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Sensory acceptance and shelf life of fresh cheese made with dry bromelain extract as a coagulating agent

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Abstract
The objective of this research was to assess the sensory acceptance and shelf life of a fresh cheese employing dry bromelain extract as a coagulating agent. Dry bromelain extract was used in three concentrations (5%, 10% and 15% w / w) and the milk utilized was both raw and pasteurized, for a total of six treatments being studied according to a factorial model 3 * 2. A sensorial analysis was conducted and the shelf life (sensorial, physicochemical and microbiological) of the treatment, with the greatest acceptance, was determined. The results showed that the cheeses obtained with the greatest sensorial acceptance were those made with 5% w / w of dry bromelain extract regardless of the type of milk used (raw or pasteurized). The shelf life of the selected product (T2), at the physicochemical level is 26 days; however, sensorially and microbiologically the quality of the product is good up to day 14. Therefore, the use of dry bromelain extract as a vegetable coagulant showed a great potential as a substitute for traditional rennet.

Keywords: sensorial acceptance; fresh cheese; dry bromelain extract.

Aceptación y vida útil sensorial de queso fresco elaborado con extracto seco de bromelina como agente coagulante

Resumen
El objetivo de esta investigación fue evaluar la aceptación sensorial y la vida útil de un queso fresco utilizando extracto de bromelina seca como agente coagulante. El extracto seco de bromelina se usó en tres concentraciones (5%, 10% y 15% p / p) y la leche se usó de forma cruda y pasteurizada, para un total de seis tratamientos en estudio bajo un modelo factorial 3*2. Se realizó un análisis sensorial y se determinó la vida útil (sensorial, fisicoquímica y microbiológica) del tratamiento con mayor aceptación. Los resultados mostraron que los quesos obtenidos con mayor aceptación sensorial fueron los elaborados con un 5% p / p de extracto seco de bromelina, independientemente del tipo de leche utilizada (cruda o pasteurizada). La vida útil del producto seleccionado (T2) a nivel fisicoquímico es de 26 días, sin embargo, sensorial y microbiológicamente, el producto es de buena calidad hasta el día 14. Por lo tanto, el uso de extracto seco de bromelina como coagulante vegetal mostró un gran potencial como Sustituto del cuajo tradicional.

Palabras clave: aceptación sensorial; queso fresco; extracto seco de bromelina.

1. Introduction

In the preparation of cheeses, the use of vegetable enzymes as coagulating agents, replacing traditional rennet, has aroused a lot of interest [22]. The extracts of vegetable juices and other parts of the plant play an important role in the appearance of aromas and textures in cheeses. Nonetheless, the use of low percentages of these coagulants is recommended to maintain the organoleptic characteristics and the degree of acceptance of cheeses made with microbial coagulants. The concentration of the enzymes affects the color, smell, taste and texture of the cheeses so the lower the concentration the greater the consumer´s acceptance [26]. In fact, depending on the concentration, we can obtain flavors that range from sweet to slighthly sweet as well as acid, slightly hot or bitter; the

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latter may be lower in matured cheese; we can also obtain intense colors ranging from intense blue to green or slightly green; furthermore, we can get acid aromas as well as softer and firmer, but creamy, textures [22-18].

On the other hand, some studies have proven that some vegetable coagulants such as green fig extract do not have a significant effect on the color of cheeses. The said coagulants are linked to the concentration and to the extract or protease employed [20].

Raw Bromelain is the name given to the main proteases obtained from the juice of the pineapple fruit (Ananas comosus) including the non usable parts thereof such as the kernel, the shell, the crown and leaves. Depending on their origin, the aforesaid parts are named as follows: fruit bromelain, skin bromelain, leaf bromelain and stem bromelain. The latter has the least proteolitic activity and its enzymatic activity is slightly less than that of the fruit bromelain; despite that, its protein content is similar to that of fruit bromelain; also, the biological value of leaf bromelain is considered as low because its enzymatic activity is very low in comparison with fruit bromelain [10-13].

In tests involving vegetable coagulants we also have to take into account the type of milk to be utilized (pasteurized or non-pasteurized) since this process interferes with the characteristics of the end product. For instance, when we employ pasteurized milk in coagulant tests, the smell and taste parameters improve while the use of non-pasteurized milk ameliorates the soft texture of the cheese due to the high proteolitic activity chracteristic of vegetable origin [20]. Also, temperature is an important factor in the making of vegetable coagulants owing to the fact that high temperatures can cause excessive proteolosis which can negatively impact both the texture and taste of cheeses [22-7].

The goal of this research is to assess, through the Institute of Food Science and Technology INTAL, the acceptance and sensorial shelf life, both physicochemical and microbiologic; of fresh cheese by using dry bromelain extract extreacted from the stem of the pineapple as a coagulant agent taking into account the incidence of physico-chemical conditions of the milk utilized in the preparation thereof as well as the concentration factors of the extract and pasteurization of the milk.

2. Materials and methods

2.1. Preparation and analysis of dry bromelain extract

2.1.1. Fruit selection

Twenty pineapples were selected taking into account the following features, pursuant to Resolution 3929 of 2013: physicochemical quality as well as acidity, pH, and optimal ripeness fit for consumption. Acidity was determined by means of titration in accordance with Colombian Technical Standard, NTC 4623 [15]; the pH was determined by means of a potentiometric assessment and the ripeness index by way of the relationship between the soluble solid concentration (Brix grades) that was the result of refractometry at room temperature and the percentage of acidity initially measured.

2.1.2. Extraction of the dry bromelain

Extraction of the juice from pineapple stems was conducted after weighing, peeling, and chopping of the fruit; then, the stems were separated and cut into small squares to facilitate the liquefying process; finally, filtering was completed. To get the powdered extract, lyophilization of the juice, at -80°C and at. vaccum pressure, was carried out; these conditions allow for the extraction of humidity and the activity of the enzyme present in the dehydrated juice is preserved. The bromeline extract was bottled in metalized resealable bags because it is sensitive to light; then, it was stored in a dessicator to prevent the product from the effects of humidity [15-8].

2.2. The cheese making process

To make the cheese, fresh milk was employed. The said milk was analyzed with the help of ECOMILK so as to determine its physicochemical quality and establish the initial working parameters. The preparation of fresh pasteurized and nonpasteurized cheeses, using dry bromelain extract, was carried out according to the procedures described by Vergara 2018. Also, the physicochemical properties of the working milk were determined; furthermore, 5% and 10 and 15% concentrations of dry coagulant were employed; in all of the cases curd cutting, draining and addition of a low amount of salt (1.5%) was performed to counter the likelihood of bitterness and low cohesiveness as a likely result of greater proteolysis characteristic of this type of coagulant. During the manufacturing process, variables such as coagulation time and curd cutting time, in minutes, were recorded maintaining strict control on the curdling temperature (63°C). Finally, the output was determined by means of the relationship between the weight of the end product and the volume of the raw material involved in the coagulation, which determined the fact that this parameter was expressed in kilograms per liter of milk [19].

2.3. Sensorial Analysis

The resulting cheeses were subjected to a sensorial acceptance test for the assessment of sensorial attributes such color, odor, taste and texture through a hedonic scale of nine points with the participation of 100 consumer judges.

2.4. Measurement of shelf life

Follow-up on the shelf life of the best treatment was carried out according to the experimental parameters studied, using the acceptability limit method [12,14] developed by the Institute of Food Science and Technology (INTAL) and recognized by Colciencias. For

the development of this stage the cheese was stored at a temperature between 4 ° C and a relative humidity of 85%; for the analysis, three components were taken into account: sensorial quality (minimum 6 concentrated assessments at the end of storage), microbiological and physicochemical parameters: initial, intermediate and final.

2.4.1. Sensorial useful lif

Quantitative descriptive tests (QDA) were carried out, with the assistance of five trained judges, under a structured scale of 10 cm. The parameters of color, taste, texture and odor / aroma were analyzed according to NTC 5328 and NTC 3932 [15].

2.4.2. Physico-chemical shelf life

Acidity and pH were determined according to A.O.A.C 947.05 and 973.41 respectively [1].

2.4.3. Microbiological shelf life

Mold and yeast counts (NTC 4132), total and fecal coliforms (NTC 4516), Staphylococcus aureus (NTC 4779), Salmonella detection (NTC 4574) and Listeria detection (NTC 4666) were conducted [15].

2.5. Statistical and experimental design

The experiment was developed under a completely random 3x2 factorial arrangement, whose factors and levels were: concentration of bromelain (5%, 10% and 15% w/w) and the use of pasteurized or unpasteurized milk in the manufacture of the cheeses, for a total of six treatments: T1 = 5% of dry bromelain extract / unpasteurized milk; T2 = 5% of dry bromelain extract / pasteurized milk; T3 = 10% dry bromelain extract / unpasteurized milk; T4 = 10% dry bromelin extract / pasteurized milk; T5 = 15% dry bromelain / unpasteurized milk extract; T6 = 15% dry bromelin extract / pasteurized milk. The hedonic test for the selection of the most accepted cheese was made under an experiment to a classification route, with a design in blocks completely at random, for the assessment of the sensorial attributes such as color, taste and smell / aroma, using a hedonic scale of nine points with the participation of 100 consumer judges. The shelf life was carried out only for the best treatment under a model in repeated measures and a completely random design, the measurements were registered in the length of time it took the product to fail with respect to the standard, whose measurement times were executed in the following way: (Initial, day 6, day 9, day 14, day 20 and day 26). The analysis of the data was developed in the statistical software R-Studio in all cases.

Table 1.
Physical-chemical properties of raw milk.

Parameter	Result ± ds
Lipid (%)	3,54±0,01
Dry degreased extract (%)	8,59±0,004
Protein (%)	3,35±0,02
Lactose (g/Dl)	4,5±0,005
Acidity (º D)	15 ±0,05
Ph	6,7±0,00
Density (g/mL)	1,0303±0,00
Mineral Salts (%)	0,73±0,02
Cryoscopic point	0,540±0,008

Source: The Authors.

Table 2.
Microbiological characterization of the dry bromelin extract.

Analysis	Resolution 3929 of 2013	Result
Count of Escherichia coli	Zero	Zero UFC/ml
Count of Coliforms	Zero	Zero UFC/ml
Count of Aerobics Coagulase (+)	N.A	<10 UFC/ml

Source: The Authors.

3. Results and discussions

3.1. Characterization of raw milk and dry bromelin extract

3.1.1. Characterization of raw milk

Table 1 shows the results of the physicochemical characterization of the milk used. As noted, the milk met the quality requirements to be used in the cheese manufacturing process according to the parameters established in Decree 616 of 2006 of the Colombian Ministry of Social Protection, which regulates the technical requirements that the raw material must meet for human consumption or for processing.

3.1.2. Characterization of the dry bromelin extract

The pineapples selected for juice extraction recorded an average acidity and pH of 0.4659% citric acid and 4.323 respectively. These results show that the fruits complied with the physicochemical quality standards established in resolution 3929 of 2013 regarding these parameters. The ripeness indices of the pineapples used were found to be (21-27) at their optimum level for consumption [23]. 3200 ml of juice were obtained for lyophilization for every 20 stems used. The yield of the lyophilization process was 11.25%. The lyophilisate obtained met the microbiological conditions by Resolution 3929 of 2013 of the Ministry of Health and Social Protection, being suitable for use in food processes as shown in Table 2.

The enzymatic activity of the bromelain dry extract was 3.68 BTU / ml, similar results obtained by Quindes et al., 2013 [18] in a study on extraction, partial purification and drying of the bromelain enzyme obtained from the heart of the pineapple.

3.2. Sensorial analysis

According to the variance analysis of the fresh

Table 3.
Acceptability of the attributes of the cheeses.

Treatment	Color	Smell	Taste	Texture
T1	7.96 a	8.06 a	7.88 a	7.75 a
T2	7.78 a	7.87 a	7.77 ab	7.58 a
T3	7.06 b	7.16 b	7.48 bc	6.84 b
T4	6.90 b	6.99 b	7.36 cd	6.70 b
T5	6.14 c	6.25 c	7.07 de	6.14 c
T6	6.04 c	6.15 c	6.84 e	5.86 c

Source: The Authors.

cheeses, there were significant statistical differences in the attributes of taste, color, smell and texture in terms of the concentration of bromelain extract ($p \leq 0.05$), as shown in Table 3. For all the sensorial descriptors, the lower the concentration of dry bromelain, as a coagulant, the higher the acceptance. The values of acceptance of the attributes such as color, smell and texture do not show significant differences with respect to the use of pasteurized or unpasteurized milk at the same concentration of bromelain extract while taste does show differences when the concentration of extract is 10% or 15%. According to the results, for the attributes such as color, smell and taste, the judgment of the tasters were placed between the terms of the hedonic scale, I like it lightly and I like it a lot, while for the texture, the qualifier was between indifferent and I like it a lot as shown in Table 3, according to this it was determined that the treatments with greater acceptance were T1 and T2.

These results are similar to those reported in an investigation in which the acceptability of the color, odor, flavor and texture attributes of a fresh cheese made with green fig milk was determined [20]. In the same way, the tendency of the results coincides with a study carried out on the use of latex from leaves, stems and papaya fruit in the production of fresh cheese, in which it was determined that for the variables color, smell and taste the acceptability, as the levels of the coagulant increase [26].

The behavior of the attributes color and texture could be given by the increase in the percentage of fat generating changes in the product matrix [26], creating a tendency to the yellow color distant from the cream white color characteristic of fresh cheese and soft textures characteristic of cheeses made with vegetable coagulants. On the other hand, the variations in the acceptance of the texture could be related to the variations presented in the hardness of the cheese since this constitutes the attribute of mechanical texture more important in the case of dairy products. In fact, hardness is one of the characteristics on which the consumer bases his decision when ingesting or accepting a product if he compares it sensory with products of the same type he has ingested [24].

Regarding the smell attribute, the sensory response of the tasters could be minimally influenced by the increase in acidity as the concentration of the coagulant used in the coagulation process increases, taking into account that these variations were very slight [24]. Regarding the flavor, it should be noted that the differences between the cheeses in terms of acceptance of this sensory attribute were not very marked and despite the fact that the use of vegetable coagulants generally causes bitter tastes (bitter peptides) in cheeses due to their high proteolysis, none of the treatments obtained showed bitterness. It is very likely that this occurred as an experimental response to the decrease in proteolysis of the extract due to the use of ripe fruits and specifically for the use of pineapple stems instead of pulp as raw material in the adequacy or lyophilized of bromelain for its employment as a coagulant [3].

Table 4.
Sensory useful life of the best treatment.

Descriptor evaluado	Evaluation Days 0	6	9	14	20	26	30
Apearance/color	9a	8,6ab	8,3b	7,5c	7,1c	6,8c	6
Characteristic dairy flavor	9a	9[a]	8,7[a]	8,3b	7,8b	7c	*
Objectionable taste	0	0	0	0	0	0	0
Characteristic smell	9a	8,8[a]	8,5ab	8bc	7,7c	7,5c	7
Objectionable smell	0	0	0	0	0	0,5	1
Cohesiveness	2,96a	2,86[a]	2,96[a]	2,90a	2,93a	2,83a	*
General quality	9,5a	9b	8,6b	8,1c	7,5c	7d	4
Acceptance (A)/ Rejection (R)	A	A	A	A	A	A	R
Salty flavor	4a	4[a]	4[a]	4a	4a	3,8a	**

* Not evaluated due to microbiological deterioration, **Loss of quality
Source: The Authors.

3.3. Shelf Life of the selected product

3.3.1. Sensorial useful life

The results of the behavior of the sensorial attributes of the selected product (T2) are recorded in Table 4 and Fig. 1.

According to the sensorial analysis conducted with the assistance of 5 trained judges, highly significant differences were found ($p \leq 0,05$) in the time of the variables such as appearance/color, characteristic dairy flavor, characteristic odor and general quality, these were initially located between the terms intense and very intense, and diminishing to the moderate and intense levels on day 26 , which indicates that the cheese produced met the requirements of fresh cheese. According to the Tukey test, the initial general quality of the product was maintained over time showing statistical differences until day 6, then the overall quality decreased without statistical differences until day 14 and also decreased until day 26. It should be noted that the sensorial characteristics of the product also began to show more marked statistical differences from day 14.

Similar results were found in research on latex use of leaves, stems and papaya fruit in the production of fresh cheese, where a shelf life of 21 days was reported. It should be noted that the percentages of humidity reported in this research were lower than those obtained in the present study, which could favor the useful life of the product [26].On the other hand, the variables such as salty taste and cohesiveness did not present significant differences in time ($p \leq 0,05$) and were located between the

Table 5.
Physicochemical useful life of the best treatment.

Storage day	Ph 20°C	Acity (% Lactic acid)	Model	Fitting MAD (RMSE)	Forecasting MAD (RMSE)
Initial	6,57[c]	0,1760[c]	SARIMA	36.11 (52.30)	51.88 (60.20)
Day9	6,67[a]	0,1894[b]	Proposed model	35.93 (50.89)	47.68 (60.06)
Day 27	6,62[b]	0,2095[a]			

Source: The Authors.

Table 6.
Microbiological useful life of the best treatment.

Parameters	Days					
	1	7	14	20	27	Specifications
MLN of Coliforms totals (MLN/g)	16	120	150	>1100	> 1100	Dosn´t apply
MLN Fecal Coliforms	6	<3	<3	36	<3	<100
Escherichia coli MLN /g	-	-	-	-	-	-
Count of S. aureus coagulase UFC/g	<100	-	<100	-	<100	1000-3000
Detection of Listeria Monocytogenes	Absence	-	Absence	-	Absence	Absence
Detection of Salmonella Spp	Absence	-	Absence	-	Absence	Absence
Count of yeasts CFU/g	500	40	180	12800	71000	100-500
Count of Molds CFU/g	<10	<10	<10	<10	<10	<10

Source: The Authors.

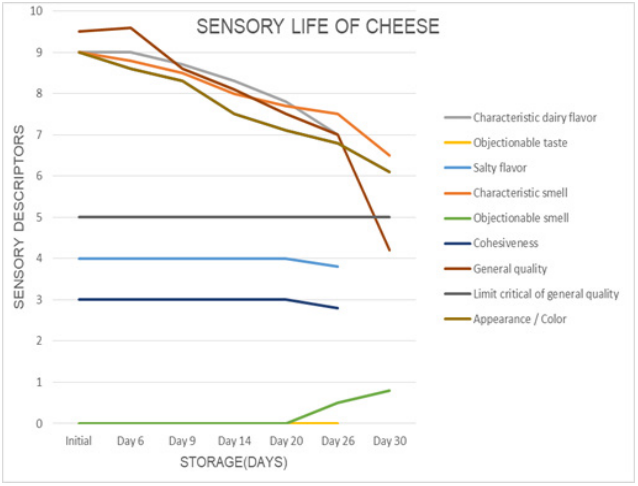

Figure 1. Sensory life cheese.
Source: The Authors.

very mild and mild terms; in the case of mild salty taste this is due to the fact that 1.5% salt was used as contingency in case of bitterness and low cohesiveness, which affects the protein matrix of cheese, making it harder and brittle.
As seen in Fig. 1, the cheese made with dry bromelain extract as coagulant showed an acceptable sensorial quality for 26 days. It should be noted that, during the time of analysis of the product, the tasters did not perceive objectionable flavors or smells, only until day 26 a slight acid smell was detected.

3.3.2. Physicochemical shelf life

According to the physicochemical assessment for acidity and pH there were highly significant differences ($p \leq 0.05$) in time for both variables, so that the acidity increased slightly during the storage of the cheese as can be observed in Table 5, probably due to the action of lactic bacteria present that degraded lactose in the form of lactic acid and as a consequence the pH decreased. According to this, it is important to note that probably the metabolic action of bacteria on lactose acidifies cheese (acidic pH) [25].

3.3.3. Microbiological shelf life

Differences in microbiological quality were found in the different times evaluated. According to the microbiological analysis, although some cheeses made with vegetable coagulants have defects in the microbiological quality, the fresh cheese made with Bromelain dry extract was microbiologically acceptable until day 14, where the causes of the failure of the product were the increase of the count of yeasts and total coliforms over the minimum allowed by the norm as shown in Table 6. It is possible that this is associated with high moisture content characteristic in fresh cheese that generally causes short shelf life, as it increases the activity of water and therefore favor microbial growth. However, in this investigation there were other factors such as low salt concentration, which probably favored the premature development of yeasts in the cheese by propitiating an acidic environment in the matrix thereof, because it extends the shelf life by slowing microbial growth it favors the decrease in water activity [26]. Yeasts are most frequently found in soft cheeses, comprising the genera *Alternaria, Aspergillus, Cladosporidium, Monilia, Mucor and Peni-Cillium and candidum yeasts Geotrichum and Candida Spp*. These influence the quality of cheeses through the production of fructifications, undesirable yeast flavors, and an unpleasant texture [19].

4. Conclusions

The dry bromelain extract at concentrations of 5% w / w has a high potential for use as a natural coagulant in the production of fresh cheeses of good sensory quality, obtained with raw or pasteurized milk. The products made with this bromelain extract reach a sensory and microbiological shelf life of 14 days.

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