Numerical optimization of probiotic Ayran production based on whey containing transglutaminase and *Aloe vera* gel

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Abstract The purpose of this study was to optimize the functional properties of probiotic Ayran. Two-level fractional factorial design with four center points was used to investigate the effect of five independent variables including, reconstructed whey protein (70–90% of milk), salt (0.5–1 g/100 g), *Aloe vera* gel (0–30 g/100 g), transglutaminase enzyme (0–14 unit/100 g) and storage time (1–21 days). The viability of *Lactobacillus acidophilus* La-5 and other physicochemical properties such as pH, acidity, viscosity, sedimentation, and color were modeled and then optimized using desirability function method. Results showed that reconstructed whey protein and *Aloe vera* gel significantly affected the viability of *L. acidophilus* La-5 and other physicochemical properties ($p < 0.05$). The viability of *L. acidophilus* La-5 and viscosity decreased by increasing of whey protein percentage from 70 to 90. Maximum *L. acidophilus* La-5 count was observed in samples with a minimum level of whey protein and maximum level of *Aloe vera* gel. Milk replacement with whey protein up to 90% caused to decrease acidity and viscosity significantly but sedimentation increased ($p < 0.05$). Optimum condition for production of functional Ayran determined as follow: *Aloe vera* gel concentration: 25.7%,

reconstructed whey protein: 70%, salt: 0.58% and transglutaminase enzyme: 5 unit/100 mL.

Keywords *Aloe vera* gel · Whey protein · Transglutaminase enzyme · *Lactobacillus acidophilus* La-5

Introduction

Whey constitutes about 85–90% of milk volume which remains after cheese making and liquid whey is composed of 5% lactose, 93% water, 0.85% protein, 0.36% fat and 0.53% minerals (Pescuma et al. 2008, 2010). This dairy effluent is heavy organic pollutant due to high BOD¹ and COD² (Ozmihci and Kargi 2007). Therefore, considerable efforts have been made to find new outlets for whey utilization and reduce environmental pollution (Pescuma et al. 2008). Availability of lactose and other nutrients for the growth of microorganisms make the whey as a potential raw material for the production of different fermented food products (Panesar et al. 2007). Beta-lactoglobulin (BLG) has most abundance in whey protein of milk, which is highly resistant to gastric digestion and a major cause of allergen in human. Lactic acid bacteria (LAB), which extensively used in the dairy fermented product, can hydrolyze BLG and the released peptides could absorb better in intestinal tract than intact protein.

Transglutaminase (TGase) is an acyltransferase enzyme which can catalyze and modify the functional properties like protein hydration by cross-linking of different proteins (Ardelean et al. 2012). The TGase polymerization effect on milk proteins has been extensively studied (Gauche et al.

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2008). Its application in yogurt production cause to increase viscosity, gel strength, water holding capacity, textural and rheological properties (Ilić et al. 2013). Numerous studies has been demonstrated that caseins can be cross-linked by TGase easily, due to their flexible and little or no secondary structure. Effect of TGase on β -casein and κ -casein confer intermolecular cross-linking in the casein micelles because κ -caseins are located near the outside of the micelle. Whey proteins have been shown to be poor substrates to TGase due to their native compact structure. However, in the presence of reducing agents, NH_2 groups in protein molecules and sulfhydryl groups increase in the active site, so whey proteins become more potential to cross-linking (Gauche et al. 2008). Sharma et al. (2002), showed that TGase can react with β -lactoglobulin and α -lactalbumin without modification of whey protein structure and β -lactoglobulin act as a substrate for TGase in a single-protein system to form analogous polymers and dimers. Presences of these polymers increase the stability and non-Newtonian behavior.

Due to the increase of demand for healthy and high-quality foods, researches are focused on functional products in the last two decades. One of the raw materials with high potential in this field is *Aloe vera*, which is a tropical or subtropical plant. It has been used in functional foods and pharmaceutical for a long time (Chang et al. 2011). *Aloe vera* is recognized as a potential source of gums or hydrocolloids. The clear understanding of physicochemical and rheological properties of natural compounds is a critical factor in the selection of new biopolymers. These properties are affected by the separation method and these can considerably change with drying process (Wang and Langrish 2009).

Dairy products are the main substrates supplemented with probiotic bacteria (Granato et al. 2010). Probiotic foods have been defined as foods including live microorganisms to enhance health by improving the gastrointestinal microflora balance (Almeida et al. 2008; Tamime et al. 2005). Genera of *Lactobacillus*, *Bifidobacterium*, *Streptococcus* and *Saccharomyces* are commonly used as probiotic. Probiotics are affected by other bacteria during long fermentation period while the growth rate is not important during short fermentation (Almeida et al. 2008).

The aims of this study were formulated and optimized a functional probiotic Ayran based on whey. Thus, the effect of five independent variables including, reconstructed whey protein (70–90% of milk), salt (0.5–1 g/100 g), *Aloe vera* gel (0–30 g/100 g), transglutaminase enzyme (0–14 unit/100 g) and storage time (1–21 day) on viability of *Lactobacillus acidophilus* La-5 and physicochemical properties such as pH, acidity, viscosity, sedimentation and color investigated using two-level fractional factorial design and optimized by numerical method.

Materials and methods

Materials

Ca^{2+} independent microbial transglutaminase was prepared from Ajinomoto (Ajinomoto, Paris, France), the enzyme activity was measured by manufacturer data. Whey protein concentrate (WPC35: Sachsenmilch, Germany) with 35% w/w protein was reconstituted using distilled water to 1% w/v of protein at 45 °C to simulate cheese whey according to the procedure used by Pescuma et al. 2010. Nonfat dry matters, total protein, fat content of reconstituted whey were 2.09%, 0.93% and 0.6%, respectively. *Aloe vera* leaves were collected from Urmia local market and gel was prepared using the method of Srisukh et al. (2008) with some modifications. HTST-treated homogenized cow milk was obtained from the local supermarket, Urmia, Iran. Nonfat dry mater, total protein, fat content and lactose of milk were 7.50, 2.95, 4 and 4.23 g/100 g, respectively. Freeze dried probiotic *Lactobacillus acidophilus* La-5 was purchased from Chr. Hansen (Chr. Hansen, Hoersholm, Denmark). DVS³ freeze-dried Ayran starter culture FVV-20 series (mixed culture of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus*) was purchased from DSM (DSM Food Specialities, Moorebank, NSW, Australia).

Preparation of Ayran

Ayran samples were prepared by the procedure that was detailed in the Turkish standard by some modification. To prepare mixtures according to the experimental design, samples were produced substituting 10%, 20% and 30% of reconstituted WPC35 by milk which followed by mixing 0, 15, and 30% of *Aloe vera* gel and then homogenized for 15 s at 17,000 rpm (Ultra Turrax T25 IKE, Germany). Achieved samples were heated to 50 °C in the water bath and TGase at levels of 0, 7 and 14 unit/100 mL were added. The samples were incubated at 50 °C for 1 h to inhibit enzymatic activity and then pasteurized at 65 °C for 30 min. The temperature was reduced to 41 °C, then starter cultures mixed with probiotic strain (12.8 g/100 kg) then incubated at 38 °C for 5.5 h to pH of 4.4–4.6 (Altay et al. 2013; TFC 2009). Samples cooled to 20 °C in ice bath, salt levels of 0.5, 0.75 and 1% added and cooled to 5 °C and stored at 4 °C. Physicochemical properties and *L. acidophilus* La-5 viability were investigated during 21 days of storage.

³ Direct vat set.

Physicochemical analysis

Total solid, crude protein, fat, lactose of initial samples and mixtures were determined according to the standard methods of AOAC⁴ (Horwitz and Latimer 2005).

The pH was determined with a digital pH meter (model pH 510; Eutech Instrument). The titratable acidity was determined by titrating with 0.1 N NaOH using a pH meter (model pH 510; Eutech Instruments) to an endpoint of pH = 8.3 ± 0.01 . It was calculated based on lactic acid as the predominant acid and was expressed as the percentage of lactic acid.

For the determination of serum separation, samples were placed in 5 ml graduated cylinders and stored at 4 °C. The volume of the separated serum at the top was measured during 21 days of storage. The amount of separated serum was measured volumetrically and its value was expressed as serum separation in percent (divided by the total volume in a bottle and multiplied by 100) (Azarikia and Abbasi 2010; Azarikia 2008).

The viscosity of samples was measured using a Brookfield viscometer (model LVDV-II+pro Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA), equipped with an LV2 NO. 61. Spindle speed was set to 30 rpm. Measurement was made using 250 mL of samples at 10 °C (Katsiari et al. 2002). Tests were performed in triplicate.

The color of samples was measured using Hunter colorimeter (Chroma Meter CR-400 Japan). Hunter CIE, L* for lightness, a* for redness and b* for yellowness were measured during 21 days of storage. During acidification and post acidification, Chroma $[(a^*^2 + b^*^2)^{1/2}]$ and hue angle $(\tan^{-1} (b^*/a^*))$ for whey and gel were calculated. ΔE as an indicator of the total color difference between two samples were calculated as: $\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$. Color measurement was performed on 50 mL samples in petri dishes against a background of white tile (Zare et al. 2011).

Microbial analysis

Enumeration of *L. acidophilus* La-5 was fulfilled in MRS-agar (Merck, Germany) + 1% bile (Sigma Aldrich, USA) at 37 °C, 72 h and aerobic condition. For microbial colony count, 1 mL of sample was suspended in 0.1% sterile peptone water and serially diluted to the desired levels; 1 mL of appropriate dilution was pour plate cultured. The results were expressed as colony forming unit per gram (CFU/g) of samples (Ghasempour et al. 2012).

Experimental design and statistical analysis

The independent variables were *Aloe vera* gel (AvG), salt (Sal), whey protein (WP), transglutaminase (TGase) and storage time (ST). Actual values of the factors are given in Table 1. Twenty Ayran samples were evaluated according to a two-level fractional factorial design with four central points. Main and interactive effects of studied factors were evaluated by analysis of variance at the significance level of 0.05. After modeling, the optimum conditions were found using the desirability function method. Design-Expert Version 7 (Stat-Ease, Int. Co., Minneapolis, MN, USA) was used for data analysis.

Results and discussion

The effect of the studied factors on pH and acidity

Gel, salt and whey ratio significantly affected pH values of Ayran samples ($p < 0.05$). Increasing of gel concentration from 0 to 30% led to an increase in pH values from 4.30 to 4.38. Changes of pH values, due to the buffering capacity of gel were negligible (Bland 1985). The effect of gel and whey on acidity were significant ($p < 0.05$). In the samples without gel, acidity was about 0.34 g/L which by increasing gel content it dropped to about 0.29 g/L. By increasing the whey replacement to milk from 70 to 90% acidity decreased from 0.35 to 0.29 g/L. Almeida et al. (2008), reported that reducing total solid content caused to increase the acidity, which was different from the results of this study. Probably due to the low concentration of lactose in WPC 35%, acid production was decreased by starter culture. The predicted equation for acidity was calculated as follows:

$$\text{Acidity (g/L)} = 0.427 - 0.003 \times \text{AvG} - 0.002 \times \text{WP} \quad (1)$$

The R^2 value and adjusted R^2 value were 0.94 and 0.93, respectively. Therefore, the calculated model was suitable for prediction.

The effect of the studied factors on viscosity

Gel and salt contents had no effect on the apparent viscosity of the samples. Whey and transglutaminase and storage time had a significant effect on apparent viscosity ($p < 0.05$). Also, the interactions between whey and enzyme levels on viscosity were statistically significant ($p < 0.05$). High contents of casein in the formulated samples (70% whey mixed with 30% milk) containing the highest amount of enzyme (14 U/100 mL) had a significant difference on viscosity (Farnsworth et al. 2006). In the

⁴ The Association of Official Analytical Chemists.

Table 1 Two-level fractional factorial design in actual levels of variables

Run	Factor 1 A: <i>Aloe vera</i> gel (%)	Factor 2 B: Salt (%)	Factor 3 C: Whey protein ratio (%)	Factor 4 D: Transglutaminase enzyme (U/100 mL)	Factor 5 E: Storage time (days)
1	0	1	90	14	1
2	30	1	70	0	21
3	30	1	70	14	1
4	15	0.75	80	7	11
5	30	0.5	70	14	21
6	0	0.5	90	14	21
7	0	1	70	0	1
8	0	0.5	90	0	1
9	15	0.75	80	7	11
10	0	0.5	70	0	21
11	0	1	70	14	21
12	30	1	90	14	21
13	15	0.75	80	7	11
14	15	0.75	80	7	11
15	30	0.5	90	14	1
16	30	0.5	90	0	21
17	0	0.5	70	14	1
18	30	1	90	0	1
19	30	0.5	70	0	1
20	0	1	90	0	21

minimum and maximum levels of the enzyme, the viscosity was 3.1 cP and 4.49 cP, respectively. With increasing whey, the apparent viscosity of the samples decreased (Fig. 1). Generally, enhancement of total solid had a positive effect on viscosity (Farnsworth et al. 2006). The effect of storage time on viscosity was significant ($p < 0.05$). The viscosity increased from 2.2 to 7.3 cP during 21 storage days. This was likely due to La-5 exopolysaccharides production (Amiri et al. 2019).

The following predictive equation for apparent viscosity in Ayran samples was calculated:

$$\text{Viscosity (cP)} = 2.491 - 0.017 \times \text{WP} + 0.478 \times \text{TGase} + 0.082 \times \text{ST} - 0.005 \times \text{WP} \times \text{TGase} \quad (2)$$

The R^2 value and adjusted R^2 value were 0.94 and 0.92, respectively. Therefore, the final model was determined suitable for prediction.

The effect of studied factors on the sedimentation

The effects of whey, storage time and interaction between whey and storage time on the samples sedimentation were significant ($p < 0.05$). During 21 days of storage, in the samples containing 70% whey, sedimentation percentage

increased from 14 to 22, while in the samples containing 90% whey, it changed from 10 to 90%.

By analyzing data, the following predictive model was obtained for sedimentation:

$$\text{Sedimentation (\%)} = 6.905 - 0.101 \times \text{WP} + 0.678 \times \text{ST} + 0.016 \times \text{WP} \times \text{ST} \quad (3)$$

The R^2 value and adjusted R^2 value were 0.97 and 0.97, respectively, so the model looking efficient.

The effect of studied factors on the Hue index

The effect of time and amount of *Aloe vera* gel and the interaction of whey and transglutaminase on Hue index was significant ($p < 0.05$). This index changed from 200 to about 194 during 21 days of storage. *Aloe vera* gel had a significant effect on Hue index ($p < 0.05$). In the samples without gel, the Hue index was about 185 and by increasing the gel contents this index increased to 210. The interaction between whey and enzyme showed a significant difference in the Hue index ($p < 0.05$). In 70% of whey and low level of enzyme (7 U/100 mL), the Hue index was about 150 which increased to 180 at high level of enzyme (14 U/100 mL) which in 90% whey it was about 210 and 200, respectively for low and high level of enzyme. By

Fig. 1 The effect of studied factors on viscosity, **a** the effect of storage time, **b** the effect of WP concentration

increasing the amount of gel, whey and transglutaminase, color of the product changed from yellow to the white, which is a desirable characteristic. The predictive equation of Hue index was calculated as follows:

$$\text{Hue} = 129.032 + 0.816 \times \text{AvG} + 1.189 \times \text{WP} + 6.964 \times \text{TGase} - 0.886 \times \text{ST} - 0.074 \times \text{WP} \times \text{TGase} \quad (4)$$

The R^2 value and adjusted R^2 value were 0.97 and 0.95, respectively, which indicates a high agreement between observed and predicted values, so it shows strong significance of the model.

The effect of studied factors on Chroma index

The interaction of gel and enzyme on the Chroma index was significant ($p < 0.05$). In samples without gel and low level of enzyme (7 U/100 mL), Chroma index was 2.7, which increased to 2.9 in high level of enzyme (14 U/100 mL). In 30% gel content of samples, this index in low and high levels of enzyme was 2.6 and 3.46, respectively. Chroma index decreased by increasing the amount of gel and so intensity of the white color decreased. The interaction between whey and enzyme on Chroma index was significant ($p < 0.05$). According to Fig. 2c, samples containing 70% whey, with low and high levels of enzyme the Chroma index was 2.75 and 2.65, respectively. Overall, the addition of whey improved the transparency of the final product.

The interaction between gel and storage time on Chroma index was significant ($p < 0.05$). Although in the samples without gel in day 1, Chroma index was lower than day of

21, but in samples with 30% gel, it was more than the day of 21. Generally, the Chroma index increased with increasing of gel concentration.

Gel and whey interaction effect on Chroma index was significant ($p < 0.05$). In the samples without gel and low whey content, Chroma index was 2.65, which in high whey content it reached to 3. In the samples with 30% of gel containing 70 and 90% whey, this index was about 2.6 and 4, respectively. Generally, in 70% whey, by increasing gel concentration, transparency decreased, but in 90% whey, transparency of the product increased (Fig. 3a–e).

The interaction between whey and storage time on Chroma index was significant ($p < 0.05$). In samples containing 70% whey, during 21 days of storage Chroma increased from 2.4 to 2.8, and in samples containing 90% whey, this index dropped from 3.6 to 2.3. In general, whey caused an increase in the transparency of the product.

The predictive equation for Chroma index was obtained as follow:

$$\begin{aligned} \text{Chroma} = & 2.291 - 0.034 \times \text{AvG} + 0.012 \times \text{WP} - 0.084 \\ & \times \text{TGase} + 0.049 \times \text{ST} + 0.001 \times \text{AvG} \times \text{WP} \\ & + 0.001 \times \text{AvG} \times \text{TGase} - 0.001 \times \text{AvG} \times \text{ST} \\ & + 0.002 \times \text{WP} \times \text{TGase} - 0.001 \times \text{WP} \times \text{ST} \end{aligned} \quad (5)$$

Considering that the R^2 value and adjusted R^2 value are 0.99 and 0.99, respectively, it confirms the model efficiency.

Fig. 2 The effect of studied factors on Hue index, **a**: the effect of storage time, **b** the effect of *Aloe vera* gel, **c** the effect of whey and enzyme interaction

The effect of studied factors on the ΔE index

According to data analysis, the effect of time and amount of gel content on ΔE index was significant ($p < 0.05$). During 21 days of storage, ΔE index dropped from 17.9 to 15.25, which means that over the time decrease in color difference between control and samples is desirable. The effect of gel on ΔE index was significant ($p < 0.05$) and it changed from 12.75 to 19.5%, respectively in control and samples containing 30% gel. The effect of whey on ΔE index was also significant ($p < 0.05$). With an increase in

whey from 70 to 90 percent, the ΔE index ranged from 10.25 to 25.75, and difference in color of the samples increased. The following predictive equation was obtained after analyzing the data for estimating ΔE index in the samples:

$$\Delta E = 5.177 + 0.210 \times AvG + 0.203 \times WP - 0.207 \times ST \quad (6)$$

The R^2 value and adjusted R^2 value for ΔE were 0.94 and 0.93, respectively, indicating the efficiency of the final model (Fig. 4a–c).

Fig. 3 The effect of studied factors on Chroma index, **a** *Aloe vera* gel and enzyme interaction, **b** interaction between whey and storage time, **c** *Aloe vera* gel and storage time interaction, **d** *Aloe vera* gel and whey interaction, **e** Whey and transglutaminase interaction

Fig. 4 The effect of studied factors on ΔE , **a** the effect of storage time, **b** the effect of *Aloe vera* gel, **c** the effect of whey

The effect of the studied factors on viability of *L. acidophilus*

L. acidophilus often shows poor survival in fermented milk products (Gueimonde et al. 2004). Acidic damage on viability of *L. acidophilus* during storage time was reported (Hekmat et al. 2009). Reduction in the viability of probiotic cells and the increase of acidity is a common characteristic of fermented milk (Martinez-Villaluenga et al. 2006). Acidity and hydrogen peroxide are the main reasons for decrease in *L. acidophilus* count during storage time (Güler-Akin and Akin 2007). The results showed that the effect of gel and enzyme on the survival of *L. acidophilus* was significant ($p < 0.05$). In the low amount of enzyme (7 U/100 mL), increasing gel up to 30% led to 2 log increase in the viability of *L. acidophilus*. In the presence of high

enzyme content (14 U/100 mL), the La-5 population only 1 log was increased. In constant concentration of gel, there was a significant difference between the low and the high amount of enzyme in the viability of probiotic bacteria, which was in opposition with the results of Benkovic et al. (2008). The interaction between gel and the storage time on the survival of La-5 was significant ($p < 0.05$). La-5 population increase during the increasing of the gel concentration may be attributed to the prebiotic properties of the gel. Panesar and Shinde (2012), showed a decrease in the population of this bacterium, which contradicts the results of this study. With increasing gel content to 30% during day 21 of storage, the La-5 count increased 3.8 log. Salt and whey had significant effect on La-5 survival ($p < 0.05$). At 0.5% salt and 70% whey probiotic count increased 2 log. In general, by increasing the amount of salt

Fig. 5 The effect of studied factors on viability of La-5, **a** the effect of *Aloe vera* gel and the enzyme interaction, **b** the effect of *Aloe vera* gel and the storage time interaction, **c** the effect of salt and whey

interaction, **d** interaction effect between salt and enzyme, **e** interaction of salt and storage time, **f** interaction between whey and storage time, **g** the interaction of enzyme and storage time

Fig. 5 continued

to 1%, the number of probiotic bacteria in both high and low concentrations of whey increased. The results of Ghasempour et al. (2012) showed that salt increases osmotic pressure in the aquatic environment, therefore, the activity and growth of starter bacteria and acidification during fermentation are reduced, finally probiotic bacteria can grow further. The high population of probiotic in 70% whey can be attributed to four reasons: first, the high buffering capacity of samples (70% whey and 30% milk) but in 90% whey and 10% milk, due to the low solids, the pH is decreased faster and the probiotic bacteria had less growth (Mortazavian et al. 2009). The second reason is the low rate of Red-Ox potential in medium with a high solids content (70% whey) in which growth and activity of probiotic bacteria increase (Shafiee et al. 2010). The third reason is that the growth nutrients in the samples are higher (70% whey) and the competition between the starting bacteria and probiotics is less. The fourth reason is the protective effect of solid matter matrix on probiotics against harmful factors in medium such as molecular oxygen, hydrogen peroxide ions, and organic acids (Mortazavian et al. 2006). The effect of salt and enzyme interaction on viability of La-5 was significant ($p < 0.05$). In 0.5% salt, the number of La-5 increased by 2 log during increase in enzyme content from minimum to maximum unit. Same result obtained in 1% salt concentration. The interaction between whey and storage time was significant on viability of probiotic bacteria ($p < 0.05$). At the first day of storage, there was no significant difference but 21 days of storage bacterial count decreased by increasing in whey concentration and it dropped about 4 log. In the 90% of whey, due to the low solids content, pH will reduce faster and so probiotic bacteria will grow slowly (Mortazavian et al. 2009). The high content of nutrients in samples with high solids (70% whey) and the reduced competition

between starter bacteria and probiotics are other reasons (Shafiee et al. 2010). The interaction of enzyme and storage time on viability of *L. acidophilus* La-5 was significant ($p < 0.05$). Increase in enzyme content caused only 0.3 log increase in *L. acidophilus* La-5 population during 21 days of storage. Farnsworth et al. 2006 reported that the enzyme has no significant effect on the survival of *L. acidophilus* La-5. The following predictive equation was obtained after analyzing the data for estimating ΔE index in the samples:

$$\begin{aligned} \text{Viability of } L. \text{ acidophilus (Log CFU/g)} \\ = 7.767 + 0.001 \times \text{AvG} + 0.445 \times \text{Sal} + 0.004 \times \text{WP} \\ + 0.060 \times \text{TGase} - 0.059 \times \text{ST} - 0.001 \times \text{AvG} \\ \times \text{TGase} + 0.001 \times \text{AvG} \times \text{ST} - 0.007 \times \text{Sal} \times \text{WP} \\ - 0.050 \times \text{Sal} \times \text{TGase} + 0.041 \times \text{Sal} \times \text{ST} - 0.001 \\ \times \text{WP} \times \text{ST} + 0.002 \times \text{TGase} \times \text{ST} \end{aligned} \quad (7)$$

The R^2 value and adjusted R^2 value for ΔE were 0.99 and 0.99, respectively, indicating the efficiency of the final model (Fig. 5a–g).

Numerical optimization

The desirability function used to optimize the Ayran samples formulation (Alizadeh et al. 2006). Optimization based on maximum viscosity and viability of probiotic bacteria at maximum storage time. Furthermore, standard values of pH and acidity were performed for optimization. The obtained optimal conditions were *Aloe vera* gel 25.53%, Salt 0.58%, whey protein 70% and Transglutaminase enzyme 5 U/100 mL, which according to these conditions, the predicted values for Ayran properties were as follow: *L. acidophilus* La-5 count (CFU/g) 7.8 log and viscosity 6.5 cP, sedimentation 20% and acidity 0.33 at maximum prediction storage time 6.18 days. In this method, the desirability was considered to be higher than 0.6, which in this study was 0.72 (Alizadeh et al. 2006).

Conclusion

In this study, Ayran formulation and production of a dairy based product with high functional properties and minimal milk use were performed using numerical optimization method and a two-level factorial design. The results of this study demonstrated that the symbiotic product with the highest levels of *Aloe vera* gel and the highest survival of *L. acidophilus* La-5 as a probiotic bacterium was formulated.

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