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Functional beverage design based on fresh milk, tarwi (*Lupinus mutabilis*) beverage and oatmeal (*Avena sativa*)

Diseño de una bebida funcional a base de leche fresca, bebida de tarwi (*Lupinus mutabilis*) y avena (*Avena sativa*)

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ABSTRACT

The tarwi is an Andean legume with a high nutritional value from which a vegetable beverage can be obtained. Cereals, like oat, have good characteristics as a prebiotic for the production of functional drinks, whose consumption is currently increasing. The objective of the research was to design a probiotic fermented beverage based on fresh milk, tarwi beverage, and oatmeal. An optimal treatment consisted of 1.9% oatmeal, 39.9% tarwi beverage, 46.2% fresh milk, 10.0% honey, and 2.0% probiotic culture; determined by applying a rotatable central composite design of surface response methodology. It had a probiotic count of 3.47×10^8 cfu/mL, a protein content of 3.75%, and overall acceptability of 7 points, which corresponds to "I like it very much". The result was experimentally validated. Likewise, the shelf life of the optimal beverage was 20 days at 5 °C with appropriate functional, nutritional, and sensory characteristics.

Key words: Fermentation; Honey; Optimization; Probiotic; Surface response methodology.

RESUMEN

El tarwi es una leguminosa andina con un alto valor nutricional a partir de la cual se puede obtener una bebida vegetal, los cereales como la avena tienen mejores características como prebióticos para la producción de bebidas funcionales, cuyo consumo está aumentando actualmente. El objetivo de la investigación fue diseñar la formulación de una bebida fermentada probiótica a base de leche fresca, bebida de tarwi y avena. Se determinó un tratamiento óptimo que consis-

tió en 1,9% de avena, 39,9% de bebida de tarwi, 46,2% de leche fresca, 10,0% de miel y 2,0% de cultivo probiótico; mediante la aplicación de un diseño compuesto central rotatable de metodología de respuesta de superficie. Se reportó un recuento de probióticos de $3,47 \times 10^8$ ufc/mL, un contenido de proteínas de 3,8% y una aceptabilidad general de 7 puntos, que corresponde a “Me gusta mucho”; el resultado fue validado experimentalmente. Asimismo, la vida útil de la bebida óptima fue de 20 días de almacenamiento a 5 °C con características funcionales, nutricionales y sensoriales apropiadas. Palabras clave: Fermentación; Metodología de superficie de respuesta; Miel; Optimización; Probiótico.

INTRODUCTION

Eating habits have changed over time, and the current trend of the consumer is the search for healthy, practical, and sensory-friendly foods¹. During the last decade, the demand for healthy food and beverages has increased in many parts of the world, due to factors such as increased health deterioration, busy lifestyles, low consumption of home-prepared foods and insufficient exercise; higher incidence of self-medication; increased awareness of the relation between diet and health due to information from health authorities and the media about nutrition; and a growing food market^{2,3}.

Functional foods are classified as whole, enriched, or improved foods that provide positive health benefits. This market is dominated by products that contain carotenoids, dietary fiber, fatty acids, minerals, prebiotics/probiotics/symbiotics, vitamins, and minerals^{4,5,6}. Functional beverages are those that have ingredients that demonstrate improving health status and reducing the risk of disease^{7,8}. Functional dairy products represent more than 40% of the food segment and growth is expected in the beverage sector until 2021, due to its acceptance in world markets^{4,9}.

Probiotics are defined as living microorganisms that, when ingested in appropriate amounts, confer health benefits to the consumer. To be beneficial, they must be present in an amount of 10^6 cfu/mL of product at the time of consumption^{10,11}. Lactic acid bacteria are commonly used in probiotic products and naturally found in traditional fermented products. The most commonly used lactic acid bacteria are *Lactobacillus acidophilus* and *Bifidobacterium animalis* subsp. *lactis* and are usually combined with *Streptococcus thermophilus* because they lack proteolytic activity^{12,13,14}.

Prebiotics are any component of plant origin that probiotic microorganisms selectively use as a substrate for their metabolism and that provide a health benefit¹⁵. Prebiotics can improve the growth and survival of probiotics in the digestive system. Cereals, legumes, fruits, and vegetables, such as wheat, oats, barley, beans, lentils, chickpeas, tomatoes, onions, garlic, chicory, vegetables, pore, asparagus, spinach, artichokes, bananas, and berries are rich in prebiotic fibers¹⁶. Prebiotics, mainly oligosaccharides, are currently used in beverages and dairy products; and in general, they can withstand thermal processes without degradation¹⁷.

The synergistic or symbiotic combination of prebiotics and probiotics that found in products such as food, medicine, and supplements are defined as a combination that beneficially affects the host by improving the survival and implementation of live microbial dietary supplements in the gastrointestinal tract. Symbiotics are more effective than probiotics or prebiotics used in isolation¹⁶.

Oatmeal, mainly as flakes, is included in the human diet for its health benefits¹⁸. It is rich in β -glucans, proteins, starch, and phenolic compounds, and it is known to have anti-cancer and hypocholesterolemic properties. The availability of soluble fibers, both oligosaccharides, and polysaccharides, have a prebiotic effect. Despite this, there is little information available on the use of oats as a possible carrier of probiotics¹⁹.

The tarwi (*Lupinus mutabilis*) is a non-widely used legume found in several regions of Peru⁶ that should be part of our diet because of its high nutrient content. Its protein content is even higher than that of soybeans, and its fat content is similar²⁰. After dehydration, it is used in different culinary dishes (mote, salads, soups, stews, desserts, and ceviche) or to obtain flour used in baking. Beverage (tarwi juice) is also produced from tarwi and consumed at breakfast¹⁰. Vegetable beverage is an aqueous extract of legumes, cereals, or seeds. A trend of these new products is the application of the fermentation process²¹.

The objective of this research was to design and optimize a fermented functional beverage based on fresh milk, tarwi beverage, and oatmeal with appropriate probiotic viability, protein content, and overall acceptability during refrigerated storage.

MATERIALS AND METHODS

Ingredients

Fresh milk was purchased from the “farm” UPAO II, located in the Barraza-Laredo-La Libertad district; debittered tarwi grains were obtained from the Palermo-Trujillo-La Libertad market; oatmeal (Quaker); honey bee (Abejas del Norte) and bottled water (Cielo), were obtained at the Plaza Veá supermarket; and the probiotic culture Lyofast SAB 446B containing strains of *Streptococcus thermophilus*, *Lactobacillus acidophilus*, and *Bifidobacterium animalis* ssp. *lactis* (Sacco-Italia) was acquired in Linros Interinsumos E.I.R.L. of Trujillo.

Tarwi beverage extraction

Debittered grains were combined with water in a 40:60 ratio and homogenized in an industrial blender (Metalmecánica Agroindustrias) of 16 L capacity, at 3600 rpm for 5 min. Grains were then filtered using a stainless-steel mesh (0.173 mm opening) and sterile gauze cloth, separating the retained solids from supernatant. This operation was carried out twice to obtain a homogeneous solution. Finally, the solution was pasteurized at 85 °C for 15 min, cooled, and stored in refrigeration at 4 °C²¹ (with author's modifications).

Preparation of the functional beverage

The fresh milk and tarwi beverage were mixed in a stainless-steel kettle with the oatmeal in the amounts indicated in the experimental design (Table 1). Afterward heated up to 70 °C, to add the honey and obtain its total solution, pasteurized at 85 °C for 15 min, and cooled to 37 °C to inoculate 2% (w/v) of the probiotic culture. Then, it was incubated at 37 °C for 5 h and finally stored in refrigeration at 4 °C for 24 h for analytical evaluations. Table 1 shows the formulations of the functional beverage with fresh milk, tarwi beverage, and oatmeal.

Physicochemical characteristics

For soluble solids²², the method of the Association

of Official Analytical Chemists-AOAC was used; for titratable acidity²³ and proteins content²⁴, the AOAC method, and dietary fiber²⁵ according to AOAC, using the Sigma TDF-100 Enzymatic Kit.

Probiotic bacteria count

De-Man Rogosa Sharpe (MRS) media used (Merck, USA) was enriched with 10% of a 0.050% L-Cysteine solution to improve anaerobic conditions in the agar. The inoculum and the functional beverage were homogenized properly and 1 mL was taken and serially diluted until reaching 10⁻⁷. After that, the pour plate method was used for the probiotic bacteria count in duplicate and incubated in anaerobiosis at 37 °C for 48 h. Results were expressed in cfu/mL¹¹.

Overall acceptability

A 9-point hedonic scale was used, anchored between 1 that is equivalent to "I dislike it very much" and 9 to "I like it very much", with intermediate point of 5 "I neither like nor dislike it". The sensory tests were performed in a laboratory room with bright natural daylight. All samples were marked with codes and were randomly provided to evaluators on white plates. The sample consisted of 20 mL of room temperature beverage in randomly numbered containers with three digits. The test was performed in two sessions with 30 untrained panelists, recruited among students and staff of Antenor Orrego University, mainly aged 20-30. In the first session, five samples were delivered, and in the second, 4. All participants involved in the evaluation were thoroughly informed about the manufacturing process and the sensory attributes of the product^{21,26}.

Statistical design

The design was based on a base formulation of acidified milk beverage consisting of 88% fresh milk, 10% honey, and 2% probiotic culture of the final volume. From this, a rotatable central composite design (DCCR) of the response surface methodology (MSR) was used to evaluate the effect of replacing fresh milk with tarwi beverage and oatmeal, and their combinations on soluble solids, titratable acidity, dietary fiber, protein content, probiotic bacteria count and overall acceptability. This resulted in a total of 9 treatments and 4 repetitions of the central point, as seen in table 1. Analysis of variance was applied with a 95% confidence level; to validate the models obtained, R² > 0.800 was considered, p<0.050, and the lack of adjustment greater than 0.05. The optimization was developed using the superposition of contours of the most relevant variables in the functional beverage, obtaining the maximum compound desirability as a statistical indicator that validates the product design. Minitab Statistical Software, version 17 (Minitab Inc., 2014) was used.

Table 1. Formulations to obtain a functional beverage with fresh milk, tarwi beverage, and oatmeal.

Formulation	Oatmeal (%)	Tarwi beverage (%)	Fresh milk (%)
F9	1.50	30.0	56.5
F3	3.00	30.0	55.0
F6	1.50	40.0	46.5
F2	3.00	40.0	45.0
F8	1.19	35.0	51.8
F5	3.31	35.0	49.7
F4	2.25	27.9	57.8
F7	2.25	42.1	43.7
F1*	2.25	35.0	50.8

*F1, central point worked with four repetitions.

Optimal beverage shelf life

Shelf life was determined using the methodology of evaluation in real time of the quality attribute lactic acidity until reaching 0.55%, correlated with the overall acceptability (data not shown).

RESULTS

Table 2 shows the results of the physicochemical characteristics; probiotic bacteria count and overall acceptability in the different treatments of the functional beverage. Soluble solids fluctuated between 15.4-17.0%. The highest values were in those formulations due to high content of oatmeal and fresh milk. The acidity varied between 0.198-0.250%, showing higher results in drinks with a higher content of oatmeal and tarwi beverage. Protein content results were between 3.62-3.86%, showing higher values in formulations with higher oatmeal and intermediate tarwi beverage. Dietary fiber showed results between 0.14-0.36%, denoting the highest

values in the formulations with the highest amount of oatmeal and tarwi beverage. The probiotic count fluctuated between 3.77×10^7 - 3.20×10^9 cfu/mL, with the formulations with highest counts being the ones with the highest amount of oatmeal and intermediate tarwi beverage. Acceptance by the panelists varied between 5.6-8.5 points, with the highest acceptance in beverages with lower content of oatmeal and higher amount of tarwi beverage.

Table 3 shows the regression models and fit of the rotatable central composite design for the variables evaluated in the functional beverage based on fresh milk, tarwi beverage and oatmeal. Table 4 shows the optimization for probiotic counts, protein content and overall acceptability. Figure 1 shows contour surface of the rotatable central composite design for probiotic counting (A), protein content (B) and overall acceptability (C). Figure 2 shows the optimization of probiotic counts, protein content and overall acceptability.

Table 2. Physicochemical characteristics, probiotic bacteria count and overall acceptability in the functional beverage.

Formulation	Soluble solids (%)	Titratable acidity (%)	Probiotic count (cfu/mL)	Protein content (%)	Dietary fiber (%)	Overall acceptability (points)
F9	16.2	0.225	1.38×10^9	3.6	0.170	8.5
F3	17.0	0.198	2.85×10^9	3.7	0.320	5.6
F6	15.8	0.216	2.66×10^8	3.7	0.180	7.4
F2	15.5	0.250	3.77×10^7	3.8	0.330	7.0
F8	16.0	0.224	5.98×10^7	3.7	0.140	7.8
F5	16.3	0.225	9.58×10^8	3.9	0.360	5.2
F4	16.8	0.207	3.20×10^9	3.6	0.240	7.7
F7	15.4	0.234	3.78×10^8	3.7	0.260	7.9
F1	16.0	0.221	8.80×10^8	3.8	0.240	5.6
F1	16.0	0.220	8.97×10^8	3.8	0.260	5.6
F1	15.9	0.221	8.90×10^8	3.8	0.250	5.6
F1	15.8	0.219	8.62×10^8	3.8	0.250	5.7
F1	16.0	0.221	9.00×10^8	3.8	0.250	5.6

Table 3. Regression models and fit of the rotatable central composite design for the variables evaluated in the functional beverage based on fresh milk, tarwi beverage and oatmeal.

Variable	Equation
Soluble solids	$y = 18.1 + 1.88 \cdot O - 0.156 \cdot TB + 0.186 \cdot O^2 + 0.003 \cdot TB^2 - 0.073 \cdot O \cdot TB$ $R^2 = 97.6$ p-value = 0.006 Lack of fit = 0.977
Titratable acidity	$y = 0.484 - 0.157 \cdot O - 0.007 \cdot TB + 0.004 \cdot O^2 - 0.001 \cdot TB^2 + 0.004 \cdot O \cdot TB$ $R^2 = 99$ p-value = 0.010 Lack of fit = 0.166
Probiotic count	$y = 18104054222 + 5937514591 \cdot O - 1194763449 \cdot TB - 341528978 \cdot O^2 + 17897344 \cdot TB^2 - 113732112 \cdot O \cdot TB$ $R^2 = 99.9$ p-value = 0.000 Lack of fit = 0.382
Protein	$y = -0.126 + 0.108 \cdot O + 0.203 \cdot TB + 0.010 \cdot O^2 - 0.003 \cdot TB^2 - 0.003 \cdot O \cdot TB$ $R^2 = 98.9$ p-value = 0.000 Lack of fit = 0.342
Dietary fiber	$y = -0.028 + 0.073 \cdot O + 0.004 \cdot TB + 0.001 \cdot O \cdot TB$ $R^2 = 99.2$ p-value = 0.000 Lack of fit = 0.962
Overall acceptability	$y = 77.9 - 10.5 \cdot O - 3.39 \cdot TB + 0.771 \cdot O^2 + 0.043 \cdot TB^2 + 0.167 \cdot O \cdot TB$ $R^2 = 99.7$ p-value = 0.000 Lack of fit = 0.147

O: oatmeal, TB: tarwi beverage.

Table 4. Optimization for probiotic counts, protein content and overall acceptability.

Response	Theoretic	Experimental	Residual (%)
Probiotic Counts (cfu/mL)	3.47×10^8	3.22×10^8	7.20
Protein (%)	3.75	3.71	1.07
Overall acceptability (points)	7	7.30	4.29
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Shelf life optimal beverage	20 days at 5 °C

Figure. 1: Contour surface of the rotatable central composite design for probiotic counting (A), protein content (B) and overall acceptability (C).

Figure 2: Optimization of probiotic counts, protein content and overall acceptability. y= Optimal result of response variable, D= Compound desirability, d=individual desirability.

DISCUSSION

Physicochemical characteristics

The treatments with the highest content of soluble solids were F3 constituted by (oatmeal 3%, tarwi beverage 30% and fresh milk 55%) and F4 (oatmeal 2.25%, tarwi beverage 27.9% and fresh milk 57.8), as seen in table 2. This is because these ingredients have more dissolved solids in their composition. Salamanca et al.²⁷ reported values of 17-24% in a fermented beverage based on

borojó juice, yogurt, and honey; Randazzo et al.²⁸ with 5.90 - 15.7% in a fermented non-dairy beverage based on Mediterranean fruit juices with kefir microorganisms in water.

The treatments with the highest titratable acidity were F2 (oatmeal 3%, tarwi 40% and fresh milk 45%) and F7 (oatmeal 2.25%, tarwi 42.1% and fresh milk 43.7); as seen in table 2. Lactic cultures have a higher rate of acidification in legume kinds of milk, such as soy,

in comparison to dairy substrates because they have lower buffer capacity, which allows *S. thermophilus*, as a homofermentative species, to produce more lactic acid²⁹. Flores et al.¹⁰ report 0.07-0.09% in a fermented beverage based on tarwi juice and kiwicha; Battistini et al.²⁹ with 0.178-0.213% in a fermented beverage based on soy milk with the addition of inulin and fructooligosaccharides; Sánchez et al.³⁰ with 0.45% in a fermented beverage based on milk, whey, sucrose, and curuba extract.

The highest protein content was found in treatments F5 (3.31% oatmeal, 35% tarwi beverage and 49.7% fresh milk) and F2 (3% oatmeal, 40% tarwi beverage and 45% fresh milk); as seen in table 2. Products derived from oatmeal such as flakes and flour denote high quantity and quality of protein that can be used in functional beverages¹⁸. Castañeda et al.²⁰ mentioned that vegetable beverage such as tarwi and soy have slightly higher amounts of protein than cow's milk, and, when combined in different proportions for the manufacture of yogurt, the final product obtains values between 3.86 and 3.93%. Kumar et al.³¹ indicated 2.21-3.23% of protein in a development of nutriceals and milk-based beverages; Wang et al.²¹ with 1.21-2.09% in a fermented beverage based on chickpea; Salamanca et al.²⁷ with 3.71% in a fermented beverage based on borojó juice, yogurt, and honey.

The highest content of dietary fiber was found in beverages made up of F5 (3.31% oatmeal, 35% tarwi and 49.7% fresh milk) and F2 (3% oatmeal, 40% tarwi and 45% fresh milk); as seen in table 2. Nionelli et al.¹⁸ mentioned that oatmeal shows impressive characteristics for food processing, in particular, due to its high fiber content and bioactive compounds beneficial to health. Buriti et al.³² indicated values between 0.620-2.25% in a fermented beverage based on goat's milk, fruit pulp, and galactomannans of *Caesalpinia pulcherrima* seeds; Din et al.³³ with 0.200-0.400% in a functional beverage with Barley β -glucan. Hernández et al.³⁴, with 0.600% in a fermented beverage based on rice flour and sesame seeds.

Probiotic bacteria count

The highest count of probiotic bacteria, which is beneficial for consumers, was obtained in beverages F4 (oatmeal 2.25%, tarwi 27.9% and fresh milk 57.8%) and F3 (oatmeal 3%, tarwi 30% and fresh milk 55%); as seen in table 2. The viability of probiotics in fermented beverages depends on factors such as their nature, the substrate, processing, and storage conditions¹⁰. Gupta et al.¹⁹ explained that the presence of prebiotic compounds and non-digestible carbohydrates present in cereals such as barley and oat, as well as, malt substrates stimulate the growth of probiotics in fermented beverages and other beneficial bacteria of the intestinal tract. The hydrolyzate kiwicha and tarwi beverage have a synergistic effect with the *Lactobacillus* and *Bifidobacterium* cultures on the

counts of these probiotics in the preparation of a fermented beverage¹⁰. Battistini et al.²⁹ reported 7.76×10^7 - 1.18×10^8 cfu/mL in a fermented beverage based on drinkable soy with the addition of inulin and fructooligosaccharides; Randazzo et al.²⁸ with 3.9×10^7 - 1×10^8 cfu/mL in a fermented non-dairy beverage based on Mediterranean fruit juices with kefir microorganisms in water; Dos Santos et al.³⁵ found values 3.10×10^7 - 2.20×10^8 cfu/mL of *Lactobacillus lactis* in drinkable soy fermentation with kefir and addition of inulin and values of 6.64×10^7 ; 3.25×10^7 and 3.07×10^7 cfu/mL were reported by Łopusiewicz et al.³⁶ in the development of non-dairy kefir-like fermented beverage based on flaxseed oil cake.

Overall acceptability

The major perception of sensory characteristics expressed as general acceptability was found in F9 (oats 1.50%, tarwi 30% and fresh milk 56.5%) and F7 (oats 2.25%, tarwi 35% and fresh milk 50.8%); as seen in table 2. Sensory results are essential and related to product quality, substrate composition and the concentration of its components¹⁰. Nionelli et al.¹⁸ mentioned that the acceptability of this type of beverage depends on the fermentation since it generates changes in the profile of its sensory characteristics such as acidity, taste, smell, and viscosity. The best formulation of a fermented beverage based on soybeans and chickpea reported by Wang et al.²¹ had 6.20 points on a 9-point scale. Skrzypczak et al.²⁶ reported a 6.80-point on a 9-point scale in a chickpea beverage; Freire et al.³⁷ reported a 5.17-point on a 9-point scale in a cassava and rice-based beverages fermented, Da Silva et al.³⁸ found values of 8-point on a 9-point scale in a drinkable soy kefir-based functional beverage and Caluza³⁹ obtained between 6-7 points on a 9-point scale in a fruity flavored drinkable soy.

Experimental design and response surface models

The results analyzed with the rotatable central composite design (Table 3), determined that the quadratic model denoted the most considerable fitted of the data for soluble solids, acidity, probiotic count, protein and overall acceptability, and the linear model for dietary fiber. Likewise, the significance of the models was evaluated through analysis of variance that presented a significant effect ($p < 0.05$). Besides regression coefficients, the equations for each response were determined.

Figure 1A shows the contours generated for the DCCR, which represents the analysis area at the limits of the independent variables, denoting that the region of interest for the probiotic count was between 1.5-3% and 30-40% of oatmeal and tarwi beverage, respectively. In figure 1B, analysis indicated that between 2.5-3% and 32.5-40% of oatmeal and tarwi beverage, respectively, was the region of interest for protein content. In figure 1C for overall acceptability, the area of interest was between 1.5-2.5% and 32.5-40% of oatmeal and tarwi beverage, respectively.

Figure 2 shows the optimization of the independent variables on the probiotic count, protein content, and overall

acceptability responses, considered as more important for the health benefit and nutritional contribution they provide to consumers. By superposition the regions of interest of the contour surfaces, it was determined that the optimal area corresponds to 1.85% oatmeal and 39.9% tarwi beverage, achieving a probiotic count of 3.47×10^8 cfu/mL, 3.75% of protein content and 7 points of overall acceptability ("I like it very much").

Table 4 presents the validation of the optimization for the probiotic count, protein content, and overall acceptability. The shelf life of the optimal formulation stored at 5 °C was tested presenting, on the 20th day of storage, a probiotic count of 3.89×10^7 cfu/mL, a protein content of 3.54%, and overall acceptability of 7.60 points, which corresponds to "I like it very much". In addition, soluble solids of 13.8%, acidity 0.550%, and dietary fiber 0.210%.

This beverage, according to Peruvian labeling regulations, would present the octagon high in sugar (using a natural sweetener, honey), but nutritionally provides the benefits of high protein content, dietary fiber, probiotic bacteria and does not contain artificial preservatives foods, that is recommended by the world health institutions, as well as, a good overall acceptability that demonstrates the intention to purchase the product for possible commercialization as a functional beverage.

CONCLUSION

The design of a functional fermented beverage containing 1.85% oatmeal, 39.9% tarwi beverage, 46.2% fresh milk, 10% honey, and 2% probiotic culture was optimized using a rotatable central composite design and experimentally validated. Likewise, the shelf life of the functional beverage was determined to be 20 days of storage at 5 °C with appropriate functional, nutritional, and sensory characteristics.

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