 2022).

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The prebiotic properties of barley's dietary fiber can help probiotic bacteria grow, enhancing the nutritional value of fermented meals. Additionally, because of its functional qualities, barley protein is thought to be an excellent candidate for value-added application as gluten-free food supplements (Suman and Sreeja, 2019). In order to manufacture functional fermented foods, barley flour (BF) can be a useful substrate for lactic acid bacteria (LAB). LAB uses the nutrients and active compounds in plant-based materials via their potent metabolic pathways and enzyme systems to achieve a combination of functionality, taste, and nutrition (Yang et al., 2024). Barley flour improves the rheological characteristics and nutritional values of final products (El-Hadary et al., 2018). Moreover, BF phytochemicals are clearly effective as antioxidants, immunomodulatory, and anti-inflammatory agents (Idehen et al., 2017).

The fermentation process associated with carbohydrates consuming in BF has been shown to efficiently enrich bioactive substances boost intestinal function, and further enhance food bioactivity. The release of short-chain fatty acids (SCFAs) eventually sets off a series of hormonal and neurological reactions that decrease appetite, encourage weight loss, and lower the risk of obesity (Jiang et al., 2024).

Barley beverage has a lot of natural nutrients that are good for LAB fermentation (Guo et al., 2023). One of the probiotics that is most commonly used in food fermentation is *Lactobacillus* because it can increase the production of flavor volatiles, improve the nutritional value, and change the physicochemical characteristics of fermented foods (Mandha et al., 2021). *Bifidobacterium* has been utilized in fruit and vegetable fermentation as well as serving as a probiotic in food for humans due to its numerous health benefits (Larsbrink et al., 2014). Furthermore, *Bifidobacterium* has the ability to release exopolysaccharides, which improves the nutritional value of fermented products (de Olmos et al., 2022). *Bifidobacterium infantis* enhances barley beverage quality (Guo et al., 2023). Barley fermented by *L. plantarum* also exhibited antioxidant activity (Zhang et al., 2022) and resulted in significantly higher levels of peptides, free phenols, soluble dietary fiber, and other healthful components (Li et al., 2020; Xiao et al., 2020). Currently, little information is available on LAB fermented barley beverages.

A synergistic fermentation of several strains can greatly increase the antioxidant property of LAB in comparison to single strain fermentation (Mongkontanawat et al., 2022), bacteriostatic capacity of plant foods, as well as produce more metabolites and form richer flavors (Nguyen et al., 2019). However, the effect of co-culture of *L. plantarum* and *B. lactis* fermentation on the properties of BF-based beverages has not been investigated so far.

Few studies have explored the potential use of BF as a nutritional supplement in beverages and pudding desserts for children, elderly and newborns, despite its beneficial properties. Traditional Egyptian beverages such as sahlab (hot drink very popular in winter) and sobia (cold drink very popular in summer) and a Middle Eastern milk pudding snack (mahalabia) are sweet and simple to swallow for children and the elderly. However, because corn flour and rice flour make up the majority of the fundamental ingredients in these traditional Egyptian foods, they are low in nutritional content. Furthermore, foods based on starch are extremely susceptible to contamination from moulds and aflatoxin due to the storage conditions (Abdullah et al., 1998). The synbiotic BF-based sobia fermented with coculture was done for the first time.

In light of these facts, the goal of this study was to **(1)** optimize the level of BF in each product formula. This was evaluated using sensory evaluation, physicochemical characterization and safety of the products after a specific storage period (aflatoxin B1 levels); **(2)** optimize the nutritional value of the different functional products, which was achieved via fermentation with a single or co-culture of *L. plantarum* and *B. lactis* for optimal growth and viability; and evaluate the most promising product for microbiological, pH and functional properties in comparison with un-fermented formulas.

2. Materials and methods

2.1. Materials

The dark BF (Giza 123) was provided by the Dr. Mirah Foundation for Reviving Ancient Egyptian Heritage and Community Development, Egypt. Corn starch, rice flour, milk powder, sugar, vanillin, and ground coconut were purchased from the local market in Egypt. The formula for powdered mahalabia was bought from the local market, which is produced by Dream Mashreq Foods S.A.E. Borg El-Arab City, Alexandria, Egypt. Riboflavin, niacin, pyridoxine hydrochloride, acetonitrile and methanol were purchased from Sigma (Sigma-Aldrich, USA). Analytical-grade compounds were employed in all experiments.

2.2. Technological methods

2.2.1. Preparation of sahlab powder formulations

Corn starch (200 g), sugar (50 g), powdered milk (50 g) and vanillin (0.1 g) were the basic constituent compositions for sahlab, as produced by traditional shops. The BF was used at 0, 20, 30, 40, and 50 % w/w replacement level in the sahlab formula. The formulations for the sahlab powder were well combined with water and then placed into 200 mL glass bottles. The sahlab drink was brought to a boil for 7 to 10 min to get the proper consistency for serving as a hot drink.

2.2.2. Preparation of sobia powder formulations

The ingredients for the sobia formula were 250 g rice flour, 100 g sugar, 100 g ground coconut, 50 g powdered milk, and 2 g vanilla. The BF was used at ratios of 0, 20, 30, 40, and 50 %, replacement level of the sobia formula. All components were combined in a Hobart mixer with 250 mL of cold water for 5 min to produce the sobia cold drink.

2.2.3. Preparation of mahalabia powder formulations

Barley flour was used at replacement levels of 0, 25, 50, 75, and 100 % in the commercial mahalabia formula obtained from Dream

Mashreq Foods. In a stainless-steel boiler, 90 g of the mahalabia formula powder was dissolved in 250 mL of cold milk that included 30 g of sugar, and the mixture was thoroughly mixed to manufacture the basic formula (control). After that, the mixture was heated to 80–85 °C while being stirred constantly until the consistency thickened, then left to cool and served as a pudding dessert.

2.2.4. Nutrient composition, phytochemicals analysis and sensory properties

Barley flour and the most preferred product formulations were analyzed for their proximate composition, including moisture, protein, ether extract, ash, and crude fiber, following the (AOAC, 1990) standard methods. Total carbohydrate content and caloric value (kcal/100 g) were calculated using the equations (Osborne and Voogt, 1978). Mineral content, specifically calcium (Ca), potassium (K), sodium (Na), iron (Fe), and zinc (Zn), was also performed in accordance with (AOAC, 1990).

The total phenolic content was determined as mg of gallic acid equivalent (GAE)/g using the Folin-Ciocalteau reagent (Singleton and Rossi, 1965). The free radical scavenging activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH) was used to evaluate antioxidant activity according to Brand-Williams et al. (1995), using a spectrophotometer (Model 91102, Laxco Inc., USA) at 517 nm.

Riboflavin (vitamin B2), niacin (vitamin B3), and pyridoxine hydrochloride (vitamin B6) were quantified using reverse-phase high-performance liquid chromatography (Shimadzu HPLC) equipped with an SPD-6AV detector, following the method of (Agostini-Costa et al., 2008). Detection was performed at 254 nm using pure reference standards obtained from Sigma-Aldrich (USA).

The pH of each product was measured using a Beckman pH meter (pH MVx 100 Beckman, USA). Viscosity of the drinks and the mahalabia formulation was determined using an oscillating laboratory viscometer (Model NDJ.93, USA) with a spindle set at 12 rpm and 25 ± 2 °C (Suwonsichon and Peleg, 1999).

Color measurements of the drinks and mahalabia powder were conducted using a Hunter Lab (Ultra Scan VIS model, colorimeter, USA), based on the characteristics of lightness, redness, and yellowness (Francis and Clydesdale, 1975).

Furthermore, a team of experts using ten panelists from the Food Science and Technology Department at Alexandria University's Faculty of Agriculture assessed the drinks' sensory qualities, taking into account factors including color, flavor, mouthfeel, and general acceptability (Kramer and Twigg, 1973).

2.2.5. Quantification and determination of the aflatoxin B1 by HPLC

In 100 mL of acetonitrile/water (60 % v/v), every 50-g sample was dissolved and filtered after 20 min of vigorous shaking at 120 RPM on a shaker. A column filled with monoclonal antibodies to aflatoxin-bound was then filled with 2 mL of filtered water and diluted with 48 mL of phosphate buffered saline (PBS, pH 7.4). The eluted aflatoxin was then purified by eluting it with a 1:1 mixture of methanol and water (Namjoo et al., 2016). Aflatoxin B1 purity was assessed using HPLC (Shimadzu) with ultraviolet detection at 265 nm (Mochamad and Hermanto, 2017).

2.3. Synbiotic beverages and mahalabia based on LAB fermentation

2.3.1. Bacterial culture

Lactobacillus plantarum DSM 20174 obtained from MIRCEN, Egypt and *Bifidobacterium lactis* BB-12, a freeze-dried probiotic culture from Chr. Hansen, Denmark, were kept at −70 °C in 30 % glycerol stocks and cultivated in Man Rogosa de Sharpe (MRS; Biolife, Italy) broth at 37 °C.

2.3.2. Preparation of fermentation media

In this investigation, distilled water was used to dissolve the powder from each product (sahlab, sobia, and mahalabia) enriched with BF, in a consistent ratio of 5, 10, and 15 % (w/v) to formulate the fermentation medium. For the purpose of partially gelatinizing starch, the suspensions were moved to volumetric flasks and thermostatically kept in a managed water bath at 95 °C for 15 min. After cooling to 37 °C, the samples were individually inoculated with either *B. lactis* or *L. plantarum*. Additionally, the controls (sahlab, sobia and mahalabia) were similarly treated and inoculated.

2.3.3. Fermentation process

In the MRS broth, *L. plantarum* or *B. lactis* were grown, collected, and then re-suspended in sterile distilled water after being centrifuged for 10 min at 6000 rpm (4 °C). After inoculating the prepared media with 2 % (v/v) starter culture ($>10^6$ CFU/mL), it was cultured for 48 h at 37 °C. The viable cell count (Log CFU/mL) was carried out using the plate count method with MRS agar (pH = 6.8) after the incubation at 37 °C for 48 h (Charalampopoulos et al., 2002). The product used in the subsequent experiment was the one that provided the highest viability for both bacteria.

2.3.4. Co-fermentation of BF-fortified sobia with L. plantarum and B. lactis via central composite design (CCD)

Response surface methodology (RSM) was applied to investigate the influence of the inoculum levels of *L. plantarum* and *B. lactis* (Table 6A) using Design Expert (Version 7) software (Stat-Ease Corporation, USA) on BF-fortified sobia. A two-factor, three-level design with five replicates at the center point was utilized in order to statistically optimize the inoculum levels and assess the main effects, interaction effects, and quadratic effects of the two components on the growth of LAB (Table 6A).

In 100 mL of distilled water, 10 g of the fortified product were dissolved. Then, for 10 min, this was heated to 95 °C in a water bath with a thermostat controlled by stirring at regular intervals. The samples were allowed to cool to 37 °C before the required quantities of starter culture were added (Table 6A). The fermentation process was conducted in 250 mL Erlenmeyer flasks with 50 mL BF-based sobia. The flasks were then incubated at 37 °C for 48 h, and the viable cell counts were expressed as Log CFU/mL.

2.3.5. Evaluation of microbiological and functional properties of BF-based sobia

The medium was made, heat sterilized, and processed in the same way without bacterial inoculums after the CCD indicated its optimal condition. Both the fermented and unfermented BF-based sobia's pH value and bacterial count were evaluated. After that, the following analysis was done.

2.3.5.1. Microbiological parameters. Microbiological safety was assessed by testing for hygiene indicators in both fermented and unfermented BF-based sobia samples. Both fermented and unfermented drinks underwent tests for hygiene indicators. *Staphylococci*, yeast and moulds, Enterobacteriaceae, and total viable count were the indicators that were analyzed. The pour plate approach, which is based on the detection of microbial proliferation after a previously outlined counting procedure, was used to calculate the total viable cell counts (Jeličić et al., 2012). A variety of enrichment agars, including nutrient agar (Sigma-Aldrich, Spain), sabouraud dextrose, mannitol salt agar, and eosin methylene blue, were employed to count the total number of bacteria, yeasts and moulds, *Staphylococci*, Enterobacteriaceae, respectively. Samples for enumeration of total viable cell count were incubated at 30 °C for 72 h, Enterobacteriaceae and *Staphylococci* samples were incubated at 37 °C for 48 h, and yeasts and moulds samples were incubated at ambient temperature for 72 h. The results were expressed as Log CFU/mL. The antibacterial activities of the cell-free supernatant (CFS) of the optimized co-fermented drink, unfermented, and monocultures (*L. plantarum* DSM, 20174, and *B. lactis* BB-12) were evaluated using an agar well diffusion assay (Schillinger and L cke, 1989). The samples were centrifuged at 3000×g for 10 min, and aliquots of the CFS were taken. The strains of *Staphylococcus aureus* 91161, *Staphylococcus sciuri* strain MH491952.1, *Bacillus subtilis, Bacillus cereus* strain 151007-R3-K09-40-27F*, Listeria monocytogenes, Pseudomonas aeruginosa* strain Kasamber5, *Salmonella typhimurium, Serratia marcescens* 98027, *Enterobacter aerogenes* 9805, and *Escherichia coli* 98082 were utilized.

2.3.5.2. Functional components. Total phenolic content and antioxidant activity were determined as described above. β-Glucan, extracellular sugars, phenolic compounds and organic acids were determined using HPLC. The analysis conditions for β-glucan and extracellular sugars (glucose, fructose, and sucrose) were mentioned (Gao et al., 2014): Agilent 1200 HPLC coupled with a refractive index detector, 5 mM sulfuric acid was employed as the eluent over 14 min at a flow rate of 1.0 mL/min on an aminex HPX-87 H column (Bio-Rad Laboratories, Hercules, USA) running at 80 °C. Utilizing the calibration standards, the measurement of sugars and β-glucan was conducted.

Phenolic compound separation and quantification were carried out utilizing an Agilent 1260 HPLC system operating at 40 °C and fitted with a 280 nm multi-wavelength detector (Stan et al., 2012). Phenolic compounds (μg/ml) in both fermented and unfermented samples were identified.

Organic acids were separated using chromatographic HPLC on an AQ-C18 HP column (4.6 mm × 150 mm i.d., 3 μm) with 0.005 N sulfuric acid as the mobile phase (Scherer et al., 2012). The results were validated as μg/mL using external standardization.

Amino acid content in sample extracts was quantified using an amino acid analyzer (Biochrom 30, UK) (Xu et al., 2010). The data collection and processing software was determined by EZ Chrome. The findings were presented as a proportion of all the amino acids.

Table 1
Nutrient composition, mineral contents, phytochemicals and vitamin of barley flour.

Component	value*
Nutrient composition (%)	
Moisture	8.13 ± 0.37
Crude protein	11.5 ± 0.43
Crude ether extract	1.91 ± 0.05
Total ash	1.9 ± 0.09
Total carbohydrate	76.56 ± 0.93
Crude fiber	3.4 ± 0.12
Total dietary fiber	17.15 ± 0.55
Mineral contents (mg/100g)	
Potassium (K)	181.61 ± 1.55
Calcium (Ca)	80.83 ± 1.38
Sodium (Na)	68.70 ± 0.62
Iron (Fe)	5.74 ± 0.16
Zinc (Zn)	3.13 ± 0.24
Phytochemicals	
Total phenolic content (mgGA/100 g)	145.21 ± 1.62
Antioxidant activity (%)	22.48 ± 0.27
Vitamins mg/100 g	
Niacin,	0.892 ± 0.09
Pyridoxine	0.542 ± 0.13
Riboflavin	0.891 ± 0.05

- Mean of three determinations ± SD (on dry weight basis).

2.4. Statistical analysis

The results are shown as the mean ± standard deviation (SD) of three replicate experiments, and the data was analyzed using SPSS 17.0 software. One-way analysis of variance (ANOVA) was used to identify significant differences, which were then compared using Duncan's test ($p < 0.05$). The data of fermented and unfermented sobia fortified with BF were analyzed using the statistical programming language R. The correlation analysis was performed using the corrplot package in R (version 0.92).

3. Results

3.1. Nutrient composition and phytochemicals of the BF

Barley flour is mostly made up of carbohydrates and protein, which account for more than 88 % of the flour (Table 1). The total carbohydrate was 76.56 % in which about 26.84 % of the BF's carbohydrates were made up of crude fiber (3.4 %) and total dietary fiber (17.15 %). The protein content was 11.5 %. The flour's quality may be preserved throughout storage due to its 8.13 % moisture content. Barley flour had low crude ether extract (crude fat, 1.9 %). Table 1 displays the mineral values of BF. The contents of K, Ca, Na, Fe, and Zn per 100 g of BF were 181.62, 80.83, 68.70, 5.74, and 3.13 mg, respectively. Barley flour is regarded as a good source of iron and zinc content, which constitute 65 % and 40 %, respectively, of the recommended daily allowances (RDAs) for each nutrient. The good concentrations of niacin (0.89 mg/100 g), pyridoxine (0.54 mg/100 g), and riboflavin (0.89 mg/100 g) in BF highlight its high nutritional value. Barley flour had phenolic content of 145.21 mg/100 g and its extract had a 22.48 % antioxidant activity, according to data in Table 1.

3.2. Sensory properties of prepared drinks and mahalabia formulation

Sensory characteristics of the fortified products were evaluated by trained panelists (Table 2). The sensory quality of both sobia and sahlab beverages remained comparable to the control samples when BF was incorporated at levels up to 30 %, with no significant statistical effect on all sensory characteristics. Furthermore, mouthfeel was significantly enhanced at the 30 % substitution level, with mean scores of 8.90 for sahlab and 8.89 for sobia, exceeding those of their respective controls. However, BF substitution beyond 30 % resulted in a significant decline ($p < 0.05$) in color and odor scores. From these results, 30 % is the maximum level of BF substitution for maintaining high sensory acceptability in both drinks.

For mahalabia samples, increasing BF levels led to a decrease in color scores, from 9.00 for the control to 7.90 at 100 % substitution, reflecting a visible darkening of the product; however, other sensory parameters were not affected up to a 75 % BF substitution level. At 50 % BF, all parameters achieved mean scores above 8.3, with no statistically significant differences from the control ($p > 0.05$), except for a slight but perceptible change in color. Importantly, the taste and mouthfeel of the 50 % BF-fortified mahalabia were rated more favorably than those of the control, contributing to a high overall acceptability score of 8.70. In general, moderate BF substitution (30 % for drinks and 50 % for mahalabia) was the most accepted, maintaining traditional sensory qualities while enhancing nutritional and functional qualities.

Table 2
Sensory evaluation of prepared drinks and Mahalabia formulation.

Replacement level with barley flour	Organoleptic properties				
	Color	Odor	Taste	Mouth feel 40 %	Acceptability Sahalab drink
Sahlab drink					
Control	8.70 ± 0.32^{a}	8.50 ± 0.42^{a}	8.50 ± 0.31^{a}	8.50 ± 0.22^{b}	8.40 ± 0.63^{a}
20 %	8.60 ± 0.47^{a}	8.45 ± 0.28^{a}	8.56 ± 0.20^{a}	8.56 ± 0.08^{b}	8.45 ± 0.78^{a}
30 %	8.55 ± 0.67^{a}	8.30 ± 0.80^{a}	8.70 ± 0.73^{a}	8.90 ± 0.32^{a}	8.45 ± 0.63^{a}
40 %	7.70 ± 0.53^{b}	7.90 ± 0.44^{b}	8.20 ± 0.73^{b}	8.90 ± 0.10^{a}	7.90 ± 0.67^{b}
50 %	7.30 ± 0.25^{b}	7.30 ± 0.33^{c}	7.60 ± 0.58^{c}	8.50 ± 0.32^{b}	7.50 ± 0.48^{b}
Sobia drink					
Control	8.90 ± 0.61^{a}	8.50 ± 0.52^{a}	8.35 ± 0.51^{a}	8.56 ± 0.22^{b}	8.50 ± 0.43^{a}
20 %	8.70 ± 0.52^{ab}	8.35 ± 0.48^{a}	8.40 ± 0.69^{a}	8.71 ± 0.18^{a} ab	8.35 ± 0.08^{a}
30 %	8.55 ± 0.35^{b}	8.20 ± 0.80^{a}	8.45 ± 0.51^{a}	8.89 ± 0.32^{a}	8.35 ± 0.43^{a}
40 %	7.90 ± 0.33^{c}	7.8 ± 0.60^{b}	7.60 ± 0.51^{b}	8.40 ± 0.10^{c}	7.80 ± 0.60^{b}
50 %	7.10 ± 0.95^{d}	7.20 ± 0.30^{c}	7.10 ± 0.44^{c}	8.10 ± 0.53^{c}	7.30 ± 0.17^{b}
Mahalabia dessert					
Control	9.00 ± 0.22^{a}	8.40 ± 0.52^{a}	8.70 ± 0.51^{a}	8.20 ± 0.22^{b}	8.60 ± 0.63^{a}
25 %	8.51 ± 0.08^{b}	8.30 ± 0.48^{a}	8.70 ± 0.69^{a}	8.25 ± 0.88^{a}	8.60 ± 0.78^{a}
50 %	8.39 ± 0.32^{b}	8.30 ± 0.84^{a}	8.80 ± 0.51^{a}	8.40 ± 0.32^{a}	8.70 ± 0.63^{a}
75 %	7.90 ± 0.10^{c}	8.25 ± 0.70^{a}	8.80 ± 0.51^{a}	8.55 ± 0.10^{a}	8.80 ± 0.67^{a}
100 %	7.9 ± 0.22^{c}	8.30 ± 0.10^{a}	8.40 ± 0.73^{b}	8.50 ± 0.10^{a}	8.30 ± 0.32^{b}

Means in the same column for each sample sharing the same letters are not significantly different at $p < 0.05$ level.

3.3. Physiochemical properties of drinks and mahalabia formula

Table 3 displays the results of the physiological features of drinks and mahalabia fortified with 30 % and 50 % BF, respectively. The data demonstrated that BF is a natural nutritious ingredient for such products that can be used to produce affordable, functional food that is readily available. The amount of moisture in the BF substituted formulas was less than that of the control sample. When compared to the control, replacing part of the starch in the original formula with BF resulted in a considerable increase in the protein and ash levels of all formulas. Additionally, results in Table 3 demonstrate that all substituted formulas with BF had lower fat content, although there was not a noticeable drop in total carbohydrate content when compared to the control samples. The energy value (K calorie) of the BF substituted formula dropped by 3.60, 2.29, and 8.67 % compared to the control products (sahlab, sobia, and mahalabia, respectively). It was clear that the viscosity increased in all formulas using BF which is mainly attributed to the dietary fiber present in BF, primarily β-glucan.

Color is a crucial feature that customers consider to be indicative of product quality (Fig. S1 and Table 3). The index (L*, a*, b*) of drinks containing 30 % BF and mahalabia containing 50 % BF as the best formulation powders was shown in Table 3. The lightness (L*) values decreased when BF was substituted for maize starch as compared to the control. Conversely, all formulations incorporating BF showed a small increase in redness (a*) values when compared to the control. Additionally, compared to the control samples, the b* values rose in the sahlab and mahalabia containing BF. The examined sensory attributes of the fortified sahlab, sobia, and mahalabia were described averaging between 8.55 and 8.39 for color and ratings exceeded 8.3 for overall acceptability. In addition, after nine months' storage of all samples at room temperature, there was no significant change in the color parameters of the BF-fortified formulas.

3.4. Aflatoxins B1 in selected BF-fortified products s after nine months' storage period

Using HPLC, aflatoxin B1 levels were assessed in the three powder formulas following 1-to 9-month storage periods. The control group contained a negligible level of AFB1 that remained within the permitted range for cereals (2–12 μg/kg), according to European regulations (El Tawila et al., 2020). Furthermore, the control samples had a greater concentration of aflatoxin B1 (Table 4) than the BF fortified samples. The control samples had aflatoxin B1 levels ranging from 0.208 to 0.351 μg/kg, while the sobia and sahlab made with 30 % BF had values of 0.038 and 0.087 μg/kg, respectively. Aflatoxin B1 was not detected in mahalabia samples. This could be the high antioxidant capacity of BF compared to the basic ingredients in all products. The length of storage had an impact on the amount of aflatoxin B1. After a nine-month storage period, there was a noticeable increase in all samples, particularly the control. AFB1 concentrations in the sahlab, sobia, and mahalabia samples increased to 1.126, 1.175, and 0.112 μg/kg, respectively; however, the concentrations of AFB1 in the samples fortified with BF were lower (0.336, 0.496, and 0.004 μg/kg). Aflatoxin B1 might be present in unprocessed foods or related materials. However, AFB1 development and output were greatly influenced by storage and processing conditions such as temperature, light exposure, and water activity. Lowering AF and avoiding product contamination might make it possible to increase sales volume, which would be profitable.

Table 3
Physiochemical properties of sahlab, sobia and mahalabia formula.

Components***	Drink type				Mahalabia	
	Sahlab		Sobia			
	Control	Fortified*	Control	Fortified*	Control	Fortified**
Moisture (%)	9.85 ± 0.34 ^{a}	8.92 ± 0.43 ^{b}	3.55 ± 0.41 ^{a}	1.12 ± 0.05 ^{b}	5.06 ± 0.25 ^{b}	7.27 ± 0.38 ^{a}
Crude Protein (%)	3.50 ± 0.41 ^{b}	7.29 ± 0.29 ^{a}	2.01 ± 0.25 ^{b}	4.18 ± 0.28 ^{a}	4.18 ± 0.36 ^{b}	9.82 ± 0.58 ^{a}
Crude ether extract**** (%)	9.94 ± 0.23 ^{a}	6.98 ± 0.32 ^{b}	8.07 ± 0.31 ^{a}	6.35 ± 0.34 ^{b}	4.89 ± 0.65 ^{a}	3.23 ± 0.19 ^{b}
Ash (%)	0.14 ± 0.04 ^{b}	0.48 ± 0.07 ^{a}	0.20 ± 0.03 ^{b}	0.52 ± 0.05 ^{a}	0.34 ± 0.07 ^{b}	1.99 ± 0.32 ^{a}
Total carbohydrates*****(%)	86.31 ± 0.26 ^{a}	85.25 ± 0.33 ^{b}	89.72 ± 0.56 ^{a}	88.65 ± 0.45 ^{a}	90.69 ± 0.54 ^{a}	79.51 ± 0.58 ^{b}
Total caloric value (kcal/100 g)	449.14 ± 0.99 ^{a}	432.99 ± 1.35 ^{b}	439.54 ± 1.43 ^{a}	428.48 ± 1.79$^{ba\ b}$	423.09 ± 4.71 ^{a}	386.39 ± 5.64 ^{b}
Color Lightness (L*)	86.93 ± 1.29 ^{a}	81.33 ± 1.83 ^{b}	87.72 ± 1.28 ^{a}	78.86 ± 1.52 ^{b}	93.66 ± 1.28 ^{a}	85.77 ± 1.72 ^{b}
Redness (a*)	−1.03 ± 0.07 ^{b}	−0.07 ± 0.02 ^{a}	−1.56 ± 0.15 ^{b}	0.18 ± 0.03 ^{a}	−0.44 ± 0.03 ^{b}	0.64 ± 0.08 ^{a}
Yellowness (b*)	7.46 ± 0.39 ^{a}	8.21 ± 0.36 ^{a}	8.35 ± 0.23 ^{a}	7.80 ± 0.26 ^{a}	4.98 ± 0.14 ^{a}	5.46 ± 0.83 ^{a}
pH	6.47 ± 0.10 ^{a}	6.42 ± 0.05 ^{a}	6.62 ± 0.02 ^{a}	6.38 ± 0.04 ^{a}	5.58 ± 0.01 ^{b}	6.43 ± 0.01 ^{a}
Viscosity (cp)	91.00 ± 0.35 ^{a}	94.00 ± 0.39 ^{a}	2.00 ± 0.53 ^{b}	4.00 ± 0.46 ^{a}	4436 ± 40.13^{b}	9388 ± 56.34^{a}

Data as mean ± SD.
* 30 % barley flour.
**50 % barley flour.
*** On dry weight basis except moisture.
****as crude fat.
*****Calculated by difference.
Means in the same rows for each sample sharing the same letters are not significantly different at $p < 0.05$ level.

Table 4
Aflatoxin B1 levels (μg/kg) in drinks and mahalabia powder formulates during storage period.

Storage periods	Sahlab		Sobia		Mahalabia	
	Control	Fortified (30 % barley flour)	Control	Fortified (30 % barley flour)	Control	Fortified (50 % barley flour)
Zero	0.208	0.038	0.351	0.087	0	0
5 months	0.579	0.069	0.673	0.130	0.0925	0.003
9 months	1.126	0.3363	1.175	0.496	0.112	0.004

3.5. Synbiotic drinks and mahalabia based on probiotic bacterial fermentation

Control and different formulation compositions (different concentrations in the BF-fortified sahlab, sobia, and mahalabia) were fermented using monocultures of *L. plantarum* and *B. lactis*. To find out if the microbes could proliferate at lower concentrations of these substrates, different concentrations of each substrate were investigated. Table 5 displays the viability for the six samples. The growth of probiotic bacteria on fortified substrates was better than control samples. At 10 % (w/v), the number of viable cells of *L. plantarum* and *B. lactis* was below or higher the number found at the highest concentration (20 %, w/v) of fortified products.

The viable cells of the sobia product ranged from 8.68 ± 0.41 to 11.62 ± 0.64 Log CFU/mL and 8.44 ± 0.06 – 10.68 ± 0.01 Log CFU/mL for *L. plantarum* and *B. lactis*, respectively. With both LAB, the viability was efficient, with the highest results (Table 5). These results showed that both LAB strains could grow in all substrates without the requirement for sugar supplementation, utilizing the substrate's naturally present sugars as a source of energy. Since the initial concentration of viable cells in the fermentation medium was about 6.0 Log CFU/mL, a higher cell multiplication (8.68 ± 0.41 - 10.34 ± 0.32 Log CFU/mL) was observed at the 5 % (w/v) concentration. The selection of the 10 % fortified sobia with BF had two reasons, as demonstrated by our findings: first, the strains of *B. lactis* and *L. plantarum* were well suited for fermentation; second, the BF-based sobia contained prebiotics, like β-glucan, supported the growth of both probiotics.

3.6. Statistical analysis of L. plantarum and B. lactis co-culture viability in BF-based sobia

The most crucial component for developing a novel functional food is the bioactive ingredients. The final viable cell population for a product made from fermented BF would be the significant bioactives. Consequently, high growth of the co-culture of *L. plantarum* and *B. lactis* was achieved by optimizing the fermentation parameters. Since both probiotics' inoculum levels that were used for optimization were the main parameters of the BF-based sobia.

The co-culture viability experimental and predicted values using the CCD were provided in Table 6A and Fig. 1. The following second-order polynomial equation linked the two factors and response values based on multiple regression analysis:

Y (Log CFU/mL) = 6.0909 + 2.06266* X1 + 1.95333*X2 - 0.18937*X1*X2 - 0.23166*X1$^{\hat{}}$2 - 0.19416 *X2$^{\hat{}}$2, where Y is the predicted co-culture viability, and X1 and X2 correspond to the inoculum levels of *L. plantarum* and *B. lactis,* respectively.

The analysis of variance (ANOVA) was presented in Table 6B. The extremely high correlation between the actual and predicted values was demonstrated by the high values of R^2 (0.9876) and R^2adj (0.9788). Furthermore, the model's p-value was less than 0.0001, which suggests that the model's variables were highly significant. The P-values for X1, X2, X1X2, X1$^{\hat{}}$2, and X2$^{\hat{}}$2 verified the highly significant difference. Additionally, the extremely low values of C.V % (1.33) significantly supported the model's adequacy. To investigate the impact of the experimental variables (*L. plantarum* and *B. lactis* inoculum level) and their interaction on the co-culture viability, response surface plots (two and three dimensions) were represented. Because of its elliptical shape, Fig. 1A and B demonstrates that the interaction of experimental variables was significant. It verified that the two probiotic species had a synergistic relationship.

Table 5
Viability of *L. plantarum* and *B. lactis* in the fermented control and fortified drinks and mahalabia formula expressed as Log CFU/mL.
Significant differences ($p < 0.05$); Very significant differences ($p < 0.01$).
Means in the same column for each sample sharing the same letters are not significantly different at $p < 0.05$ level.

LAB strains	Control (%)			Fortified (Replacement level%)		
	5	10	20	5	10	20
Sahlab						
L. plantarum	9.43 ± 0.14 ^{ab}	9.59 ± 0.21 ^{ab}	9.50 ± 0.17 ^{a}	10.34 ± 0.32 ^{a}	10.70 ± 0 ^{ab}	10.44 ± 0.37 ^{ab}
B. lactis	8.82 ± 0.30 ^{ab}	8.86 ± 0.39 ^{bc}	8.91 ± 0.12 ^{b}	9.13 ± 0.69 ^{b}	9.19 ± 0.71 ^{c}	9.31 ± 0.16 ^{c}
Sobia						
L. plantarum	8.68 ± 0.41 ^{b}	9.50 ± 0.14 ^{ab}	9.89 ± 0.28 ^{a}	9.58 ± 0.03 ^{ab}	11.62 ± 0.64 ^{a}	11.28 ± 0.27 ^{a}
B. lactis	9.35 ± 0.38 ^{ab}	8.44 ± 0.06 ^{c}	9.72 ± 0.03 ^{a}	10.26 ± 0.35 ^{a}	10.48 ± 0.33 ^{ab}	10.68 ± 0.01 ^{a}
Mahalabia						
L. plantarum	9.53 ± 0.18 ^{a}	9.27 ± 0.25 ^{b}	9.62 ± 0.20 ^{a}	9.96 ± 0.29 ^{ab}	9.63 ± 0.27 ^{bc}	9.61 ± 0.40 ^{bc}
B. lactis	9.58 ± 0.34 ^{a}	10.15 ± 0.49 ^{a}	9.53 ± 0.02 ^{a}	10.23 ± 0.31 ^{a}	9.76 ± 0.41^{bc}	9.71 ± 0.52 ^{bc}
F-value	3.08	8.09 *	8.19 *	3.03	7.32*	10.02 **

Table 6
RSM of CCD of fortified sobia (A) and ANOVA for Response Surface (B).

(A)

Run	Inoculum level of *L. plantarum* (%)	Inoculum level of *B. lactis* (%)	Log CFU/ml	
			Actual value	Predicted value
1	3	3	12.60	12.60
2	3	5.83	11.63	10.45
3	0.17	3	10.61	11.67
4	3	0.17	10.39	10.43
5	3	3	12.60	12.60
6	3	3	12.60	12.60
7	5	1	11.6	11.42
8	1	5	11.79	11.89
9	1	1	9.39	9.49
10	3	3	12.60	12.60
11	5.83	3	10.81	11.04
12	5	5	10.97	10.79
13	3	3	12.60	12.60

(B)

Source	Sum of Squares	df	Mean Square	*F-Value*	*p-value*	
Model	13.21	5	2.64	111.94	<0.0001	significant
A-(X1)	0.35	1	0.35	14.82	0.0063	
B-(X2)	1.55	1	1.55	65.73	<0.0001	
AB	2.3	1	2.3	97.21	<0.0001	
$A^{\hat{}2}$	5.97	1	5.97	253.01	<0.0001	
$B^{\hat{}2}$	4.2	1	4.2	177.73	<0.0001	
Residual	0.17	7	0.024			
Lack of Fit	0.17	3	0.055			
Pure Error	0	4	0			
Cor Total	13.38	12				
	$R2 = 0.9876$; $R^2_{Adj} = 0.9788$; Pred $R^2 = 0.9122$; C.V.% = 1.33					

Note: df: degree of freedom.

The model indicated that 3 % of both *L. plantarum* and *B. lactis* would be the ideal inoculum level for co-culture viability. The co-culture viability under these conditions was 12.60 Log CFU/mL, which was in line with the estimates. As so, the model in this study was precise, useful, and efficient. The viability of the fermentation process with monocultures of *L. plantarum* and *B. lactis* was 11.28 ± 0.27 and 10.68 ± 0.01 Log CFU/mL, respectively. This was the first report to show how the viability of the co-culture of *L. plantarum* and *B. lactis* was influenced by the inoculum level. During the fermentation process of sobia fortified with BF, the microbiological parameters and the contents of sugar, polyphenols, organic acids, and amino acid compounds were done in comparison with the unfermented sample in the following part.

3.7. Microbiological analysis and pH

The microbiological characterization of BF-fortified co-fermented and unfermented sobia is summarized in Table 7. Total aerobic bacteria (non-LAB), yeast, moulds, Enterobacteria, and *Staphylococci* were examined in the samples. The fermented sobia was completely devoid of any yeast, moulds, Enterobacteria, or *Staphylococci*. On the other hand, the unfermented product exhibited non-LAB counts of 3.22 ± 0.64 Log CFU/mL and yeast and moulds counts of 2.54 ± 1.02 Log CFU/mL. The final pH values of the fortified sobia fermented by LAB co-culture were 4.06 ± 0.33 while the beginning value was 6.38 ± 0.04 (Table 7). The pH value was 4.06, and yeasts were absent from the fermented products.

3.8. Antibacterial activity

Preparing potent antibacterial agents is crucial to the problem's solution since it protects human health and food safety. This study examined the antibacterial activity of the co-culture CFS against a range of bacterial pathogens.

The results for the antibacterial activity of the co-fermented CFS, unfermented BF-based sobia and monocultures of *B. lactis* and *L. plantarum* are shown in Fig. 2A, according to the inhibition diameter. Every CFS showed the ability to impede the development of tested pathogens in varying proportions. The fermented product exhibited more antibacterial activity in comparison to the monoculture strains of *L. plantarum* and *B. lactis*, but the unfermented product lacked any antibacterial activity.

The antibacterial activity was recorded as strong growth inhibition against *St. aureus* (41.95 ± 1.4 mm), *Ps. aeruginosa* (33.85 ± 1.63 mm), *S. typhimurium* (35 ± 2.82 mm) for the fermented product. While the lowest antimicrobial activity of *L. plantarum* was recorded with *Ent. aerogenes* (13.65 ± 1.91). In our case, the pH change on CFS was likely the result of the accumulation of fermentative compounds, which inhibited the growth of microorganisms in both monocultures and fermented products.

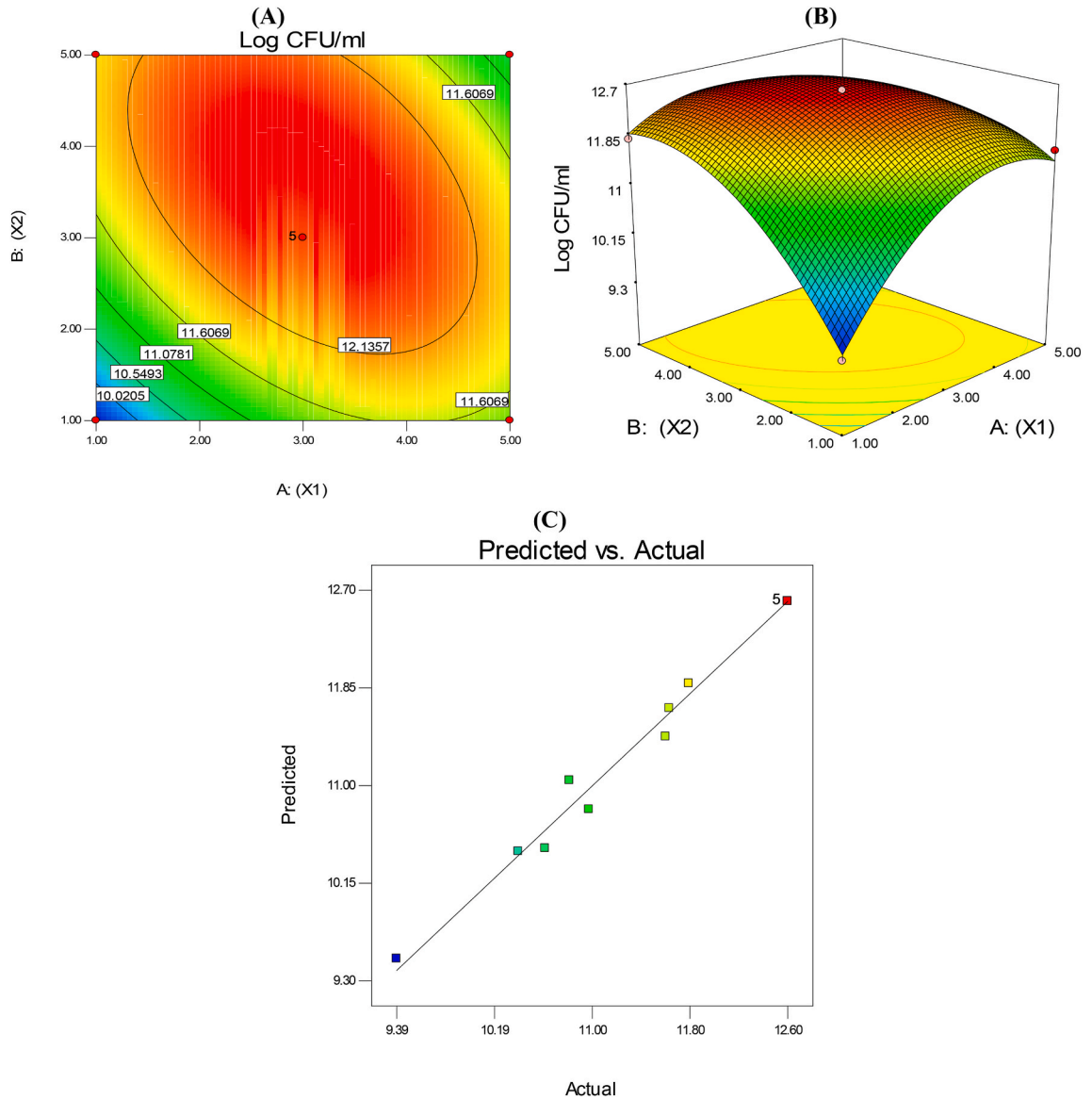

Fig. 1. Response surface 2D **(A)** and 3D **(B)** contour plots for co-culture of *L. plantarum* and *B. lactis*. according to inoculum size of *L. plantarum* (X1) and *B. lactis* (X2) at its optimum point. Predicted and actual response of co-culture viability **(C).**

Table 7
Microbiological analyses, pH, DPPH and total phenolic content of fermented and un-fermented sobia fortified with barley flour.

Analysis	Un-fermented sobia fortified with 30 % barley flour	Fermented sobia fortified with 30 % barley flour
Microbiological parameters (Log CFU/ml)		
LAB	-ve	12.60 ± 0.14
Total aerobic bacteria (Non LAB)	3.22 ± 0.64	-ve
Enterobacteriaceae	-ve	-ve
Yeasts and moulds	2.54 ± 1.02	-ve
Staphylococci	-ve	-ve
pH	6.38 ± 0.04	4.06 ± 0.33
DPPH inhibition activity (%)	23.54 ± 0.72	24.86 ± 0.37
Total phenolic content (mg GA/100 g)	131.70 ± 0.48	149.55 ± 0.69

-ve means negative results.

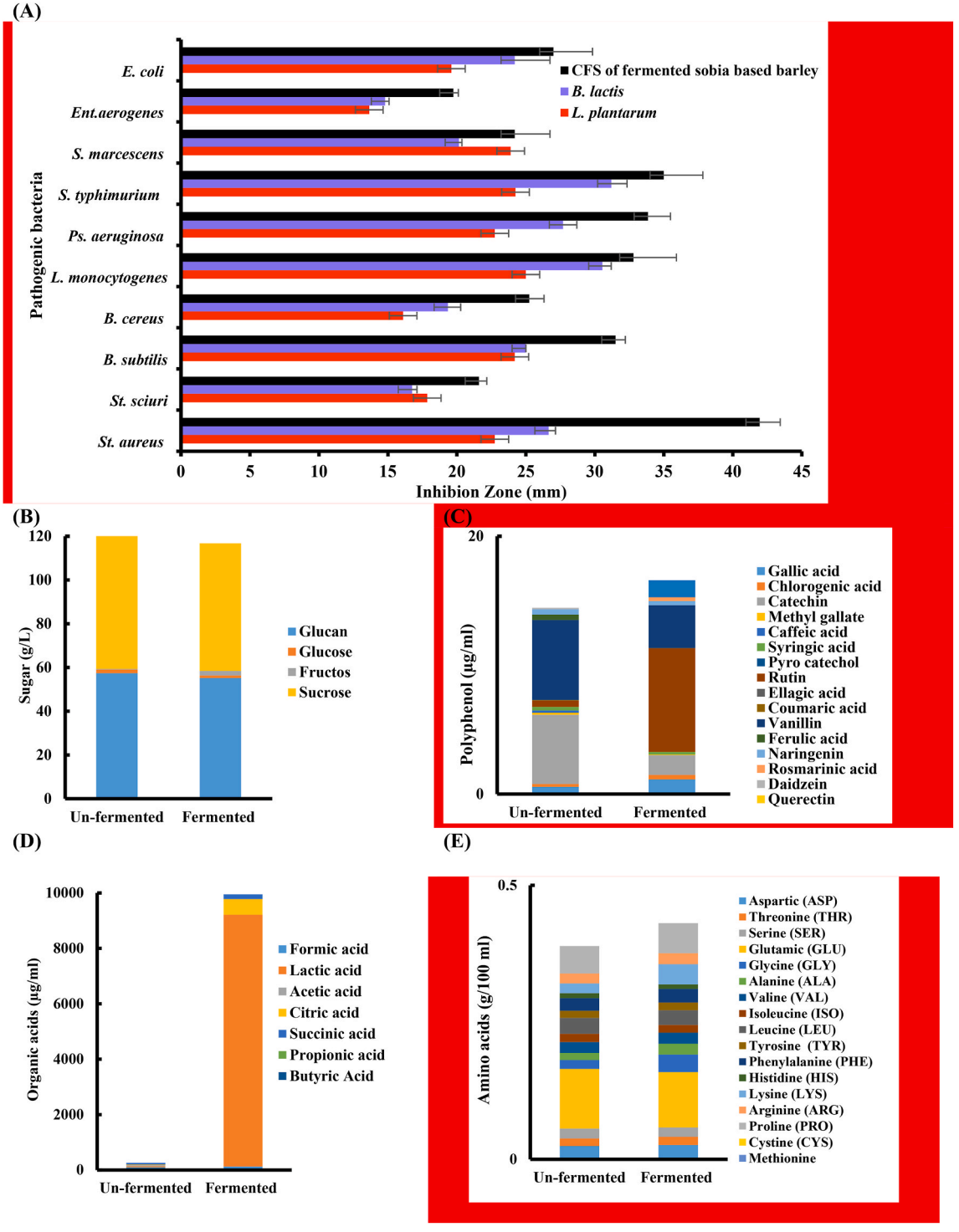

Fig. 2. Antibacterial activity of CFS of *L. plantarum*, *B. lactis* and fermented sobia fortified with BF (note that the CFS of un-fermented sobia fortified with BF had no antibacterial activity) **(A)**. Profiles of metabolic compounds identified in unfermented and fermented sobia fortified with barley (**B-E**). Changes in sugar (**B**), polyphenols (**C**), organic acid (**D**), and amino acid nitrogen (**E**).

3.9. Antioxidant activity and total phenolic content

A slight increase in antioxidant activity was observed in the fermented sobia. The drink's percent DPPH inhibition activity rose from 23.54 ± 0.72 to 24.86 ± 0.37 % (Table 7), due to the release of free bioactive chemicals caused by biological acidification and microbial enzyme activity (Pontonio et al., 2019). Fermentation has been shown to increase the antioxidant activity of sobia fortified with BF.

The initial amount of 131.70 ± 0.48 mg/100 g of phenols was increased by 13.55 %. The rise in reactive hydroxyl groups might be the cause of that. The results showed that the total phenolic content and antioxidant activity were positively correlated. The phenols were partially dissolved by the fermentation.

3.10. Sugar analysis

The main fermentable sugars in BF fortified sobia were β-glucan, glucose, fructose, and sucrose. When comparing the fermented product to unfermented fortified sobia, there was a drop of 3.83, 30.54, and 15.48 % in the levels of β-glucan, glucose, and sucrose. Additionally, Fig. 2B shows that the glucose content of co-culture bacterial growth in 10 % sobia enriched with BF was 1.16 g/L.

The hydrolysis of the sucrose molecule to fructose and glucose was demonstrated (Fig. 2B) by the bacterial co-culture. Fructose accumulation was visible in the fermentative matrix, and after 48 h, it reached 2.06 g/L. In the fermentation medium, sucrose continued to be present at 58.33 g/L (Fig. 2B).

3.11. Polyphenols component

Fig. 2C and Fig. S2 list the 19 phenolic compounds found in the samples. According to the HPLC analyses, the content of gallic acid, chlorogenic acid, rutin, rosmarinic acid, and hesperetin in sobia fortified with BF increased during fermentation while the content of them decreased in un-fermented sobia. Quantitative results indicated that vanillin was the most abundant compound in un-fermented sobia fortified BF, followed by catechin. Additionally, compared to the un-fermented sobia fortified BF, the content of catechin, methyl gallate, caffeic acid, syringic acid, coumaric acid, vanillin, ferulic acid, naringenin, and daidzein was decreased in fermented product.

3.12. Organic acids profile

Fig. 2D and Fig. S3 show the production of organic acids during the fermentation of bacterial co-culture (*L. plantarum* and *B. lactis*). There was a natural occurrence of formic, lactic, acetic, citric, and succinic acids in the unfermented product, therefore most of them are detected in the fermented product. The co-fermented product showed higher production of lactic acid (9086.43 μg/mL) and formic

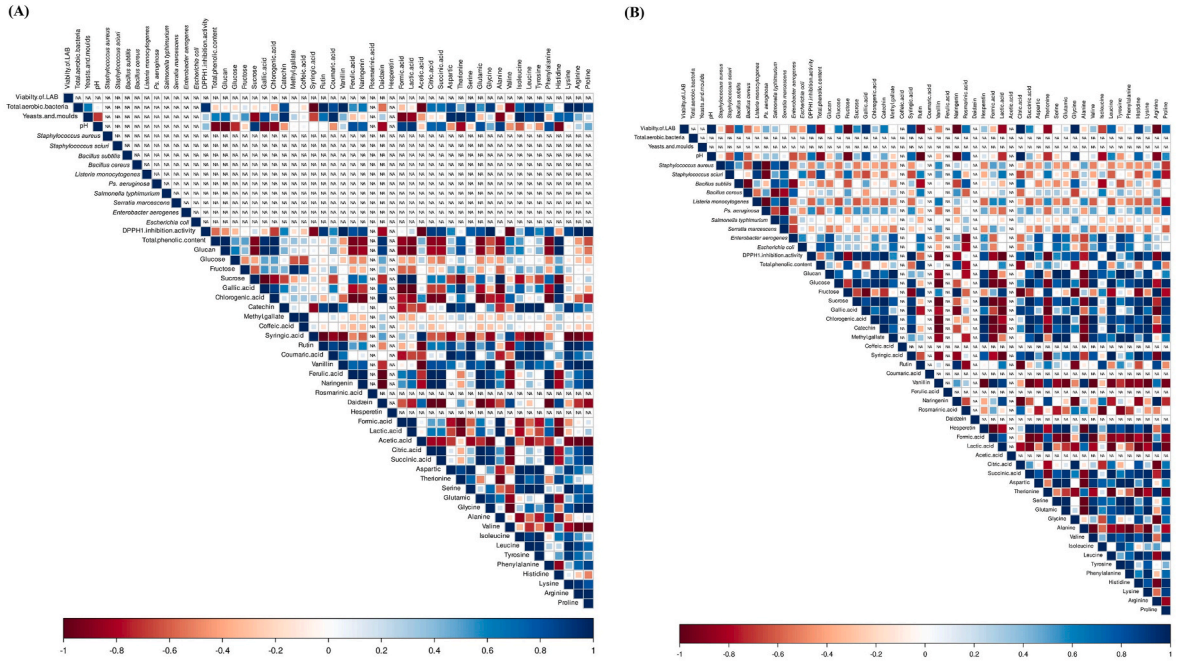

Fig. 3. Correlation between the different microbiological parameters and metabolic profile of unfermented (**A**) and fermented (**B**) for sobia based barley (**B**). (Note to correlation key and the scale reads: positive—blue, negative—red; smaller square—lesser significance; bigger square—greater significance; non—significant values—blank square; NA—no correlation available.

acid in a lower proportion (124.29 μg/mL) after 48 h, whereas, the acetic acid production was not detected. The amount of acetic acid that was naturally present in the unfermented product was completely eliminated after the 48-h fermentation period. The absence of acetic acid in the matrix might be explained by the description of the co-culture of *L. plantarum* and *B. lactis* as being capable of metabolizing acetic acid.

3.13. Amino acids

Nitrogen is an essential indication that is frequently used to assess the degree of fermentation of a fermented product. It is known that LAB are auxotrophic to a large variety of vital amino acids. It attracted our interest in the amino acid composition of sobia-fortified BF fermented by the co-culture. According to Fig. 2E, the fermented product (0.431 g/100 mL) contained slightly higher total amino acids than the un-fermented product (0.389 g/100 mL). Amino acids were vital to the growth of LAB. The most abundant amino acids in BF were glutamic acid, valine, leucine, and proline. The contents of most amino acids increased after fermentation, such as aspartic acid, threonine, glycine, alanine, tyrosine, phenylalanine, lysine, arginine, and proline, whereas, the others decreased, which might be because during fermentation amino acids break down into small molecules of ammonia.

3.14. Correlation analysis

To reveal the interaction between the different variance, the relationship between all microbiological parameters and nutrient components in fermented and the unfermented sobia-based BF was performed. Regarding the unfermented product, a number of positive and negative correlation relationships were revealed by the correlation analysis (Fig. 3A). The significant positive correlations were observed between total aerobic bacterial count and most of the amino acids. Yeast and moulds counts showed negative correlations with pH. However, the blank square did not show significant correlations.

In the fermented sobia based BF, co-culture viability was correlated positively with pH, DPPH, *Ps. aeruginosa*, *S. typhimurium*, and *B. subtilis* and negatively with *S. aureus*, *S. sciuri, B. cereus, L. monocytogenes, En. aerogenes*, *E. coli* indicating their competitive or antagonistic relationships. The co-culture viability and pH have a positive correlation, which could be attributed to the fermentation process. Because of their lactic acid fermentation, pH showed a negative correlation with the other indicator microorganisms and a positive correlation with *Ps. aeruginosa, S. typhimurium*, and *B. subtilis*. DPPH was found to be positively correlated with pH, viability of co-culture, *Ps. aeruginosa*, *S. typhimurium* and *B. subtilis* and negatively with most pathogenic indicators. In particular, certain indicators did not show an obvious correlation. Probiotic bacteria had a significant ability to catabolize and use carbon sources, which explains the positive association that was found between co-cultured bacteria and glucose. In addition, lactic acid also exhibited an obvious negative correlation with co-culture viability, pH, DPPH, glucose, sucrose, and some polyphenols (Fig. 3B).

However, it's important to note from Fig. 3B that glucose and sucrose had a positive association with pH because the co-cultured bacteria used different carbohydrates, which led to the production of numerous organic acids and a decrease in the pH of the sobia. The correlation analyses showed that citric and succinic acids were significantly positively correlated with LAB, suggesting that LAB may be majorly responsible for the fermentation of sobia-based BF. Significantly positive correlations were seen between the viability of LAB and glycine, leucine, histidine, and proline. This suggests that LAB was either the creator of biogenic amines or could be involved in the digestion of proteins during sobia-based BF fermentation.

4. Discussion

4.1. Nutritional value and functional enhancement

Results in Table 1 shows that BF has promising potential as a nutritious functional ingredient in traditional foods as previously mentioned by several researchers (Geng et al., 2022; Huang et al., 2020; Lahouar et al., 2017; Suman and Sreeja, 2019; Yang et al., 2024). This is attributed to its high content of dietary fibers, proteins, total phenolics, and total flavonoids, which also contribute to both the functional and sensory advantages of BF. Overall, the results of the nutrient composition analysis of BF agree with other studies (Aly et al., 2021; Elhadidy et al., 2020; Maray et al., 2018), who reported total carbohydrates from 69.0 to 85.18 %, protein content from 10.92 to 11.30 %, crude fat from 1.81 to 2.69 %, ash content from 1.28 to 2.81 %, crude fiber content from 2.54 to 4.32 %, dietary fiber content from 8.16 to 21.46 %. Barley flour is regarded as a good source of minerals, especially iron and zinc content which constitute 65 % and 40 %, respectively, of the recommended daily allowances (RDAs) for each nutrient.

According to the National Health and Nutrition Survey (2019), men should consume 8.3 ± 3.3 mg of Fe per day, while women should consume 7.5 ± 3.2 mg. Our results were higher than those reported by Elhadidy et al. (2020), who found that the levels of Ca, K, Fe, and Zn in BF were 21.40, 160, 2.0, and 2.1 mg/100 g, respectively. These variances could be related to some factors such as genotypes, maturity stages, agricultural sites, and environmental factors (Obadi et al., 2021). According to data in Table 1, BF achieved the daily nutrient requirements (PRD/AI) for children, adolescents, and the elderly with high levels of B3 (0.892 mg/100 g), B6 (0.891 mg/100 g), and B2 (0.542 mg/100 g). Studies of (Huang et al., 2020; Raj et al., 2023) showed that barley and BF are regarded as good sources of B-complex vitamins and vitamin E (tocotrienols and tocopherols), which help prevent many diseases like heart disease, hypertension, diabetes, and obesity, they can be employed as functional ingredients in food production. The good concentration of phenolic compounds and B-complex vitamins in BF enhances the nutritional quality of the starchy products when replacing part of the original formula by the BF. Hamli et al. (2017) reported anti-inflammatory, anti-oxidative, and anti-cancer properties for barley extract. Also, other studies (Obadi et al., 2021; Šimić et al., 2019) reported a preventative effect of barley extract against degenerative

diseases by improving or delaying oxidative damage due to its abundance of bioactive carbohydrates (β-glucan), phenolic substances with high antioxidant capacity, minerals and vitamins.

4.2. Sensory evaluation and comparison with traditional preparations

The results presented in Table 2 showed that drinks fortified with 30 % BF and mahalabia with 50 % BF had the highest taste scores, indicating that BF enhanced the flavor and the mouthfeel of all the fortified products without overpowering the traditional sensory quality of the fortified products compared to control. This is because BF contains β-glucan, which has a thickening effect on the product when heated in water. The 30 % substitution of BF in the drinks (sahlab and sobia) was selected based on the low score of aroma due to the strong or unfamiliar aromas that the panelists experienced when the BF level was increased more than 30 %. Also, lightness (L*) value decreased with increasing BF substitution in the original formula. This may be attributed to the dark pigments present in BF. A ranking of drink quality attributes indicates that taste is the most important item followed by homogeneity, flavor, and color (Routray and Mishra, 2012). Nakov et al. (2022) found that substituting hulless BF for wheat flour during the cookie-making process increased a* values and decreased L* values, giving the cookies a faint purple color. Hulless barley's anthocyanins have a major role in the color of BF, contributing to its purple-blue hues (Shaveta et al., 2019). The viscosity of the different fortified products was investigated as an indicator of the viscosity-related functional properties of BF in the formulations (Table 3). It was clear that the high dietary fiber content in BF (which is mainly β-glucan), which serves as a stabilizer due to its high capacity to bind water in the formulation, causes viscosity to increase in all formulas having BF (Nakov et al., 2022). Barley β-glucan can be utilized in the production of calorie-reduced foods by substituting fat or sugar, adding a fatty mouthfeel, and boosting the product's ability to hold water (Sereti et al., 2023). It can also be utilized as a source of dietary fiber to create functional foods (Lazaridou et al., 2022; Onacik-Gür et al., 2016). These fortified nutritious products had excellent sensory characteristics and improved nutritional value. BF significantly enhanced the protein, fiber, and mineral content of the fortified products, making them more nutritious and beneficial for overall health. Its high levels of bioactive compounds, including polyphenols, flavonoids, and β-glucan, contribute to improved antioxidant activity, better gut health, and reduced risks of chronic diseases such as diabetes and obesity (Geng et al., 2022; Raj et al., 2023).

4.3. Product stability and aflatoxin reduction

Aflatoxins are carcinogenic to humans and can lead to digestive issues that can have major health consequences (Ajmal et al., 2022). Barley flour demonstrated a significant reduction in aflatoxin contamination and improved shelf stability of the fortified products, ensuring better food quality over extended storage periods. Moreover, its fermentation with probiotics resulted in increased antibacterial activity, making BF-fortified products not only nutritious but also protective against harmful pathogens.

4.4. Functional bioactivity and fermentation effects

Microorganisms and substrates are the two main components of a fermentation system (Septembre-Malaterre et al., 2018). Probiotic LAB viability in acidic environments is positively impacted by the high sugar content of cereal extracts (Charalampopoulos et al., 2003). An 8-h fermented oat-based functional drink with 5.5 % oat flour, 1.25 % sugar, and 5 % probiotic inoculum levels had a significantly higher count (10.4 Log CFU/mL) of *L. plantarum* ATCC8014 (Gupta et al., 2010).

This study demonstrated that using co-cultures of various bacteria could enhance process performances. In comparison to other research on oat-based fermented functional beverages (Angelov et al., 2006; Gupta et al., 2010) and barley-milk composite-based fermented drinks (Ahuja et al., 2017), the growth of our probiotic cultures in the BF-sobia formula was higher.

Some *Lactobacillus* species have been shown to be impacted by inoculum concentration in their growth. A prior study (Alvarez et al., 2010) discovered that the amount of inoculum employed at the beginning controls the final biomass concentration. Considering that higher initial biomass concentrations implied faster formation and accumulation of lactic acid, which exhibited inhibitory effects with a lower biomass net production, larger inoculum concentrations inversely led to lower final biomass production. Our research was in line with the findings of (Alvarez et al., 2010), which demonstrated that the co-culture's biomass output was stimulated by the combination of the two initial inoculum concentrations. The pH of the fermentative matrix was dropped. Similar outcomes during the fermentation of a malt-based beverage by *Bifidobacterium breve* were documented (Rozada et al., 2009). Cereal-based products' pH values reduced to 3.4–4.4 (Helland et al., 2004). A fermented beverage typically needs a pH level above 4.0 (Gupta et al., 2010). Non-LAB is a measure of the amount of microorganisms contaminating the sample that may come from different bacterial genera (Angelidis et al., 2006). The fermented product's low pH values led to a drop in the non-LAB count, indicating good fermentation quality. Krungleviciute et al. (2017) observed similar trends and stated that the total aerobic bacteria count in the substrate was decreased by LAB-fermented barley and wheat bran. The pH value dropping below 3.8, which inhibited the majority of food spoilage bacteria, can be used to explain this assumption (Ávila and Carvalho, 2020).

4.5. Antibacterial and antioxidant properties

Bacterial transmission is a major global health concern that gives rise to serious food safety issues, ultimately threatening human health (Dickerson et al., 2021). Human health benefits greatly from the antibacterial activity of LAB cultures, which inhibits pathogenic intestinal microorganisms and protects against the invasion of dangerous pathogens (Ahuja et al., 2017). LAB are known to produce a variety of metabolic end products that have the ability to prevent the growth of specific undesirable microorganisms in food

systems (Alvarado et al., 2006). In fact, the pH drop caused by organic acids was responsible for the majority of the inhibitory action displayed by LAB strains. Furthermore, it has been proposed that acid products disrupt the plasmic membrane's permeability and increase its diffusion, which stops metabolic processes and inhibits the activity of sensitive microorganisms (Piard and Desmazeaud, 1991). Barley grain extract was added to increase the antibacterial activity of probiotic *Lactobacillus* sp. against pathogenic bacteria such as *E. coli, S. aureus, Salmonella paratyphi A, Shigella dysenteriae*, and *Vibrio cholerae* (Sheela and Suganya, 2012). Based on these results, it concluded that co-cultured sobia fortified with BF consistently inhibited the growth of both gram negative and positive bacterial species.

Depending on the bacterial strain or combination used as the inoculum, the released phenolic compounds were changed (Zamfir et al., 2022). The antioxidant activity measured after fermentation was higher in our strains than in the unfermented sample. Studies employing other cereal substrates found the same impact (Shumoy et al., 2017). A similar result had been reported by Xiao et al. (2022) for barley powder, Mao et al. (2020) for the wheat bran and Chu et al. (2019) for the millet bran. Additionally, it has been suggested that LAB strains may possess antioxidant potential on their own since they produce antioxidant enzymes like superoxide dismutase as well as antioxidant substances such exopolysaccharides, peptides, glutathione, benzoic acid, and benzaldehyde (Zhang et al., 2019). According to earlier studies, the elevated total phenolic content enhanced the antioxidant activity (Xiao et al., 2022; Zhu et al., 2015). Following fermentation, the total phenol content increased dramatically and was 39.10 % greater than that of the raw wheat bran (Mao et al., 2020).

4.6. Sugar analysis

Research had indicated the significance of β-glucan in dietary matrices and its impact on the development of various probiotic strains, including *L. fermentum, L. plantarum*, and *L. acidophilus* (Arena et al., 2014). The presence of β-glucan was found to significantly affect only the development of *L. plantarum*, according to these investigators. Additionally, due to its ability to stimulate the growth of probiotics as an excellent prebiotic, β-glucan is significant during the first 24-h of fermentation (Arena et al., 2014). β-glucan promotes *Lactobacillus* growth through generating short chain fatty acids. Probiotic growth product is enhanced by BF-derived β-glucan, making it a viable material for symbiotic product development (Kristek et al., 2018). Cereal with β-glucan content can lower its glycemic index (GI), which can be employed in a healthy diet to lower blood sugar, cholesterol, and blood fat levels in patients (Xiao et al., 2022). To the best of our knowledge, there was no information on the minimal concentration of glucose needed for LAB development; nonetheless, its presence in the medium may affect the LAB's absorption and glycolytic activity (Papagianni et al., 2007). The main carbon/energy source for the fermentation processes of *Bifidobacteria* and *Lactobacilli* is glucose (Wang et al., 2003).

The microbe in the glucose-containing media utilized fructose as an electron acceptor (Mauro and Garcia, 2019). Fructose is thought to increase LAB metabolic activity and growth rate, however there is currently no evidence to support this theory (Zaunmüller et al., 2006). Nevertheless, fructose might be a necessary sugar for LAB development. When fructose is used as an electron acceptor (Zaunmüller et al., 2006). Enzymes could have converted sucrose to fructose, which would explain the higher fructose concentration during fermentation. Here, adding more fructose could improve the viability and growth of the LAB. Levansucrase or β-glucosidase is responsible for the conversion of sucrose to fructose and glucose in both homolactic and heterolactic LAB (Bonestroo et al., 1992; Li, 2004).

4.7. Polyphenol components, organic acids and amino acids

It was commonly known that the majority of phenolic acids found in cereals were bound and linked to cellulose, proteins, or lignin through ester, ether, or acetal bonds. Enzymes like proteases and esterases that develop during fermentation cause these bonds to break (Xiao et al., 2022). A similar result has been reported for the fermented rice bran (Liu et al., 2017).

Relevant research has demonstrated that organic acids preferentially suppress pathogenic microbes during the initial phases of fermentation, leaving beneficial microbes unaffected in terms of their growth and metabolism. The fermentation environment ought to quickly stabilize, encouraging the variety of aroma constituents and a well-balanced flavor profile (Wu et al., 2021). Pallin et al. (2016) linked the increased lactic acid concentrations to *L. reuteri*. After the fermentation process was completed, the co-culture's synthesis of lactic acid reached a maximum concentration that was 299.39 times higher than that of the unfermented product. Because the bacterium may have employed the glycolytic pathway, it is therefore feasible to explain why the mixed culture in the fermented product produced a higher amount of lactic acid (Mauro and Garcia, 2019).

The synthesis of hydrogen peroxide, organic acids, and short-chain fatty acids (SCFA) benefits the human body. These benefits include the production of neurotransmitters, improvement of intestinal barrier functions, immunomodulation of the host's body, and control of the immune response in the gut (Ma et al., 2022; Plaza-Diaz et al., 2019). Furthermore, these metabolites interact with intestinal epithelial cells and attract macrophages and mononuclear cells, thereby increasing the production of anti-inflammatory cytokines (Petruzziello et al., 2023).

The production of microbial proteases during the fermentation of the bacterial co-culture was the main cause of the rise in amino acid nitrogen. The process of hydrolyzing proteins releases amino acids and gives microorganisms nutrients, both of which enhance the nutritional value and sensory appeal of fermented foods (Wang et al., 2020). In addition to providing cells with the nitrogen they need to grow, amino acids are also required for the decarboxylation that allows cells to react to acid stress. The process of lysine and glutamate decarboxylation aids in increasing intracellular pH. While ammonia, which is produced by deamination, raises the medium pH, arginine catabolism produces ATP, which provides an alternate energy source to LAB (Azcarate-Peril and Klaenhammer, 2010; Pessione, 2012). The proportion of important amino acids, such as threonine, valine, and isoleucine increased during fermentation (Hu

et al., 2019). Methionine and cysteine grew by 3.15-fold and 0.33-fold, respectively, in wheat bran (Mao et al., 2020). For numerous LAB strains, glutamic acid was discovered to be necessary (Costello et al., 2015; Terrade and de Orduña, 2009). Free amino acids like valine, isoleucine, leucine, and arginine can greatly enhance the growth of *Lactobacillus*, an amino acid-deficient species (Chen et al., 2024).

5. Conclusion

The food sector faces a hurdle in using BF as a functional ingredient in the baking industry. Therefore, the aim of this study was to incorporate BF in some traditional Egyptian drinks (sobia and sahlab) that are mainly consumed in the winter by many people and one type of dessert (mahalabia) that is very preferred by children and the elderly to maximize their nutritional values with minimum effect on their sensory properties. Incorporation of BF at 30 % for beverages and 50 % for mahalabia improved the nutritional value—particularly protein, dietary fiber, vitamins, and minerals while maintaining desirable sensory characteristics. The fortified formulations also exhibited improved viscosity, improved color stability, elevated antioxidant activity, and excellent storage stability, with notably reduced aflatoxin levels following extended storage. Monocultures of *L. plantarum* and *B. lactis* showed good cell viability. The CCD design could achieve 12.6 Log CFU/mL. The combination of the starters was found to be the most effective choice for the fermentation of a BF-based sobia. This study demonstrates that BF-fortified sobia can be synergistically fermented to produce functional fermented cereal products and synbiotic foods, further increasing its value in health-promoting diets. Clinical research and animal experiments are needed to find out the potential health effects of co-fermented sobia fortified with BF.

CRediT authorship contribution statement

Ebtehag A.E. Sakr: Writing – review & editing, Writing – original draft, Validation, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Mona I. Massoud:** Writing – review & editing, Writing – original draft, Validation, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Saadia M. Hashem:** Writing – review & editing, Writing – original draft, Validation, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Ethics approval and consent to participate

All experiments were approved by the Faculty of Agriculture, Alexandria University (Alex. Agri. 152404311).

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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Data availability

Data will be made available on request.

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