



Development of a preservative for white fresh cheese from the addition of Peruvian Tara gum *Caesalpinia spinosa*

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Abstract

Over the time, fresh cheese undergoes changes in its physicochemical and sensorial properties, mostly associated to the loss of moisture, which directly affects the texture and yield. In this study, we elaborated an alternative formulation for the preparation of cheese with Peruvian tara gum *Caesalpinia spinosa*, for moisture retention increasing, without loss of sensory acceptance. Different formulations were prepared by modifying amounts of salt and tara gum to achieve the highest sensory acceptability, without loss of texture, water and weight. Microbiological tests were performed in agreement with Peruvian regulations, showing limits of mold, yeast, coliform, *Salmonella sp* and bacteria below the established limit. Cheese with addition of tara gum presented a moisture content of $59.8 \pm 0.6\%$, ensuring the physical properties of the cheese. One-hundred untrained individuals experienced the sensory acceptability test. In a nine-point hedonic scale, the acceptance for salt and texture were 8.2 for salt, while the appearance achieved. The optimal formulation for the preparation was 0.05% tara gum with a shelf life of 14 days in refrigeration. The prepared cheese is a promising alternative for production of fresh cheese with greater yield and sensory acceptability.

Keywords: fresh cheese; tara gum; sensory acceptance; microbiological analysis; moisture retention.

Practical Application: Preparation of a fresh cheese with addition of a natural product, Peruvian Tara gum, to obtain a final product with more shelf life and moisture

1 Introduction

Cheese is a dairy product consisting mainly of proteins, carbohydrates, lipids, and vitamins (Perry, 2004). Its composition varies according to the properties of milk and manufacturing process (Walstra et al., 1999, 2006). Moisture levels and pH are also important for producing a fresh cheese (Ramírez-Navas et al., 2017). A fresh cheese must meet some requirements: moisture greater than 46%, amount of fat greater than 40% and be within the microbiological technical standards, that depend on the country of origin of manufacture (Hernández, 2007). In general, fresh cheese should have a shelf life of five to nine days; after this period the cheese begins to lose moisture and increase its microbiological values, making it unhealthy for consumption (Perry, 2004; Hernández, 2007). Process of making cheese consists of some stages such as: milk coagulation, curd cutting, molding, salting and packaging (Perry, 2004). The process of salt addition is important since it enables the replacement of divalent calcium ions, determining the stability of the renneting process (Ramírez-Navas et al., 2017). Additives such as gums and hydrocolloids are used in the final product to modify the texture, consistency and appearance of the cheese, so the water retention (Saha & Bhattacharya, 2010). Gums frequently used to produce dairy products are carrageenans, Xanthan gum,

carboxymethylcellulose and galactomannans (Tara, guar and Locust-bean). This wide range of hydrocolloids and stabilizers are used in the food industries to perform various technological and innovative functions that meet the consumer needs (Saha & Bhattacharya, 2010).

Tara gum is a natural additive obtained by grinding the endosperm of the seeds of *Caesalpinia spinosa*, of the Leguminosae family, also called Peruvian carob, which is a yellow-white powder partially soluble in cold water. The leguminous is used as food additive, according to Food Chemicals Codex, and works mainly as a thickener and stabilizer (Saha & Bhattacharya, 2010). This gum, typical of the Cordillera region of Peru and Bolivia, is also useful as an absorption reagent and hydrogen binder to mineral and cellulose surfaces (Ferrari et al., 2013), which provides higher viscosities compared to other galactomannans (Hernández, 2007).

To maintain the product validity, it may offer levels of acceptable or uniform quality, without losing the characteristics of a traditional manufactured good (Leite, 1993). Sensory analysis is an important tool for measuring the quality of a food in quality control programs and development of new products

Received 18 Jan., 2018

Accepted 22 Oct., 2018

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(Machado et al., 2004), stating the use of the hedonic scale (Dongowski, 1986; Moskowitz, 1988; Peryam & Pilgrim, 1957; Stone et al., 2012).

In this study, moisture levels and microbiological parameters were analyzed for fresh cheese and fresh cheese with Peruvian tara gum.

2 Materials and methods

Nine formulations were made with the addition of different amounts of tara gum and salt, in order to verify the different levels of moisture and sensorial acceptability in relation to fresh cheese, finding an optimal formulation to achieve high acceptability levels. The cheese was prepared from milk came from cows in a barn of the Universidad Nacional Agraria La Molina, Lima, Peru. Tara gum, Sodium chloride, calcium chloride and chymosin. All the reagents were obtained from CRH Hansen S.A (Lima, Peru).

2.1 Cheese preparation

The production followed established procedures (Law & Tamime, 2010) with some modifications, since tara gum was incorporated to the formulation. Thirty liters of previously pasteurized (65 °C, 10 minutes) filtered cow milk (1:1.99; fat:protein) was cooled down to 50 °C before calcium chloride addition, and maintained at 39 °C. to replace the amount lost in mass of the pasteurization process. The preliminary step was to develop a cheese with different concentrations of salt that was acceptable by the hedonic scale. The concentrations accepted by the hedonic scale were 14, 16 and 18 g/L of salt. In relation to the addition of tara gum, the same preliminary test was performed, obtaining percentages of 0.05, 0.10 and 0.15%, being these concentrations acceptable by the hedonic scale. Tara gum and salt were added as shown in Table 1. Then, 0.65 g of chymosin was added, the mixture futher stirred for 1 minute, and left to rest for 80 minutes at 39 °C. Finally, the process of draining and pressed (Law & Tamime, 2010) was performed. The fresh white cheese with tara gum produced was stored between 4-8 °C.

2.2 Microbiological analysis

Microbiological analysis was carried out according to described protocols (Tuesta et al., 2015) for the optimal formulation of cheese with tara gum. The samples were

Table 1. Formulations for preparation of cheese with tara gum.

Formulations	Salt (g/L)	% Tara gum
F1	14	0.10
F2	18	0.15
F3	18	0.05
F4	16	0.15
F5	16	0.05
F6	14	0.15
F7	18	0.10
F8	16	0.10
F9	14	0.05

compared and analyzed following parameters established in the Peruvian technical standards NTP 202.195 2004 (Guzmán et al., 2015) (Table 2).

2.3 Moisture analysis

Moisture and weight analyzes were performed according to the protocols published elsewhere (Latimer, 2016). Samples (n=9) masses were between 2-5 g and placed in ovens at temperatures between 98-100 °C for 5 h. All the samples were dried and weighed at room temperature. The percentage of moisture was calculated using the following equation 1:

$$\%H = \frac{PI - PF}{PI - PC} \times 10 \tag{1}$$

Where:

- % H = Percentage of moisture.
- PI = Container weight with fresh sample (g).
- PF = Container weight with dry sample (g).
- PC = Container weight without sample (g).

2.4 Water retention

Water retention analysis followed protocols described in the literature (Flores et al., 2013), and was calculated according to (2):

$$RA = \frac{\%H}{\%PQ} \tag{2}$$

Where:

- RA: Water retention in cheese.
- % H: Percentage of moisture.
- % PQ: Percentage of protein in cheese.

The percentage of protein was calculated as follows (3):

$$\%PQ = \frac{PQ}{MQ} \times 100\% \tag{3}$$

Where:

- PQ: Milk protein
- MQ: Cheese weight

Table 2. Microbiological requirements for fresh cheese (Guzmán et al., 2015).

Microbial Agent	Unit	Limit
Total coliform	UFC/g	5 x 10 ²
<i>Salmonella sp</i>	P o A/25 g	Absence
<i>Escherichia coli</i>	NMP/g	3-10
<i>Staphylococcus aureus</i>	UFC/g	10-10 ²
Mesophilic/aerobic		Not Specified

2.5 Sensorial analysis

The sensorial analysis was performed based on the number of people required for acceptability tests. One-hundred (50 men and 50 women) untrained and random people were selected, the same who were recruited for being consumers of the product (Flores et al., 2013; Tuesta et al., 2015). About 30 g of cheese (n=4 cheese per person) were analyzed by each person; they evaluated, during 15 min, the taste of salt, appearance and texture of the nine formulations prepared. Each sample was in small plates that were offered to the participants. Each of them answered a brief survey with alternatives of acceptability level for the three sensory attributes according to the 9-hedonic scale (Espinosa, 2007).

2.6 Statistical analysis

For the test performed, we used parametric analysis through analysis of variance (ANOVA). For moisture, we used the Tukey post hoc test, and for Sensorial Analysis, we used Friedman test and Wilcoxon test (Non-Parametric Statistical). Analyses were performed using Sigma Stat for windows Ver 3.10 and Statgraphics Centurion Ver. XVI, with a significance level of 0.05.

3 Results and discussion

Tara gum was incorporated in the fresh cheese (0.05%-0.15%) to verify if there was an increase of moisture and water retention. This incorporation was successfully performed, as it provides resistance when found in percentages lower than 0.2%. Above this value, the gum separates from the proteins and polysaccharides, decreasing the firmness, through a double compression test of samples with constant weight, separating in phases between the gum and sodium paracaseinate present in mature cheeses. The firmness was measured as a whole, where firmness and consistency are already contemplated. It was measured by sensorial analysis linear hedonic type - unstructured and validated with statistical analysis. (Hernández, 2007; Saha & Bhattacharya, 2010). By adding tara gum to milk, the polysaccharide binds to the protein through the interactions that exist between the hydroxyl groups of tara gum and the functional groups of the protein (Flores et al., 2013). These properties have been exploited and used in the food industry, mainly to provide qualities on the viscosity and texture (Hernández, 2007; Hatanaka, 2009). Another advantage provided by the tara gum is the formation of high viscosity, being little affected by the pH, ions and temperature (Sittikijyothin et al., 2005), owing to the great variability in the composition of the galactose and its distribution along the mannose chain and the origin for the different rheological characteristics (Chandrasekara & Radhaa, 1998). That is, the greater the mannose/galactose ratio (3:1, tara gum), the greater the likelihood of branching free chain sections. These chains may align and interact with proteins and other hydrocolloids to give higher viscosity, such as locust gum (4:1), or lower viscosity such as guar gum (2:1) (Hatanaka, 2009). Thus, the tara gum was chosen to be incorporated into the cheese, as it provides intermediate viscosity (5.649 Pa.s) to the product, not affecting its physical properties when compared to fresh cheese. With the presence of tara gum, it was also noticed that the microbiological parameters were not altered, indicating that

the cheese remains fresh and excellent for consumption. These results are displayed in Table 3.

Microbiological parameters of the cheese with tara gum (Table 3) are within the specified values according to Peruvian legislation (Guzmán et al., 2015). This shows that the addition of tara gum, in cheese preparation, does not significantly alters any of the microbiological levels. According to the legal parameters, a cheese is fresh since it presents low levels of molds and yeasts ($10 \cdot 10^3$ UFC/g), coliforms ($5 \times 10^2 \cdot 10^3$ UFC/g), *Staphylococcus aureus*/other bacteria ($10 \cdot 10^2$ UFC/g) and *Salmonella sp* (absent).

According to studies in Cream cheese (Franco & Landgraf, 2002), the analysis of coliforms in food provides information on hygienic conditions of the product. Low presence of coliforms and absence of *Salmonella sp*, as shown in Table 3, indicates adequate manufacture of the product, which is consistent with current legislation (Guzmán et al., 2015). Thus, the cheese with tara gum meets all the requirements specified by the Peruvian standard NTP 202.195 2004, being considered a product that can be consumed by the public without health risks (Guzmán et al., 2015). Tara gum was added to the cheese preparation to ensure that it had a tendency to water retention, making it more hydrated than the control cheese. This feature is important because it tolerates a longer shelf life and also a greater acceptance in the market (Flores et al., 2013).

Results obtained in Figure 1 show that the moisture content of fresh cheese ($58.6 \pm 0.9\%$) and cheese with tara gum ($59.8 \pm 0.6\%$) did not show significant differences ($p = 0.2$), but the cheese with tara gum presents a trend in increasing moisture since it has a high water retention parameter. This phenomenon is due to the interactions of the hydroxyl groups of tara gum

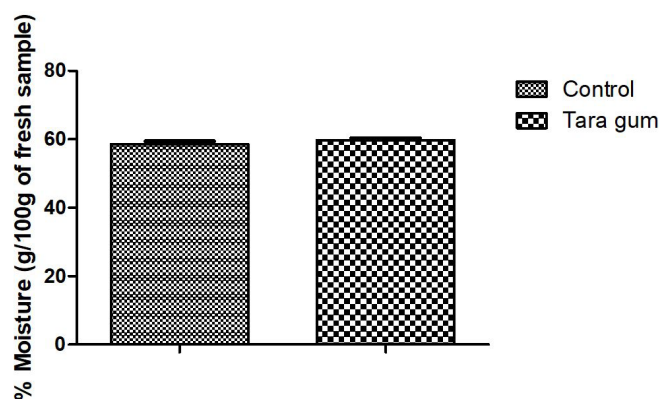


Figure 1. Moisture percentage in relation to fresh cheese control and cheese with tara gum.

Table 3. Comparison of the microbiological parameters of fresh cheese and cheese with tara gum.

	Control	Cheese with tara gum
Viable aerobic mesophilic (UFC/g)	not specified	350
Molds and yeasts (UFC/g)	not specified	< 10
Total coliforms (UFC/g)	5×10^2	< 3
<i>Staphylococcus aureus</i> (UFC/g)	$10 \cdot 10^2$	< 10
<i>Salmonella sp</i> (P o A/25g)	Absent	Absent

(mannose and galactose) with water, through hydrogen bonds (Badui Dergal, 2006). There are polymeric interactions that lead to complexes formation or phase separation, being able to have exceptionally miscibility (Syrbe et al., 1998). In addition, the entropic contribution of the polymer mixture is generally greater than the enthalpic, with phase separation as a general rule for such blends (Doublier et al., 2000). When hydrocolloids are added to the milk, these polysaccharides are not the only ones in the medium. They are a pseudo-ternary solution composed of milk proteins, polysaccharide and water (Hatanaka, 2009). Several researches studied applications of hydrocolloids in dairy products, e.g., yogurt, whipped yogurt, milk drinks, cream cheese, to improve rheological characteristics and increase water retention capacity. Keogh & O’Kennedy (1998) have shown the effect of the fat content, protein addition and hydrocolloids on the consistency and syneresis of the yogurt beaten, showing that the addition of gums improved the consistency without significantly affecting the syneresis of the product (Keogh & O’Kennedy, 1998). Other studies showed the effect of three combinations of gums in petit-Suisse cheese on the texture of the product, for which different formulations of xanthan, carrageenan and pectin were tested, showing firmness for 21 days of product storage, obtaining a better result for gum xanthan, due to its high viscosity. It was also used in yogurt at concentrations of 0.01%, obtaining null syneresis due to the increased interaction of the hydrocolloid with the milk protein (Maruyama et al., 2006). Another study on the effect of adding locust gum (0.002%-0.02%) on yogurt, showed that the viscosity and water retention capacity in the final product were positively affected by the increasing in solids content (10% to 14%). Displaying that, for gum concentrations above 0.02%, yogurt showed higher viscosity and syneresis and lower water retention capacity, being the best proportion with 14% solids and 0.02% locust gum (Hatanaka, 2009). Therefore, moisture content is directly related to yield, so higher yields are related to higher moisture content and water retention (Moore et al., 1986). Higher the content of hydrocolloids (gums) in cheeses, higher the moisture content as well (Koca & Metin, 2004; Rahimi et al., 2007; Volikakis et al., 2004). This was shown for the cheeses Oaxaca (Rahimi et al., 2007), cool white to spread (Lluch Rodríguez et al., 1982), white, cottage and cheddar (Manning et al., 1986). This same phenomenon

can also be seen in Table 4, where different concentrations of tara gum and salt were added to the cheese, resulting in nine formulations, to verify in which concentration there is a greater retention of water and moisture. With the increasing of gum concentration, there was an increase in moisture and water retention observed by formulation 4 (0.15% tara gum). In the same way, the interactions of the hydroxyl groups of the tara gum with the water, through the hydrogen bonds, allow a greater retention of water (Badui Dergal, 2006), also increasing the moisture of the product, in this case the cheese.

Figure 2 shows the percentage of cheese moisture in different concentrations of tara and salt gum. The graph shows that the moisture increases with increasing concentration of tara gum, wherein the concentration of 0.15% has a higher percentage of moisture compared with the concentration of 0.05%, this being a significant result ($p < 0.05$). However, when adding different concentrations of salt, there was an increase in moisture and water retention, but this increase was not significant. Thus, it is suggested that the water retention and moisture increasing are related to the variation of the gum concentration, the salt being only used to flavor the cheese curd and improve the process (Law & Tamime, 2010).

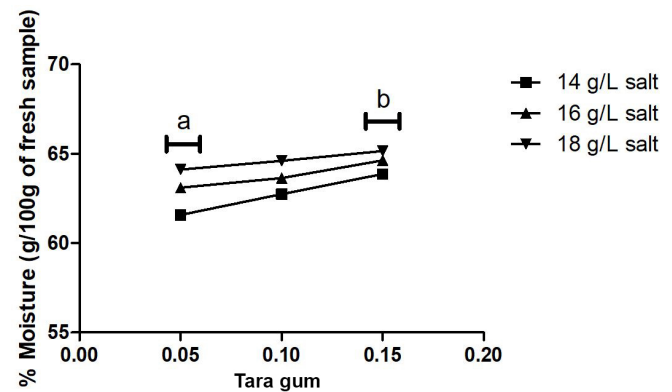


Figure 2. Moisture percentage in relation to different concentrations of salt and tara gum (a, b letters represent the significant difference, $p < 0.05$).

Table 4. Percentage of moisture, retained water and % weight increase by tara gum for the nine cheese formulations with tara gum, compared to control (without tara gum).

Formulation	Weight (kg)	Moisture (%)	Retained water	% Weight increase by tara gum
Control	4.932	58.544	4.221	0.0
F1	4.981	62.740	4.569	7.1
F2	5.627	65.161	5.343	22.7
F3	4.977	64.121	4.619	8.1
F4	5.745	64.636	5.393	23.8
F5	4.943	63.110	4.546	6.8
F6	5.441	63.868	5.097	17.8
F7	4.971	64.611	4.727	10.3
F8	5.166	63.639	4.775	11.2
F9	4.977	61.584	4.511	5.9

Table 5. Optimum concentrations of salt and tara gum.

	Taste of salt	Appearance	Texture
Salt concentration (g/L)	15.7	16.4	15.9
Tara gum concentration (%)	0.05	0.05	0.05
Sensory acceptability (hedonic scale)	8.2	8.5	8.2

Finally, nine formulations developed through different concentrations of tara gum and salt were evaluated by 100 untrained individuals, to verify which of these formulations would be more acceptable to the public, besides presenting all physical and microbiological characteristics previously reported.

Table 5 shows the optimum concentrations of salt and tara gum for the maximum values of acceptability with respect to taste, appearance and texture attributes. The optimum formulation was determined through the sensorial evaluations of all the formulations developed, with formulation five being the most accepted (lower concentration of tara gum, 0.05%, and medium amount of salt, 16 g), and the less accepted formulations were the higher concentration of tara gum and salt.

Literature states that the use of stabilizing agents in the preparation of cheeses modifies their characteristics, i.e., chemical and rheological parameters (Lobato-Calleros et al., 2008), affecting sensorial properties and acceptance (Rahimi et al., 2007). Research has shown that the sensory properties and acceptability by “consumers” is greater in cheeses that have stabilizing agents (gums) compared to cheeses without addition (Hernando et al., 1999; Gammariello et al., 2008; Lobato-Calleros et al., 2008). It is important to note that the results obtained showed that cheeses made with tara gum have a higher retained mass (Table 4) and a linear decrease in cheeses, but do not correlate with a higher sensory acceptance when increasing tara gum when compared to cheese control.

4 Conclusion

The incorporation of Peruvian tara gum into the alternative formulation of fresh cheese was feasible. The optimum percentage of tara gum was 0.05% and salt was 16 g/L of milk during the pasteurization and coagulation process at 40 °C. The increasing in weight of cheese production was 6.8% due to the higher moisture retention, making the cheese softer, with a shelf life of 14 days (loss of weight and moisture in relation to time, in days) at temperatures between 4-8 °C. Finally, the sensory analysis obtained an acceptance of 8.2 for salt, 8.5 for appearance and 8.2 for texture in a nine-hedonic scale. This formulation in the preparation of cheese may propose an alternative manufacturing for artisan and industrial producers, to improve the storage conditions, yield and to generate greater returns production.

Acknowledgements

The authors acknowledge Grupo de Investigación en Alimentos the Facultad de Ingeniería Química y Textil of the Universidad Nacional de Ingeniería (GIA-FIQT-UNI) and the Universidad Nacional Agraria La Molina for the laboratory facilities.

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