MedCrave
Step into the World of Research

MOJ Food Processing & Technology

Research Article

Open Access

CrossMark

Development, characterization, and stability of a functional beverage from whey

Abstract

Functional foods have become an important part of human diet, due to the contribution of these foods for health improving; being those products added with probiotics an example of functional foods. Whey is a by-product of cheese production considered as a waste, despite its protein content and nutritive value, whereas inulin has been recently used in food industry by adding it to foods in combination with certain probiotics, it is found naturally in the agave as part of dietary fiber. Then the objective of this research was to obtain a functional whey drink added with inulin and probiotic microorganisms. Therefore, different formulations of this beverage were obtained varying the content of inulin (0, 2, 4, 6 and 8% w/w) and the type of microorganism (*Lactobacillus casei*, *L. plantarum and L. reuteri*). Some properties and viability of microorganisms in the different systems were evaluated during 30 days of refrigerated storage, in which the physicochemical properties, flow parameters, and microbial stability were determined. Results showed that pH and acidity varied among different systems; pH values of whey beverages ranged between 4.75 and 6.18, decreasing with increasing of inulin content; in which there was a significant difference for initial pH. Acidity values ranged from 1.743 to 1.987 g/L, and also exhibiting significant difference. Soluble solids and moisture of beverages were stable through the storage period in contrast to acidity, color, pH, and flow properties. Whey beverages behaved as non-Newtonian fluids of pseudoplastic nature, better fitted by the Power Law equation, in which consistency increased with inulin content. All systems had the minimal level of BAL (lactic acid bacteria), and their growth was related to inulin content.

Keywords: functional beverage, whey, probiotics, inulin, properties

Volume 12 Issue 2 - 2024

Teresa Hernández, Jorge F Vélez-Ruiz
Department of Chemical and Food Engineering, Universidad de las Américas, Puebla, Mexico

Correspondence: Jorge F Vélez-Ruiz, Biochemical Engineer, Master in Food Science, Ph. D. in Food Engineering, Tel +222.2370117, Email jorgef.velez@udlap.mx

Received: July 16, 2024 | **Published:** August 07, 2024

Introduction

Functional foods have become an important part of human diet, due to awareness of consumers about contribution of these foods to improve health.[1–4] Such products have been developed by modifying traditional formulations of foods, replacing some ingredients,[5,6] and with the addition of some compounds such as soluble fiber, vitamins and probiotic microorganisms that benefit the intestinal flora. Currently there are already, different types of functional foods, such as energy drinks, high fiber foods, foods fortified with vitamins and/or minerals, and probiotic products.[7,8] Dairy products are important food medium to obtain functional foods.[3,4,9–12]

Prebiotics are defined as ingredients that have a beneficial effect on the body by stimulating the growth and activity of one or more strains of bacteria in the colon, hence improving health. Among prebiotics are included dietary fiber, inulin, and fructo-oligosaccharides.[2,13] Some of the probiotic bacteria most widely used, are *Lactobacillus casei*, *Lactobacillus acidophilus* and *Bifidobacterium bifidum*, among others.[6,12]

On the other hand, whey is a by-product of cheese, it is considered as a waste, however it has good nutritional and health benefits due to its composition, with high protein content. Its use has not been completely exploited in food industry, even though a part of the effluent is still polluting. Since years ago, some alternatives have been explored for its management and utilization in food development.[5,14] Whey has already been used in the preparation of soft drinks flavored with fruit juice in which, their nutritional value has increased with the addition of probiotic microorganisms, providing more pleasant sensory characteristics, better acceptability, and a longer shelf life. Other investigations that have been completed report the use of whey isolate or concentrate but not the use of serum. In case that whey is utilized as a liquid, it generates an important energy saving, because a drying process is not needed. Serum could be used as a base for preparation of functional drinks, that will have the additional benefit of saving water, in comparison to most of methods that have been carried out in some investigations.[11,12,15–17]

Additionally, inulin is a fructan (polymer of fructose), naturally found in roots, tubers, and rhizomes of certain flowering plants such as chicory, jicama, and agave, it is part of the dietary fiber.[18] Some years ago, it begun to be used in food industry, adding it to foods in combination with certain probiotics, but still needs to be investigated. The inclusion of this carbohydrate in functional beverages, requires the knowledge of its effect on the growth of microorganisms.[1,10,19] Different aspects on the addition of probiotic microorganisms in beverages made from whey, also with the addition of inulin in various food products has been researched, but the application of prebiotics, probiotic bacteria, and inulin studies, still continue. There is not enough information about the addition of both, inulin and probiotic, on whey beverages.[6,20–22]

By combining characteristics of whey with inulin and probiotic microorganisms, a low cost fermented beverage, with important nutritional characteristics could be obtained. Therefore, it is considered of great importance to obtain a functional beverage using serum, fortified with inulin and probiotics, that is the purpose of this research. Thus, our investigation was divided into three stages: formulation development, characterization of the prepared beverages and stability evolution, completed with a sensory evaluation of selected systems; in order to suggest a selected formulation, considered as the best combination.

Material and methods

To achieve the proposed objectives, several materials and standard methodologies were utilized, in a work of several months.

Materials

Whey was provided by local cheese factories, interested in this project, whereas three strains of bacteria (*Lactobacillus casei*, *Lactobacillus plantarum* and *Lactobacillus reuteri*) were taken from the laboratory of Microbiology at the Chemical and Food Engineering Department (Universidad de las Américas, Puebla). Whereas the inulin (Agaviótica brand) was taken from other laboratory, other ingredients were acquired in a local supermarket. or taken from laboratories (Chemical and Food Engineering Department).

Microbial and beverages preparation

Lactic bacteria reactivation

Lactic bacteria were reactivated into MRS broth to be sown in agar plates and placed at 37°C through 48 hours at anaerobic conditions. Subsequently, the bacteria were preserved at 4°C. The wedges were reseeded every 2 weeks to avoid inactivation of microorganisms.

Preparation of probiotics

50 mL of MRS broth were prepared, inoculating with a crop roast, for each microorganism. Then the broths were incubated at 35 °C through 24 hours; each broth was distributed in one centrifuge tube (each tube for 25 mL, approximately). After that, they were centrifuged at 12000 rpm for 5 min, at 15 °C. The precipitates were resuspended in 2 mL of sterile water to be added at the whey, that was thermically treated at 75 °C for 15 min.

Enumeration of bacteria

One gram of beverage was taken, and serial tenfold dilutions were prepared in a solution of sterile peptone water (0.1% w/v), and suitable dilutions were planted on appropriate media.

Preparation of functional beverages

Thermically treated whey (300 mL) were added with inulin powder (0, 2, 4, 6 y 8 % w/w), following an experimental design. Samples were well mixed with a blender, the specific probiotic microorganisms were incorporated, and the fermentation stage was developed at 35°C, through 4 hours. Once the beverages were prepared, they were preserved at low temperature (4°C) up to analysis requirement. The systems are specified in the results section. And for the final sensory evaluation, the selected fermented beverage with *L. reuteri* was added with sugar and inulin at the same concentration (4% w/v).

Methodology

Physicochemical determinations

Acidity was quantified by titration with NaOH (0.1N), using 5 g of sample and phenolphthalein as indicator. This is an accepted methodology,[23] in which next relationship was used, to express lactic acid:

$$Acidity\ (\%) = \frac{V \times N(of\ NaOH, mL)}{SV\ (mL)} x100 \qquad \text{(Eq. 1)}$$

Where: V is the used NaOH volume (mL), N is the normality of NaOH, meq: are the

milliequivalents of lactic acid, and SV is the sample volume (mL)

For pH determination, a digital potentiometer was utilized, it was a Corning meter 445 (Cole Parmer Co., USA), previously calibrated with buffers of pH 4 and pH 7, by immersion of the electrode in the liquid sample.

Color was measured with a Gardner Color System 05 colorimeter (Hunter Labs, Reston VA, USA), previously calibrated with two references (white and black plates, having standardized reflectance), and using a tristimulus scale with 20 mL of beverage sample. The differences between samples (ΔL*, Δa*, Δb*) were evaluated, using L*, a* and b* CIELAB color parameters, as well as chrome (C) and tone (H), and net change of color by using equations 2,[24] with 10 g of sample placed in quartz cells.

$$H = \tan - 1(b* / a*) \qquad \text{(Eq. 2a)}$$

$$C = \sqrt{a*^2 + b*^2} \qquad \text{(Eq. 2b)}$$

$$\Delta E = \left(\Delta L*^2 + \Delta a*^2 + \Delta b*^2\right)^{1/2} \qquad \text{(Eq. 2c)}$$

Where: H is the hue or tone, C is the chrome, and ΔE is the net change of color, ΔL* is the luminosity parameter change, Δa* is the red parameter change, and Δb* is the yellow parameter change.

Total Solids or Moisture was quantified by weight loss through evaporation of water, introducing 2 mL of sample into an oven at 100°C for 4 hours, according to the method 17.007.12.[25] Computing the correspondent weight differences.

Proximal Analysis was completed determining the presence of fat by using a mix of ethyl ether, petroleum ether, and ethanol,[23] and computing weight differences after evaporation of solvents. Whereas protein was quantified by Kjeldahl methodology[23] based on nitrogen determination.

Determination of flow properties

Measurements for flow characterization of beverages with 10 mL of sample, were completed in a digital Brookfield Viscometer.[26] The viscometer was adjusted to zero, a small sample cell (20 mL) and a spindle LV with a specific torque, were set in the instrument.

The flow parameters were determined using those relationships (Equations 3) given by the company.[26] Thus, from the rotation speed and torque, the correspondent shear rate (g) and shear stress (t) as two fundamental parameters were computed:

$$\gamma = \frac{2\omega R_c^{\ 2}}{(R_c^{\ 2} - R_b^{\ 2})} \qquad \text{(Eq. 3a)}$$

$$\omega = \left(\frac{2\pi}{60}\right) N \qquad \text{(Eq.3b)}$$

$$\tau = \frac{M}{2\pi R_b^{\ 2} L} \qquad \text{(Eq. 3c)}$$

Where: g is the shear rate (s^{-1}), w is the angular velocity of the spindle (rad/s), R_c is the internal bowl radius (0.9525 cm), R_b is the spindle radius (cm), N is the rotational velocity of the spindle (rpm), τ is the shear stress (N/m^2), M is the torque of the apparatus, 6.737 x 10^{-5} times the instrument reading, in percentage (N·m), and L is the effective length of the spindle (cm).

In this research, two flow models were used to fit experimental data, Power Law and Herschel-Bulkley relationships, both models are expressed by equations 4a and 4b respectively (Ramírez-Sucre and Vélez-Ruiz, 2011; Vélez-Ruiz, 2017).

$$\tau = K\gamma^{n} \quad \text{(Eq. 4a)}$$

$$\tau = \tau_{o} + K\gamma \quad \text{(Eq. 4b)}$$

Where: τ is the shear stress (Pa), γ is the shear rate (s^{-1}), n is the flow behavior index (dimensionless), K is the consistency coefficient ($Pa \cdot s^{n}$), and τ_{o} is the yield stress (Pa).

In order to determine the best fitting, when both models were applied, two goodness relationships were applied: the percentage of mean relative error (PEM, Eq. 5a) and the square root of mean error (RMSE, Eq. 5b).[27,28]

$$PEM = \frac{100}{n}\sum_{i=1}^{n}\left(\frac{\left|\tau_{\exp} - \tau_{pred}\right|}{\tau^{1}\exp}\right) \quad \text{(Eq. 5a)}$$

$$RMSE = \left[\frac{1}{n}\sum_{i=1}^{n}\left(\tau_{\exp} - \tau_{pred}\right)^{2}\right]^{\frac{1}{2}} \quad \text{(Eq. 5b)}$$

Where: *n* is the number of determinations, τ_{exp}: is the experimental shear stress (Pa), and τ_{pred}:

is the predicted shear stress, by the model (Pa).

Microbiological count

The functional beverages were analysed for aerobic bacteria, fungi, and yeast counting, by following the correspondent Mexican norms (NMX-092 and 111-SSA1-1994). These analyses were performed by preparing serial dilutions in peptone water (0.1% w/v) and plating samples by duplicated, in nutrient agar (Merck, Mexico) at 25°C-48 h for mesophilic bacteria, in potato dextrose agar (Merck, Mexico) at 35°C-120 h for fungi and yeasts, and in bright violet red bile agar (Merck, Mexico) for total coliforms, before enumeration.

Lactic bacteria determination

Samples of 10^{-6} dilutions were utilized, using agar MRS and the Autoplate 4000 Spiral Biotech (Advanced Instruments, Inc. MA, USA). Incubating at 35 °C for 48 hours (aerobic condition) in an Imperial Counter III (Lab Line Instruments) and doing the count of lactic bacteria in a Spiral Biotech Q-Count.

Sensorial analysis

Sensory characterization (acidity and global acceptability) of some samples, that were previously selected, was carried out with a non-trained panel of 50 persons, applying two tests, a non-structured lineal hedonic scale, and a structured scale by categories.[29] This sensory evaluation of three selected items with 15 mL of sample by an untrained panel, was based on affective tests by using a 9-point hedonic scale, 1 for dislike extremely and 9 for like extremely.[27] The group of panelists worked individually, with 3 samples per session.

A final comparison of two beverages was carried out, one was that original sample that was most accepted, and the other was this same beverage sweetened with 4% (p/V), in this case a group of 25 non-trained panelists was utilized.

Statistical analysis

Experimental data were subjected to analysis of variance and Tukey test to determine significant differences between the studied systems, with a confidence level of 95%, using the MINITAB software v.16® (Minitab Inc., State College, PA, USA).

Research evolution

In order to complete this study, firstly three industrial whey were analyzed, to have information of this by-product. Next, the formulation of the prepared beverages was experimentally standardized; in which their important properties were measured and characterized. Thirdly, a stability analysis was completed.

Results and discussion

As it was mentioned, the experimental part was divided in three stages, next are the results, observations, and comments, respectively.

Beverages formulation

Analysis and characterization of whey

Three samples of whey, supplied by three cheese plants of the region, were analyzed, and one of them was selected as the most convenient, due to its composition, physicochemical, and microbial characteristics. Composition of the selected whey is shown in Table 1:

Table 1 Composition and physicochemical properties of whey

Component or property (% p/v)	Quantity* (Mean and st dev.)
Fat	0.032 ± 0.006
Protein	0.875 ± 0.062
Total solids	7.080 ± 0.128
Brix degrees	5.525 ± 0.120
Acidity (% of lactic acid)	0.103 ± 0.011
pH	6.650 ± 0.004

* Mean of three determinations

As it was expected, the content of solids is low (7.080%), but with a good content, mainly represented by lactose (5.825 °Bx); protein and fat are very lows (< 1%). Acidity and pH are normal for this milk byproduct.

Microbial counts

Whey was subjected to a thermal treatment or pasteurization of 75°C during 15 min, to obtain a satisfactory raw liquid. Table 2 expresses the original counts and post-treatment whey counts.

Table 2 Microbial counts* before and after thermal treatment

Microorganisms	Before treatment (CFU/mL)	After thermal treatment (CFU/mL)
Mesophilic bacteria	3.70 E+05	< 10
Yeast and fungi	1.90 E+04	< 10
Total coliforms	2.00 E+02	< 10

*Average of three determination4A.3 formulation of functional beverages

After the treatment the number of microorganisms was under a Mexican Norm (NOM-184-SSA1-2002)[30] and under universal acceptation, since the microbiological viewpoint. Thus, the whey was utilized as a main ingredient of the beverage formulation.

Formulation of functional beverages

Once the whey was available (analyzed, and pasteurized), it was considered appropriate for preparation of the functional beverages. Fifteen samples were formulated, including five levels of inulin and three probiotic microorganism (*Lactobacillus sp.*), Table 3 shows the composition of prepared beverages to be studied.

Table 3 Formulation of functional beverages

Number	Inulin (%)	Probiotic microorganism	Code
1	0	L. casei	0 C
2	0	L. plantarum	0 P
3	0	L. reuteri	0 R
4	2	L. casei	2 C
5	2	L. plantarum	2 P
6	2	L. reuteri	2 R
7	4	L. casei	4 C
8	4	L. plantarum	4 P
9	4	L. reuteri	4 R
10	6	L. casei	6 C
11	6	L. plantarum	6 P
12	6	L. reuteri	6 R
13	8	L. casei	8 C
14	8	L. plantarum	8 P
15	8	L. reuteri	8 R

In this experimental design, those formulations with 0% of inulin (systems 1, 2, 3), are properly considered the controls or reference systems with respect to the other beverages with the same probiotic microorganism.

Characterization of prepared beverages

Several properties of the prepared functional beverages were measured through a month of storage, taking determinations of physicochemical and flow properties on days 0, 5 10, 15, 20, 25 and 30, whereas microorganism viability was determined on days 0, 5, 10, 15, 18, 21, 24, 27 and 30, to know their evolution through this second, detailed, and important part of the study.

Physicochemical determinations

Acidity and pH, these parameters that are related, showed a normal evolution. As expected, the quantity of lactic acid increased with storage time and additionally, it was related with inulin concentration and with microorganisms. The acidity determined through the storage time for all the formulated beverages is shown next, in Table 4.

Table 4 Acidity of functional beverages through storage time

Percentage of acidity (% lactic acid) *							
Day:	**0**	**5**	**10**	**15**	**20**	**25**	**30**
0-C	0.072	0.075	0.079	0.082	0.108	0.099	0.110
0-P	0.077	0.078	0.081	0.083	0.122	0.131	0.135
0-R	0.079	0.08	0.081	0.082	0.119	0.142	0.141
2-C	0.116	0.128	0.139	0.146	0.162	0.169	0.176
2-P	0.116	0.123	0.132	0.139	0.147	0.164	0.169
2-R	0.118	0.124	0.134	0.141	0.153	0.167	0.173
4-C	0.135	0.147	0.152	0.165	0.171	0.189	0.195
4-P	0.147	0.156	0.163	0.187	0.203	0.212	0.227
4-R	0.143	0.156	0.176	0.197	0.204	0.216	0.235
6-C	0.145	0.161	0.177	0.188	0.196	0.207	0.245
6-P	0.149	0.162	0.176	0.193	0.203	0.213	0.229
6-R	0.151	0.166	0.178	0.184	0.198	0.215	0.224
8-C	0.149	0.161	0.172	0.179	0.184	0.198	0.209
8-P	0.156	0.175	0.187	0.199	0.211	0.217	0.233
8-R	0.158	0.168	0.186	0.201	0.214	0.221	0.238

* Mean of three determinations

The storage time had a significant effect on increasing acidity, observing two groups, one with determinations through day 15 in a group (0.072-0.201%), and acid determinations for the other half month, in the second group (0.108-0.238%); being similar to a trend reported by Paseephol et al.,[31] on set yogurt. Whereas with respect to inulin level, beverage systems similarly, exhibited two trends, 4, 6 and 8% showed similar higher acidities, than those measured in beverages with 0 and 2% of inulin, with lower acidity. Additionally, the quantity of lactic acid showed a general increasing trend with respect to probiotic microorganisms; similar observations were reported by Akalin et al.,[32] when fructo-oligosaccharides were added to a probiotic yogurt. This increasing in lactic acid, was also reported for analogous beverages by De Castro et al.,[33] and Maity et al., [21] No significant difference was observed between the acidity generated by *L. plantarum* and *L. reuteri*, but it was a significant difference ($P < 0.05$) between these two mentioned microorganisms and *L casei*.

The pH of beverages was determined within a wide range, from 6.18 to 3.79 as a function of the formulation and storage time. Samples without presence of microorganisms (1-3) showed highest pH at day 0, being 6.14, 6.18 and 6.11, that decreased with storage, at day 30, to 5.35, 5.32 and 5.33, correspondingly. Samples with medium levels of inulin (2-6) exhibited a pH range of 5.26 to 4.85 at day 0 and a pH range of 4.51 to 3.85 for day 30, with values also related to storage time. And systems with highest content of inulin (8%), had a pH range of 4.92 to 3.99 for system 13, 4.86 to 3.97 for system 14, and 4.76 to 3.79 for system 15, at day 0 and 30, respectively. This evolution of a notable decrease may be related to a higher presence of probiotic bacteria, favored by the higher content of inulin; that it was also mentioned by Kip et al.,[34] in their research with low-fat yoghurts. Additionally, the pH decreasing has been observed in other works; Burns et al.,[20] observed a pH decrease of 7.1 to 3.9 in a fermented whey beverage. Perea and Brito[35] also observed a pH decreasing of 6.6 to 4.2 on lactic fermentation in a dietetic yoghurt, that was related to the probiotics activity.

The statistical analysis of studied parameters on pH, indicated a significant difference between beverages with *L. reuteri* and those with *L. casei* and *L. plantarum*, being a different effect, than that obtained for acidity Similarly, it was a significant difference between beverages with 0 and 2%, with respect to those beverages with 4, 6, and 8% of inulin. And the significant effect of the storage time, was most important and notable during the first ten days.

For color evaluation, those color parameters (*L*, a*, b**) were also determined every five days through storage, in which no significant effect of time was detected ($P > 0.05$). Magnitudes of color parameters are included in Table 5.

Table 5 Color parameters from hunter scale for prepared beverages at day 0

Sample	L*	a*	b*
1 (0-C)	53.18^{ab}	-4.11^{b}	7.26^{a}
2 (0-P)	53.66^{b}	-4.17^{b}	7.19^{a}
3 (0-R)	53.27^{ab}	-4.12^{b}	7.32^{ab}
4 (2-C)	53.19^{ab}	-4.10^{b}	7.63^{ab}
5 (2-P)	53.55^{b}	-4.21^{ab}	7.24^{a}
6 (2-R)	52.97^{a}	-4.05^{b}	7.31^{a}
7 (4-C)	53.24^{ab}	-4.72^{a}	7.22^{a}
8 (4-P)	53.33^{ab}	-4.19^{ab}	7.53^{ab}
9 (4-R)	53.88^{b}	-4.21^{ab}	7.66b
10 (6-C)	53.64^{b}	-4.35^{ab}	7.41^{ab}
11 (6-P)	53.44^{b}	-4.17^{b}	7.61^{ab}
12 (6-R)	53.66^{b}	-4.17^{b}	7.19^{a}

Citation: Hernández T, Vélez-Ruiz JF. Development, characterization, and stability of a functional beverage from whey. *MOJ Food Process Technols*. 2024;12(2):140–147. DOI: 10.15406/mojfpt.2024.12.00310

Table 5 Continued..

Sample	L*	a*	b*
13 (8-C)	53.08[ab]	-4.18[ab]	7.59[ab]
14 (8-P)	53.53[b]	-4.27[ab]	7.81[b]
15 (8-R)	53.27[ab]	-4.12[b]	7.32[ab]

Different superscripts as a meaning of significant differences between simples ($P < 0.05$).

These values indicated a medium luminosity and a little tendency to yellow color. Similar determinations of *L*, a** and *b** were obtained for our beverages systems, even though it was difference between samples, that may be attributed to beverage formulation and a natural phase separation. Dello Staffolo et al.,[36] as well as Aryana et al.,[37] reported that color parameters were not affected by both ingredients, inulin and oligofructose on probiotic yoghurt.

To have an idea of beverages appearance, Figure 1 shows three of them, with a milky color (medium luminosity and low yellow intensity) in which two phases are present, after some time of repose. The color of the upper phase is similar to, that whey used for beverages preparation.

Figure 1 General appearance of functional beverages.

Microorganisms viability

As a very important determination, the microorganism viability of prepared beverages, was also carried out through one month of storage, completing the determinations each five days. Table 6 shows the microorganisms counts during a month of storage.

Table 6 Viability of acid-lactic bacteria* in prepared beverages through storing

(CFU/mL) at day									
Sample	**0**	**5**	**10**	**15**	**18**	**21**	**24**	**27**	**30**
1	3.25E+09	1.47E+10	2.62E+10	5.62E+11	6.72E+11	1.07E+12	1.27E+11	9.67E+10	1.96E+10
2	6.54E+09	7.88E+10	1.51E+11	2.52E+11	1.36E+12	1.30E+12	2.68E+11	1.17E+11	6.63E+10
3	4.74E+09	3.30E+10	6.13E+10	1.36E+11	1.19E+12	1.20E+12	2.16E+11	1.53E+11	1.13E+10
4	3.91E+10	5.98E+10	8.04E+10	1.37E+11	1.25E+10	4.20E+11	3.50E+11	2.98E+11	2.66E+11
5	3.41E+10	3.85E+10	4.28E+10	1.84E+11	1.68E+10	6.07E+11	4.11E+11	4.40E+11	3.37E+11
6	3.58E+09	2.49E+10	4.62E+10	8.10E+10	8.93E+10	3.58E+11	3.25E+11	3.43E+11	7.49E+11
7	2.58E+10	4.61E+10	6.64E+10	2.16E+10	6.78E+10	1.58E+11	8.92E+11	8.26E+11	4.37E+11
8	1.60E+10	2.60E+10	3.60E+10	1.37E+11	1.54E+11	2.49E+11	2.91E+11	1.69E+11	9.38E+10
9	1.34E+10	2.78E+10	4.21E+10	7.77E+11	3.15E+11	4.43E+11	6.44E+11	7.96E+11	5.61E+11
10	3.38E+10	3.08E+10	2.78E+10	3.91E+10	1.27E+10	2.97E+10	5.58E+10	3.48E+10	1.59E+10
11	2.67E+10	2.55E+10	2.43E+10	6.54E+10	6.54E+10	2.11E+11	1.53E+11	9.70E+11	7.43E+10
12	3.46E+10	3.01E+10	2.55E+10	1.26E+12	1.39E+12	2.26E+12	2.63E+12	3.33E+12	2.96E+12
13	2.44E+10	7.67E+10	1.29E+11	7.55E+11	8.71E+11	1.33E+12	1.56E+12	1.94E+12	8.28E+11
14	3.68E+10	1.36E+11	2.35E+11	1.05E+12	1.48E+12	1.74E+12	1.68E+12	1.12E+12	1.03E+12
15	5.33E+09	5.57E+10	1.06E+11	1.62E+12	1.69E+12	2.69E+12	4.55E+12	7.37E+12	4.72E+12

* Mean of three determinations

In Table 6, a higher growth of BAL may be observed in beverages with high content of inulin, achieving a maximum of $7.37 * 10^{12}$ CFU/mL, on day 27. This observation is in agreement with other works.[38,39] However, all systems have the minimal level of BAL (10^6 CFU/mL) needed to obtain health benefits, as it was cited by Ouwehand et al.,[40] The good development of bacteria of this kind has also been reported by other authors, De Castro et al.,[33] reached 10^6 CFU/mL in a fermented beverage based on whey, where oligofructose was added. Similarly, Maity et al.,[21] determined a total count of $1.86 * 10^9$ CFU/mL on whey beverages fermented with different BAL.

The studied variables had different influence on microorganism growth, however all the analyzed systems may be considered as functional beverages, they exhibited a minimal count of 10^7 CFU/

mL.[38,41,42] Even though BAL increased as a function of inulin content, there was not significant effect (P > 0.05) on 0, 2 and 4 % systems, but it was on 6 and 8 % p/p; in according with the results reported by Kalyani[43] and Perea.[35] Storage time was also related to probiotics augment, in which the significant effect started at day 18. Arango-Bedoya et al.,[44] and Barclaya et al.,[45] reported the presence of an enzyme (inulinase) from BAL microorganisms that favored hydrolysis and absorption of inulin.

With respect to growth of other microorganism, the studied systems did not exhibit their presence in beverages with inulin, in which the production of lactic acid by BAL has been related to the inhibition of undesirable species, such coliforms, fungi, mesophilic and yeasts.[21,35] For those beverages without inulin, the presence of yeasts was detected on day 20 of refrigerated storage. These determinations can help to decide the shelf life of functional beverages, without use of chemical preservatives.

Evaluation of flow properties

As other important part of this research, the flow properties of prepared beverages were determined and fitted, characterizing two or three flow parameters, and applying two rheological models, Power Law and Herschel and Bulkley; using two goodness criteria to decide the best fitting, being square root of the mean error (RSME) the most important one.

From shear rate and shear stress data, two for the first and three parameters for the second model were quantified., in which the best fitting corresponded to Power Law model, where the RSME was lower. Magnitudes of the flow behavior index (n) and the consistency coefficient (K) for prepared beverages through storage obtained with Power Law equation, are included in Table 7.

Table 7 Flow parameters (*) for prepared functional beverages from power law model through thirty days of storage

System	Storage day (n: dimensionless, K: Pa s^n)											
	0		5		10		15		20		30	
	n	K	n	K	n	K	n	K	n	K	n	K
1	0.543	4.37	0.460	5.47	0.465	5.99	0.424	5.80	0.637	6.09	0.617	6.44
2	0.501	6.03	0.463	6.14	0.44	6.19	0.449	5.93	0.340	6.10	0.616	6.34
3	0.430	6.08	0.415	6.15	0.411	6.26	0.453	6.31	0.600	6.60	0.569	6.90
4	0.516	6.22	0.477	6.47	0.457	6.87	0.505	6.44	0.634	6.52	0.573	7.05
5	0.538	6.32	0.507	6.36	0.455	6.42	0.473	6.54	0.639	6.54	0.616	6.99
6	0.462	5.93	0.482	6.02	0.517	6.42	0.477	6.37	0.632	6.62	0.615	7.86
7	0.497	7.14	0.532	7.31	0.555	7.27	0.558	6.84	0.645	6.99	0.633	7.36
8	0.504	7.32	0.514	7.38	0.511	7.43	0.502	7.36	0.610	7.46	0.622	7.53
9	0.445	6.84	0.469	6.98	0.475	7.38	0.558	7.41	6.07	7.99	0.583	8.66
10	0.487	7.30	0.511	7.41	0.507	7.54	0.518	7.66	0.626	7.66	0.652	8.02
11	0.562	7.29	0.521	7.32	0.517	7.60	0.518	7.96	0.623	8.21	0.667	8.98
12	0.530	7.87	0.49	8.36	0.483	8.51	0.446	8.7	0.602	8.82	0.603	9.05
13	0.536	8.38	0.576	8.64	0.521	8.84	0.49	9.14	0.603	9.67	0.588	10.1
14	0.527	8.71	0.547	8.93	0.512	8.99	0.472	10.3	0.598	10.1	0.616	10.4
15	0.499	8.93	0.500	9.64	0.507	9.82	0.662	10.2	0.544	12.3	0.651	13.2

All systems behaved as pseudoplastic materials, that is an expected behavior for this type of beverages in which the solid and liquid components interact, generating a non-Newtonian flow response, this behavior has been also reported by Oliveira et al.,[46] for a fermented milk beverage added with inulin. Both parameters exhibited a diversity of values but, without a general trend.

With respect to time as an important factor, both flow parameters had an increasing trend, with next ranges at three times: 0.445-0.543 for n and 4.37-8.93 for K at day 0; 0.424-0.558 for n and 6.80-8.70 for K at day 15, and 0.573-0.667 for n and 6.34-9.05 Pa s^n for K at day 30, indicating a higher Newtonian trend due to flow index augment, and an increasing in viscosity or consistency due to K increasing, with respect to storage time.

And with respect to composition, K was higher in beverages with *L. reuteri*; that was significantly different (P < 0.05) to those beverages added with *L. casei* and *L. plantarum*. Similarly, the consistency augmented with inulin content as expected, due to the increasing of solids in the liquid phase, that is in agreement with Villegas and Costell,[47] who observed an increasing in viscosity of lactic beverages and yoghurt added with inulin. Those beverages with. 6 and 8 %, were significantly different to those samples with 0, 2 and 4%.

From the flow parameters, a linear correlation equation (Eq. 6) was deducted (R^2=0.995) between the consistency coefficient and inulin content, that is next:

$$K = 0.0004\ \%\ \text{inulin} + 0.0054 \quad \text{(Eq. 6)}$$

Where:

K: is the consistency coefficient (Pa s^n)

Inulin in percentage w/w

Similarly for the flow behavior index, there was a significant difference between those beverages with *L. reuteri*, with respect to those prepared with *L. casei* and *L. plantarum*. Beverages with 0-4% inulin behaved similar, but different that the rest of the systems. And with respect to storage, n was relatively constant up to the day 20, in which a decreasing trend was observed and related with the grow of microorganisms. Lately to day 20, an light increasing in *n,* or tendency to Newtonian behavior was observed. The effect of the studied variables is complex, and they influenced the flow response in a different importance degree, such it was mentioned by Wehrle et al.,[48] and Völker and Fritz,[49] among others.

Selection of beverages by sensory assessment

To continue with this research work and taking into account the previous results; in which the most important factor was the bacteria grow, some beverages were considered as best, distinction that corresponded to systems prepared with *L. reuteri*. Therefore, three beverages with 2, 4 and 6% of inulin were subjected to a sensory evaluation with 50 judges, applying two types of tests, a non-structured linear scale for general acceptation, and another with structured scaling for acidity. From the first sensory tests, next means and standard deviations were quantified, 6.00 (+ 2.54) for 2%, 8.00 (+ 2.22) for 4%, and 3.00 (+ 2.34) for 6% beverages, the system with 4% was preferred for the judges, clearly. The threes systems showed high standard deviations, with significant difference between them; 2 and 4% were significantly different, with respect to system 6%. Thus, the beverage with 4% was considered the best.

On the other hand, for acidity sensorial evaluation of beverages, it was observed a relation between inulin level and acidity perception, in which 3.00 (+ 0.72) was measured for 2%, 4.00 (+ 0.71) for 4%, and 5.00 (+ 0.77) for 6% of inulin. There was significant difference between them, and they may be considered as lightly acid, acidic, and very acid, respectively.

Finally, and just to obtain more information, two beverages were subjected to an extra sensory assessment (a linear structured type); one of them with 4%, in comparison with another one, with 4% inulin also, but with sugar added. And although the sensory evaluation showed small differences in a range of 6-9 points, some statistical conclusions were obtained: odor and color did not show significant difference, but texture, flavor and general acceptability did, that was attributed to the sugar presence in the corresponding functional beverage.[50,51]

Conclusion

Functional beveragaes based on whey, were formulated, studied, and stored. All prepared beverages may be considered as functional, because the number of BAL meets the required quantity (>10^7), exhibiting a higher grow in those prepared with *L. reuteri*, and also with content of inulin. The presence of BAL inhibited the development of deteriorative microorganisms.

Measured physicochemical characteristics corresponded to a functional beverage in which the acidity was higher and pH lower, in systems fermented with *L. reuteri* than with the other two microorganism. Also, both parameters were related to inulin content and storage time. Color parameters were stable through storage time at low temperature.

With respect to flow response, beverages exhibited a non-Newtonian behavior of pseudoplastic nature, better fitted by the Power Law model. Both flow properties were affected by the type of microorganism, inulin content and storage time. The viscosity of prepared beverages augmented with presence of *L. reuteri*, inulin content and storage time.

In general, these functional beverages were stable through the storage period and well accepted in a sensory evaluation by non-trained panelists; particularly, system with 4% inulin was considered as the best, in which sugar adding, contributed to a better acceptability.

This research showed the characteristics and potential benefits of a formulated and studied functional beverage, it may contribute to the knowledge of scientists, people, and developers of foods.

Acknowledgments

None.

Conflicts of interest

Authors declare that there is no conflict of interest.

Funding

None.

References

1. Madrigal L, Sangronis E. Inuline and its derivatives as key ingredients in foods. *Archivos Latinoamericanos de Nutrición.* 2007;57(4):387–395.
2. Paiboon T, Tipparat H, Kongkarn K, et al. Prebiotics – a review. *Songklanakarin Journal of Science and Technology*. 2009;31(4):401–408.
3. Aguilar-Raymundo VG, Vélez-Ruiz JF. Physicochemical and rheological properties of a dairy dessert, enriched with chickpea flour. *Foods*. 2018;7(2):1–25.
4. Aguilar-Raymundo VG, **Vélez-Ruiz JF. Yoghurt type beverage with partial substitution of milk by a chickpea extract: effect on physicochemical and flow properties.** *International Journal of Dairy Technology.* 2019;72(2):266–274.
5. Haque ZU, Ji T. Cheddar whey processing and source: II. Effect on non-fat ice cream and yoghurt. *International Journal of Food Science and Technology*. 2003;38:463–473.
6. Mudgil D, Barak S. *Chapter 3 Dairy Based Functional Beverages*. Milk-Based Beverages, In: Grunezescu AM et al., editors. Wiidhead Publishing. Elsevier. 2019. p. 67–93.
7. Foda MI, El-Aziz MA, Awad AA. Chemical, rheological and sensory evaluation of yogurt supplemented with turmeric. *International Journal of Dairy Science.* 2007;2(3):252–259.
8. Koh L, Jiang B, Kasapis S, et al. Structure, sensory and nutritional aspects of soluble-fibre inclusion in processed food products. *Food Hydrocolloids*. 2011;25(2):159–164.
9. Duarte C, Hickmann S. The influence of additives on the rheological and sensory properties of nonfat yogurt. *International Journal of Dairy Technology*. 2007;60(4):68–75.
10. González-Tomás L, Bayari S, Costell E. Inulin-enriched dairy desserts: Physicochemical and sensory aspects. *J Dairy Sci.* 2009;92(9):4188–4199.
11. Ozer BH, Kirmaci HA. Functional milks and dairy beverages. *International Journal of Dairy Technology.* 2010;63(1):266–274.
12. Turkmen N, Akal C, Ozer B. Probiotic dairy-based beverages: A review. *Journal of Functional Foods*. 2019;53:62–75.
13. Patel P, Parekh T, Subhash R. Development of probiotic and symbiotic chocolate mousse: a functional food. *Biotechnology*. 2008;7(4):769–774.
14. Williams-Zambrano MB, Dueñas-Rivadeneira AA. Alternatives for the use of whey: research background and traditional uses. *La Técnica Revista de las Agrociencias.* 2021;11(2):39–50.
15. Glibowski P. Rheological properties and structure of inulin-whey protein gels. *International Dairy Journal*. 2009;19(8):19–24.
16. Tobin JT, Fitzsimons SM, Kelly AL, et al. Microparticulation of mixtures of whey protein and inulin. *International Journal of Dairy Technology*. 2010;63(1):32–40.

17. Walsh H, Ross J, Hendricks G, et al. Physicochemical properties, probiotic survivability, microstructure and acceptability of a yogurt-like symbiotic oats-based product using pre-polymerized whey protein as a gelation agent. *J Food Sci*. 2010;75(5):327–337.

18. Cherbut C. Inulin and oligofructose in the dietary fiber concept. *Br J Nutr*. 2002;87(2):159–162.

19. Kelly ND. Inulin-type prebiotics a review: Part 1. *Altern Med Rev*. 2008;13(4):315–329.

20. Burns P, Vinderola G, Molinari F, et al. Suitability of whey and buttermilk for the growth and frozen storage of probiotic lactobacilli. *International Journal of Dairy Technology*. 2008;61(2):156–164.

21. Maity TK, Rakesh K, Misra AK. Development of healthy whey drink with *Lactobacillus rhamnosus*, *Bifidobacterium bifidum* and *Propionibacterium freudenreichii* subsp. *shermanii*. *Mljekarstvo.* 2008;58(4):315–325.

22. Tárrega A, Costell E. *Effect of inulin addition on rheological and sensory properties of fat free starch-based dairy desserts*. Instituto de Agroquímica y Tecnología de Alimentos. Valencia. Spain. 2008.

23. AOAC. *"Official Methods of Analysis"*. 14th edn. Association of Official Analytical Chemists. Inc. Washington, D.C. EE.UU. 1984.

24. Díaz-Jiménez B, Sosa-Morales ME, Vélez-Ruiz JF. Effect of addition of fiber and fat reduction on physicochemical properties of yogurt. *Revista Mexicana de Ingeniería Química.* 2004;3:287–305.

25. AOAC. *"Official Methods of Analysis"*. 17th edn Association of Official Analytical Chemists. Inc. Washington, D.C. EE.UU. 2000.

26. Brookfield Engineering Laboratories, Inc. *Brookfield Instruction Manual.* DVIII. Massachussets, USA. 1985.

27. Ramírez-Sucre MO, **Vélez-Ruiz JF.** The physicochemical and rheological properties of a milk drink flavored with *cajeta*, a Mexican caramel jam. *International Journal of Dairy Technology.* 2011;64(2):294–304.

28. Vélez-Ruiz JF. *Notes for Food Engineering Course*. Universidad de las Américas, Puebla. México. 2017.

29. Lardmond E. *"Méthodes d'appréciation sensorielle des aliments en laboratoire"*. Research Branch, Agriculture Canada, Ottawa, Canada. 1977.

30. Secretary of Health in Mexico. Mexican Official Norm (NOM-184-SSA1-2002) for different microorganisms' growth.

31. Paseephol T, Small DM, Sherkat F. Rheology and texture of set yogurt as affected by inulin addition. *Journal of Texture Studies.* 2008;39(6):617–634.

32. Akalin AS, Tokusoglu Ö, Gönc S, et al. Occurrence of conjugated linoleic acid in probiotic yoghurts supplemented with fructo-oligosaccharide. *International Dairy Journal*. 2007;17(9):1089–1095.

33. De Castro FP, Cunha TM, Barreto PL, et al. Effect of oligofructose incorporation on the properties of fermented probiotic lactic beverages. *International Journal of Dairy Technology*. 2008;60(4):270–276.

34. Kip P, Meyer D, Jellema RH. Inulins improve sensoric and textural properties of low-fat yoghurts. *International Dairy Journal*. 2006;16:1098–1103.

35. Perea J, Brito AI. Evaluation of lactic fermentation in a dietetic yoghurt. *Ciencia y Tecnología de Alimentos*. 2008;18(1):20–25.

36. Dello Staffolo M, Bertola N, Martino M, et al. Influence of dietary fiber addition on sensory and rheological properties of yogurt. *International Dairy Journal.* 2004;14(3):263–268.

37. Aryana KJ, McGrew P. Quality attributes of yogurt with *Lactobacillus casei* and various prebiotics. *Food Science and Technology*. 2007;40(10):1808–1814.

38. Shah N. Functional food from probiotics and prebiotics. *Food Technology*. 2001;55(11):46–53.

39. Bengmark S, Gil A. Bioecological and nutritional control of sickness: prebiotics, probiotics and symbiotics. *Nutr Hosp*. 2006;21(2):73–86.

40. Ouwehand AC, Salminen SE, Isolauri E. Probiotics: an overview of beneficial effects. *Antonie Van Leeuwenhoeck*. 2002;82(4):279–289.

41. Gomes MPA, Malcata FX. *Bifidobacterium spp*. and *Lactobacillus acidophilus*: biological, biochemical, technological, and therapeutical properties relevant for use as probiotics. *Trends in Food Science and Technology*. 1999;10(4–5):139–157.

42. Kushal R, Anand SK, Chander H. In vivo demonstration of enhanced probiotic effect of co-immobilized *Lactobacillus acidophilus* and *Bifidobacterium bifidum*. *International Journal of Dairy Technology.* 2006;59(4):265–271.

43. Kalyani N, Thompkinson DK. Optimization of ingredients for the formulation of a direct acidified whey based lassi-like beverage. *International Journal of Dairy Technology*. 2008;61(2):199–205.

44. Arango-Bedoya A, Cuarán GP, Fajardo JF. Extraction, crystallization, and characterization of inulin from yacon (*Smallanthus sonchifolius*) for to be used in food and pharmaceutic industry. *Facultad de Ciencias Agropecuarias*. 20086:14–20.

45. Barclaya T, Ginic-Markovica M, Cooperb P, et al. Inulin - a versatile polysaccharide with multiple pharmaceutical and food chemical uses. *Journal of Excipients and Food Chemistry*. 2010;1(3):27–50.

46. Oliveira MN, Sodini I, Remeuf F, et al. Effect of milk supplementation and culture composition on acidification, textural properties and microbiological stability of fermented milks containing probiotic bacteria. *International Dairy Journal.* 2001;11(11–12):935–942.

47. Villegas B, Costell E. Inulin milk beverages: sensory differences in thickness and creaminess using R-index analysis of the ranking data. *Journal of Sensory Studies*. 2007;22(4):377–393.

48. Wehrle K, Grau H, Arendt EK. Effects of lactic acid, acetic acid and table salt on fundamental rheological properties of wheat dough. *Cereal Chemistry*. 1997;74(6):739–744.

49. Volker TH, Fritz HG. Rheological characterization of fermentation broths. In: "*Theoretical and Applied Rheology*". 1st edn. P Moldenaers, R Keunings, Editors. Elsevier. London. England. 1992. p. 725.

50. Chavez-Ugalde Y, **Vélez-Ruiz JF. Characterization of a Mexican hot sauce (from "**chile de arbol") and changes through storage. *MOJ Food Processing and Technology.* 2024;12(1):12–20.

51. Sekhon BS, Jairath S. Probiotic, prebiotic and symbiotic: An overview. *Journal of Pharmaceutical Education and Research*. 2010;1(2):13–19.