JSR Publications

Available Online

J. Sci. Res. **15** (3), 769-781 (2023)

JOURNAL OF SCIENTIFIC RESEARCH

www.banglajol.info/index.php/JSR

Analysis of Physiochemical and Functional Properties of Oat-Based Synbiotic Beverage Enriched with Phytonutrients

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Received 16 November 2022, accepted in final revised form 11 May 2023

Abstract

Synbiotic food and beverages incorporate a synergistic combination of probiotics and prebiotics to foster enhanced health benefits compared to their individual counterparts. The present study analyzed the physiochemical and functional properties of a synbiotic beverage prepared with a combination of *Lactobacillus casei* as probiotic and oats as the prebiotic substrate versus *L. casei* (5 %) in the dairy matrix as control (C). This low-fat and hypo-caloric product was enriched with whey proteins and natural colors to enhance its favorable effects. Sensory analysis depicted variation 2 (V2) to manifest increased acceptance (8.0±0.2) and the highest count of viable probiotics (8.5×10^{11}±82) versus the other variants. V2 exhibited a significant content of proximate components, dietary fiber (TDF), and polyphenols along with an optimum organic acid production (88±0.3), titratable acidity (1.6±0.3), pH (4.3±0.2), and enhanced antioxidant capacity portrayed by reducing power (25 ± 0.9 mg/mL) and H_2O_2 scavenging abilities (157.5±6.1 µg/mL) as well as improved survival in the *in vitro* simulated gastrointestinal environment compared to the control. Additionally, V2 revealed a shelf life of 28 days at refrigerated storage (4-6 °C) with suitable retention of organoleptic characteristics, acidity, and viability. Hence, the developed synbiotic and polyphenol-enriched beverage may impart beneficial health effects through its nutritional and functional properties.

Keywords: Antioxidant; Health; Nutrients; Phytochemical; Prebiotic; Probiotic; Synbiotic.

doi: http://doi.org/10.3329/jsr.v15i3.62823 J. Sci. Res. **15** (3), 761-781 (2023)

1. Introduction

The rise in health awareness, as well as recent developments in the functional food sector, have heightened the demand for probiotic, prebiotic as well as synbiotic food and beverages amongst consumers. Synbiotic products are usually formulated with a careful combination of probiotics and prebiotics to exert a synergistic effect [1]. Probiotics, living microorganisms capable of significantly contributing to health benefits when ingested in appropriate numbers, and prebiotics, indigestible dietary fibers preferentially utilized by probiotic organisms, have both been conferred with the improvement of digestive health along with alleviation of several ailments, including cardiovascular diseases, obesity, diabetes and cancers, especially, colon cancers [2]. Prebiotic carbohydrates resist

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digestion in the upper gastrointestinal tract, thereby allowing their selective fermentation in the lower GIT by specific beneficial microorganisms, including members of *Lactobacillus* and *Bifido bacterium*. These fibers enhance the activity of the probiotic strains by boosting their metabolism as well as survival, hence exacerbating the effectiveness of the food products harboring them [3]. When amalgamated in a careful combination, the metabolism of prebiotic carbohydrates by the probiotics generates metabolites inclusive of short-chain fatty acids (SCFAs), chiefly acetate, propionate, and butyrate along with carbon dioxide, which has been associated with several advantages such as weight management, DNA repair, cancer prevention as well as improvement of digestive processes through enhanced water absorption and muscular activity in the large intestine [4]. Noteworthy, probiotic activity facilitated by prebiotics has also been reported to promote optimum metabolism, enhance nutrient absorption, and reduce insulin resistance [5]. Interestingly, the administration of synbiotics has been observed to improve gut microbial diversity and benefit host health in clinical trial studies [6]. Moreover, the antimicrobial action of probiotic microbes, fostered by the synbiotic combination, aids in improving the quality of food and enhancement of its nutritive value and organoleptic properties [7].

Most of the population often prefers plant-based foods due to several social and religious constraints associated with the consumption of their animal-based counterparts and the latter's environmental impact, thereby urging the requirements of heightened efforts toward developing novel plant-based functional foods. Oats, *Avena sativa*, is a popular plant-based product that has been utilized for fermentation by probiotic microorganisms, especially members of the Lactic Acid Bacteria (LAB) group, for increasing their nutritional and therapeutic potential, hence being considered as a prebiotic ingredient [8]. Indeed, oats have been recognized as a good source of prebiotic substrates, chiefly β glucans (3-7 %), along with fructose-oligosaccharides (FOS) and resistant starch (RS), which have been noted to bestow numerous benefits inclusive of immunoregulation, anti-inflammatory effects, hypocholesterolemic properties, control of blood glucose levels, and antioxidant actions [9]. Un-branched β glucans, mainly those connected by β 1,3 and 1,4 glycosidic linkages, are a class of soluble fibers that have been attributed to the majority of the prebiotic-mediated health benefits of oats [10]. These carbohydrates also increase the bulk of the stool and bind to bile acids and cholesterol, hence relieving digestive discomforts as well as cardiovascular disorders [11]. Nonetheless, processing and growth conditions influence these glucans' structural composition and branching, thus impacting their usefulness [12]. Additionally, FOS comprising of β 2,1 linked fructose units with a terminal glucose residue and the RS components reported in oats have been attributed to boosting the prebiotic potential of the substrate through modulation of the gut microbiome [13,14].

Lactobacillus casei, a LAB family member, are gram-positive, facultatively anaerobic, non-sporulating bacilli that are known to chiefly produce lactic acid in addition to ethanol and carbon dioxide upon sugar metabolism. These catalase and oxidase-negative bacteria have been observed to modulate intestinal pH and promote their survival

through selective fermentation of dietary fibers in the colon [15]. Moreover, their capability to synthesize superoxide dismutase and antimicrobial peptides aids host health by suppressing oxidative stress and pathogenic multiplication, respectively [16]. Noteworthy, the low pH generated as a result of *Lactobacillus*-mediated prebiotic utilization discourages the multiplication of most pathogenic microorganisms [17]. Furthermore, SCFAs produced through fermentation of the prebiotic fibers mediated by these organisms benefit the host by regulating immunity mediators and inflammatory cascades, helping reduce the risk of several acute and chronic illnesses [18].

Cruciferous vegetables, especially red cabbages, are a potent source of phytonutrients and antioxidants that impart several health advantages [19]. Interestingly, the phytochemical components, including anthocyanins and other polyphenols, have been studied to serve as significant sources of colorants often utilized in food products owing to their natural origin and low toxicity compared to synthetic ones. Moreover, these have also been known to protect against a range of ailments through their antioxidant and anti-inflammatory effects [21]. Therefore, the present study aimed to develop a synbiotic beverage with probiotic *L. casei* in combination with oats as a prebiotic substrate along with the addition of red cabbage-derived natural colors. This blend was selected to improve the product's health benefit through the enhancement of viability and the affectivity of the probiotic species through the prebiotic carbohydrates present in oats. Additionally, the product was enhanced with natural extracts to further ameliorate its overall functionality and efficiency. Regular utilization of this product may help improve the population's health through the combined action of probiotics, prebiotics as well as natural phytochemicals.

2. Materials and Methods

2.1. *Starter culture*

The probiotic strain — *Lactobacillus casei* (ATCC 334) that qualified as one of the most potent strains in previous studies was used in the development of the synbiotic drink [22]. The identity of the probiotic microorganism isolated and used for product development was conducted by studying their gram-staining behavior and sugar-metabolizing properties. The isolated microbes manifested gram-positive characteristics owing to the retention of the primary stain, gram's crystal violet leading to the appearance of purple-colored rod-shaped cells. These microbes also confirmed the production of lactic acid through the fermentation of glucose and displayed catalase and negative oxidase patterns, thereby confirming them as *Lactobacillus casei*. To check the cell count of the probiotic strain, the bacteria were isolated from beverages and grown on MRS-broth at 37 °C for 48 h. The cultures were maintained at 4–6 °C. The culture was centrifuged (4500 g, 10 min, 4 °C), washed in autoclaved water, and re-suspended culture media. The cell count of the culture used for product development and analysis was 10^{11} CFU/mL.

2.2. *Product development*

2.2.1. *Preparation of oat-based substrate*

The oat substrate was prepared using varying combinations of oat (*Avena sativa*) mash, whey proteins, sucrose, saccharin (E954), and natural colors, as shown in Table 1. The slurry was then heated at 95 °C for 10 min and cooled to 37 °C.

Table 1. Preparation of oat-based substrate.

Products	Oat mash (%)	Whey protein (%)	Sucrose (%)	Saccharin E954 (%)	Natural food color (%)
Variation 1 (V1)	4	5	1	0	10
Variation 2 (V2)	5.5	7	1.5	0.25	20
Variation 3 (V3)	7	10	2	0.75	30

2.2.2. *Extraction of natural food color*

Red cabbage leaves were taken as a source of natural color. 55 g of finely cut red cabbage leaves were dissolved in 60 mL of extraction medium (purified water) in a ratio of 1:2 and filtered. The filtrate was centrifuged at 6000 rpm for 15 min to remove the fine suspended particles, and the supernatant was utilized for addition into beverages. Since red cabbage polyphenols are mainly derived from cyanidin glycoside, quantitative data were expressed as cyanidin-3-glycoside [23]. The natural color concentration was estimated using the pH differential method, employing the following equation [24]:

Pigment concentration (mg/mL) = (A × Mw × DF × L) / ε
Where, A = (A530 − A700) pH 1.0 − (A530 − A700) pH 4.5,
Mw represents the molecular weight of anthocyanin (449.2 g/mol),
DF is the dilution factor,
ε represents the extinction coefficient (26,900 L/cm mol) and
L = The path length (1 cm).

2.2.3. *Fermentation and storage*

A dairy beverage containing *Lactobacillus casei* (5 %) was used as a source of starter culture. The oat mash was inoculated with 1 %- 10 % (v/v) starter culture from dairy beverage, which gave inoculation levels between 9.8×10^{7} to 7.5×10^{8} CFU/ mL. Fermentation was carried out at 37°C for 8–10 h. Storage observations were carried out at 4–6 °C for 28 days.

2.3. *Evaluation of sensory properties*

The developed synbiotic beverages were evaluated for sensory parameters, including appearance, taste, and texture, using the 9-point hedonic scale method by 50-panel

members comprising of adults aged between 20-45 due to the evidence regarding the health consciousness of this group along with their readiness to accept health-promoting food and beverages [25]. The selected probiotic strain in the dairy beverage matrix was used as the control.

2.4. *Viable cell enumeration*

Enumeration of viable cells of *L. casei* was performed by the Total Viable Count (TVC) method through the estimation of microbial colonies on MRS-agar plates (medium pH 5.7) after incubation at 37 °C for 48 h. A colony count in the range 10^5-10^7 CFU/mL was considered to be the optimum range of probiotics, capable of showing the desirable effects.

2.5. *Determination of proximate composition*

2.5.1. *Estimation of carbohydrate content*

The carbohydrate content of the sample drinks was estimated by the Anthrone method [26]. 100 mL of the sample was hydrolyzed by keeping it in a boiling water bath for 3 h with 2.5 N HCl and was cooled to room temperature. After neutralization with sodium carbonate, the volume was made up to 100 mL and was centrifuged. The supernatant was diluted and mixed with Anthrone reagent, followed by absorbance measurement at 630 nm. D-Glucose (1 mg/mL) was used as the standard solution. The carbohydrate content of the sample was calculated with respect to the standard curve.

2.5.2. *Estimation of protein content*

Protein was estimated by the Biuret Method. Bovine Serum Albumin (BSA) 1 mg/mL was used as the standard protein. Different concentrations of the standard or test protein solutions were mixed with required volumes of distilled water along with Biuret reagent and incubated for 30 min at room temperature, followed by absorbance measurement at 550 nm in a UV-Visible spectrophotometer (Hitachi, Japan) against blank.

2.5.3. *Estimation of fat content*

5 mL of the drink sample was dissolved in petroleum ether by thorough mixing and was centrifuged at 3000 rpm for 5 min. The supernatant ether extract of the sample was separated from the suspended matter in a pre-dried, pre-weighed petri plate and allowed to evaporate. The fat content was calculated by the loss of weight post-evaporation with respect to the initial weight.

2.6. *Analysis of total dietary fiber (TDF)*

AOAC-approved method No. 985.29 through the FOSS Analytical Fibertec E 1023 system was used for determining the total dietary fiber in the samples. The samples were devoid of their fat content, dried, and prepared to a final particle size lower than 0.5 mm. After weighing, each sample was enzymatically digested with α amylase and incubated at 100 °C followed by digestion with protease and amylo-glucosidase at 60 °C. The process used for the measurement of TDF is displayed in Fig. 1. After digestion, the total fiber content was precipitated by adding 95 % ethanol, followed by filtration and collection of fiber which was thereafter dried and weighed. The protein and ash content were determined to correct any of these substances which might remain in the fiber (Fig. 1). TDF content was calculated as follows:

Content of fiber = residue weight–the weight of (protein+ash)

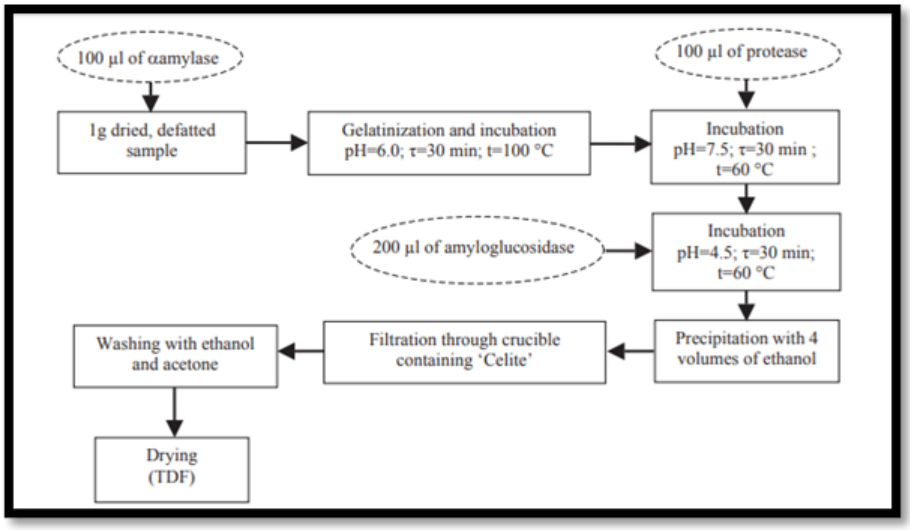

Fig. 1. Procedure for estimation of Total Dietary fiber (TDF).

2.7. *Estimation of polyphenol content*

The total phenolic content of the synbiotic drink was determined using the Folin Ciocalteau method. Gallic acid solutions in different concentrations were taken for the preparation of standards. 40 µL of standard, test sample or blank was mixed with 3 mL of water followed by 200 µL of the Folin- Ciocalteu reagent. Solutions were allowed to stand for 5 min, followed by the addition of 600 µL of the sodium carbonate solution. The solutions were left at 40 °C for 30 min, and then the absorbance of each solution was determined at 765 nm against the blank.

2.8. Determination of organic acid production, titratable acidity, and pH

Organic acid production was determined through the titrimetric method using phenolphthalein as an indicator. The pH meter measured the samples' pH (EI Self pH Meter, India). Titratable acidity (TA) was determined by titrating 10 mL samples with 0.1 N NaOH with phenolphthalein as an indicator. TA was expressed as °N (degrees Neuman) [27].

2.9. *Determination of antioxidant properties*

2.9.1. *Estimation of reducing power*

The reduction property of the extracts was assessed according to the method of Oyaizu [28]. Different concentrations (0.2– 1.0 mg/mL) of standard (ascorbic acid) or sample extracts were added to 1 mL of distilled water and then mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1 % potassium ferrocyanide. The mixture was incubated at 50 °C for 20 min before the addition of 2.5 mL of trichloroacetic acid. The obtained mixture was centrifuged at 10 min for 3000 rpm. After this, 2.5 mL of the supernatant was mixed with an equal amount of distilled water and 0.5 mL of 0.1 % $FeCl_3$. The color change of the resulting solution was then estimated by measurement of absorbance at 700 nm.

2.9.2. *Hydroxyl radical inhibitory potential*

The ability of the sample beverages to stop Fe^{2+}/H_2O_2-induced decomposition of deoxyribose was assayed using the modified method of Oboh and Rocha [29]. 40 mL of the freshly prepared extracts (0.2–1.0 mg/mL) was added to a reaction mixture containing 20 mL of 20 mM deoxyribose, 80 mL of 0.1 M phosphate buffer, 500 mM $FeSO_4$ (10 mL), and the volume was made up with distilled water to 200 mL. The reaction mixture was initiated at 37 °C for 30 min and stopped by adding 50 mL of 2.8 % TCA (trichloroacetic acid). This was followed by the addition of 50 mL of 0.6 % thiobarbituric acid solution. The mixture was then incubated in boiling water for 20 min, and absorbance was read by a spectrophotometer at 700nm. The standard was taken as ascorbic acid (1 mg/mL).

2.10. *Preparation of simulated gastrointestinal (GIT) juice*

Simulated gastrointestinal juice was developed by mixing NaCl (2.05 g/L), glucose (3.5 g/L), KH_2PO_4 (0.60 g/L), KCl (0.37 g/L), and $CaCl_2$ (0.11 g/L) followed by adjustment to pH 2.0 and autoclaving (121 °C for 15 min). To prepare the bile solution, 10 g oxgall (Difco) was dissolved in 90 mL distilled water. This solution was then used to prepare 0.5 % and 2.0 % concentrations of bile. All solutions were sterilized at 121 °C for 15 min. Pancreatic juice solution contained NaCl (125.0 mM), $CaCl_2$ (0.6 mM), $MgCl_2$ (0.3 mM),

trypsin (activity 13,800 U/mg of protein), a-chymotrypsin (activity 40 U/mg of protein) were added to pancreatic juice so that the final enzyme concentrations were the following: pancreatic lipase (590 U/mL), trypsin (11 U/mL), a-chymotrypsin (24 U/mL).

2.11. *Estimation of shelf life*

The shelf life of the fermented oat drink was defined as the period of refrigerated storage (4–6 °C) during which pH remained above 4.0, and the number of viable cell counts was above 10^6 CFU/ mL. Refrigerated storage was carried out for 28 days with periodical observations of pH, TA, sensory attributes, and the viability of the starter culture.

2.12. *Analysis of data*

Data were quantified and represented as mean±s.e.m. of N≥3 discrete experiments. Statistical evaluation was conducted via the Data Analysis Software pack of Microsoft Excel 2010. Data were also examined by using the ANOVA data analysis [30]. Only a P value <0.05 was considered statistically significant.*, **, *** signified p value < 0.05, < 0.01 and < 0.001 respectively.

3. Results and Discussion

1.1. *Sensory evaluation and total viable count (TVC)*

Table 2. Sensory evaluation and TVC of the developed products.

Samples	Sensory parameters				TVC (CFU/mL)
	Appearance	Taste	Texture	Overall Acceptability	
Control (C)	$6.0\pm0.5^{*}$	$7.0\pm0.2^{**}$	$7.8\pm0.3^{**}$	$7.0\pm0.3^{**}$	$9.8\times10^{9}\pm76^{***}$
Variation 1 (V1)	$5.0\pm0.4^{*}$	$6.4\pm0.4^{*}$	$7.0\pm0.2^{**}$	$6.1\pm0.3^{*}$	$6.8\times10^{10}\pm65^{***}$
Variation 2 (V2)	$8.5\pm0.4^{**}$	$8.2\pm0.3^{**}$	$7.2\pm0.3^{**}$	$8.0\pm0.2^{**}$	$8.5\times10^{11}\pm82^{***}$
Variation 3 (V3)	$6.5\pm0.2^{**}$	$6.9\pm0.5^{*}$	$6.8\pm0.4^{*}$	$6.2\pm0.3^{*}$	$5.5\times10^{10}\pm93^{***}$

Synbiotic beverages were developed with *Lactobacillus casei* (ATCC 334), which has been observed as one of the most functional strains in earlier studies in combination with varying proportions of oats, whey protein, and sucrose. The addition of sugar was replaced partially by an artificial sweetener, saccharin, to keep the drink diabetes-friendly and low-calorie. It was observed that the use of the artificial sweetener did not affect the growth and survival of the probiotic (data not shown). Artificial sweeteners were used in the drink within the range of acceptable daily intake (ADI; 5mg/Kg IBW/day). The drink was enriched with red cabbage extract to augment the beverage with natural color and plant-based phytochemicals. The sensory evaluation results displayed that variation 2 (V2) had the highest hedonic score regarding appearance, taste, and overall acceptability. The textures of all the products, except control (C), were a little grainy due to the presence

of oats which may be responsible for obtaining lower scores for texture in the developed products (7.0±0.2, 7.2±0.3, and 6.8±0.4 in V1, V2, and V3, respectively) versus the control (7.8±0.3). The appearance of V2, however, was brighter and more appealing (8.5±0.4) than the rest of the variations. Moreover, this variation also scored the highest in terms of taste (8.2±0.3) compared to the other products. Overall, variation 2 was chosen to be the most accepted variation by the panel members (Table 2). The developed products were also checked for the presence of viable probiotic species by growth on MRS media. All the products displayed an adequate number of viable probiotic microbes, with the highest count in V2 (8.5×10^{11}±82). Noteworthy, the administration of probiotics in food matrices in the range of 10^9-10^{11} has been documented to portray positive health effects [31]. Therefore, subsequent physiochemical analyses, including nutrient content, antioxidant properties, survival in the simulated gastrointestinal environment, and shelf-life studies, were conducted in variation 2 versus the control owing to the highest acceptability of the former through sensory evaluation along with the presence of the greatest number of viable probiotics in the same.

3.2. *Proximate composition, TDF, and polyphenols*

The synbiotic drink (V2) was assayed for proximate composition with respect to the control. Variation 2 was found to exhibit carbohydrate, protein, and fat content of 12.07±1.7 g/100 mL, 3.5±0.08 g/100 mL, and 0.52±0.02 g/100 mL compared to 7.1±1.1 g/100 mL, 1.0±0.09 g/100 mL and 0.41±0.05 g/100 mL in C respectively (Table 3). The presence of the increased amount of oats in the developed product may be responsible for the higher carbohydrate concentration in this product, along with increasing the bulk of the drink. Furthermore, the protein content of V2 may be attributed to the addition of whey protein (7%) in the same. The product was observed to exhibit a minimal fat content since no fat-containing ingredients were used in its preparation with the aim of developing a healthy low-fat product. The samples were also analyzed for their TDF content to estimate their health-promoting properties. It was observed that V2 displayed a TDF amount of 5±0.2 g/100 mL compared to 1.2±0.06 g/100 mL in control (Table 3). A significant TDF content in the developed products may aid in not only the improvement of gastrointestinal health but also the prevention of cardiovascular diseases and cancer, and various other chronic ailments [32]. Dietary fiber has also been shown to improve glucose tolerance and help in weight management. Polyphenols are potent plant-based phytochemicals that are known for their functional and physiological effects, including antioxidant-mediated management of various chronic disorders. Moreover, previous studies have implicated dietary fiber to interact with polyphenols and deliver positive health benefits [33]. Furthermore, polyphenols individually have also been associated with significant beneficial biological properties. Hence, supplementation of the drink with red cabbage extracts may enhance the beneficial effects via phytochemicals, including polyphenols. Therefore, the prepared samples were also investigated for their polyphenol content. Results revealed the presence of 11.4±1.1 mg/mL polyphenols in V2 compared to

0.05±0.01 mg/mL (Table 3). Dietary fibers and polyphenols have been associated with prebiotic action, which may further elevate the functionality of the developed synbiotic beverage. Hence, the developed synbiotic beverage may serve as a good source of nutritional and functional components for the advancement of health.

Table 3. Proximate composition, TDF, and polyphenol content of the beverages.

Sample	Carbohydrate (g/100 mL)	Protein (g/100 mL)	Fat (mg/100 mL)	TDF (g/100 mL)	Polyphenols (mg/mL)
Control (C)	7.1±1.1*	1.0±0.09	0.41±0.05*	1.2±0.06*	0.05±0.01
Variation 2 (V2)	12.07±1.7**	3.5±0.08*	0.52±0.02*	5±0.2*	11.4±1.1**

3.3. *Acid production and antioxidant properties*

To determine their efficacy, the developed products were assayed for organic acid production, titratable acidity, and pH. Organic acid production is an important parameter not only for the determination of probiotic viability and action but also for the manifestation of functional effects, especially anti-pathogenic and immune-modulatory actions. Moreover, these acids have also been implicated in the improvement of gut barrier function and GIT physiology. Experimental results displayed V2 to reveal enhanced organic acid production (88±0.3; $P<0.05$) along with favorable pH (4.3±0.2) and titratable acidity (1.6±0.3°N), hence indicating its prospective role in benefiting host health (Table 4). The synbiotic beverage was also examined for its antioxidant properties. Estimation of reducing power and H_2O_2 scavenging activity were used for the determination of the antioxidant capability of the probiotic. The results showed the developed beverage (V2) to portray 25 ± 0.9 reducing power compared to 14±1.2 in control, thereby signifying its potential to terminate free radical chain reaction (Table 4). However, the reducing power of the sample drink was comparable to that of ascorbic acid ($p> 0.01$) (data not shown). Furthermore, the H_2O_2 scavenging ability of the synbiotic drink (V2) was calculated as 157.5±6.1 ug/mL versus 101.2±4.5 in Control. The antioxidant properties of the developed product exhibited by their reducing potential and H_2O_2 scavenging capabilities may be attributed to the enhanced action of probiotic *Lactobacillus* due to the existence of a synbiotic environment and the presence of phytochemicals including polyphenols in the sample. Hence, consumption of this beverage may mitigate free radical-mediated damage. Moreover, incorporating probiotics in foods, especially in combination with appropriate prebiotic combinations, can provide a good strategy for administering dietary antioxidants.

Table 4. Acid production and antioxidant properties of the beverages.

Sample	Organic acids (mg/mL)	TA (°N)	pH	Reducing Power (mg/mL)	H_2O_2 Scavenging Potential (µg/mL)
Control (C)	76±0.5**	1.0±0.2*	6.0±0.4*	14±1.2**	101.2±4.5*
Variation 2 (V2)	88±0.3**	1.6±0.3*	4.3±0.2*	25 ± 0.9**	157.5±6.1*

3.4. *Survival in vitro gastrointestinal environment*

Survival of the prepared oat-based synbiotic beverage was checked in a simulated gastrointestinal environment (gastric, bile, and intestinal digestion) to estimate its effectiveness in the human gastrointestinal tract. V2 was observed to display improved viability in this environment compared to C, as manifested by increased microbial counts, especially with respect to resistance against bile and intestinal digestive conditions (Table 5). The proportion of oats and whey matrix used in the preparation of V2 may be responsible for providing enhanced protection to *Lactobacillus* along with a favorable environment for its multiplication and viability, thereby ameliorating the survival of the former compared to C. However, the developed drinks were observed to perform better at 24 h versus the latter time points, which may be due to the microbes entering the stationary phase beyond 24 h ($P>0.05$). Nonetheless, the survival of V2 was significantly elevated versus C at all time points. Hence, the developed oats-based synbiotic and antioxidant-enriched beverage may promote health in humans through its nutritional and functional properties.

Table 5. Survival *in vitro* gastrointestinal environment.

Time (Hours)	Survival after *in vivo* gastric digestion (O. D 600 nm)		Survival after *in vivo* bile digestion (O. D 600 nm)		Survival after *in vivo* intestinal digestion (O. D 600 nm)	
	C	V2	C	V2	C	V2
24	$0.88\pm0.04^{**}$	$0.99\pm0.07^{*}$	$0.67\pm0.04^{*}$	$0.98\pm0.05^{**}$	$0.69\pm0.06^{*}$	$0.86\pm0.05^{**}$
48	$0.86\pm0.02^{**}$	$0.98\pm0.04^{*}$	$0.62\pm0.03^{**}$	$0.89\pm0.04^{**}$	$0.65\pm0.05^{*}$	$0.84\pm0.06^{*}$
72	$0.84\pm0.05^{*}$	$0.98\pm0.05^{*}$	$0.59\pm0.03^{**}$	$0.88\pm0.03^{**}$	$0.64\pm0.03^{**}$	$0.75\pm0.05^{**}$

3.5. *Shelf life*

Since the developed product (variation 2) was observed to display improved sensory attributes, nutrient content, acid production, and antioxidant properties compared to the control beverage, V2 was further evaluated for its shelf life with respect to the number of viable bacteria, pH, TA, and sensory attributes. V2 was noticed to retain sufficient viable *Lactobacillus* numbers required for positive health outcomes for a period of 28 days at refrigerated storage (4-6^{o}C). Nonetheless, viable cell counts decreased with about 1 log order (from 8.5×10^{11} to 9.3×10^{9} CFU/mL). Moreover, the TA and pH of the drink remained within the desired ranges for 28 days. Sweeteners were observed to have a negligible effect on the viability of the starter culture during storage. V2 was also found to retain the desired sensory characteristics manifested by the overall acceptability of the product as evaluated through the 9-point hedonic scale (Table 6). Therefore, the developed oat-based synbiotic beverage was observed to retain the desired characteristics under 28 days of storage at 4-6 °C.

Table 6: Shelf-life analysis of the synbiotic beverage (V2).

Time (Days)	TVC (CFU/mL)	TA (°N)	pH	Overall Acceptability
7	$8.5\times10^{11}\pm34^{***}$	$1.6\pm0.3^{*}$	$4.3\pm0.3^{*}$	$8.0\pm0.2^{**}$
14	$4.5\times10^{10}\pm23^{***}$	$1.1\pm0.1^{*}$	$5.5\pm0.1^{*}$	$7.5\pm0.3^{*}$
21	$9.9\times10^{9}\pm33^{***}$	$1.0\pm0.1^{*}$	$5.9\pm0.2^{*}$	$7.2\pm0.4^{*}$
28	$6.3\times10^{9}\pm43^{***}$	$0.8\pm0.002^{*}$	$6.1\pm0.3^{*}$	$7.1\pm0.2^{**}$

4. Conclusion

The present study was designed to develop an oat-based synbiotic beverage with *Lactobacillus casei*, a strain that has been documented to deliver desirable benefits through food matrices. The product utilized oats, a known prebiotic and gut-promoting substrate. The drink was further fortified with whey protein and was kept hypocaloric with the use of artificial sweetener. Furthermore, the above was enriched with the natural color to enhance the phytochemical and antioxidant content mediated efficacy. Variation 2 was found to display the most acceptable sensory characteristics compared to the other developed products. This variation also portrayed enhanced dietary fiber, polyphenols, and proximate components versus the control. Moreover, V2 revealed increased antioxidant potential manifested by their reducing capacity and H_2O_2 foraging ability. V2 was also observed to tolerate gastrointestinal digestion and maintain the desired viability in an *in vitro* simulated environment. The developed product exhibited a shelf life of 28 days at 4-6°C with suitable retention of organoleptic characteristics, acidity, and viability. Therefore, variation 2 may foster the right proportion of prebiotic and probiotic combinations for enhanced benefits. Consumption of this product may aid in health promotion owing to its nutritional and functional quality.

Acknowledgment

The authors acknowledge the laboratory facilities of J. D. Birla Institute, Kolkata.

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