

# Sensorial Evaluation and Physical Chemical Characterization of a Beverage Based on Whey and $\beta$ -Glucans as a Potential Prevention of Nonalcoholic Steatohepatitis

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## Abstract

Whey and B-glucans are functional food ingredients that contribute to the prevention of non-communicable diseases. The objective of the study was to develop a functional beverage based on whey, B-glucans, and blackberry concentrate for the prevention of nonalcoholic steatohepatitis, determining its sensory, physicochemical, and nutritional qualities. The following phases were developed: 1) formulation with 75%, 85%, and 90% whey and 25%, 15%, and 10% blackberry concentrate, with a fixed amount of 0.243 g  $\beta$ -glucans in 100 ml; 2) microbiological analysis to ensure the safety of the beverage; 3) sensory analysis with untrained panelists to assess the degree of preference (appearance, color, odor, flavor, viscosity, acidity, sweetness), and general acceptance, with a seven-point hedonic scale to rate the level of acceptability; 4) physicochemical and nutritional characterization of the beverage with the highest acceptance. An experimental design using complete random blocks was employed. Data analysis was performed with variance (ANDEVA) and mean DUNCAN separation at a 95% confidence level, using SAS<sup>®</sup>. A correlation study was conducted on acceptance analysis. A nutritional label was developed for the control beverage and the treatment with the highest acceptance. The formulation with 75% whey, 25% blackberry concentrate, and 0.243 g of  $\beta$ -glucan obtained the highest acceptance, with flavor and sweetness being the attributes that contributed to its acceptance. Nutritionally, the beverage with 80 cal, would be considered fortified with fiber (2 g), free of cholesterol, sodium, and saturated fat, according to the Central American Technical Regulation on Nutritional Labeling (Version 67.01.60:10). It is recommended that the effect of this beverage be measured to determine the

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benefits.

## Keywords

Fatty Acids, Fatty Liver, Glucans, Whey

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## 1. Introduction

In recent decades, nonalcoholic steatohepatitis (NASH) has become the leading cause of liver disease in the world, affecting 25% of the population [1]-[3]. NASH is a clinically distinguished syndrome characterized by lipid accumulation and macro-vesicular changes, greater than 5% of total liver weight [4] [5]. The severity and incidence of NASH are related to body mass index (BMI), waist circumference, hyperinsulinemia, hypertriglyceridemia, cholesterol, obesity, type 2 diabetes, and insulin resistance [6] [7]; where obesity is considered the main cause, so far, studies have shown that 76% of people with this disease did not consume alcohol but were obese [8]. Physical activity and a healthy diet control the prevalence of NASH, especially the consumption of dietary fibers,  $\beta$ -glucans, and micronutrients, anthocyanins, and vitamins [9]. The development of functional products with  $\beta$ -glucans constitutes a favorable option to combat the prevalence of diseases such as NASH.

The food industry plays a fundamental role in the nutrition of people, so it is crucial that it contributes to the creation and innovation of products that favor the prevention of disease, especially in the context of conditions such as NASH. As NASH has emerged as the leading cause of liver disease worldwide with a prevalence of 25% in the population, it has become increasingly important to explore how the food industry can address NASH-related health concerns [10]. Functional foods, known for their beneficial and nutritional effects, offer a promising avenue for improving health and preventing diseases [11]. Several investigations have highlighted the importance of different natural ingredients, such as whey and  $\beta$ -glucans, which are extraordinarily useful in disease prevention [12].

Whey, a byproduct of cheese production, not only has economic and environmental advantages, but also offers potential health benefits, including a connection to NASH and other health problems. Whey is typically discarded during cheese making, leading to economic losses and environmental pollution [13]. In the cheese industry, a significant portion of milk, approximately 85% - 95%, is disposed of as whey [14]. Interestingly, whey retains around 55% of total milk ingredients, such as lactose, soluble proteins, lipids, and mineral salts [15] [16]. These components found in whey have been associated with various health benefits. For example, they have the potential to prevent cardiovascular disease, lower triglyceride levels, and improve glucose intake tolerance [17]. Moreover, whey can serve as a valuable raw material to make various products. By incorporating other compounds such as  $\beta$ -glucans, polyphe-

nols, vitamins, and minerals, the health benefits of whey can be further enhanced, particularly in the context of the prevention of non-communicable diseases such as NASH. This is a liver condition closely related to metabolic health and is often associated with conditions such as obesity and insulin resistance. The beneficial components in whey may contribute to improving metabolic health and potentially aid in the prevention or management of NASH and related health problems. Therefore, the potential health benefits of whey, such as its positive impact on cardiovascular health and glucose tolerance, can be harnessed while simultaneously addressing environmental concerns and reducing waste in the cheese industry. Furthermore, the incorporation of additional health-promoting compounds can extend the potential benefits of whey, including its role in the prevention of non-communicable diseases such as NASH.

$\beta$ -glucans are glucose polymers that act as intestinal fiber and are naturally found in sources such as bacteria, cereals, yeasts, and fungi [18]. The beneficial effects of  $\beta$ -glucans depend on the molecular weight and source, and will affect the physical properties, solubility and viscosity when present in a food [19]. Consumption of  $\beta$ -glucans is associated with hypolipidemic, hypoglycemic, satiating, and prebiotic characteristics; consequently, they act by reducing the glycemic index and serum cholesterol [20] [21]. It has been shown that  $\beta$ -glucans, which come from the *Ganoderma lucidum* fungus, have been shown to improve insulin sensitivity, metabolic complications, and promote the balance of cholesterol levels [22] [23]. These bioactive compounds have been associated with reduced levels of low-density cholesterol (LDL), which contributes to overweight and obesity in the population [23].

Similarly, blackberries possess anthocyanins that have a beneficial effect in the prevention of diabetes, cancer, inflammation, cardiovascular disease, and obesity [24]. This is because these polyphenol nutrients improve glucose and lipid metabolism by increasing antioxidant and anti-inflammatory activities and regulating the intestinal microbiota [25]. Therefore, bioactive compounds such as  $\beta$ -glucans and anthocyanins are postulated as a solution for the prevention of some diseases through foods containing them.

Regular consumption of functional foods is an effective alternative for its prevention taking advantage of co-products from the cheese industry and bioactive compounds present in foods [26]. Therefore, the consumption of a beverage with whey and  $\beta$ -glucans can contribute to the prevention of NASH and the mitigation of environmental impact, working particularly in accordance with the Sustainable Development Goals (SDG) of “Climate Action” (SDG 13) and “Health and Well-being” (SDG 3). Thus, the specific objectives set for this study are to determine the formulation and sensory acceptance of a functional beverage with whey,  $\beta$ -glucans and blackberry concentrate, assess the physicochemical properties of the beverage, and evaluate the nutritional quality of the most preferred beverage based on sensory analysis.

## 2. Materials and Methods

### 2.1. Preparation of the Beverage

Three treatments were carried out with the same amount (grams) of  $\beta$ -glucans, obtained from the *Ganoderma lucidum* fungus, but with different proportions of sweet whey, with a pH of  $5 \pm 0.5$  and  $7 \pm 1$  °Brix, and blackberry concentrate, with a pH of  $1.5 \pm 0.5$  and  $40 \pm 1$  °Brix. A control treatment without  $\beta$ -glucans and with water was also used. To define the amount of  $\beta$ -glucans, the recommendation of Calderon (2022) was used, who estimated that a health benefit will be achieved by consuming 3.56 mg of  $\beta$ -glucans per kilogram of body weight. Equation (1) was used to define the number of  $\beta$ -glucans that a 100 ml serving of beverage should contain [27]. The average weight of the target population used was 68.14 kg, in this case university students between 18 and 26 years of age [28], who were asked to perform the sensory evaluation of the beverage.

$$\text{Daily dose (g)} = \frac{\text{mg of } \beta\text{-glucans}}{\text{kg}} \times \text{kg of body weight} \quad (1)$$

With data substitution to obtain the equivalent daily dose:

$$\begin{aligned} \text{Daily dose (g)} &= 3.56 \frac{\text{mg of } \beta\text{-glucans}}{\text{kg}} \times 68.14 \text{ kg} \\ &= 242.58 \text{ mg of } \beta\text{-glucans} \end{aligned} \quad (2)$$

To prepare the treatments (T1, T2, T3), 75, 85 and 90 ml of whey were mixed for 2 minutes with 25, 15 and 10 ml of blackberry concentrate, respectively, and the  $\beta$ -glucans were dissolved. Also, 75 ml of water was mixed with 25 ml of blackberry concentrate for control. All preparations were pasteurized in an InstantPot Duo-Nova slow cooker model at 65°C for 30 min. The beverages were then cooled by being exposed to 10°C with a water bath in cold water and ice for 10 minutes. Finally, the samples were stored in polyethylene terephthalate plastic bottles in the refrigerator.

### 2.2. Microbiological Analysis

According to the Peruvian Sanitary Standard [29], the International Commission on Microbiological Specifications for Foods (ICMSF) [30] and the Central American Technical Regulation (RTCA) 67.04.50:17 [31], it was imperative to perform microbiological tests for aerobic mesophilic bacteria (AMB), total coliforms (tC), and molds and yeasts (MY) to ensure the safety of the beverage before proceeding with sensory analysis. To achieve this, 10 ml of each sample was placed in a sterile bag with 90 ml of phosphate buffer solution and homogenized for 2 minutes using the Stomacher IUL instrument. This process produced a dilution of 10<sup>1</sup>, which was further diluted to obtain dilutions of 10<sup>2</sup> and 10<sup>3</sup>. For the analysis of AMB, 1 ml of the dilution was placed in a Petri dish and 15 ml of Agar Standard Count (ACE) culture medium was added, followed by incubation at 25°C for 2 days.

For tC, the most probable number method was used, where 1 ml of each dilu-

tion was added to a test tube containing 9 ml of Lauryl Tryptose Broth (LTC) and then incubated at 25°C for 24 and 48 hours. MY analysis used the pour plate method, using only the 10-fold dilution, which was plated onto a Petri dish. Subsequently, 15 ml of Rose Bengal Agar culture was added and the samples were incubated for 5 days at 25°C.

### 2.3. Sensory Analysis

An untrained sensory panel composed of 100 individuals (university students between the ages of 18 and 26 years) was assembled for sensory evaluation. Each panelist received the four samples, including each treatment and control, along with a ballot for evaluation. An acceptance analysis was performed to assess the degree of preference for the attributes of appearance, color, odor, flavor, viscosity, acidity, sweetness, and general acceptance. The panelists used a seven-point hedonic scale to rate the level of acceptability. An experimental design using complete random blocks (RCB) was employed and data analysis was performed using the SAS® program, using analysis of variance (ANDEVA) and mean DUNCAN separation at a 95% confidence level. Furthermore, a correlation study was conducted on acceptance analysis.

### 2.4. Physical-Chemical Analysis and Nutritional Quality

Soluble solids, pH, viscosity, and color analyzes were performed on all treatments, while the nutritional label (**Table 1**) was exclusively prepared for control and treatment with the highest acceptance, based on the results of the sensory analysis.

**Table 1.** Physical-chemical and nutritional quality properties evaluated of the beverage.

Analysis	Property	Type of analysis
Physical and chemical	Color	AN 1018.00 [32]
	pH	AOAC 981.12 [33]
	Total soluble solids (°Brix)	AOAC 983.17 [34]
	Viscosity	ISO 1652:2004 [35]
	Moisture	AOAC 945.15 [36]
	Ash	AOAC 923.03 [37]
Nutritional	Protein	AOAC 2001.11 [38]
	Carbohydrates	CFR 101.45 [39]
	Dietary fiber	AOAC 985.29 [40]
	Fat	AOAC 983.23-1984 [41]
	Fatty acid profile	AOCS Ce 2b-11 [42] & Ce 2-66 [43]
	Sugars	AOAC 982.14 [44]
	Minerals	AOAC 923.03 [45] & AOAC 985.35 [46]

AOAC: Association of Official Analytical Chemists; ISO: International Organization for Standardization. AOCS: American Oil Chemists Society; CFR: Code of Federal Regulations.

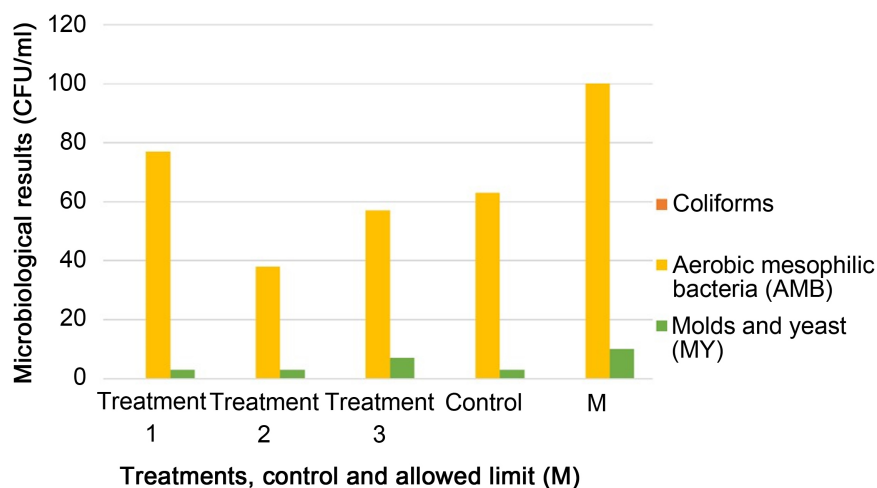
For attributes such as pH, total solids, color, and viscosity, a completely randomized experimental design (CRD) was used to evaluate the characteristics of all samples. Data were analyzed in the SAS® program using ANDEVA analysis and DUNCAN mean separation with a significance level of 95%. Specifically, the treatment with the highest acceptance and the control were considered, totaling two variables and two replications, resulting in four experimental units.

A nutritional label was developed for the control beverage and the treatment with the highest acceptance to determine the nutritional quality of the beverage, following the guidelines outlined in the Central American Technical Regulation (RTCA) on Nutritional Labeling Version 67.01.60:10 [47].

### 3. Results

#### 3.1. Microbiological Analysis

As shown in **Figure 1**, the results of the microbiological analysis were considered satisfactory since they did not exceed the allowed limits (M) for each of the microorganisms examined. To ascertain the presence of coliforms, observations were made across various dilutions in each of the test tubes, and no gas production was evident in the Durham hood for any of the dilutions in the samples. Consequently, all results were recorded as indicating the absence of coliforms.



**Figure 1.** Results of microbiological analysis of each treatment (T1, T2, T3) and control of the beverage and the allowed limit (M).

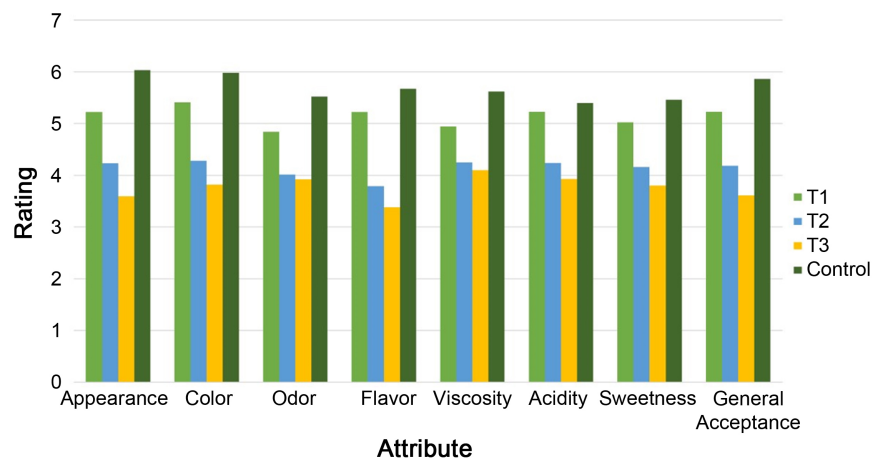
For the determination of aerobic mesophilic bacteria (AMB), colonies were counted in Petri dishes. According to the colony-forming unit (CFU) counting rules, the most accurate data were utilized and adjusted for the dilution factor. However, given the scarcity of colonies (fewer than 25) in the samples, only an estimated value could be provided. In all replicates of each sample, the values obtained for AMB were below 100 CFU, well within the allowed limit.

Likewise, the colonies that developed in the Petri dishes during mold and yeast (MY) analysis were minimal and did not exceed the allowable limit of 10

CFU. Therefore, it can be inferred that the beverage was handled appropriately, ensuring its safety, and thus allowing subsequent sensory analysis.

### 3.2. Sensory Analysis

Affective sensory acceptance tests were conducted to evaluate taste and general agreement, considering each panelist's perception of specific attributes. **Figure 2** shows the means of sensory attributes for each formulation, assessed using a 7-point hedonic scale. In general, the formulations exhibited significant statistical differences, with the control being the most widely accepted, followed by treatment 1. The high acceptance of the control was to be expected because it was a beverage with which the panelists were already familiar as it was frequently consumed in the student cafeteria.



**Figure 2.** Sensory analysis results from the acceptance test of each treatment and control of the beverage.

Although both control and treatment 1 contained the same amount of blackberry concentrate (25%), the presence of whey in treatment 1 altered the striking color of the concentrate. Furthermore, the unfamiliar odor of whey was generally disliked by panelists. Many preferred its absence or its reduced quantity. Similarly, in terms of taste, the evaluators were unfamiliar with the taste of whey, but the increased content of blackberry concentrate masked it. Some participants mentioned their preference for sweeter drinks and could perceive differences in sweetness in treatments 2 and 3. The panelists generally favored the level of acidity provided by the 25% blackberry concentrate more than those with lower concentrations.

The general acceptance attribute was used to gauge overall satisfaction with all the aspects previously evaluated. The control received the highest acceptance scores, likely because study participants regularly consumed blackberry drinks in the student cafeteria. Treatment 1 was the second highest rated, followed by treatment 2, and finally treatment 3. Furthermore, a correlation analysis was performed (**Table 2**), revealing positive associations between

all attributes and general acceptance. In this study, flavor and sweetness showed the highest correlation with overall acceptance, with a high correlation coefficient ( $>0.80000$ ). The other attributes exhibited medium Pearson correlations ( $>0.50000 - 0.79000$ ).

**Table 2.** Results of the correlation analysis of the attributes evaluated in the sensory analysis of the beverage.

	Color	Odor	Appearance	Flavor	Viscosity	Acidity	Sweetness
r	0.71141	0.73694	0.76853	0.87034	0.76573	0.76699	0.82597
P >  t	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

r: Pearson correlation; Pr > |t|: probability.

**Table 3** presents the results of the physicochemical analysis. First, it shows the color results for all samples on the L\*, a\*, and b\* scales. The L\* value, which represents lightness, indicated that treatment 3 was the lightest, while control was the darkest due to varying concentrations of blackberry concentrate. The presence of whey also influenced luminosity, as it presented a greenish-yellow color that counteracted the effect of antioxidants.

**Table 3.** Results of the physicochemical properties of the beverage.

Properties	T1	T2	T3	Control	CV%	Pr >  t
Color	L	26.19 ± 0.34c	43.16 ± 1.05b	52.06 ± 1.47a	11.28 ± 0.38d	1.67 <0.0001
	a*	16.91 ± 0.42a	12.74 ± 0.25ab	10.03 ± 0.31b	14.57 ± 0.21ab	10.58 0.0586
	b*	2.08 ± 0.12c	4.35 ± 0.16b	6.17 ± 0.37a	2.70 ± 0.22c	8.76 0.0035
°Brix	21.87 ± 0.86a	19.87 ± 0.40b	15.87 ± 0.60d	17.93 ± 0.45c	3.64	0.0018
pH	3.59 ± 0.18c	4.24 ± 0.24b	4.56 ± 0.06a	2.78 ± 0.18d	12.11	0.0023
Viscosity (mPa·s)	30.6	14.6	13.3	19	-	-

a, b, c, d: indicate significant differences between treatments ( $P < 0.05$ ). Pr > |t|: probability. CV%: Coefficient of variation. Control: blackberry juice. T1: treatment 1, 75% whey and 25% blackberry concentrate. T2: treatment 2, 85% whey and 15% blackberry concentrate. T3: treatment 3, 90% whey and 10% blackberry concentrate. L: luminosity, white to black (100 to 0). a\*: chromaticity, red to green (+60 to -60). b\*: yellow to blue (+60 to -60).

The value of a\*, reflecting the red-green spectrum, showed a predominance of red colors in all samples. Significant differences were observed, with treatment 1 showing the most intense red color. As for the b\* coordinate, which indicates yellow-blue tendencies, positive values suggested a tendency toward yellowish

colors. Treatment 3 had the most significant yellow component, followed by treatment 2. In contrast, control and treatment 1 exhibited a more pronounced blue component.

The number of soluble solids showed a statistically significant difference between the treatments. Treatment 1 had the highest °Brix value. Blackberry concentrate was the main contributor to soluble solids, as a decrease in concentrate and an increase in whey content was correlated with a decrease in °Brix. The control had the lowest °Brix value.

The pH values showed a significant difference between all treatments, with the order from most acidic to most alkaline being control, treatment 1, treatment 2, and treatment 3. This indicates that higher concentrations of blackberry concentrate corresponded to higher acidity.

In terms of viscosity, treatment 1 exhibited the highest viscosity, followed by control, treatment 2, and treatment 3 (**Table 3**). Samples containing 25% blackberry concentrate, in both treatment 1 and treatment 3, had the highest viscosity.

### 3.3. Nutritional Quality

The results for both the control and treatment 1, which exhibited the highest acceptance, are displayed in **Table 4**. Surprisingly, the control displayed the lowest nutritional data. Although both contained the same amount of concentrate, it was the addition of whey and  $\beta$ -glucans that contributed to increased levels of macro and micronutrients. A comparison was also drawn between the nutritional composition obtained from chemical tests, as presented in **Table 5**, and the data from commercial products. The data were remarkably similar. Furthermore, a comparison was made between treatment 1 and two beverages available on the market. In particular, commercial products had a long list of ingredients and there was a substantial difference in the calorie content of each beverage, with treatment 1 having the highest calorie count. It is important to mention that the two beverages containing whey (treatment 1 and Lattella Classic Mango) had a high sodium content. However, the commercial beverage contained more than double the amount of sodium compared to the treatment. Similarly, treatment 1 provided a higher carbohydrate content compared to the other beverages (100 ml each one).

## 4. Discussion

### 4.1. Microbiological Analysis

Coliform bacteria serve as safety indicators during the food manufacturing process and should not be present in pasteurized beverages that have undergone additional manipulation after heat treatment [48]. This is how the adherence to hygiene practices during beverage processing is verified.

According to the FDA guidelines, optimal conditions for MY growth include a pH range of 2 to 9, a temperature range of 10°C to 35°C and appropriate humidity levels [49]. Consequently, they pose a potential risk to the beverage, leading

**Table 4.** Results of the nutritional quality of the control and the most accepted beverage formulation.

Nutrient	Control			T1		
	Chemical Analysis			Chemical Analysis		
	Mean ± S.D.	CV	%DV	Mean ± S.D.	CV	%DV
Energy (kcal)	66.12	n/a	3	78.44		4
Protein (g)	0.04 ± 0.00	1.4	0	0.65 ± 0.01	1.23	1
Total fat (g)	0.06 ± 0.00	6.26	0	0.69 ± 0.06	8.45	1
Saturated fat (g)	0 ± 0.00	0	0	0.31 ± 0.02	7.62	2
Cholesterol (mg)	0	n/a	0	0		0
Carbohydrate (g)	16.37	n/a	5	17.41		6
Sugar (g)	9.23 ± 0.54	5.84	18	11.17 ± 0.19	1.74	22
Fiber (g)	0.85 ± 0.11	9.21	3	1.91 ± 0.25	9.69	8
Sodium	8 ± 0.31	3.88	0.5	39 ± 0.64	1.64	2
Moisture	82.55 ± 1.03	1.25	n/a	78.9 ± 1.32	1.67	n/a
Ash	0.14 ± 0.01	9.71	n/a	0.44 ± 0.04	8.42	n/a

Kcal: calories. g: grams. mg: milligrams. n/a: not applicable. a b c d: indicate significant differences between treatments. S.D.: Standard Deviation. CV: Coefficient of Variation. Control: blackberry juice. T1: treatment 1, 75% whey and 25% blackberry concentrate. S.D.: Standard Deviation. %VD: Percent Daily Value.

**Table 5.** Comparison of the best-accepted beverage proposed and some similar products on the market (100 ml).

Nutrient	T1	MO	LS
Energy (kcal)	80	60	37
Protein (g)	<1	2	<1
Total fat (g)	1	1	1
Saturated fat (g)	0.5	0	0
Cholesterol (g)	0	0	-
Carbohydrates (g)	18	10	9
Sugar (g)	11	3	9
Fiber (g)	2	1	-
Sodium (mg)	40	5	90
Ingredients	Whey, blackberry concentrate and $\beta$ -glucans.	Water, organic oats, organic cane sugar, pectin, natural organic vanilla flavor, organic reishi, live and active cultures.	Sweet whey, mango puree, sugar, acidity regulator, sodium citrate, lactic acid, ascorbic acid, and flavorings.

Kcal: calories. g: grams. T1: treatment 1 of this study. MO: Commercial drink containing *Ganoderma lucidum* "MSHRM OAT REISHI VANILLA". LS: Commercial buttermilk drink "Lattella Classic Mango".

to varying degrees of decomposition. Furthermore, the characteristics of the beverage provide an environment conducive to their growth. The results of the plate casting method using Rose Bengal agar culture medium were found to be within the allowed limits. As illustrated in **Figure 1**, the formulations adhered to the maximum acceptable contamination limits established for MY. Therefore, heat treatment, carried out at 63°C - 65°C for 30 minutes using an instant pot machine, effectively pasteurized batches, contributing to the destruction or inactivation of yeast spores or fungi. Although the Pan American Health Organization [50] attributes the use of heat treatment with the right temperature and time as a preventive measure against the growth of pathogenic microorganisms, it is essential to consider other contributing factors.

According to ANMAT and ReNaLOA (2014) [51], the AMB analysis reflects the sanitary quality of the analyzed product. As a result, counts below the maximum allowed limit for these pathogenic bacteria were observed when the plate-cast method with ACE culture medium. This justifies the adequacy of heat treatment and material handling [52].

## 4.2. Sensory Analysis

While the presence of whey affected the color rating, it also offers the opportunity to prevent the degradation of anthocyanins, which are responsible for the purple or red hues and various health benefits [52] [53]. This is crucial because color is essential to determine the level of acceptance and whether consumers find the appearance of a food appealing [54]. Generally, the deeper the color, the higher the rating [55] [56].

The perception of odors is influenced by volatile compounds released by food [57]. Populations rely on olfactory memory to associate familiar scents with unknown ones and evaluate their acceptance [58]. In the assessed population, whey was relatively unknown and was typically considered a waste product. In a study by Amador *et al.* (2020), a beverage containing 70% whey and 30% blackberry was rated as having an unpleasant smell [59]. However, in our study, for treatment 1, which contained a similar amount of both ingredients, it was predominantly perceived to have a mildly attractive odor. Undoubtedly, whey imparts a slightly acidic odor that may not be pleasant to some panelists [60]. On the other hand, blackberry provides a slightly sweet aroma [61]. As described by Muñoz *et al.*, treatments with lower amounts of whey were generally better accepted [62].

The flavor results aligned with what Vivas *et al.* (2016) reported, where the best score was associated with the treatment that contained the highest amount of blackberry pulp [63]. Furthermore, in our study, as the whey content increased, the acceptance of the taste of the beverage decreased. This can be attributed to the fact that whey intensifies the sensation of astringency due to the acidity and residual flavor it provides [64]. In terms of viscosity, according to Gavilanes López *et al.* (2018) [54], 50% whey treatment was rated as “I like it a little,” while 70% whey were classified as “I dislike it” [53]. The differences between this study and others were due to the varying amounts of whey added and

the presence of  $\beta$ -glucans, which contribute to the formation of more viscous solutions when added to liquids [65] [66].

Blackberries increase the acidity of the beverage due to their high content of organic acids and antioxidants [67]. Likewise, sweet whey can contribute a certain degree of acidity to the beverage, although it has a low acidity level that does not exceed 2 g/L of lactic acid [68]. The ability of a person to detect sweetness is one of the factors that influence food acceptance [69]. Similarly, whey contains lactose, which is a disaccharide with low sweetening power. However, when present in high concentrations, it can produce an unpleasant taste [70].

Regarding general acceptance, Campos (2019), in his review of the literature, reported that beverages with lower whey content tend to be preferred, as they also had higher acceptance ratings for color, taste, and odor attributes [71]. Furthermore, Gavilanes-López *et al.* (2018) suggested that an increase in whey content negatively affected overall acceptance [54]. Vivas *et al.* (2016) also noted that a stabilizer was necessary to maintain homogeneous consistency, as its absence negatively impacted acceptance [55] [63]. Turkmen *et al.* (2019) pointed out that the attributes with the highest correlation were odor and flavor due to the amount of whey added in the beverages [72]. As described by Muñoz I *et al.* (2020), sweetness is undeniably a key factor, as most people tend to prefer sweet-tasting foods and beverages [73]. Montoya and Alcaraz (2016) also pointed out that people tend to prefer sweet products [74]. According to sensory analysis, the control had the highest acceptance of sweetness, followed by treatment 1 [74]. These results are consistent with the Campos study, where the beverage with the lowest whey content was the most preferred, although in that case it contained 40% whey and 60% orange juice [71].

### 4.3. Physicochemical Analysis

The predominant shades were due to the presence of anthocyanins, which are natural colorants that provide red, purple, or black colors [75]. According to Rodoni *et al.*, the value of  $b^*$  is the most affected by heat treatment, leading to color degradation [76]. As mentioned by Solórzano *et al.* (2015), a lower brightness corresponds to a higher  $a^*$  value, and a higher  $b^*$  value results in a higher concentration of solids [77]. The research by Robles (2016) [78] and Alava Viteri *et al.* (2014) [79] reported that blackberry concentrate has around 23 °Brix, while whey has 6.5 °Brix. The increase or decrease in any of the ingredients influenced the number of soluble solids. In the case of the control, the concentrate was diluted in water, resulting in a lower °Brix reading because it lacked the contribution provided by the whey.

According to RTCA 67.04.48:08 [80], if a beverage contains 25% juice or pulp of any fruit, it can be considered nectar. This regulation also states that the pH of nectar must not exceed 4.5. In this context, both control and treatment 1 meet both conditions, while treatment 2 only meets the pH condition, and treatment 3 does not. When comparing the values obtained (see **Table 3**) with the classification provided by Guevara (2015), the control pH is considered highly acidic (pH

between 2.5 and 3.5), treatment 1 has medium acidity (pH between 3.5 and 4.2), and treatments 2 and 3 have low acidity (pH greater than 4.2) [81]. Additionally, Guevara suggests that the acidity of juices or nectar should be below a pH of 3.8; otherwise, it may require regulation with citric acid [81]. However, the sweet whey itself should have a pH greater than 5.6 [82]. These findings can be attributed to the acidity contributed by blackberry concentrate, which is the raw material with theoretically the highest pH. As noted by Jin and Kirk (2018), pH levels below 4 reduce the growth of different pathogenic microorganisms, since most thrive at pH levels greater than 4 [83].

The presence of  $\beta$ -glucans theoretically leads to a higher viscosity in treatments that contain them [84]. Some factors that contribute to viscosity differences between samples may include the absence of  $\beta$ -glucans in the control, variations in temperature, and different numbers of soluble solids between samples, leading to varying molecular associations in the solutions [85]. Based on viscosity readings performed at different revolutions per minute, viscosity in all samples follows a Newtonian fluid behavior, with relatively constant viscosity even when samples are forced to flow faster or slower through a channel [86]. Furthermore, some variations may be attributed to factors such as temperature, air flow within the room where the viscometer is located, or the timing of the viscosity readings [87].

#### 4.4. Nutritional Quality

Beverages can be a good choice to provide energy, facilitate physiological processes, and incorporate a wide variety of foods such as fruits [78]. A series of analyses were conducted on the treatment with the highest acceptance and on the control. Both control and T1 provide fewer than 100 kcal per serving (see **Table 4**), which is considered a moderate calorie content per serving. The number of calories supplied by a serving of this beverage is less than 4% of the total daily calories recommended for a diet of 2000 calories (see **Table 4**).

According to the RTCA guidelines for nutritional labeling [47], T1 can be classified as fiber-fortified, since it contains more than 1.5 grams per 100 ml. The control, with no more than 0.5 g of fat and saturated fat, falls under the category of being free of these nutrients. Additionally, the control contains less than 35 mg of sodium, which makes it very low in sodium. Furthermore, T1 is considered free from cholesterol and saturated fat. Both control and T1 adhere to international recommendations that recommend making appropriate food choices by limiting saturated fat and controlling sodium intake [73].

According to Annex G of the RTCA for nutrition labeling [73], a statement indicating the amount of  $\beta$ -glucans present in this product could be included. In one study, the effects of  $\beta$ -glucans from various sources were examined, and it was concluded that those from mushrooms required lower doses to achieve the same effects as those of other sources, which was attributed to their chemical structure and higher molecular weight [80].

A comparison of the nutritional composition of the data obtained from chemical tests and products available on the market was made (see **Table 5**). To perform this comparison, nutritional information from “MSHRM OAT REISHI VANILLA” was used, as it is a beverage containing *Ganoderma lucidum* among its ingredients. In addition, “Lattella Classic Mango,” an Austrian drink based on sweet whey, was included. The nutritional quality varies significantly between these products due to the differences in their ingredients. The functional beverage with the highest acceptance in this study contained more calories, available carbohydrates, and sugar compared to the others. Furthermore, the beverage in this study had a higher fiber content, attributed to the addition of  $\beta$ -glucans and blackberry concentrate.

## 5. Conclusions

Treatment with the highest acceptance comprised 75% whey, 25% blackberry concentrate, and  $\beta$ -glucans. The sensory attributes that had the most significant impact on overall acceptance were flavor and sweetness. The addition of whey had effects on the °Brix value, pH, and viscosity, while blackberry concentrate reduced the brightness of the treatments and introduced red tones. Furthermore, incorporation of  $\beta$ -glucans increased the content of dietary fiber. This specific treatment, consisting of 75% whey, 25% blackberry concentrate, and  $\beta$ -glucans, can be classified as fiber-fortified, very low in sodium, and free of saturated fat and cholesterol.

For future research, it is recommended to expand the physicochemical evaluations, including the quantification of anthocyanin levels and the performance of a shelf-life study. In addition, exploring the creation of a drink similar to the one proposed using whey, and other more potent flavors that could more effectively mask the strong taste of whey. Evaluating the benefits of regular consumption of this drink in the population could be a valuable avenue of research.

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## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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