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Determination of Proximate Composition, Phytochemical Content and Gut-health-promoting Efficacy of Developed Sorghum-based Functional Beverage

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Abstract

Considering the steady rise in gut-related issues worldwide, the study determined the nutritional composition, phytochemical content, and gut-health-promoting properties of a developed functional beverage containing Sorghum in combination with oats, barley, and whey versus the basic beverage (B) without Sorghum. Moreover, the functionality was assayed in vitro in a simulated human gastrointestinal environment to predict its efficacy in a human host. Variation 2 (V2), ranked highest through sensory acceptance (8.8), exhibited high fiber (43 %) and proteins (72.2 mg/dL), low reducing sugar and fat, a significant concentration of minerals (calcium, iron, and phosphorus), and phytochemicals (17.82 mg/dL alkaloids, 170.94 mgQE/dL flavonoids, and 85.5 mg GAE/dL polyphenols), which may propagate gut health. Moreover, V2 manifested potent antioxidant capacity (76.05 % DPPH radical inhibition and 35.2 µg/dL reducing potential) and prebiotic abilities, all of which may bestow gut-health promoting abilities. Interestingly, the phytochemical content and functional potentials were found to be retained even after an in vitro human gastrointestinal digestive treatment, indicating their effectiveness inside the human body. Therefore, intake of Sorghum in the form of V2 beverages may benefit gut health and supplement a healthy lifestyle owing to their biochemical composition and functional potential.

Keywords: Beverages; Gut-Health; Nutrient; Phytochemicals; Sorghum.

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1. Introduction

Multifunctional millet *Sorghum bicolor* is widely cultivated mainly because of its drought resistance, nutritional and health-promoting properties, and cost-effectiveness. It constitutes the world's fifth-most produced grain, with Asia qualifying as one of the largest cultivators [1]. In fact, the rise of sorghum production has been called the country's second 'Green Revolution' [2]. Prevalent in six varieties, these crops are important with respect to health and nutrition and represent a viable option for promoting food and nutritional security [3]. Its high energy and protein content make it an ideal option for malnourished individuals and those with varied acute and chronic health issues [4]. Sorghum is commonly eaten with the testa, which retains the majority of the nutrients and

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beneficial constituents. Even though its demand had subsided in the past with consumers opting for finer cereals such as rice and wheat, millets, especially Sorghum, has drawn particular attention in recent years given its balanced nutritional value and possible health advantages such as antioxidant potential as well as protection against dyslipidemia and other lifestyle diseases [5].

A new era in the food and health sectors has dawned with the realization of the critical role of the forgotten organ, the gut microbiome, in maintaining well-being. The interaction between gut bacteria and the host is mutually beneficial, as the bacteria contribute to a variety of metabolic processes and aid in maintaining the digestive health of the host [6]. Interestingly, although Sorghum has been traditionally cultivated for protein and energy, recent studies have indicated its usefulness in maintaining a healthy gut. Dietary fibers derived from the above help in the growth of beneficial gut microbes like *Bifidobacterium* and *Lactobacillus* species [7]. Moreover, the vitamin content of this millet assists in metabolism and digestion and can be used by patients who have Celiac disease owing to its gluten-free properties [8]. The phytochemical composition, particularly in the bran layer, and other beneficial compounds represent a promising opportunity to be exploited as a functional food to reduce the risk of non-communicable chronic diseases [9].

Several reports have documented a steady rise in incidences of gut abnormalities such as constipation, inflammatory bowel disease (IBS), and diarrhea, mainly amongst young adults, fostered by improper dietary patterns, inappropriate nutrition, inactive living, and anxiety [10]. Consumption of millets, particularly Sorghum, has been indicated for alleviation of the above. Nonetheless, regular utilization of these grains is not often preferred owing to limited awareness, unique flavor, and a dearth of attractive recipes [11]. In light of the health and ecological benefits of Sorghum, along with indications of its low popularity as a result of its unacceptable taste and other sensory attributes, pronounced efforts are required for food product development and analysis of therapeutic potentials to promote the consumption of these grains among the masses. Interestingly, the advantages of millet consumption have led the FAO to declare 2023 the International Year of Millet [12]. Hence, the preparation and propagation of millets in the form of convenience foods may elevate their utilization within the population, thereby aiding in relieving numerous health concerns. Therefore, the present study focused on the development and analysis of sorghum-based functional beverages to not only add to the manifold ways of incorporating millets, especially Sorghum, in one's diet but also to portray the nutritional and health advantages that may be obtained from the same, mainly its potential in promoting gut health in view of the ever-increasing gut-related issues in present times stimulated by sedentary lifestyles and poor eating habits.

2. Materials and Methods

2.1. *Materials*

Quantitative analysis kits for determination of iron, calcium and phosphorus were purchased from Tulip Diagnostics Private Limited, Coral Clinical Systems, India. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was procured from Sigma-Aldrich Chemicals Private Limited, India. All the other analytical reagents and chemicals were procured from Loba Chemie, India.

2.2. *Development of beverages and sensory assessment*

The measured amount of Sorghum was blended into a fine powder, and the other ingredients were taken according to the amounts mentioned in Table 1. The ingredients were constantly stirred until a uniform consistency was obtained. The products were standardized to balance taste, texture, and other organoleptic properties. Whey, jaggery, and vanilla essence were added in different variations (variation 1 to variation 4) to improve the consistency and taste of the products. The developed beverages were subjected to sensory assessment by a panel of 35 untrained participants (college-going adults) for various parameters, namely, appearance, color, taste, texture, odor, and overall rating. The 9-point hedonic scale was used to carry out the sensory evaluation process [13].

Table 1. Product variations of sorghum-based beverages.

Ingredient (%)	Basic Beverage (B)	Variation 1 (V1)	Variation 2 (V2)	Variation 3 (V3)	Variation 4 (V4)
Sorghum (*Sorghum bicolor*)	0	10	20	30	40
Oats	50	40	30	20	10
Barley	20	20	20	20	20
Whey	10	10	10	10	10
Jaggery	5	5	5	5	5
Vanilla Essence	5	5	5	5	5
Water	10	10	10	10	10
Total	100	100	100	100	100

2.3. *Analysis of proximate composition and micronutrient profile*

The moisture content of the product was measured using the hot air drying method in a hot air oven (YSI-431, Yorco, India), while the ash content was determined using the hydrometric method by placing the developed products in a furnace (I:S: 1248-68, Cosmo, India) at 600^{o}C. Carbohydrate content was determined using Anthrone's method, in which carbohydrates were dehydrated with concentrated H_2SO_4 to form furfural, which reacted by condensation with anthranol to form a bluish-green complex whose absorbance was measured at 620 nm. The protein was determined using the Biuret colorimetric method. The fat content of the products was determined according to the Folch method. The samples were homogenized with chloroform: methanol (2:1) (1 g in 20 mL of mixed solvents), followed by centrifugation and removal of the supernatant [14]. The

micronutrient analysis of the products was carried out using Tulip Group India's chemical analysis kits. The iron content was estimated using the ferrozine method, the calcium content by the OCPC (o-cresol phthalein complexone) method, and the phosphorus content by the UV molybdate method [15]. A UV-Vis spectrophotometer (U2910, Hitachi, Japan) was used to determine the optical density (OD) of the nutrients, viz. iron, calcium, and phosphorus, at 540 nm, 570 nm, and 340 nm, respectively.

2.4. *Determination of crude fibre*

After fat extraction, samples were boiled with 200 mL of (0.3 N) H_2SO_4 followed by (0.3 N) NaOH for 30 min with boiling chips. Thereafter, they were filtered through muslin cloth and washed with boiling water until they were no longer acidic or alkaline. Samples were transferred to a muffle furnace (I: S: 1248-68, Cosmo, India) and kept at 600 °C overnight for conversion to ash. Lastly, the samples were cooled and weighed again [15]. Crude fiber content was calculated by using the following formula:

Percentage (%) of crude fiber = Weight of sample residue remaining after acid & alkali digestion - the weight of ash.

2.5. *Phytochemical evaluation*

Sample preparation and procedure for tests were done according to protocols recommended by Singh *et al*. to qualitatively determine the presence of phenols, flavonoids, and alkaloids [16]. The beverages were also quantified for their alkaloid, flavonoid, and phenol contents. Using the phenol reagent developed by Folin-Ciocalteau, the total phenols were calculated through spectrophotometric determination at 765 nm with gallic acid as standard and expressed as gallic acid equivalent (GAE/dL). The total flavonoid content at 510 nm was calculated using the aluminum chloride ($AlCl_3$) technique with quercetin as a reference, and results were expressed as quercetin equivalents (mg QE/dL) [17]. The total quantity of alkaloids was determined using the titrimetric technique. Alkaloids were solubilized in a separating funnel with 0.1 (N) HCl. The extract was titrated against 0.1 (N) NaOH until the crimson color turned pale yellow, and the total alkaloid content was calculated by using the formula:
1 mL 0.1N HCI = 0.0162 g alkaloid.

2.6. *Analysis of antioxidant properties*

2.6.1. *DPPH radical scavenging activity*

The ability of the extracts to scavenge free radicals was tested using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging test (1898-66-4, Sigma-Aldrich Chemicals Private Limited, India). 2.4 mL of DPPH solution was mixed with 1.6 mL of alcoholic extracts of samples (12.5-150 µg/mL) and left in the dark for 30 minutes, followed by an

absorbance measurement at 517nm against ascorbic acid as a standard [18]. The antioxidant potential of the samples was calculated according to the given formula:

DPPH radical scavenging activity (%) = [(AC−AS)/AC] × 100, where AC=absorbance of the control, AS=absorbance of the sample or standard

2.6.2. *Reducing power assay*

For the reducing power assay, aliquots of standard and test sample extracts (10 to 100 µg/mL) were mixed in 1.0 mL of deionized water with 2.5 mL (pH 6.6) of phosphate buffer and 2.5 mL (1 %) potassium ferrocyanide, followed by incubation in a water bath for 20 min at 50 °C. This was succeeded by the addition of 2.5 mL (10%) of trichloroacetic acid and centrifugation at 3000 rpm for 10 minutes. 5 mL of the upper layer was mixed with 2.5 mL of distilled water and 0.5 mL (0.1 %) of a freshly prepared ferric chloride solution, accompanied by an OD measurement at 700 nm [18].

2.7. *Analysis of prebiotic properties*

The prebiotic potential of the products was estimated by inoculating probiotic bacterial species (*Lactobacillus rhamnosus* GG, ATCC 53013) in MRS media containing peptone (1 %), beef extract (1 %), yeast extract (0.5 %), glucose (2 %), polysorbitate 80 (0.1 %), ammonium citrate (0.2 %), sodium acetate (0.5 %), magnesium sulfate (0.01 %), manganese sulfate (0.05 %), dipotassium hydrogen phosphate (0.2 %), agar (1.2 %), and measuring the growth of the bacterial species in a spectrophotometer at 660nm. Both the standard and sample(s) were inoculated into a culture broth containing *Lactobacillus sp*. in MRS media, and the growth of these species was measured every hour. The prebiotic potential was estimated by determining the increase in growth upon the addition of the standard/sample products compared to the control (without beverages) [19].

2.8. *In vitro gastrointestinal digestion*

A simulated gastric juice was prepared by dissolving pepsin (12,000 U/L), glucose (3.5 g), NaCl (2 g), KCl (0.4 g), $CaCl_2$ (0.1 g), KH_2P0_4 (0.5 g) in 1 L distilled water with pH of 1.5 adjusted using 0.1 M HCl. A simulated intestinal digestive solution was prepared with 125.0 mM NaCl, 0.6 mM $CaCl_2$, 0.3 mM $MgCl_2$, trypsin (11 U/mL), α-chymotrypsin (24 U/mL), and pancreatic lipase (590 U/mL). The gastric and intestinal digestive solutions were sterilized through membrane filtration (0.2 µm) [20]. Five grams of standard or developed products were subjected to subsequent treatment with the above solutions for 60 minutes and thereafter assayed for their functional characteristics.

2.9. *Shelf-life studies*

Shelf life was analyzed using the microbiological spread plate method. 0.1 mL of the samples stored at room temperature (25 °C) and refrigeration temperature (4 °C) were

plated onto a pre-sterilized nutrient agar plate, Sabourand's dextrose agar, and Mac Conkey's Agar for the determination of Total Plate Count (TPC), fungi, and coliforms, respectively. Cultured plates were incubated for 24-48 h at 37 °C. Results were expressed as Colony-forming units per mL (CFU/mL) [20].

2.10. *Analysis of data*

The data obtained were computed and evaluated using Microsoft Office Excel (version 16.63.1) software. All results were expressed as Mean±SEM of $N \geq 3$ experiments. To determine the significance of the data, p scores were also obtained using the t-test. Only p values with $p \leq 0.0001$ = ***; $p \leq 0.001$ = **; $p \leq 0.01$ = * were considered significant with Confidence Interval (CI) = 95 %.

3. Results and Discussion

3.1. *Sensory assessment of the developed functional beverages*

Table 2. Sensory evaluation of the sorghum-based functional beverage.

Parameters	Basic Beverage (B)	Variation 1 (V1)	Variation 2 (V2)	Variation 3 (V3)	Variation 4 (V4)
Appearance	$7.1\pm0.11^{**}$	$8.1\pm0.13^{**}$	$9.1\pm0.13^{**}$	$7.1\pm0.11^{**}$	$7.2\pm0.11^{*}$
Color	$7.5\pm0.12^{**}$	$7.5\pm0.14^{**}$	$7.9\pm0.62^{**}$	$7.3\pm0.12^{**}$	$7.1\pm0.15^{**}$
Taste	$8.5\pm0.78^{**}$	$8.2\pm0.20^{**}$	$9.2\pm0.11^{*}$	$7.8\pm0.78^{**}$	$6.4\pm0.12^{*}$
Texture	$7.2\pm0.52^{**}$	$8.7\pm0.19^{**}$	$9.5\pm0.10^{*}$	$6.9\pm0.52^{**}$	$6.2\pm0.32^{*}$
Odor	$6.8\pm0.31^{**}$	$8.1\pm0.16^{**}$	$8.3\pm0.02^{**}$	$6.8\pm0.31^{**}$	$6.7\pm0.21^{*}$
Overall ranking	$7.4\pm0.55^{**}$	$8.1\pm0.18^{**}$	$8.8\pm0.12^{**}$	$7.2\pm0.55^{**}$	$6.7\pm0.12^{*}$

Note: ***, **, * represents $p \leq 0.0001$, $p \leq 0.005$ and $p \leq 0.01$, respectively at 95 % CI

Functional beverages were developed by the addition of different proportions of Sorghum, oats, barley, and whey owing to evidence of gut-enhancing properties associated with them [21]. Jaggery and vanilla essence were added as sweeteners and mild flavoring agents to enhance the taste of beverages. In contrast to earlier research that majorly focused on the utilization of Sorghum for the development of alcoholic beverages, the current study developed a non-alcoholic and easy-to-prepare beverage to not only cater to a wider population belonging to different socioeconomic categories and age groups but also be alluring because of the inclusion of readily available ingredients and a simple preparation method. The amount of Sorghum was progressively increased by 10g in each variation, ranging from 10 g in Variation 1 to 40 g in Variation 4, to understand the taste, flavor, texture, and overall viscosity of the beverage with different quantities of this grain. The beverages were assessed for their sensory characteristics to analyze consumer receptivity regarding the developed products [13]. Results showed that variation 2 (V2) was ranked highest in terms of taste, texture, and overall acceptability (8.8) compared to the basic beverage (B) (7.4), which may be credited to the incorporation

of optimum amounts of Sorghum in the former leading to a balance in the appearance, taste, texture, and aroma of the same, in contrast to higher quantities used in V3 and V4, (Table 2). Consequently, V1 also displayed an acceptability of 8.1 due to the addition of moderate amounts of Sorghum along with other ingredients. The color of all products received a similar ranking due to the creamish hue of most ingredients. V3 and V4 received a reduced rating, which may be attributed to the addition of higher amounts of Sorghum, which might have overpowered the taste when used in higher quantities. Moreover, higher amounts of sorghum addition also disturbed the texture, appearance, and color, owing to their coarse nature and suboptimal balance with the other ingredients. The findings of this study corroborate those from earlier studies, showing that Sorghum in beverage form is easier to consume and a suitable product for adolescents as an on-the-go food [11]. Hence, the results of the sensory analysis showed that V2, containing a combination of oats, Sorghum, and barley in a ratio of 2:3:2, was the most appreciated beverage.

3.2. *Analysis of proximate composition and micronutrient profile*

Table 3. Proximate composition and micronutrient profile of the functional beverage.

Component	Basic Beverage (B)	Variation2 Beverage (V2)	Component	Basic Beverage (B)	Variation2 Beverage (V2)
Energy (Kcal)	721.1	812.2	Crude fiber (%)	28±2.4	43±3.1**
Total Carbohydrate (mg/dL)	13.9 ±2.1	30.3±2.75**	Calcium (mg/dL)	16.0±13.2	26.5±11.1*
Reducing sugars (mg/dL)	10.1±0.01	11.2±0.01***	Iron (µg/dL)	120.2±10.1	187.4±10.5**
Protein (mg/dL)	31.8±3.2	72.2±5.3**	Phosphorus (mg/dL)	3.24±0.01	6.4±0.01**
Fat (mg/dL)	1.19±0.03	3.03±0.02**			

Note: ***, **, * represents $p \leq 0.0001$, $p \leq 0.005$ and $p \leq 0.01$, respectively at 95 % CI

Since V2 was observed to be the product with the highest acceptance with respect to sensory parameters, these beverages were further evaluated for proximate components and gut health-promoting properties with reference to B. As depicted in Table 3, V2 was found to harbor a higher content of total carbohydrates (30.3 mg/dL) versus the basic beverage (13.9 mg/dL). Although the basic beverage contained oats and barley, which are known to be rich in carbohydrates, the additional presence of optimum amounts of Sorghum in V2 may have contributed to the elevated carbohydrate proportion in this product. Notably, these products were observed to hold a low concentration of reducing sugars, hence indicating a greater proportion of the total carbohydrate content to be non-reducing sugars, which may encourage maintaining gut health by supporting the growth of beneficial microflora and relieving constipation without markedly elevating the blood glucose levels upon being consumed. V2 was also found to encompass a greater concentration of proteins (72.2 mg/dL) and dietary fats (3.03 mg/dL) compared to B, which may also be because of the additional presence of sorghum grains in the former. The kafirin and non-kafirin proteins present in Sorghum may promote gut health through

modulation of the number and diversity of the human gut microbiota [22]. Moreover, dietary fats may support the wellness of the gut via their influence on digestion, absorption, and anti-bacterial properties, mediated mainly by medium-chain fatty acids [23]. Furthermore, the developed products were noted to contain important minerals such as iron, calcium, and phosphorus, with their content higher in V2 (187.4 µg/dL, 26.5 mg/dL, and 6.4 mg/dL) versus B. These minerals, especially iron and phosphorus, may assist in promoting gut health through their role in supporting the gut microbial population, including *Lactobacillus sp*. [24]. Additionally, the beverages were assessed for their fiber content due to the utilization of Sorghum, oats, whey, and barley, which are known to be good fiber sources, and because of the importance of dietary fibers in maintaining gut health. As given in Table 3, the crude fiber content of the basic product was found to be 28 %, while that of the best variation of the product (V2) was 43 %. The higher fiber proportion in V2 can indeed be attributed to Sorghum, which has been known to encompass 7 g of dietary fiber per 100 g, most of which are insoluble fibers such as cellulose and prebiotic β-glucan, which have been documented to have a significant impact on the diversity, richness, and composition of the gut microbiome, along with restoring digestive abnormalities and gut related disorders [25]. Even though Sorghum has been previously reported to be utilized for the production of beverages, the present study focused on the development of a sorghum-based beverage with a combination of ingredients that are not only economical and readily available but also hold the potential to improve gut health which is a potent problem in modern times owing to dietary and lifestyle factors. Hence, consumption of V2 may aid in gut health due to the significant presence of proximate components, including macronutrients, fiber, and important minerals.

3.3. *Phytochemical content of the developed beverages*

Table 4. Phytochemical content of the functional beverage.

Phytochemical	Qualitative		Quantitative			
	B	V2	B		V2	
			Before digestion (mg/dL)	After digestion (mg/dL)	Before digestion (mg/dL)	After digestion (mg/dL)
Alkaloid (mg /dL)	++	++	8.1±1.6	6.4±1.18**	17.82±2.6**	12.2±2.3*
Flavonoid (mg QE/dL)	++	+++	80.08±2.01	60.04±5.01**	170.94±9.05**	150.36±5.03**
Polyphenols (mg G.A.E./dL)	++	+++	51.1±9.1	42.4±5.1**	85.5±6.1**	52.2±4.2**

Note: ***, **, * represents $p \leq 0.0001$, $p \leq 0.005$ and $p \leq 0.01$, respectively at 95 % CI; ++ & +++ denotes moderate and high qualitative presence.

The prepared beverages were evaluated for the presence of important phytochemicals via both qualitative and quantitative estimations. In contrast to similar studies conducted on the analysis of sorghum-based products, the present study estimated the presence of those phytochemicals that have been known to aid in the maintenance of gut homeostasis

in addition to other beneficial functions. As given in Table 4, both B and V2 beverages displayed the presence of phytochemicals, including polyphenols, flavonoids, and alkaloids. Importantly, V2 beverages were observed to harbor higher concentrations of the aforementioned components compared to the basic product. Quantitative estimation manifested the presence of 170.94 mgQE/dL, 85.5 mg GAE/dL, and 17.82 mg/dL of flavonoids, polyphenols, and alkaloids, respectively, in V2 compared to 80.08 mgQE/dL, 51.1 mgGAE/dL, and 8.1 mg/dL in B. As also noted in earlier studies, the presence of optimum amounts of Sorghum in addition to oats, barley, and whey, which have all been acknowledged as sources of bioactive compounds, may be responsible for the higher content of the above components in V2 versus B [26]. In contrast to previous reports on the analysis of Sorghum and its food products, the present investigation additionally evaluated the availability of the above phytochemicals after a simulated *in vitro* gastrointestinal digestion to further estimate their effectiveness. As tabulated in Table 4, a significant amount of these phytochemicals were found to be retained in both products, especially V2, even after *in vitro* gastrointestinal digestion, indicating sufficient bioavailability of these components for the desired functional effects. Notably, the detected phytochemicals have been recognized to improve gut dysbiosis and promote gut health through various mechanisms, including antioxidant, bile acid metabolism, anti-inflammatory, anti-microbial, and prebiotic functions, along with influencing the microbiota of the gastrointestinal tract [27]. Therefore, intake of the developed sorghum-based functional beverages may assist in the improvement and maintenance of health, including gut equilibrium, due to their nutritional properties as well as phytochemical composition.

3.4. *Analysis of antioxidant properties*

Considering the presence of significant amounts of polyphenols, flavonoids, and alkaloids in the beverages that have previously been associated with antioxidant potential and because of the inclusion of Sorghum along with oats, barley, and whey that have been noted for antioxidant abilities, the samples were assayed for their antioxidant properties through DPPH and reducing power estimations. As tabulated in Table 5, V2 beverages showed a greater potential to scavenge DPPH radical (76.05 %) versus B (57.04 %), hence depicting a noticeable antioxidant potential in the former, which may benefit health, including improvement of gut homeostasis. The higher radical scavenging capacity may be attributed to the presence of adequate amounts of Sorghum in addition to whey, barley, and oats in this variation versus the presence of only whey, barley, and oats in the basic beverage. This was in concert with earlier reports where Sorghum was observed to possess a greater concentration of phenolics as well as a higher antioxidant capacity compared to several grains such as wheat and barley [28]. Additionally, the beverages were also assessed for reducing power to estimate their antioxidant potential further. The best variation of the sorghum-based product (V2) was observed to manifest greater reducing power compared to B, as given in Table 5. Notably, the functional effects of

numerous food products have been noted to diminish post-gastrointestinal digestion. Therefore, the present work determined the retention of antioxidant properties after an *in vitro* simulation of gastric and intestinal digestion. Results showed that although there was a minor reduction in both DPPH scavenging and reducing power after being subjected to the above treatment, the beverages, especially V2, were found to retain the antioxidant properties sufficiently (64.12 % DPPH scavenging and 21.01 µg/dL) versus B. Hence, intake of the developed sorghum-based functional beverages (V2) may promote gut health, mainly due to the significant display of antioxidant properties even after being subjected to both gastric and intestinal digestion.

Table 5. Antioxidant properties of the functional beverage.

Product	DPPH Assay (% inhibition)	Reducing power assay (µg/dL)
Basic beverage (B)	57.04±2.3	$12.1\pm0.11^{***}$
Basic beverage (B) after *in vitro* gastrointestinal digestion	$43.05\pm5.31^{*}$	$8.1\pm0.21^{***}$
Variation 2 (V2)	$76.05\pm7.2^{*}$	$35.2\pm0.1^{***}$
Variation 2 (V2) after *in vitro* gastrointestinal digestion	$64.12\pm2.5^{**}$	$21.01\pm0.2^{***}$

Note: ***, **, * represents $p \leq 0.0001$, $p \leq 0.005$ and $p \leq 0.01$, respectively at 95 % CI

3.5. *Analysis of prebiotic potential*

The beverages were also assayed for their prebiotic potential owing to the importance of the former in promoting gut health and because of the occurrence of important phytochemicals and fiber in them that have been formerly associated with prebiotic effects. As depicted in Fig. 1, the basic (B) and V2 beverages elevated the growth of the probiotic organism, viz., *Lactobacillus rhamnosus* GG, relative to the control (without beverage). This could be due to the presence of ingredients such as Sorghum, oats, barley, and whey in the developed products that have formerly been acknowledged to have prebiotic potential. Variation 2 of the product (V2) showed a prolonged log phase (OD_{660nm} 0.2 to 0.9) followed by the basic product (OD_{660nm} 0.1 to 0.58) compared to the control before entering the stationary phase, thereby indicating their prebiotic abilities. Hence, although both B and V2 displayed probiotic upliftment capabilities, this potential was observed to be greater in the latter. Notably, only a few studies have analyzed the effectiveness of sorghum-based products after gastrointestinal digestion. Importantly, the current analysis showed that the prebiotic properties of the beverages were significantly retained even after *in vitro* gastric and intestinal digestion, indicating their effectiveness in the human body. As observed in Fig. 1, V2 manifested an OD_{660nm} of 0.78 versus the OD_{660nm} of 0.37 displayed by B at 24 hours after being subjected to an *in vitro* simulated gastrointestinal digestion treatment. All samples were observed to enter the stationary phase beyond 36 hours of incubation and thereafter slowly infiltrate into the declining phase. Although a marginal reduction in the prebiotic abilities was detected post *in vitro* simulated human gastrointestinal digestion, a significant potential was found to be maintained, with the power higher in V2 compared to B after being subjected to the above

treatment (Fig. 1). Most of the prebiotic potential of the beverage may be attributed to its polyphenol content, considering the positive correlation between polyphenol content and antioxidant potential with the prebiotic capacity of natural ingredients [29]. Moreover, the alkaloid and flavonoid content may additionally help in bestowing this effect. Although studies in the past have documented the prebiotic effects of Sorghum, the current report further displays its potency when incorporated into an easy-to-prepare and cost-effective concoction, even after gastrointestinal digestion. Consequently, sorghum-containing V2 functional beverages may serve as gut-healthy products owing to their prebiotic and antioxidant potential in addition to their nutritional and phytochemical content.

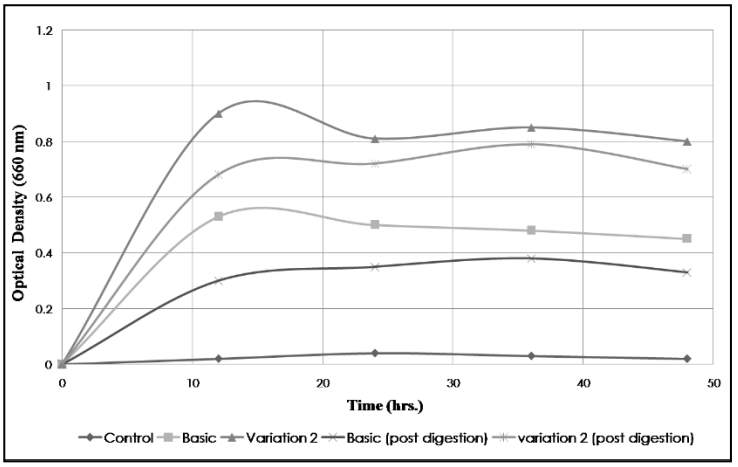

Fig. 1. Prebiotic property of the functional beverage.

3.6. *Shelf life*

Table 6 shows that negligible to minimal microbial growth occurred within 6 days of storage at both room temperature (25 °C) and refrigeration temperature (4 °C). However, growth was observed after 12 days of storage in both conditions. Moreover, low growth was visible when beverages were stored at refrigeration temperature (4 °C), compared to room temperature, hence recommending storage of the beverages at temperatures at or below 4 °C. The products displayed minimal growth of yeasts and molds (< 5-10), and no coliforms were detected in any of the samples until 12 days of storage. This is in concert with previous studies that have also observed microbial contamination beyond 6 days of storage [30]. The absence of any added preservatives may be responsible for the observed shelf life of the products. Nonetheless, being easy to prepare from readily available and low-cost ingredients, these beverages may be consumed within a short duration of their preparation in order to derive their nutritional and functional benefits without any safety risks.

Table 6. Shelf life of functional beverages.

Products	Basic beverage (B)				Sorghum-containing functional beverage (V2)			
Time (Days)	6 days		12 days		6 days		12 days	
Temperature (°C)	(25°C)	(4°C)	(25°C)	(4°C)	(25°C)	(4°C)	(25°C)	(4°C)
TPC (CFU/mL)	<05	<05	57±03*	27±02*	<05	<05	68±05*	32±02*
Yeasts and molds (CFU/mL)	<05	<05	10±02	<05	12±02	<05	<10	<05
Coliforms (CFU/mL)	0	0	0	0	0	0	0	0

4. Conclusion

The present study focused on the development of *Sorghum bicolor*-based easy -to-prepare and ready-to-consume functional beverages along with an assessment of their nutritional composition and functional effects to address the rising incidences of gut anomalies and provide novel ways of assimilation of this multifunctional millet. Importantly, the functional effects were assayed both before and after a simulated *in vitro* human gastrointestinal digestion treatment to estimate its effectiveness in a human host. Variation 2 (V2) was ranked highest in terms of taste, texture, appearance, and overall acceptability compared to all other variations (V1, V3, and V4) versus the basic beverage (B). This variation (V2) also portrayed a significant presence of minerals (iron, phosphorus, and calcium), fiber, and phytochemicals (polyphenols, flavonoids, and alkaloids) in addition to optimum macronutrients, which may aid in gut health maintenance by augmenting important metabolic and physiological pathways. Moreover, V2 displayed a higher antioxidant potential manifested by a greater DPPH radical rummaging capacity and reducing power, in addition to a stronger prebiotic potential exhibited by the ability to accelerate the growth of *Lactobacillus rhamnosus* GG compared to B. Noteworthy, the above phytochemicals, and functional effects, though marginally lowered, were found to be adequately retained even after simulated *in vitro* human gastrointestinal digestion, hence signifying the usefulness of the prepared beverages in promoting well-being, especially gut health via reduction of oxidative stress and supporting probiotic flourishment. Nevertheless, the limited shelf life due to the absence of any added preservatives recommends its safe consumption, preferably within 6 days and definitely within 12 days of refrigerated storage. Therefore, consumption of Sorghum in the form of V2 beverages may benefit gut health and foster general well-being owing to their nutritional and functional properties.

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References

1. C. W. Mundia, S. Secchi, K. Akamani, and G. Wang, Sustainability **11**, 2135 (2019). https://doi.org/10.3390/su11072135
2. A. R. L. E. Nelson, K. Ravichandran, and U. Antony, Ethn. Food **6**, 8 (2019). https://doi.org/10.1186/s42779-019-0011-9
3. M. S. Hossain, M. N. Islam, M. M. Rahman, M. G. Mostofa, and M. A. R. Khan, J. Agric. Food Res. **8**, ID 1003002022 (2022). https://doi.org/10.1016/j.jafr.2022.100300
4. C. R. Abah, C. N. Ishiwu, J. E. Obiegbuna, and A. A. Oladejo, European J. Nutr. Food Saf. **12**, 101 (2020). https://doi.org/10.9734/ejnfs/2020/v12i530232
5. W. Khalid, A. Ali, M. S. Arshad, F. Afzal, R. Akram et al., Int. J. Food Prop. **25**, 1045 (2022). https://doi.org/10.1080/10942912.2022.2071293
6. Z. Y. Kho and S. K. Lal, Front Microbiol. **9**, 1835 (2018). https://doi.org/10.3389/fmicb.2018.01835
7. Y. Chen, R. Zhang, J . Xu, and Q. Ren, Front Nutr. **9**, ID 965687 (2022). https://doi.org/10.3389/fnut.2022.965687
8. H. I. Mohamed, E. M. Fawzi, A. Basit, R. Lone, and M. R. Sofy, Phyton Int. J. Experiment. Bot. **91**, 1303 (2022). https://doi.org/10.32604/phyton.2022.020642
9. J. Xu, W. Wang, and Y. Zhao, Foods **10**, 1921 (2021). https://doi.org/10.3390/foods10081921
10. H. Vivier, E. J. Ross, and J. E. Cassisi, B.M.C. Gastroenterol. **20**, 326 (2020). https://doi.org/10.1186/s12876-020-01478-7
11. J. K.-Potaka, S. Anitha, T. W. Tsusaka, R. Botha, M. Budumuru et al., Front. Sustain. Food Syst. **5**, 680777 (2021). https://doi.org/10.3389/fsufs.2021.680777
12. A. Poshadri, H. W. Deshpande, and R. B. Kshirsagar, AATCC Rev. **2023**, 7 (2023). 0.58321/AATCCReview.2023.11.01.02
13. C. Ruiz-Capillas and A. M. Herrero, Foods **10**, 582 (2021). https://doi.org/10.3390/foods10030582
14. C. Breil, M. AbertVian, T. Zemb, W. Kunz, and F. Chemat, Int. J. Mol. Sci. **18**, 708 (2017). https://doi.org/10.3390/ijms18040708
15. A. D. Pal, Int. J. Food Sci. Nutr. **2**, 173 (2017).
16. P. K. Singh, J. Singh, T. Medhi, A. Kumar, ACS Omega **7**, 33067 (2022). https://doi.org/10.1021/acsomega.2c03117
17. B. Debnath, M. J. Uddin, P. Patari, M. Das, D. Maiti, and K. Manna, Int. J. Pharm. Pharm. Sci. **7**, 223 (2015).
18. A. D. Pal and R. Basistha, J. Sci. Res. **13**, 1011 (2021). https://doi.org/10.3329/jsr.v13i3.53443
19. K. Śliżewska and A. Chlebicz-Wójcik, Nutrients **12**, 1272 (2020). https://doi.org/10.3390/nu12051272
20. A. D. Pal and S. Bhowal, J. Sci. Res. **15**, 769 (2023).
21. H. K. A. H. Gamage, S. G. Tetu, R. W. W. Chong, J. Ashton, N. H. Packer, and I. T. Paulsen, Sci. Rep. **7**, 14312 (2017). https://doi.org/10.1038/s41598-017-14707-z
22. V. A. Balderrama-Pérez, J. G. Gómez-Soto, T. C. Reis de Souza, E. R. Rodríguez, and G. Mariscal-Landín, Anim Nutr. **5**, 124 (2019). https://doi.org/10.1016/j.aninu.2019.01.002
23. E. Xu, C. Chen, J. Fu, L. Zhu, J. Shu et al., AnimNutr. **7**, 1337 (2021). https://doi.org/10.1016/j.aninu.2021.09.010
24. Y. Y. Miao, C. M. Xu, M. Xia, H. Q. Zhu, and Y. Q. Chen, Chin Med. J. (Engl). **131**, 2792 (2018).
25. T. M. Barber, S. Kabisch, A. F. Pfeiffer, and M. O. Weickert, Nutrients **12**, 3209 (2020). https://doi.org/10.3390/nu12103209
26. A. García-Castro, A. D. Román-Gutiérrez, A. Castañeda-Ovando, R. Cariño-Cortés, O. A. Acevedo-Sandoval et al., Foods **11**, 3231 (2022). https://doi.org/10.3390/foods11203231
27. R. Yin, H. C. Kuo, R. Hudlikar, D. Sargsyan, S. Li et al., Curr Pharmacol Rep. **5**, 332 (2019). https://doi.org/10.1007/s40495-019-00196-3

28. N. Arouna, M. Gabriele, and L. Pucci, Foods 9, 1218 (2020). https://doi.org/10.3390/foods9091218
29. Y. Zhang, Y. Li, X. Ren, X. Zhang, Z. Wu, and L. Liu, Food Chem. **402**, ID 134231 (2023). https://doi.org/10.1016/j.foodchem.2022.134231
30. D. S. Unhale, B. K. Sakhale, R. C. Ranveer, and V. D. Pawar, Int. Food Res. J. **19**, 733 (2012). https://doi.org/10.5281/zenodo.1076092