Original Article
Food Science and Technology

ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)

DOI: https://doi.org/10.1590/fst.07022

Prebiotic effect of D-allulose and β-glucan on whey beverage with *Bifidobacterium animalis* and investigation of some health effects of this functional beverage on rats

Jerina RUGJI[1], Zühal ÇALIŞKAN[1], Ahmet Hulusi DINÇOĞLU[2*], Mustafa ÖZGÜR[2], Zeki EROL[1], Elif Büşra ÖZGÜR[1]

Abstract

To meet consumer requirements and expectations, innovative approaches to combining whey with other ingredients are being explored. The demand for health-promoting drinks containing vitamins, probiotics, prebiotics, minerals, and bioactive components (antioxidants) has risen, propelling market expansion. The purpose of this study was to develop a synbiotic functional whey beverage supplemented with *Bifidobacterium animalis*, D-allulose, and β-glucan and evaluate its microbiological, physicochemical, and influence on several health indicators in a Wistar rat model. The beverage supplemented with D-allulose had the highest average viable counts of *B. animalis* (9.20 $\log_{10}$CFU/g) and was the second most preferred in terms of of taste, texture and general acceptability compared to β-glucan-containing beverage. The highest TAS and lowest TOS values were determined in the serum samples of rats belonging to group WA, WG and WAG, respectively. This study might lead to additional studies focusing on specific variables and the relevance of utilizing D-allulose in dairy product processing.

Keywords: *Bifidobacterium animalis*; β-glucan; D-allulose; functional beverage; whey.

Practical Application: Whey beverage combining β-glucan, D-allulose, and Bifidobacterium has the potential to play an essential role in promoting health and preventing illnesses, particularly in terms of decreasing total cholesterol, enhancing blood lipid profile, and reducing or maintaining body weight.

1 Introduction

The importance of the intestinal microbiota in health has grown in recent years (Quigley, 2019). Dietary supplements, such as prebiotics, are one common approach for altering the composition of the gut microbiota. Prebiotics are defined as:" selectively fermented ingredients that result in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host" (Gibson et al., 2017). As a result, there is an increasing interest in interventions that can regulate the microbiota and its interactions with the host. In this sense, prebiotics are one of the most used substances to maintain a healthy microbiome or to restore its equilibrium when bacterial homeostasis is disrupted (Farias et al., 2019). Food components with prebiotic characteristics include non-digestible carbohydrates such lactulose, inulin, and others (Al Saqqa, 2021; Colantonio et al., 2020; Mohanty et al., 2018). These components have been proven to enhance the growth of Bifidobacterium populations in vitro and in randomized controlled trials (Roberfroid et al., 1998; Sawicki et al., 2017). Although probiotics are commonly defined as live and active microorganisms, Zendeboodi et al. (2020) recommend conceptualization of probiotics in 3 classes, which are 'true probiotic' referring to viable and active cells, 'pseudo-probiotic' referring to viable and inactive cells in the forms of vegetative or spore and 'ghost probiotic' referring to dead/nonviable cell, in the forms of intact or ruptured. The Bifidobacterium genera are the most often utilized probiotics in the food industry. They are also common occupants of the human gut and have a long history of safe use in the food industry (Dinkçi et al., 2019). The survivability of *Bifidobacterium* spp. in foods is affected by factors such as shelf life, preservation temperature, and the presence of O_2 (Siró et al., 2008).

International Society of Rare Sugars (ISRS) defines the term "rare sugars" as "monosaccharides and their derivations that are rare in nature." Natural D-allulose, which is a C-3 epimer of D-fructose ($C_6H_{12}O_6$) is one of the uncommon sugars found in nature (Chung et al., 2012). Dietary D-allulose has a calorie-reduction impact on the body, which improves insulin resistance, antioxidant capacity and hypoglycemia control, as well as diabetes and obesity management (Han et al., 2016; Itoh et al., 2015). In addition, D-allulose can be utilized as a prebiotic in synbiotic mixtures (Do et al., 2019). β-glucan is an important dietary fiber found in cereal crops, such as barley, oats, and mushrooms (Ahmad et al., 2012). It is an appealing glucose polymer and a physiologically functional component with a wide range of health effects, including anti-inflammatory, antioxidant, anti-tumor, immunomodulation, and glycemic and blood cholesterol regulation (Zanon et al., 2020). β-glucan has been reported to act as a substrate for microbiological fermentation and selectively stimulate growth and activity of

Received 02 Jan., 2022
Accepted 28 Feb., 2022
[1] *Department of Food Hygiene and Technology, Institute of Health Sciences, Burdur Mehmet Akif Ersoy University, Burdur, Turkey*
[2] *Department of Nutrition and Dietetics, Faculty of Health Sciences, Burdur Mehmet Akif Ersoy University, Burdur, Turkey*
*Corresponding author: adincoglu@mehmetakif.edu.tr

beneficial bacteria such as Bifidobacterium and Lactobacillus resulting in the formation of beneficial short chain fatty acids (SCFA) (Nordlund et al., 2012; Shen et al., 2012). *Lactobacillus fermentum* Lf2 can produce large quantities of exopolysaccharides consisting of high molecular mass β-glucan (Ale et al., 2020).

Whey is the dairy industry's greatest volume co-product, and over the last two centuries, a great deal of effort has gone into determining its chemical makeup and better understanding the biological actions of whey components (Tsermoula et al., 2021). Whey beverages have long been studied as carriers for different prebiotics (Guimarães et al., 2018, 2019). Whey-based beverages have a high nutritional value due to the high concentration of essential and branched-chain amino acids (Yadav et al., 2015). Consumption of this type of ready-to-drink beverage is widespread, and its nutritional value is enhanced by the addition of compounds such as probiotics and prebiotics (Chavan et al., 2015). The purpose of this study was to investigate the link between oat β-glucan, D-allulose, and survival of *Bifidobacterium animalis* in whey beverage during cold storage, as well as the influence of probiotic supplemented D-allulose and β-glucan diet on some quality parameters, both separately and in combination, in a rat model.

2 Materials and methods

2.1 Materials and preparation of whey beverage

The whey powder, D-allulose, and β–glucan used in the preparation of the whey beverage were purchased commercially from *Mirel® Süt Ürünleri, Demireller Akaryakıt Nak. Tic. ve San. Ltd. Şti., Astraea*, *Matsutani Chemical Industry Co., Ltd*. and *Hammaddedepo Beta Glucan* (80%), respectively. *Bifidobacterium animalis* (ATCC 25527) was supplied from the culture collection of Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Department of Food Hygiene and Technology.

Whey beverage was prepared using 90% demineralized skimmed whey powder. The whey powder was dissolved in sterile dH_2O at 65 °C for 15 min to approximately 20% of total solids. It was cooled to 35 °C immediately after the heat treatment. Afterwards, *B. animalis* and prebiotics were inoculated under aseptic conditions and dispersed in equal volume containers in the appropriate groups. The study consisted of four groups. The sole ingredients in the control group (W) were whey and *B. animalis*. Group WA had whey, *B. animalis*, and D-allulose (1.2%), whereas group WG contained whey, *B. animalis* and β–glucan (1.2%). Group WAG was made up of whey, *B. animalis*, D-allulose (0.6%) and β–glucan (0.6%) combination. *B. animalis* was inoculated at the 0.5 McFarland level. The beverage was distributed in equal volume containers and stored at 4 °C until the analysis days. Production and analysis were performed in triplicates.

2.2 Methods

Determining the microbiological quality of whey beverage

Total aerobic mesophilic counts (TAMC) were determined by using plate count agar (Merck KGaA, Darmstadt, Germany, VM748563624). Incubation was done at 37 °C for 48 h (International Organization for Standardization, 2003). Total aerobic psychrotrophic counts were determined by using plate count agar (Merck KGaA, Darmstadt, Germany, VM748563624). Incubation was done at 6.5 °C for 10 days (International Organization for Standardization, 2019). Yeast and mould counts were determined by using potato dextrose agar (Biolife, LOT CH1803). Incubation was done at 25 °C for 5 days (International Organization for Standardization, 2008). Coliform counts were determined by using violet, red bile dextrose agar (Merck KGaA, Darmstadt, Germany, VM730375616). Incubation was done at 37 °C for 24 ± 2 h (International Organization for Standardization, 2006). *B animalis* counts were determined with the use of a selective culture medium MRS-NNLP by using the pour plate method. It consisted of MRS Agar Iso Formulation, produced by Biolife (LOT HC5002) supplemented with filter sterilized solutions of nalidixic acid (PhytoTechnology Laboratories, 15 mg/L), neomycin sulfate (PhytoTechnology Laboratories©, 100 mg/L), lithium chloride (Merck KGaA, Darmstadt, Germany, 3.0 g/L), paromomycin sulfate (Cayman Chemical Company, Batch0538278-4, 200 mg/L), and L-cysteine hydrochloride (Merck KGaA, Darmstadt, Germany, 0.5 g/L). Incubation was done in anaerobic conditions as above mentioned at 37 °C for 72 h (Man et al., 1960).

Determining the physicochemical quality of whey beverage

Titratable acidity, dry matter, total nitrogen content and total protein content was evaluated in accordance with Association of Official Analytical Chemists (Williams, 1984). pH was measured directly with a pH meter (704 pH Meter, Metrohm, Netherlands). A tristimulus chromatometer (Minolta CR-400, Osaka, Japan) was used to measure color, which was calibrated with a white reference plate and CIELAB L*a*b* and E values. Minolta measures were represented as L* for lightness, a* for redness, and b* for yellowness (Kristensen et al., 2000). Viscosity of beverage was measured using a rotary viscometer (DVII+Pro; Brookfield Engineering, Middleboro, MA, USA). Results obtained after 10 s are expressed as cP (Gassem & Frank, 1991).

Sensory analysis of whey beverage

A panel of ten expert analysts conducted sensory evaluations on days 0, 7, 14, 21, and 28. The goal of sensory evaluation was to determine if fermentation of whey would produce a drink with acceptable sensory qualities. The evaluation was carried out on a 9-point hedonic scale (9 being extremely excellent and 1 being extremely poor). Color, texture, aroma, taste and general acceptability were all rated by the panelists (International Organization for Standardization, 2016).

Animals and experimental design

All experimental procedures were approved by the institutional animal care committee in accordance with the guidelines of the Experimental Animal Production and Experimental Research Center (Protocol no: 813). A total of 50 male Wistar rats (6-8 weeks) were purchased from the laboratory of the Experimental Animal Production and Experimental Research Center. Only males

weighing 250-300 g were used in the study. After a week of acclimatization, the animals were randomly separated into five groups (n = 10 animals per group) and housed in a room under standard conditions of humidity (50-60%), temperature (25 ± 2 °C) and on a 12 h light/dark cycle. A diet with standard laboratory pellet and water *ad libitum* was provided. The research consisted of five groups including a negative control (NC) group (dH_2O). Beverages were given to each member of the group (1 mL per 300 g animal). Prior to the administration of the beverages the rats were weighed. The weightings were repeated on the 7, 14, 21 and 28th days, respectively. After the prepared products were given to the rats for 28 days. Stool samples were taken from the rats on the 7, 14 and 28th days. Coliform counts were determined by using violet, red bile dextrose agar (Merck KGaA, Darmstadt, Germany, VM730375616). Incubation was done at 37 °C for 24 ± 2 h (International Organization for Standardization, 2006). *E. coli* counts were determined on TBX (Oxoid CM 945) agar at 30 °C for 4 h, then at 44 °C for 18 h, *Lactococcus* spp. (Dolci et al., 2020), *Lactobacillus* spp. (Man et al., 1960), *Streptococcus* spp. (Delgado-Fernández et al., 2020) and *Bifidobacterium animalis* (Dave & Shah, 1996) counts were analyzed from stool samples. In addition, biochemical analyzes were performed from stool and blood samples taken on days 14 and 28th, respectively. Analyzes were performed in triplicates.

Biochemical analysis

On the 14th day, blood samples were collected from 5 rats in each group while they were under chemical anesthesia (ketamine 85 mg/kg-xylazine 10 mg/kg). Serum glucose, total cholesterol, triglyceride, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were determined. A commercial kit (*Rel Assay Diagnostic*) was used to determine the levels of total oxidant status (TOS) and total antioxidant status (TAS) of the serum samples.

2.3 Statistical analysis

All analyses were carried out in triplicate. The results were subjected to one-way variance analysis (ANOVA) using the Statistical Package for the Social Sciences (SPSS) (Version 26.0; SPSS, Chicago, IL, USA). The statistical procedure, Tukey's test, was used to determine significant differences among the mean values. Mann Whitney U test, which is the non-parametric equivalent of the independent sample T-test, was used in the analysis of the difference between days in biochemical analyses and $p < 0.05$ was considered statistically significant in all analyses. The results were expressed as mean ± standard deviation (SD).

3 Results and discussions

3.1 Microbiological properties of whey beverage

The beverage was pasteurized at 65 °C for 30 min. The primary objective of this heat treatment was to prevent or eliminate microorganisms that may compromise the quality of the food product. Thermal treatment was successful since no bacteria were present (day 1). Table 1 illustrates the changes in counts of *B. animalis* (at 7-day intervals) in synbiotic whey beverages containing D-allulose and oat-based β-glucan during 28 days of storage at 4 °C. *B. animalis* counts in probiotic whey beverage supplemented with D-allulose (WA) were substantially higher than in beverage supplemented with β-glucan. Dairy products are the primary carriers of probiotics, and it has been indicated that the minimum counts of probiotic bacteria in fermented milk products at the time of ingestion should be 10^6-10^7 cfu/mL to impart health benefits (Kurtuldu & Ozcan, 2018). Obtained results show that the viability *of B. animalis* was significantly enhanced by D-allulose supplementation. The beverage with D-allulose had the highest average viable count of *B. animalis* (9.20 $\log_{10}$CFU/g), which was greater than in β-glucan-containing beverage. The current study found that viable counts of *B. animalis* on day 7 were substantially greater than viable counts on previous storage days ($P < 0.05$).

3.2 Physicochemical properties of whey beverage

Table 2 shows the changes in chemical contents of synbiotic whey beverage samples held at refrigerator temperature (4 °C) for 28 days. pH is one of the most important factors impacting probiotic viability. The pH of the drinks altered over time, with the *B. animalis* and D-allulose-containing groups having a slightly higher average pH than the other groups. The initial titratable acidity in D-allulose and β-glucan samples was 0.38 and 0.42%, respectively, and gradually increased after 28 days of storage. The initial viscosity for all beverages was around 8 mPa.s except for

Table 1. Microbiological properties of whey beverage samples during storage.

	1	7	14	21	28
TAMC					
W	8.69 ± 0.03^{Cb}	8.81 ± 0.03^{Aa}	8.67 ± 0.02^{Cb}	8.79 ± 0.01^{ABa}	8.58 ± 0.01^{Bc}
WA	8.89 ± 0.01^{Aa}	8.83 ± 0.01^{Ac}	8.85 ± 0.01^{Aab}	8.78 ± 0.01^{Bd}	8.86 ± 0.01^{Ab}
WG	8.77 ± 0.04^{Ba}	8.69 ± 0.03^{Bb}	8.64 ± 0.01^{Cb}	8.76 ± 0.01^{Ca}	8.49 ± 0.02^{Dc}
WAG	8.87 ± 0.02^{Aa}	8.81 ± 0.01^{Ab}	8.76 ± 0.02^{Bc}	8.81 ± 0.01^{Ab}	8.53 ± 0.03^{Cd}
B.animalis					
W	8.83 ± 0.3^{Aa}	8.54 ± 0.01^{Cc}	8.70 ± 0.01^{Cb}	8.71 ± 0.04^{Bb}	8.81 ± 0.01^{Ba}
WA	8.84 ± 0.01^{Ae}	9.20 ± 0.01^{Aa}	8.90 ± 0.01^{Ab}	8.88 ± 0.01^{Ac}	8.86 ± 0.01^{Ad}
WG	8.77 ± 0.01^{Ba}	8.75 ± 0.02^{Bab}	8.61 ± 0.01^{Dc}	8.74 ± 0.01^{Bb}	8.76 ± 0.01^{Dab}
WAG	8.80 ± 0.01^{ABa}	8.75 ± 0.06^{Ba}	8.80 ± 0.04^{Ba}	8.77 ± 0.01^{Ba}	8.78 ± 0.01^{Ca}

Values are expressed as mean ± Standard Deviation (SD). A-D = values with different superscripts in the same column are significantly different ($P < 0.05$); a-e = values with different superscripts in the same line are significantly different ($P < 0.05$). W = control; WA = D-allulose 1.2%; WG = β-glucan 1.2%; WAG = D-allulose 0.6% and β-glucan 0.6%.

Table 2. Physicochemical properties of whey beverage samples during storage.

	1	7	14	21	28
pH					
W	4.85 ± 0.01^{Aa}	4.63 ± 0.06A^{Bb}	4.63 ± 0.01^{Ab}	4.66 ± 0.01^{Bb}	4.41 ± 0.01^{Cc}
WA	4.87 ± 0.01^{Aa}	4.70 ± 0.01^{Abc}	4.63 ± 0.09^{Ac}	4.77 ± 0.01^{Aab}	4.74 ± 0.01^{Ab}
WG	4.83 ± 0.01^{Ba}	4.55 ± 0.02^{Bc}	4.53 ± 0.01^{Acd}	4.61 ± 0.01^{Cb}	4.49 ± 0.04^{Bd}
WAG	4.86 ± 0.02^{Aa}	4.64 ± 0.01^{Bc}	4.58 ± 0.01^{Ad}	4.68 ± 0.01^{Bb}	4.49 ± 0.02^{Be}
Dry mater					
W	17.69 ± 0.01^{Ba}	17.69 ± 0.04^{Ba}	17.67 ± 0.01^{Ba}	17.69 ± 0.01^{Ba}	17.70 ± 0.01^{Ba}
WA	18.65 ± 0.01^{Aa}	18.64 ± 0.01^{Ab}	18.63 ± 0.01^{Ab}	18.63 ± 0.01^{Ab}	18.65 ± 0.01^{Aa}
WG	18.65 ± 0.01^{Aa}	18.65 ± 0.01^{Aa}	18.65 ± 0.02^{Aa}	18.65 ± 0.02^{Aa}	18.66 ± 0.01^{Aa}
WAG	18.64 ± 0.01^{Aab}	18.63 ± 0.01^{Aab}	18.62 ± 0.02^{Ab}	18.64 ± 0.01^{Aab}	18.65 ± 0.01^{Aa}
Titratable acidity					
W	0.39 ± 0.01^{BCd}	0.54 ± 0.01^{Bb}	0.54 ± 0.01^{Db}	0.51 ± 0.01^{Bc}	0.71 ± 0.01^{Aa}
WA	0.38 ± 0.01^{Ce}	0.50 ± 0.01^{Db}	0.55 ± 0.01^{BCa}	0.42 ± 0.01^{Cd}	0.44 ± 0.01^{Cc}
WG	0.42 ± 0.02^{Ad}	0.60 ± 0.01^{Ab}	0.57 ± 0.01^{Abc}	0.55 ± 0.01^{Ac}	0.66 ± 0.01^{Ba}
WAG	0.41 ± 0.01^{ABd}	0.52 ± 0.01^{Cc}	0.57 ± 0.01^{ABb}	0.51 ± 0.01^{Bc}	0.66 ± 0.01^{Ba}
Total protein content					
W	1.81 ± 0.04^{Cc}	1.94 ± 0.02^{Bc}	2.21 ± 0.06^{Bab}	2.31 ± 0.07^{Ba}	2.12 ± 0.06^{Ab}
WA	1.90 ± 0.02^{Bc}	2.16 ± 0.01^{Ab}	2.26 ± 0.03^{ABb}	2.48 ± 0.07^{Aa}	2.21 ± 0.06^{Ab}
WG	1.97 ± 0.02^{Ae}	2.11 ± 0.04^{Ad}	2.33 ± 0.01^{Ab}	2.41 ± 0.03^{ABa}	2.21 ± 0.03^{Ac}
WAG	1.93 ± 0.01^{ABd}	2.15 ± 0.01^{Ac}	2.26 ± 0.02^{ABb}	2.37 ± 0.03^{ABa}	2.11 ± 0.07^{Ac}

Values are expressed as mean ± Standard Deviation (SD). A-D = values with different superscripts in the same column are significantly different ($P < 0.05$); a-e = values with different superscripts in the same line are significantly different ($P < 0.05$). W = control; WA = D-allulose 1.2%; WG = β-glucan 1.2%; WAG = D-allulose 0.6% and β-glucan 0.6%.

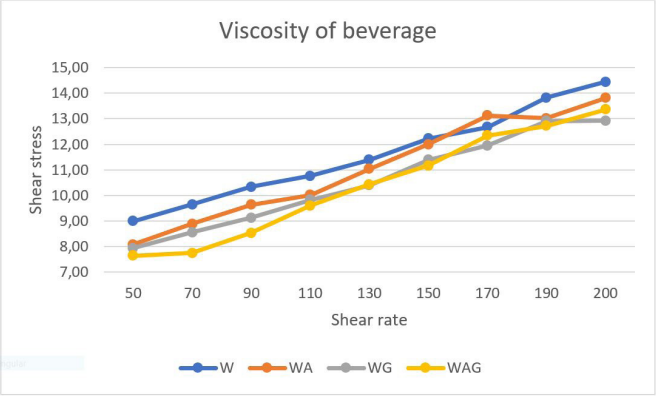

Figure 1. Shear stress versus shear rate for whey beverages. W= control; WA = D-allulose 1.2%; WG = β-glucan 1.2%; WAG = D-allulose 0.6% and β-glucan 0.6%.

the control group (9 mPa.s) (Figure 1). All drinks demonstrated an increase in viscosity with time, with the control group having the highest value (14.5). The similar trend of pH and viscosity was reported by Ryan & Foegeding (2015). When compared to the other groups, the WG and WAG groups, appeared to have the highest amount of protein (Table 2).

Table 3 shows the evaluation of the color parameters such as L^* (lightness), a^* (redness: green to red), and b^* (yellowness: blue to yellow) of the samples. Analyzing L^* parameter, control group (W) seemed to be darker meanwhile the highest L^* value was determined in group WG ($P > 0.05$). On the first day of storage, the L^*, a^* and b^* values of the control group were 58.16-8.03 and 8.55, respectively. After 28 days of shelf life, these values changed to 73.76-8.66 and 15.50. After 28 days of shelf life, these values changed as 73.76-8.66 and 15.50.

3.3 Sensory evaluation of whey beverage

In sensory evaluation of whey beverages prepared with β-glucan, D-allulose and *B. animalis,* control group (W) on the 14th day resulted to have the highest scores in terms of taste, aroma, color, texture, and general acceptability. Meanwhile, WG and WAG groups containing β-glucan received the lowest scores in terms of taste, texture and general acceptability. Kurtuldu & Ozcan (2018) reported that yoghurt samples containing β-glucan had a softer and rougher structure than the groups that did not contain β-glucan. This situation may be related with the secondary metabolites released because of synbiotic activities (Kurtuldu & Ozcan, 2018). In terms of general acceptability, it was seen that the group W and WA were not statistically different ($P > 0.05$). Results are related with the findings in other studies (Costa et al., 2020). Because of their functional and technical properties, integration in the food matrix of synbiotics may be of great interest for improving the texture of food products. Especially, prospects for the development of innovative functional foods with appealing sensory features that are accepted by consumers are created, in addition to the possibility of developing healthy products for persons with dietary restrictions (Guimarães et al., 2020). According to the sensory evaluation results, the most liked group was the group W, followed by the group WA. However, large-scale further research is needed in the evaluation of consumer perceptions in new functional products, as in the study of Silva et al. (2021).

While the second most liked group was WA, it was seen that the group W and WA were not statistically different in terms of general acceptability ($P > 0.05$). Similar results were found by Kim & Han (2019), where the overall acceptability score of soy yogurt made using allulose received the highest score when compared to the groups using less allulose.

3.4 Animal experiments

The effect of probiotic and prebiotics on body weight

The effect of the combined use of functional whey beverages enhanced with D-allulose and β-glucan containing *B. animalis* on some health and microbiological parameters in rats were investigated for 4 weeks. The body weight changes are shown in Table 4. The total weight gains of the groups were 70.4 ± 27.4, 58.5 ± 20.2, 63.3 ± 11.4, 63.9 ± 14.2, and 63.4 ± 29.3 g, respectively, over 28 days. Although the weight gain of the negative control (NC) group was higher than the groups containing functional beverages, no statistically significant difference was found ($P > 0.05$). Similarly, although the experimental group receiving only *B. animalis* (W) gained at least (58.5 ± 20.2) for 28 days, there was no statistically significant difference ($P > 0.05$). To the knowledge of the authors, no study was found to investigate in a beverage the prebiotic effects of both D-allulose and β-glucan together or separately in the same product. Therefore, this study is the first scientific research in this context. Choi et al. (2018) investigated the effects of D-allulose as a synbiotic mixture with *Lactobacillus sakei* LS03 and *Leuconostoc kimchii* GJ2 probiotics on diet-induced obese mice. At the end of the study, they reported that groups containing D-allulose and probiotic bacteria significantly reduced weight loss at the end of 12 weeks. In the current study, healthy rats were fed with synbiotic whey beverages. Therefore, it was necessary to comment on weight gain rather than weight loss.

Do et al. (2019) investigated the effects of D-allulose on impaired blood glucose and blood lipids with a high-fat diet in C57BL/6J mice and reported that the group containing D-allulose showed less weight gain than the control group. Ke et al. (2019) investigated the synbiotic effects of β-glucan in high cholesterol diet on wistar rats; it has been shown that rats fed with a synbiotic mixture containing β-glucan and *B. animalis* gained lower weight. However, in this study, unlike our study, the synbiotic mixture was given to the experimental animals as a supplement, not in the food matrix.

Table 3. Changing on color parameters of whey beverage samples during storage.

	1	28
L*		
W	58.16 ± 2.11^{Ab}	73.76 ± 0.5^{Aa}
WA	61.31 ± 3.54^{Ab}	73.17 ± 0.5^{ABa}
WG	63.85 ± 0.23^{Ab}	72.27 ± 0.5^{Ba}
WAG	60.80 ± 2.68^{Aa}	62.01 ± 0.5^{Ca}
a*		
W	-8.03 ± 0.02^{Bb}	-8.66 ± 0.05^{Ca}
WA	-8.46 ± 0.32^{Ba}	-9.19 ± 0.05^{Da}
WG	-7.00 ± 1.19^{Aa}	-7.17 ± 0.05^{Ba}
WAG	-7.13 ± 0.36^{Aa}	-6.86 ± 0.05^{Aa}
b*		
W	8.55 ± 0.20^{Ab}	15.50 ± 0.5^{Ba}
WA	10.16 ± 1.37^{Ab}	16.30 ± 0.10^{Aa}
WG	9.45 ± 0.70^{Ab}	13.79 ± 0.02^{Ca}
WAG	7.86 ± 1.26^{Aa}	10.20 ± 0.02^{Da}

Values are expressed as mean ± Standard Deviation (SD). A-D = values with different superscripts in the same column are significantly different ($P < 0.05$); a-e = values with different superscripts in the same line are significantly different ($P < 0.05$). W = control; WA = D-allulose 1.2%; WG = β-glucan 1.2%; WAG = D-allulose 0.6% and β-glucan 0.6%.

Biochemical analysis of blood samples

The blood glucose, lipids, AST-ALT enzymes, TAS and TOS capacity of the rats on the 14th and 28th days are shown in Table 5. When the mean plasma glucose levels the lowest and highest plasma glucose levels varied between 112.0 ± 3.0 and 123.3 ± 1.3, but there is no significant difference ($P > 0.05$). The evaluation of the plasma glucose levels revealed that this parameter remains regular for 4 weeks. The lack of significant differences between the groups in this study can be explained by the fact that healthy experimental animals were studied, and the plasma glucose levels were not impaired by diet or any drug.

Triglyceride and total cholesterol level examination revealed no statistically difference in mean triglyceride levels between the groups and between the days within the same group ($P > 0.05$). On the other hand, total cholesterol levels of the groups on the 14th day revealed that the highest total cholesterol level was in the negative control (NC) group, and the lowest total cholesterol level was in the group that received a synbiotic mixture containing D-allulose + β-glucan (WAG), and this difference was statistically significant ($P < 0.05$). At the end of the 4th week, the total cholesterol level was found to be highest

Table 4. Weight changes of the experimental groups during the study.

Parameters	Experimental Groups (Mean ± Std. Deviation)				
	NC	W	WA	WG	WAG
Initial body weight (g)	227.1 ± 30.8^{Ab}	243.4 ± 29.1^{Ab}	218.1 ± 26.4^{Ab}	231.1 ± 21.6^{Ac}	225.7 ± 32.5^{Ab}
7. day body weight (g)	255.7 ± 34.9^{Aab}	268.2 ± 31.2^{Aab}	240.4 ± 27.8^{Aab}	255.7 ± 22.1^{Abc}	252.6 ± 33.6^{Aab}
14. day body weight (g)	270.4 ± 38.1^{Aab}	286.5 ± 32.7^{Aa}	259.1 ± 30.1^{Aab}	275.8 ± 24.7^{Aab}	264.6 ± 36.0^{Aab}
21. day body weight (g)	285.3 ± 27.0^{Aa}	283.5 ± 20.3^{Aab}	271.0 ± 33.3^{Aa}	286.1 ± 22.9^{Aab}	285.2 ± 14.0^{Aa}
28. day body weight (g)	297.5 ± 27.0^{Aa}	301.9 ± 20.4^{Aa}	281.4 ± 33.5^{Aa}	295.1 ± 24.4^{Aa}	289.1 ± 19.8^{Aa}
Weight gain (g)	70.4 ± 27.4^{A}	58.5 ± 20.2^{A}	63.3 ± 11.4^{A}	63.9 ± 14.2^{A}	63.4 ± 29.3^{A}

Differences in analyzes between groups are shown in capital letters; differences in analyzes between days are shown in lowercase letters. NC = negative control; W = whey beverage; WA = whey+D-allulose; WG = whey+β-glucan; WAG = whey+D-allulose+β-glucan; g = gram; Std. = standart.

Table 5. Results of some biochemical parameter analyzes of the experimental groups.

Parameters	Day*	Experimental Groups (Mean ± Std. Deviation)				
		NC	W	WA	WG	WAG
Glucose (mg/dL)	14	112.0 ± 3.0^{Aa}	116.0 ± 2.0^{Aa}	114.0 ± 2.0^{Aa}	115.0 ± 5.0^{Aa}	113.3 ± 1.3^{Aa}
	28	115.0 ± 6.6^{Aa}	115.3 ± 0.0^{Aa}	118.0 ± 0.0^{Aa}	111.3 ± 4.7^{Aa}	123.7 ± 7.0^{Aa}
Triglyceride (mg/dL)	14	52.3 ± 21.6^{Aa}	42.7 ± 2.7^{Aa}	25.6 ± 6.7^{Aa}	46.1 ± 0.7^{Aa}	53.1 ± 14.3^{Aa}
	28	33.5 ± 7.5^{Aa}	36.0 ± 3.0^{Aa}	31.0 ± 3.0^{Aa}	46.5 ± 8.5^{Aa}	40.5 ± 4.5^{Aa}
Total cholesterol (mg/dL)	14	34.5 ± 5.6^{Aa}	34.3 ± 1.2^{ABa}	29.1 ± 3.5^{ABa}	32.0 ± 4.1^{ABa}	24.6 ± 2.5^{Ba}
	28	34.0 ± 2.0^{ABa}	31.0 ± 5.0^{Ba}	32.0 ± 3.0^{Aa}	32.5 ± 4.5^{Ba}	27.0 ± 3.0^{Ba}
AST (U/L)	14	91.5 ± 10.2^{Aa}	121.8 ± 1.4^{Aa}	81.9 ± 5.1^{Aa}	102.8 ± 32.9^{Aa}	134.4 ± 24.3^{Aa}
	28	217.1 ± 9.4^{Ab}	187.7 ± 32.7^{Aa}	191.4 ± 34.6^{Ab}	188.3 ± 18.1^{Aa}	143.4 ± 14.1^{Aa}
ALT (U/L)	14	53.8 ± 11.5^{Aa}	72.6 ± 13.9^{Aa}	70.8 ± 25.0^{Aa}	48.4 ± 6.7^{Aa}	61.0 ± 2.0^{Aa}
	28	57.4 ± 16.7^{Aa}	58.9 ± 10.6^{Aa}	56.8 ± 11.0^{Aa}	51.6 ± 3.3^{Aa}	53.4 ± 4.4^{Aa}
TAS (mmol/L)	14	1.1 ± 0.7^{Aa}	1.0 ± 0.2^{Aa}	0.8 ± 0.1^{Aa}	0.8 ± 0.1^{Aa}	0.7 ± 0.7^{Aa}
	28	1.0 ± 0.3^{Aa}	0.8 ± 0.3^{Aa}	1.0 ± 0.1^{Aa}	1.0 ± 0.1^{Ab}	1.6 ± 0.1^{Bb}
TOS (µmmol/L)	14	4.4 ± 0.8^{Aa}	7.6 ± 2.5^{Aa}	2.9 ± 0.6^{ABa}	9.6 ± 1.6^{BCa}	5.9 ± 0.7^{Ca}
	28	9.4 ± 5.3^{Aa}	3.8 ± 2.1^{Ab}	2.8 ± 0.7^{Aa}	2.5 ± 0.4^{ABb}	2.5 ± 0.1^{Bb}

*Mann Whitney U test was used. Differences in analyzes between groups are shown in capital letters; differences in analyzes between days are shown in lowercase letters. NC = negative control; W = whey control; WA = whey+D-allulose; WG = whey+β-glucan; WAG = whey+D-allulose+β-glucan; AST = aspartate aminotransferase; ALT = alanine aminotransferase; Std. = standart; mg/dL = milligram/deciliter; U/L = unite/liter; mmol/L = millimole/liter; µmmol/L = micromole/liter.

in the WA group and lowest in the WAG group. Although the effect of the synbiotic mixture containing D-allulose + β-glucan on total cholesterol levels is higher, the total cholesterol levels of all groups are lower than the reference values.

Regarding the administration of prebiotics, most meta-analyses revealed minor or no changes in body weight as well as improvements in total cholesterol level as in the present study (Vallianou et al., 2020). Colonization in the human gut of the Bifidobacteria, which has many beneficial health effects, prevents hypercholesterolemia (Rosburg et al., 2010). β-glucan and D-allulose commonly metabolized by Bifidobacterium reduce energy intake and lower cholesterol levels by altering the lipid metabolism and increasing the excretion of fecal lipids (Fernandez-Julia et al., 2021; Han et al., 2020). These effects were clearly demonstrated in the present study. Likewise, Guimarães et al. (2022) observed that a 15 days of prebiotic soursop whey beverage consumption was sufficient to cause significant alterations in healthy Wistar rats' health indices.

There was no statistically significant difference between the days in the TAS and TOS values ($P < 0.05$) (Table 5). When the TAS values are examined, it is seen that there is a decrease from the 14 to the 28th day in the NC and W groups, while there is an increase in the WA, WG and WAG groups. While there was an increase in TOS values in the NC group, there was a decrease in all other groups. As a result, it is thought that WA, WG and WAG groups increase TAS in experimental animals while decreasing TOS. Therefore, it can be said that D-allulose and β-glucan in beverages have antioxidant effects. Suna & Tokuda (2020) reported that D-allulose prevents phthalic acid-induced testicular damage by inhibiting reactive oxygen species. Alp et al. (2012) reported that β-glucan supplementation increased TAS levels and decreased TOS levels in diabetic rats. It is seen that the combined effect of D-allulose and β-glucan causes higher TAS level and lower TOS level compared to the groups in which they are administered separately.

Choi et al. (2018) studied the effects of D-allulose on the plasma lipid profile in their study on diet-induced obese rats using D-allulose as a synbiotic mixture. It has been reported that the plasma triglyceride, non-HDL and apolipoprotein A1 values of the group given *Lactobacillus sakei* LS03 and *Leuconostoc kimchii* GJ2 and D-allulose decreased significantly. It has been reported that supplementation of *Leuconostoc kimchii* GJ2 with D-allulose is associated with lower triglyceride and total cholesterol levels. As a result, it has been shown that D-allulose, which has anti-obesity effects, can be used in synbiotic mixtures. Do et al. (2019) investigated the effects of high-fat diet and high-fat diet containing D-allulose on blood glucose and blood lipids in C57BL/6J mice; They reported that plasma blood glucose, triglyceride, AST and ALT enzyme levels were significantly lower in the group containing D-allulose at the end of 8 weeks. In a study in which β-glucan was given to rats as a synbiotic mixture with *B. animalis*, it was reported that the synbiotic mixture reduced fasting blood sugar levels more than the control group and the groups in which prebiotics and probiotics were given separately.

Microbiological analysis of faeces

Table 6 displays the findings of microbiological assessment conducted on fecal samples collected from individuals of each group on the 7, 14, and 28th days. Except for the coliform counts in the WG group, the obtained findings were nearly similar for all parameters. On the 28th day, this group showed a statistically significant decline ($P < 0.05$). *E. coli* counts were found to be identical to coliform counts. The decrease in the WK and WG groups was statistically significant only on the 14th day, although it was discovered that this decline tended to increase again on the 28th day. Initially *Lactobacillus* spp. counts increased with the highest counts on the 14th day in all groups; but it started to decrease again on the 28th day. On the 7th day, the highest *Lactobacillus* spp. counts were found in the WA group, then in

Table 6. Microbiological analysis of fecal samples.

Parameters	Day	Experimental Groups (Mean ± Std. Deviation)				
		NC	W	WA	WG	WAG
Coliform	7	5.50 ± 0.07^{Ba}	5.71 ± 0.07^{ABa}	5.81 ± 0.02^{Aa}	5.42 ± 0.16^{Ba}	5.68 ± 0.15^{ABa}
	14	5.62 ± 0.15^{Aa}	5.71 ± 0.09^{Aa}	5.70 ± 0.15^{Aa}	5.57 ± 0.52^{Aa}	5.40 ± 0.28^{Aa}
	28	5.45 ± 0.29^{Aa}	5.51 ± 0.39^{Aa}	5.59 ± 0.23^{Aa}	5.09 ± 0.00^{Ab}	5.51 ± 0.46^{Aa}
E. coli	7	5.75 ± 0.12^{Aa}	5.80 ± 0.50^{Aa}	5.61 ± 0.20^{Aa}	5.71 ± 0.00^{Aa}	5.53 ± 0.82^{Aa}
	14	5.94 ± 0.04^{Aa}	5.29 ± 0.10^{Bb}	5.91 ± 0.53^{Aa}	5.09 ± 0.88^{Bb}	5.55 ± 0.38^{ABa}
	28	5.90 ± 0.50^{Aa}	5.79 ± 0.16^{Aa}	5.79 ± 0.11^{Aa}	5.40 ± 0.30^{Aa}	5.55 ± 0.34^{Aa}
***Lactobacillus* spp.**	7	8.44 ± 0.56^{Cb}	8.82 ± 0.88^{Bb}	9.00 ± 0.43^{Aa}	8.87 ± 0.23^{ABa}	8.92 ± 0.01^{ABa}
	14	9.42 ± 0.22^{Aa}	9.48 ± 0.25^{Aa}	9.36 ± 0.26^{Aa}	9.12 ± 0.81^{Aa}	9.15 ± 0.56^{Aa}
	28	8.50 ± 0.24^{Ab}	8.39 ± 0.17^{Ac}	8.30 ± 0.13^{Ab}	8.26 ± 0.20^{Ab}	8.38 ± 0.15^{Ab}
B. animalis	7	8.47 ± 0.67^{Bb}	8.88 ± 0.29^{Ab}	9.12 ± 0.15^{Aa}	8.94 ± 0.15^{Ab}	9.08 ± 0.13^{Ab}
	14	9.23 ± 0.14^{Ba}	9.39 ± 0.12^{ABa}	9.18 ± 0.53^{Ba}	9.24 ± 0.88^{Ba}	9.53 ± 0.96^{Aa}
	28	8.33 ± 0.84^{ABb}	8.45 ± 0.54^{Ac}	8.10 ± 0.17^{Cb}	8.16 ± 0.52^{Cc}	8.94 ± 0.59^{BCc}
***Lactococcus* spp.**	7	6.97 ± 0.16^{Aa}	7.31 ± 0.21^{Aa}	7.03 ± 0.00^{ABa}	6.90 ± 0.04^{ABa}	7.63 ± 0.45^{Ba}
	14	7.89 ± 0.02^{Ab}	7.79 ± 0.96^{Aa}	7.93 ± 1.02^{Aa}	8.17 ± 0.39^{Ab}	7.80 ± 0.07^{Aa}
	28	7.05 ± 0.39^{Aa}	7.57 ± 0.70^{Aa}	7.03 ± 0.07^{Aa}	8.72 ± 0.07^{Bb}	8.69 ± 0.0^{Bb}
***Streptococcus* spp.**	7	8.09 ± 0.03^{Aa}	8.91 ± 0.17^{Ba}	8.91 ± 0.17^{Ba}	8.76 ± 0.1^{Ba}	8.92 ± 0.10^{Bab}
	14	8.36 ± 0.13^{Aa}	8.77 ± 0.16^{Aa}	8.71 ± 0.07^{Aa}	8.80 ± 0.43^{Aa}	8.71 ± 0.17^{Aab}
	28	8.61 ± 0.39^{A}	8.01 ± 0.06^{Ab}	8.04 ± 0.04^{ABb}	9.16 ± 0.32^{Ba}	9.21 ± 0.20^{Bac}

Differences in analyzes between groups are shown in capital letters; differences in analyzes between days are shown in lowercase letters. NC = negative control; W = whey control; WA = whey+D-allulose; WG = whey+β-glucan; WAG = whey+D-allulose+β-glucan; Std. = standart.

the WAG and WG groups. *B. animalis* used as a probiotic in the study was found to be in the lowest NK group, which is an expected result.

4 Conclusion

Although the health benefits of β-glucan, D-allulose and Bifidobacterium species have been demonstrated in vivo and in vitro studies, no study has been conducted on a diet modeling using all of them at once, and on revealing their effects on experimental animals. For this purpose, a functional whey beverage was prepared by using these components in different combinations and the effects of this beverage on some health parameters were investigated on a rat model.

In view of obtained data we can safely say that the whey beverage prepared with the combination of β-glucan, D-allulose and Bifidobacterium is a high-quality product in terms of the tested parameters. The obtained results revealed that whey beverage containing these components is a candidate to play an important role in promoting health and protecting against diseases, especially for lowering total cholesterol, improving blood lipid profile, reducing, or maintaining body weight.

Conflict of interest

The authors report no conflict of interest.

Author contributions

During the study's preparation, all authors contributed equally.

Funding

This study received no financial support.

References

Ahmad, A., Anjum, F. M., Zahoor, T., Nawaz, H., & Dilshad, S. M. R. (2012). Beta glucan: a valuable functional ingredient in foods. *Critical Reviews in Food Science and Nutrition*, 52(3), 201-212. http://dx.doi.org/10.1080/10408398.2010.499806. PMid:22214441.

Al Saqqa, G. S. (2021). Some functional foods and benefits of their bioactive components. *Journal of the Saudi Society for Food and Nutrition*, 14(1), 1-11.

Ale, E. C., Batistela, V. A., Olivar, G. C., Ferrado, J. B., Sadiq, S., Ahmed, H. I., Reinheimer, J. A., Vera-Candioti, L., Laws, A. P., & Binetti, A. G. (2020). Statistical optimisation of the exopolysaccharide production by Lactobacillus fermentum Lf2 and analysis of its chemical composition. *International Journal of Dairy Technology*, 73(1), 76-87. http://dx.doi.org/10.1111/1471-0307.12639.

Alp, H., Varol, S., Celik, M. M., Altas, M., Evliyaoglu, O., Tokgoz, O., Tanrıverdi, M. H., & Uzar, E. (2012). Protective effects of beta glucan and gliclazide on brain tissue and sciatic nerve of diabetic rats induced by streptozosin. *Experimental Diabetes Research*, 2012, 230342. http://dx.doi.org/10.1155/2012/230342. PMid:22291696.

Chavan, R. S., Shraddha, R. C., Kumar, A., & Nalawade, T. (2015). Whey based beverage: its functionality, formulations, health benefits and applications. *Journal of Food Processing & Technology*, 6(10), 1-8.

Choi, B.-R., Kwon, E.-Y., Kim, H.-J., & Choi, M.-S. (2018). Role of synbiotics containing D-allulose in the alteration of body fat and hepatic lipids in diet-induced obese mice. *Nutrients*, 10(11), 1797. http://dx.doi.org/10.3390/nu10111797. PMid:30463250.

Chung, M. Y., Oh, D. K., & Lee, K. W. (2012). Hypoglycemic health benefits of d-psicose. *Journal of Agricultural and Food Chemistry*, 60(4), 863-869. http://dx.doi.org/10.1021/jf204050w. PMid:22224918.

Colantonio, A. G., Werner, S. L., & Brown, M. (2020). The effects of prebiotics and substances with prebiotic properties on metabolic and inflammatory biomarkers in individuals with type 2 diabetes mellitus: a systematic review. *Journal of the Academy of Nutrition*

and Dietetics, 120(4), 587-607.E2. http://dx.doi.org/10.1016/j.jand.2018.12.013. PMid:30827823.

Costa, G. M., Paula, M. M., Costa, G. N., Esmerino, E. A., Silva, R., Freitas, M. Q., Barão, C. E., Cruz, A. G., & Pimentel, T. C. (2020). Preferred attribute elicitation methodology compared to conventional descriptive analysis: a study using probiotic yogurt sweetened with xylitol and added with prebiotic components. *Journal of Sensory Studies*, 35(6), e12602. http://dx.doi.org/10.1111/joss.12602.

Dave, R. I., & Shah, N. P. (1996). Evaluation of media for selective enumeration of Streptococcus thermophilus, Lactobacillus delbrueckii ssp. bulgaricus, Lactobacillus acidophilus, and Bifidobacteria. *Journal of Dairy Science*, 79(9), 1529-1536. http://dx.doi.org/10.3168/jds.S0022-0302(96)76513-X. PMid:8899517.

Delgado-Fernández, P., Hernández-Hernández, O., Olano, A., Moreno, F. J., & Corzo, N. (2020). Probiotic viability in yoghurts containing oligosaccharides derived from lactulose (OsLu) during fermentation and cold storage. *International Dairy Journal*, 102, 104621. http://dx.doi.org/10.1016/j.idairyj.2019.104621.

Dinkçi, N., Akdeniz, V., & Akalin, A. S. (2019). Survival of probiotics in functional foods during shelf life. In C. M. Galanakis (Ed.), *Food quality and shelf life* (pp. 201-233). London: Academic Press. http://dx.doi.org/10.1016/B978-0-12-817190-5.00006-9.

Do, G. Y., Kwon, E.-Y., Kim, Y. J., Han, Y., Kim, S.-B., Kim, Y. H., & Choi, M.-S. (2019). Supplementation of non-dairy creamer-enriched high-fat diet with D-allulose ameliorated blood glucose and body fat accumulation in C57BL/6J mice. *Applied Sciences*, 9(13), 2750. http://dx.doi.org/10.3390/app9132750.

Dolci, P., Ferrocino, I., Giordano, M., Pramotton, R., Vernetti-Prot, L., Zenato, S., & Barmaz, A. (2020). Impact of Lactococcus lactis as starter culture on microbiota and metabolome profile of an Italian raw milk cheese. *International Dairy Journal*, 110, 104804. http://dx.doi.org/10.1016/j.idairyj.2020.104804.

Farias, D. P., Araújo, F. F., Neri-Numa, I. A., & Pastore, G. M. (2019). Prebiotics: trends in food, health and technological applications. *Trends in Food Science & Technology*, 93, 23-35. http://dx.doi.org/10.1016/j.tifs.2019.09.004.

Fernandez-Julia, P. J., Munoz-Munoz, J., & van Sinderen, D. (2021). A comprehensive review on the impact of β-glucan metabolism by Bacteroides and Bifidobacterium species as members of the gut microbiota. *International Journal of Biological Macromolecules*, 181, 877-889. http://dx.doi.org/10.1016/j.ijbiomac.2021.04.069. PMid:33864864.

Gassem, M. A., & Frank, J. F. (1991). Physical properties of yogurt made from milk treated with proteolytic enzymes. *Journal of Dairy Science*, 74(5), 1503-1511. http://dx.doi.org/10.3168/jds.S0022-0302(91)78310-0.

Gibson, G. R., Hutkins, R., Sanders, M. E., Prescott, S. L., Reimer, R. A., Salminen, S. J., Scott, K., Stanton, C., Swanson, K. S., Cani, P. D., Verbeke, K., & Reid, G. (2017). Expert consensus document: the International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nature Reviews Gastroenterology & Hepatology*, 14(8), 491-502. http://dx.doi.org/10.1038/nrgastro.2017.75. PMid:28611480.

Guimarães, J. T., Almeida, P. P., Brito, M. L., Cruz, B. O., Costa, N. S., Ito, R. V. A., Mota, J. C., Bertolo, M. R. V., Morais, S. T. B., Cucinelli, R. P. No., Tavares, M. I. B., Souto, F., Bogusz, S. Jr., Pimentel, T. C., Stockler-Pinto, M. B., Freitas, M. Q., & Cruz, A. G. (2022). In vivo functional and health benefits of a prebiotic soursop whey beverage processed by high-intensity ultrasound: study with healthy Wistar rats. *Food Chemistry*, 380, 132193. http://dx.doi.org/10.1016/j.foodchem.2022.132193. PMid:35093650.

Guimarães, J. T., Balthazar, C. F., Silva, R., Rocha, R. S., Graça, J. S., Esmerino, E. A., Silva, M. C., Sant'Ana, A. S., Duarte, M. C. K. H., Freitas, M. Q., & Cruz, A. G. (2020). Impact of probiotics and prebiotics on food texture. *Current Opinion in Food Science*, 33, 38-44. http://dx.doi.org/10.1016/j.cofs.2019.12.002.

Guimarães, J. T., Silva, E. K., Alvarenga, V. O., Costa, A. L. R., Cunha, R. L., Sant'Ana, A. S., Freitas, M. Q., Meireles, M. A. A., & Cruz, A. G. (2018). Physicochemical changes and microbial inactivation after high-intensity ultrasound processing of prebiotic whey beverage applying different ultrasonic power levels. *Ultrasonics Sonochemistry*, 44, 251-260. http://dx.doi.org/10.1016/j.ultsonch.2018.02.012. PMid:29680610.

Guimarães, J. T., Silva, E. K., Ranadheera, C. S., Moraes, J., Raices, R. S. L., Silva, M. C., Ferreira, M. S., Freitas, M. Q., Meireles, M. A. A., & Cruz, A. G. (2019). Effect of high-intensity ultrasound on the nutritional profile and volatile compounds of a prebiotic soursop whey beverage. *Ultrasonics Sonochemistry*, 55, 157-164. http://dx.doi.org/10.1016/j.ultsonch.2019.02.025. PMid:30853535.

Han, Y., Han, H. J., Kim, A.-H., Choi, J.-Y., Cho, S.-J., Park, Y. B., Jung, U. J., & Choi, M.-S. (2016). D-allulose supplementation normalized the body weight and fat-pad mass in diet-induced obese mice via the regulation of lipid metabolism under isocaloric fed condition. *Molecular Nutrition & Food Research*, 60(7), 1695-1706. http://dx.doi.org/10.1002/mnfr.201500771. PMid:26920079.

Han, Y., Park, H., Choi, B.-R., Ji, Y., Kwon, E.-Y., & Choi, M.-S. (2020). Alteration of microbiome profile by D-allulose in amelioration of high-fat-diet-induced obesity in mice. *Nutrients*, 12(2), 352. http://dx.doi.org/10.3390/nu12020352. PMid:32013116.

International Organization for Standardization – ISO. (2003). *ISO 4833:2003. Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of microorganisms — Colony-count technique at 30 C*. Geneva: ISO.

International Organization for Standardization – ISO. (2006). *ISO 4832:2006. Microbiology of food and animal feeding stuffs — horizontal method for the enumeration of coliforms — colony-count technique.* Geneva: ISO.

International Organization for Standardization – ISO. (2008). *ISO 21527-1:2008. Microbiology of food and animal feeding stuffs — horizontal method for the enumeration of yeasts and moulds — part 1: colony count technique in products with water activity greater than or equal to 0,95*. Geneva: ISO.

International Organization for Standardization – ISO. (2016). *ISO 13299:2016. Sensory analysis – methodology – general guidance for establishing a sensory profile.* Geneva: ISO.

International Organization for Standardization – ISO. (2019). *ISO 17410:2019. Microbiology of the food chain — horizontal method for the enumeration of psychrotrophic microorganisms*. Geneva: ISO.

Itoh, K., Mizuno, S., Hama, S., Oshima, W., Kawamata, M., Hossain, A., Ishihara, Y., & Tokuda, M. (2015). Beneficial effects of supplementation of the rare sugar "D-allulose" against hepatic steatosis and severe obesity in Lep^{ob}/Lep^{ob} mice. *Journal of Food Science*, 80(7), H1619-H1626. http://dx.doi.org/10.1111/1750-3841.12908. PMid:26012374.

Ke, X., Walker, A., Haange, S. B., Lagkouvardos, I., Liu, Y., Schmitt-Kopplin, P., von Bergen, M., Jehmlich, N., He, X., Clavel, T., & Cheung, P. C. K. (2019). Synbiotic-driven improvement of metabolic disturbances is associated with changes in the gut microbiome in diet-induced obese mice. *Molecular Metabolism*, 22, 96-109. http://dx.doi.org/10.1016/j.molmet.2019.01.012. PMid:30792016.

Kim, H. J., & Han, M. J. (2019). The fermentation characteristics of soy yogurt with different content of d-allulose and sucrose fermented by lactic acid bacteria from Kimchi. *Food Science and Biotechnology*,

28(4), 1155-1161. http://dx.doi.org/10.1007/s10068-019-00560-5. PMid:31275715.

Kristensen, D., Orlien, V., Mortensen, G., Brockhoff, P., & Skibsted, L. H. (2000). Light-induced oxidation in sliced havarti cheese packaged in modified atmosphere. *International Dairy Journal*, 10(1-2), 95-103. http://dx.doi.org/10.1016/S0958-6946(00)00028-5.

Kurtuldu, O., & Ozcan, T. (2018). Effect of β-glucan on the properties of probiotic set yoghurt with Bifidobacterium animalis subsp. lactis strain Bb-12. *International Journal of Dairy Technology*, 71, 157-166. http://dx.doi.org/10.1111/1471-0307.12414.

Man, J. C., Rogosa, M., & Sharpe, M. E. (1960). A medium for the cultivation of lactobacilli. *The Journal of Applied Bacteriology*, 23(1), 130-135. http://dx.doi.org/10.1111/j.1365-2672.1960.tb00188.x.

Mohanty, D., Misra, S., Mohapatra, S., & Sahu, P. S. (2018). Prebiotics and synbiotics: recent concepts in nutrition. *Food Bioscience*, 26, 152-160. http://dx.doi.org/10.1016/j.fbio.2018.10.008.

Nordlund, E., Aura, A. M., Mattila, I., Kössö, T., Rouau, X., & Poutanen, K. (2012). Formation of phenolic microbial metabolites and short-chain fatty acids from rye, wheat, and oat bran and their fractions in the metabolical in vitro colon model. *Journal of Agricultural and Food Chemistry*, 60(33), 8134-8145. http://dx.doi.org/10.1021/jf3008037. PMid:22731123.

Quigley, E. M. M. (2019). Prebiotics and probiotics in digestive health. *Clinical Gastroenterology and Hepatology*, 17(2), 333-344. http://dx.doi.org/10.1016/j.cgh.2018.09.028. PMid:30267869.

Roberfroid, M. B., van Loo, J. A. E., & Gibson, G. R. (1998). The bifidogenic nature of chicory inulin and its hydrolysis products. *The Journal of Nutrition*, 128(1), 11-19. http://dx.doi.org/10.1093/jn/128.1.11. PMid:9430596.

Rosburg, V., Boylston, T., & White, P. (2010). Viability of Bifidobacteria strains in yogurt with added oat beta-glucan and corn starch during cold storage. *Journal of Food Science*, 75(5), C439-C444. http://dx.doi.org/10.1111/j.1750-3841.2010.01620.x. PMid:20629865.

Ryan, K. N., & Foegeding, E. A. (2015). Formation of soluble whey protein aggregates and their stability in beverages. *Food Hydrocolloids*, 43, 265-274. http://dx.doi.org/10.1016/j.foodhyd.2014.05.025.

Sawicki, C. M., Livingston, K. A., Obin, M., Roberts, S. B., Chung, M., & McKeown, N. M. (2017). Dietary Fiber and the human gut microbiota: application of evidence mapping methodology. *Nutrients*, 9(2), 125. http://dx.doi.org/10.3390/nu9020125. PMid:28208609.

Shen, R. L., Dang, X. Y., Dong, J. L., & Hu, X. Z. (2012). Effects of oat β-glucan and barley β-glucan on fecal characteristics, intestinal microflora, and intestinal bacterial metabolites in rats. *Journal of Agricultural and Food Chemistry*, 60(45), 11301-11308. http://dx.doi.org/10.1021/jf302824h. PMid:23113683.

Silva, W. P., Pimentel, T. C., Silva, C. B. F., Pagani, M. M., Cruz, A. G., Freitas, M. Q., & Esmerino, E. A. (2021). Story completion technique: a useful methodology to evaluate the risk perception of consumers from different regions of Brazil about cheeses sold at open markets. *Journal of Sensory Studies*, 36(6), e12702. http://dx.doi.org/10.1111/joss.12702.

Siró, I., Kápolna, E., Kápolna, B., & Lugasi, A. (2008). Functional food. Product development, marketing and consumer acceptance—a review. *Appetite*, 51(3), 456-467. http://dx.doi.org/10.1016/j.appet.2008.05.060. PMid:18582508.

Suna, S., & Tokuda, M. (2020). Effects of D-allose and D-allulose on DEHP toxicities in rats. *International Journal of Pharmaceutical Sciences and Research*, 11, 21-26.

Tsermoula, P., Khakimov, B., Nielsen, J. H., & Engelsen, S. B. (2021). Whey - the waste-stream that became more valuable than the food product. *Trends in Food Science & Technology*, 118, 230-241. http://dx.doi.org/10.1016/j.tifs.2021.08.025.

Vallianou, N., Stratigou, T., Christodoulatos, G. S., Tsigalou, C., & Dalamaga, M. (2020). Probiotics, prebiotics, synbiotics, postbiotics, and obesity: current evidence, controversies, and perspectives. *Current Obesity Reports*, 9(3), 179-192. http://dx.doi.org/10.1007/s13679-020-00379-w. PMid:32472285.

Williams, S. (1984). *Official methods of analysis of the Association of official analytical chemists* (14th ed.). Arlington: Association of official analytical chemists.

Yadav, J. S. S., Yan, S., Pilli, S., Kumar, L., Tyagi, R. D., & Surampalli, R. Y. (2015). Cheese whey: a potential resource to transform into bioprotein, functional/nutritional proteins and bioactive peptides. *Biotechnology Advances*, 33(6 Part 1), 756-774. http://dx.doi.org/10.1016/j.biotechadv.2015.07.002. PMid:26165970.

Zanon, E. O., Pimentel, T. C., Gomez, R. J. H. C., & Fagnani, R. (2020). Development of a whey protein spread enriched with β-glucan: an alternative for whey valorization. *Journal of the Science of Food and Agriculture*, 100(4), 1711-1717. http://dx.doi.org/10.1002/jsfa.10186. PMid:31803934.

Zendeboodi, F., Khorshidian, N., Mortazavian, A. M., & Cruz, A. G. (2020). Probiotic: conceptualization from a new approach. *Current Opinion in Food Science*, 32, 103-123. http://dx.doi.org/10.1016/j.cofs.2020.03.009.