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Research Article

Evaluation of the Physicochemical and Structural Characteristics of Oaxaca Cheese Made with Three Organic Acids (Acetic, Citric and Malic)

Suyapa Ramírez-Nolla[1] and Jorge F. Vélez-Ruiz[1, 2,*]

[1]*Departamento de Ingeniería Química e Ingeniería en Alimentos Universidad de las Américas Puebla San Andrés Cholula, Puebla, C.P. 72810. MEXICO*

[2]*Food Network ConsultoresInstituto de Diseño e Innovación Tecnológica,Blvd. del Niño Poblano 2901. Unidad Territorial AtlixcayotlPuebla, Puebla, C.P. 72420. MEXICO*

***Corresponding author**

Jorge F. Vélez-Ruiz, Departamento de Ingeniería Química e Ingeniería en Alimentos Universidad de las Américas Puebla San Andrés Cholula, Puebla, C.P. 72810. MEXICO, Tel: 222.2370117; E-Mail: jorgef.velez@udlap.mx

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Abstract

Oaxaca cheese is an important dairy product of high consumption in Mexico. The use of organic acids to make cheeses, like the Oaxaca cheese type, it is a common practice that aims to reduce manufacture times; however, these acid compounds have important effects on physicochemical and physical characteristics. In this work we conducted an evaluation of the characteristics of Oaxaca cheese made conventionally and with three organic acids: acetic, citric, and malic. As part of the results some changes were observed, the color of acidified cheese had an important variation showing less luminosity (< 70), compared to cheese made with lactic cultures (76), attributed to some precipitation of whey proteins caused by the addition of acids and an applied temperature of 85°C, through the preparation process. Likewise, there were changes in the yield of cheeses, malic acid presented a yield of 13.2% while cheeses made with acetic acid and citric presented yields below 9.9%, differences that were related to moisture content (51.8%, 48.0, and 50.0, respectively). From the results and tested acids, the malic acid proved to show better physicochemical properties and sensorial attributes. A stability study with the four manufactured cheeses was also completed through three weeks of storage.

INTRODUCTION

Food industry is researching and developing products that are based on improved manufacturing processes, therefore, it is convenient to know how the incorporation of new components; such is the case of Oaxaca cheese, in which the use of organic acids affects food properties and perception by consumers. Furthermore, the excessive demand for Oaxaca cheese has led to search alternatives for the manufacture of this dairy product; as is the addition of organic acids, such acetic. Even if the addition of acids reduces the time of acidification in milk for cheese making, promoting abrupt precipitation of proteins, with some desired and undesired changes, that have been observed and measured, such as texture, color, pH, moisture, water activity, acidity and decrease in yield [1-4], and also, as expected, the microstructure is also modified; there is not enough information.

There are few studies related with this dairy product, due to its proper characteristics and production, both artisanal and industrial. However, some of them may be mentioned De Oca-Flores et al. [3]. Completed a work to characterize the physicochemical properties of Oaxaca cheese manufactured at different times and temperatures, measuring its composition and a pH of 4.9-5.8. A sensory evaluation study of Oaxaca cheese, involving different trained panel was conducted by Ramón et al. [5] in order, to identify characteristics of this cheese and the abilities of sensorial panels. In an interesting and subjective work, Villanueva et al. [6], identified the flavor and texture profiles of Oaxaca cheese, attributing a 51.9% of flavor variance to the origin of the milk, whereas sensory chewiness, cohesiveness and springiness were highly correlated (0.60 - 0.83). Caro et al. [7], focused their research to identify the probiotic microorganisms from Oaxaca cheese, characterizing the lactobacilli strains in this Mexican dairy product. Whereas Sandoval-Copado et al. [8] completed their research preparing artisanal an industrial Oaxaca cheese to correlate sensory and physicochemical properties. González et al. [9] presented a review paper to identify artisan Mexican cheeses, including the Oaxaca type, emphasizing their characteristics, such as the used milk type and manufacturing process, among others. In other study, carried out by De Oca-Flores et al. [4], they analyzed the effect of milk, collected at different times of the year, they related several properties of the Oaxaca cheese, with the raw fluid, obtaining interesting conclusions. In which differences by season were observed in milk properties (fat and protein contents, acidity level, coagulation time, curd firmness and yield), and the season also affected physicochemical and technological characteristics of milk, and consequently cheese composition.

In the case of Oaxaca cheese, that is very similar to that identified as "quesillo" in South America; it is convenient to analyze alternatives for acid incorporation, several acids have been tested, to know if they can provide similar characteristics, to those of a cheese made with milk acidified conventionally through the making process. Although, it is convenient and it has been recommended, the use of organic acids, like citric and malic organic acids, among others, with the idea of time reduction in milk acidification and time reduction through Oaxaca cheese manufacturing, it is necessary to research into the effects and changes on physicochemical, rheology and structure of the product.

Therefore, this work, on a very important dairy product, such is the Oaxaca cheese; analyzed and studied how the incorporation of three organic acids; then, acetic, citric, and malic, were utilized during the acidification stage to know how they affect the process and product characteristics. The acids were selected taking into account, the provision facilities and previous tests, developed by us. Both aspects, process, and product properties, are very important for the food industry, in comparison with a traditional manufacturing process [10, 11, 4,]. It was also researched, its evolution during storage to establish differences and similarities.

This work properly covered three stages, a first one to characterize commercial items, in order to have a reference frame; the second and longest stage in which the different cheese samples were formulated, manufactured, and studied; and the last part where some selected cheeses were stored and analyzed, to know their changes through shelf life.

MATERIALS AND METHODS

Materials

Commercial pasteurized whole milk (Alpura®, Alpura, Coahuila, Mexico) was acquired in a local supermarket, the milk was standardized at 2.6% of fat content, doing a mass balance and mixing commercial milk, defatted milk, and water. Distilled water was provided by the Universidad de las Americas Puebla, both were used for manufacturing of the Oaxaca cheese.

Acids were acquired from commercial suppliers, acetic acid (85%) from Alquimia Mexicana, Mexico; and citric and malic acids (powders) from Cosmopolita Drugstore, Mexico. Three acid solutions (8 N) were prepared, and from them other solutions of 500 mL were obtained and used for cheese elaboration.

Cheeses Elaboration

The Oaxaca cheeses were prepared applying four process variants, directly acidified with acetic, citric, and malic acids, as well as the natural one (conventionally acidified by lactic acid bacteria). The manufacturing process is visualized in Figure 1 [12] that is a complex one, in which some stages were not necessary, such the recollection and preparation (filtration) of the milk batch.

Oaxaca cheese is considered a fresh, cooked paste and acidified dairy product by the Health Secretary of Mexico [13, 14, 12, 3, 4]. It is also mentioned, such as a pasta filata cheese, due to its important melting capacity.

Process and Physicochemical Determinations

Process parameters and physicochemical determinations were evaluated, by following standard methodologies for milk, commercial cheeses and manufactured Oaxaca cheeses, as liquid and solid samples, respectively. Methodology was taken from literature, and the employed methods are mentioned next.

Process Parameters: Coagulation time was measured by following the methodology of Huppertz et al. [15], in which liquid rennet (CHY-MAX Plus, Chr. Hansen, Mexico) was added to 15 mL of milk in glass tubes with cap. They were placed in a thermostatic bath at 32°C, after the lumpiness by enzymatic action, the coagulation time was recorded.

Cheese yield was quantified as the ratio between the mass of produced cheese, with respect to the used mass of milk. Yield was reported as the mean of three determinations, obtaining the ratio of the weight of Oaxaca fresh cheese obtained, in relation to the used acidified milk (Equation 2) [16, 17].

$$\text{Yield } (\%) = [\text{cheese produced (g)}/\text{used milk (g)}] * 100 \qquad (1)$$

Physicochemical Determinations: Bromatological analysis, in milk, was carried out with a specialized instrument (Lactoscan MCCW, Milkotronic Ltd., Bulgaria), that is based on ultrasonic analysis of cow´s milk, moisture, fat and protein were quantified.

Acidity was quantified by titration using NaOH (0.1N) and phenolphthalein as indicator, with 10 mL of the milk or 25 mL of filtered sample from each cheese batch (10 g mixed with 50 mL of distilled water at 40°C, shaken and filtered) (method 920.124) [18].

pH was measured with a digital potentiometer (Conductronics, Mexico), previously calibrated with buffers of pH 4.0 and pH 7.0, by immersing the electrode in a mixture of 10 g of grated cheese and 50 mL of distilled water [19].

Color was measured with a Gardner Color System 05 colorimeter (Hunter Labs, Reston VA), previously calibrated with a reference, white plate, having standardized reflectance Y=86.6, x=0.3168, y=0.3242; in reflection mode and using a tristimulus scale. The net differences among samples were evaluated using L*, a* and b* CIELAB color parameters, as well as the net change of color by using equation 1 [Díaz-Jiménez et al., 20; Bermúdez-Aguirre et al., 21], in which a fresh cheese was used as reference.

$$\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{1/2} \qquad (2)$$

Moisture was quantified by weight loss through evaporation, introducing samples into an oven at 135°C for 2 hours, according to the method 948.12 [22].

Water activity measurement was carried out using a commercial instrument (Decagon CX-1 model, Pullman, Washington), previously standardized with activated carbon at a_w 0.530.

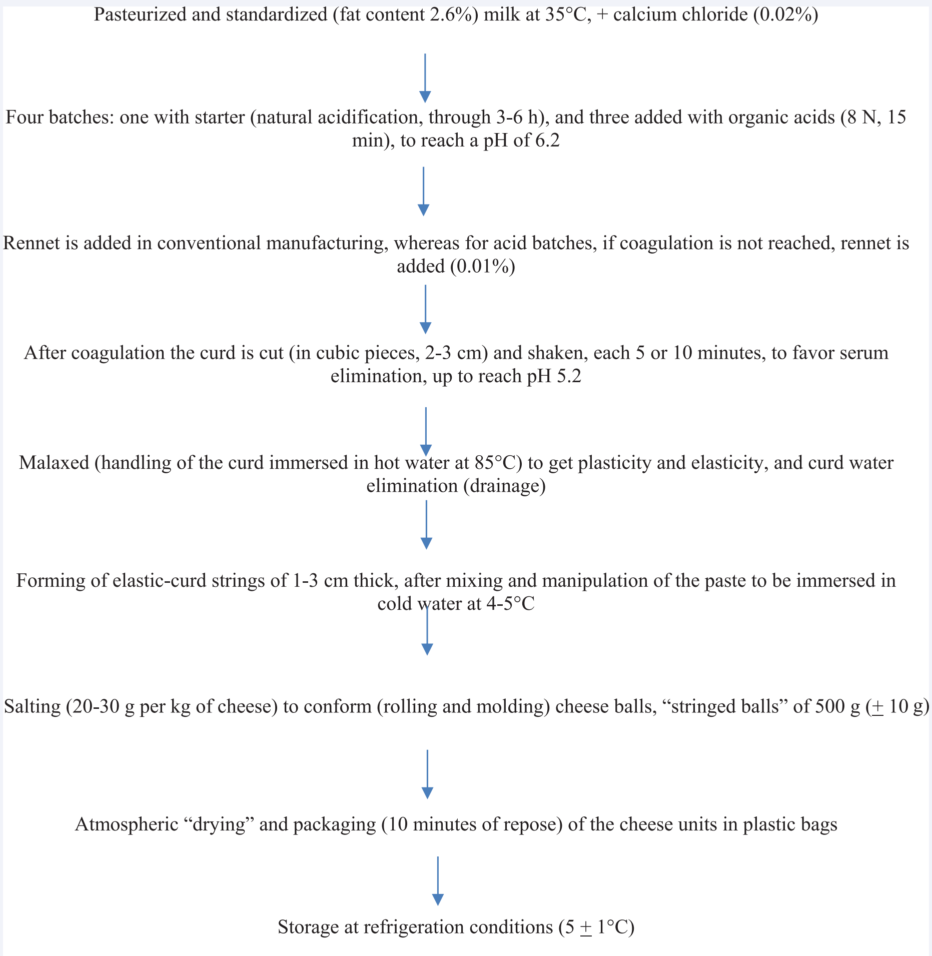

Figure 1 Schematic methodology for Oaxaca cheese manufacturing (SAGARPA, 2000).

Water holding capacity was determined, following the method of Zobrist et al. [23], measuring the solids weight recovered from the centrifugation of coagulated milk, in which rennet was used. 25 mL of milk are placed in Falcon tubes, rennet is added, and the sample was placed in a thermostatic bath, subjected to light agitation through 30 minutes. After that, the sample was centrifuged at 5000 rpm during 55 min and then the weight of solids was completed and recorded.

Melting capacity was determined using the method of Schreiber, modified by Kosikowski, in which, cheese cubes of 2 cm side, were placed on a glass plate and melted at 110° C during 15 min, measuring the area occupied by the cheese after molten. The result was reported in percentage of increase in that area [3].

Rheological and Textural Characterization: Measurements were carried out in a rheometer of controlled stress (model AR-2000; TA Instruments) at 25°C. Samples were allowed to rest for 15 minutes before measurement and a fresh sample was loaded for each measurement; both tests, stress and frequency swept were applied to get the corresponding rheological parameters [24].

The stress swept, applied a range of 0 to 2700 Pa (5-1000 μN-m at a constant frequency of 1 Hz, 6.28 rad/s), with parallel plates of 6 cm diameter and 6.89 mm gap. Whereas the frequency swept applied a range of 1-100 rad/s at a constant stress.

Texture was monitored through two tests, compression, and penetration by triplicate, using the TA.XT2 Texture Analyzer (Texture Technologies Corp., Scarsdale, NY, USA).

For compression test, cylinder samples of 2 cm diameter and 1.5 cm height, were subjected to 50% compression of its original size, by applying a plate of 3.8 cm diameter at a speed of 0.5 mm/s [10, 25]. Force or stress was measured.

Penetration test, conducted also on cylindrical samples with the same dimensions, that were subjected to penetration with a needle of 0.3 cm diameter at a speed of 1.0 mm/s up to 50% depth [16, 24]. Forces were measured.

Scanning Electron Microscopy: Samples for electron microscopy were prepared by applying the freeze-drying process (LABCONCO Equipment). After dehydration, the dried samples were fractured and the fragments were mounted on metal stubs, rendered conductive by coating with gold (JEOL JSMD-5600 LV, Denton Vacuum, Tokyo, Japon.), and examined in the Hitachi electron microscope (model JSM 5600 LV, Tokio, Japón) operated at 15 KV. In which the micrographs were taken by a computer program attached to the SEM. These observations were completed in a research center, by a technician.

Sensory Assessment: For the sensory evaluation, a preference test was carried out with 50 non trained panelists (students and workers). Testing the coded cheese samples and expressing the perceived values for each one, using a scale of 9 points hedonic scale (0 for dislike extremely, and 9 for like extremely).

Statistical Analysis: All systems were analyzed after preparation, fresh and stored. The response variables identified as process parameters and physicochemical properties were statistically examined with the Minitab software (V. 16, Minitab Inc, Pennsylvania, USA), using analysis of variance (ANOVA) and 95% of significance. Also, Tukey test was applied for multiple comparisons of the mean values.

RESULTS AND DISCUSSION

The three stages of this work are covered next.

Commercial Cheeses

In order, to have a context of proper characteristics and compare our manufactured cheeses, with them; five brans of Oaxaca cheese were acquired and analyzed. The same standard methods were used for these samples. Next are the main results of this first stage, showing four properties of the cheeses.

In moisture determination, the range of values, were between 47.1 and 49.9% that corresponded properly to fresh cheeses [26]. The mean and standard deviation of five commercial brands are included in Table 1; this moisture content is in agreement with other values, reported by García [27], in which a moisture of 52.8-46.5 were measured. As consequence of high moisture, the recorded water activity (0.969 to 0.979), was also high,

On the other hand, the acidity and pH, with 0.31-0.45% and 5.31-6.38 respectively, exhibited, also a fresh dairy product (Table 1), with low acidity and few days of storage, not controlled by us. More variability was observed in moisture, acidity, and pH, in that order. The observed difference in the measured characteristics may be attributed to the manufacture process of each brand and storage of the samples.

For color parameters of the different commercial cheeses, it was a general trend to a white-light yellow color that was recorded by the measurement instrument. Brightness was high, with a wide range of 63.54 to 93.81, being comparable to those values reported by García [27]. The lowest value (63.54) corresponded to the Alpura cheese brand, whereas the highest value (93.81), corresponded to the "Volcanes". The redness a* parameter had a range of - 5.14 to -2.55, showing some effect of the green color; and the yellowness parameter b*, showed a range of 15.13 to 19.35 with a clear trend, to yellow color presence. Table 2 include all the measured color parameters, including the magnitude computed for the net change of color (< 6.9), being low, when the commercial cheeses were compared with a fresh one; indicating that the commercial cheeses did not have too much time of elaboration.

Finally, the melting capacity of the tested commercial Oaxaca cheeses was also determined, recording a range of 356-463%, with specific values of 356% for "Villita" brand, 425% for "Volcanes",457% for "Lala", 460% for "Alpura", and 463% for "Nestlé", indicating a very high capacity of these cheeses for sample extension or meltability, that is a very important characteristic of pasta filata cheeses. Some brands use to add melting salts to the Oaxaca cheese, to favor this important property.

Then, as expected, a wide range of properties was measured in the commercial Oaxaca cheeses.

Manufactured Cheeses

Next are the results and observations related with the manufacturing, characterization, and analysis of the four prepared cheese Oaxaca variants.

Table 1: Properties* in Five Brands of Commercial Oaxaca Cheeses.

Brand	Moisture	Aw	Acidity	pH
"Volcanes"	47.92 ± 0.38	0.969 ± 0.002	0.45 ± 0.09	5.42 ± 0.005
"Villita"	49.89 ± 0.40	0-972 ± 0.001	0.31 ± 0.04	6-39 ± 0.025
"Lala"	48.07 ± 0.49	0.975 ± 0.001	0.31 ± 0.04	5.67 ± 0.010
"Alpura"	48.70 ± 0.65	0.979 ± 0	0.45 ± 0	5.55 ± 0.015
"Nestlé"	47.16 ± 0.28	0.972 ± 0.001	0.40 ± 0.04	5.49 ± 0.012

* Mean and standard deviation

Table 2: Color Parameters* in Five Brands of Commercial Oaxaca Cheeses.

Brand	Lightness (L*)	Redness (a*)	Yellowness (b*)	ΔE
"Volcanes"	93.81 ± 0.10	- 3.95±0.05	15.21±0.08	4.257
"Villita"	81.59 ± 0.78	- 2.26±0.28	19.35±0.30	5.653
"Lala"	74.55 ± 0.13	- 5.14±0.05	18.38±0.08	6.397
"Alpura"	63.54 ± 0	- 03.49±0.49	14.48±0.82	6.810
"Nestlé"	71.42 ± 0.12	- 3.86±0.45	15.13±0.82	6.286

* Mean and standard deviation

Composition and Coagulation Capacity: Previous, to manufacturing of Oaxaca cheese samples in our labs, a couple of analysis were completed; a bromatological profile of used milk and a coagulation time of each cheese. The used milk was a commercial one (pasteurized milk, “Lala” brand), in which next composition was obtained: fat 2.9%, protein 3.2% and pH 6.6, indicating that is a milk with good quality, in which the measured composition corresponded to the reported in the product´s nutritional label; as it was previously mentioned, the fat level was standardized at 2.6%; in which this milk was mixed with defatted milk and water.

For the coagulation time, important differences were found; 10.0 (±0.62)[a] min for natural, 1.16 (±0.15)[c] min for acetic cheese, 6.06 (±0.15)[c] min for citric cheese, and 8.13 (±0.20)[d] min for malic cheese. Times were completely different ($p \geq 0.05$), the use of the organic acids in the acidification stage reduced notably the coagulation time. The incorporation of acetic acid resulted 8.6 times faster with respect to the natural acidification, as expected. The differences in the proteinic matrix and structure could be appreciated in a microscopic study, and thus, the manufactured cheese exhibited a diversity of properties, strongly related with the acid used through the acidification process.

Cheese Determinations: With respect to performance and yield, the manufactured Oaxaca cheeses exhibited the following percentages, 10.10% (± 0.14) for natural cheese, 9.20 (± 0.14) for cheese prepared with citric acid, 9.90 (± 0.14) for cheese prepared with acetic acid, and 13.15 (± 0.21) for cheese prepared with malic acid. Showing significative difference between acetic and citric cheeses, being lower than natural and malic acid cheeses; these determinations were not related with moisture content. Qualitatively the yield may be related to the correspondent cheese protein matrix. This yield is very important for industrial production.

The physicochemical properties of the manufactured four cheeses, are included in Table 3. With values of 46.5 to 51.8 for moisture, and a range of 0.934 to 0.974, for water activity. Higher moisture was exhibited by cheeses prepared with citric and malic acids; being significantly differents ($p < 0.05$) to the other two cheeses. Moisture is inside the range reported by De Oca-Flores et al. [3,4], and by Montes de Oca et al. [4]. The same significant difference was recorded for water activity of the cheeses; lower a_w was measured in cheeses with acetic and citric acids. It is notable the lowest value for a_w in acetic acid cheese, that was not the case for moisture, whereas the highest a_w and moisture was measured in malic acid cheese.

For acidity and pH, that are correlated properties, the four cheeses showed ranges of 0.69 to 0.82% of lactic acid, and a range of 5.1 to 5.8 for pH. Highest acidity and consequently lowest pH corresponded to the natural cheese, attributed to the presence of starter cultures (thermophilic bacteria) that survive to the malaxed processing and have longer action. On the other hand, lower acid was present in the other three cheeses, in which part of the acid is lost through the curd handling at 85°C. A significant difference was observed for acidity, between the two couples of cheeses, natural and acetic acid with respect to malic and citric cheeses; whereas for pH, the first three (natural, citric and acetic cheeses) were statistically different to malic acid. These differences in acidity and pH are due to the manufacturing process in good grade, and particularly to the acidification stage, in which each acid has also a particular effect, as expected. The acidity and pH of the manufactured Oaxaca cheeses are in according with those values recorded by De Oca-Flores et al. [3,4], and by Montes de Oca et al. [4]. Even though there exist differences in process and other variables, in these cited works.

The water holding capacity, as a measure of the matrix structure and how the solids are retained in that structure, exhibited two couples of responses. Malic and natural cheeses with higher retention (> 6. 7%) and acetic and citric with lower capacity (< 6.1%), both couple of cheeses with significant difference. Considering only this property, there is a great difference between acetic cheese with only 1.16%, and malic acid with 8.13%, practically 7 times higher, and better even that the natural one. This result is very important to decide the manufacturing with the malic acid and not with acetic acid.

Table 3: Physicochemical properties* in four manufactured Oaxaca cheeses.

Cheese	Moisture (%)	a_w	Acidity (%)	pH	Water holding capacity (%)
Natural	46.51 ± 0.06[b]	0.962±0[b]	0.82±0.02[c]	5.11±0[a]	6.76±0.22[a]
Citric acid	50.01 ± 0.44[a]	0.955±0[b]	0.73±0.02[b]	5.42±0.02[a]	6.06±0.26[b]
Acetic acid	48.00 ± 0.29[b]	0.934±0[c]	0.80±0.02[b]	5.35±0[a]	1.16±0.35[b]
Malic acid	51.80 ± 0.78	0.974±0[a]	0.69±0.02[a]	5.79±0.02[b]	8.13±0.15[a]

* Mean and standard deviation

Table 4: Color Parameters* in Four Manufactured Oaxaca Cheeses.

Cheese	Lightness (L*)	Redness (a*)	Yellowness (b*)	ΔE
Natural	76.42 ± 0.97[a]	- 5.14 ± 0.04[a]	26.47 ± 2.45[a]	0
Citric	69.78 ± 3.84[a]	- 4.12 ± 0.21[a]	14.44 ± 1.34[b]	14.33
Acetic	48.30 ± 0.22[b]	- 4.96 ± 2.87[a]	14.19 ± 0.91[b]	30.70
Malic	53.58 ± 0.60	- 3.08 ± 0.02[a]	10.29 ± 0.09[b]	28.40

* Mean and standard deviation

And the color parameters in the manufactured cheeses are included in Table 4. Observing notable differences in lightness with respect to commercial ones, indicating a lower whiteness. This lightness decreasing, has been also reported by Metzelger et al. [28]), who observed a low lightness in Mozzarella cheese, when organic acids where added, and with differences with respect to commercial cheese. The other two, color parameters, although are different in manufactured samples, have some similarity to commercial items. It is notable that natural cheese showed the highest values in redness and yellowness. For the net change of color, in this case, the natural one was taken as reference, then acetic and malic acid cheeses are the double of different than citric acid cheese. In general, color parameters are comparable to those reported by Caro et al. [29], mainly for L and b, being the yellowness different, they reported positive values.

Additional properties, such meltability of manufactured cheeses, exhibited a range of 97 to 363% of expanded area with heating; being lower in general for manufactured samples, than the commercial ones. Particularly acid and citric cheeses, in which cheese, from citric acid was the lowest, 97.0 ± 39.60%, followed by acetic acid cheese with 125 ± 0%, and 282.5 ± 82.73 for natural one, being the highest meltability that corresponded to cheese made with malic acid, 362.5± 30.45%. Some interesting observations for properties may be mentioned, natural and malic cheeses were not statistically different, in contrast to the other two cheeses. In three of them, the variability for this physicochemical parameter was very high, indicating that the manufacturing process had notable effects. Metzelger et al. [28] observed a clear decreasing in melting capacity of Mozzarella cheese, when citric acid was incorporated in the preparing process, and it was attributed to the alterations or changes in protein-protein interactions and calcium loss.

For the rheological characterization of the four cheeses, a viscoelastic nature was observed, in which the storage modulus was higher than the loss modulus, as expected for this type of dairy product, and a significant difference was observed between the natural and the acidified cheeses, for the elastic response. Rogers et al. [30] mentioned a moisture-protein relation as responsible of a stronger structure; that was the case of the cheese acidified with probiotic bacteria. Figure 2 and Table 5, include, the graphic response and some specific moduli, as representative of the change with time. May be observed that acetic and citric acid cheeses have closer magnitudes of the G´ parameter; the similarity in structure of these two cheeses was also reflected in textural parameters. The cheese elaborated with malic acid had an "intermediate" structure, between natural and citric/acetic cheeses.

Some differences in the structure characteristics may be observed in the micrographs of the manufactured cheeses, on Figure 3; where notable differences were detected at 370 augments, at the cheese surface. Natural cheese (N) exhibits a more orderly proteinic and rough surface with notable strings and numerous corpuscles, whereas in citric acid, an agglomerated

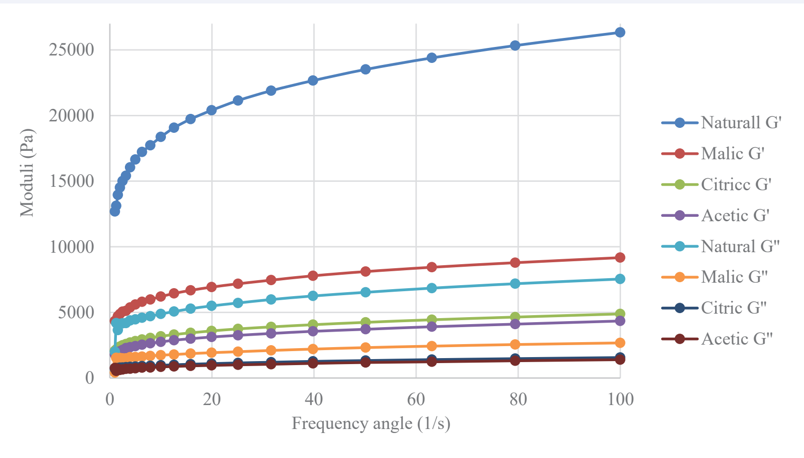

Figure 2 Viscoelastic (elastic and viscous moduli) data for fresh manufactured Oaxaca cheese.

Table 5: Viscoelastic data (storage, G´, and loss, G´´,moduli) for manufactured Oaxaca cheese, fresh and stored.

Cheese	Frequency Angle (s^{-1})	First day G´ (kPa)	Fifteen day	
			G´´(kPa)	G´(kPa)
Natural	1	13	4.13	8.04
	2.5	15	4.14	8.7
	5	16.4	4.15	10
	10	18	5	11.52
	25	21.1	5.87	13.26
	50	23.7	6.74	15
	100	26.5	7.83	16.91

Malic	1	4.13	2.2	8.04
	2.5	5	2.6	8.7
	2	6.1	2.8	10
	10	6.5	3	11.52
	25	7.6	3.1	13.26
	50	7.8	3.5	15
	100	8.9	4.3	16.91
Citric	1	2.4	1.2	5
	2.5	2.6	1.4	5.4
	2	2.8	1.5	5.9
	10	3.3	1.9	6.9
	25	3.7	2.1	8.5
	50	4.3	2.4	9.3
	100	5	2.6	10.4
Acetic	1	2.2	0.89	3.9
	2.5	2.6	0.9	4.8
	5	2.8	0.91	5
	10	3	0.93	5.1
	25	3.1	0.96	7.2
	50	3.5	1.3	7.8
	100	4.3	1.5	9.3

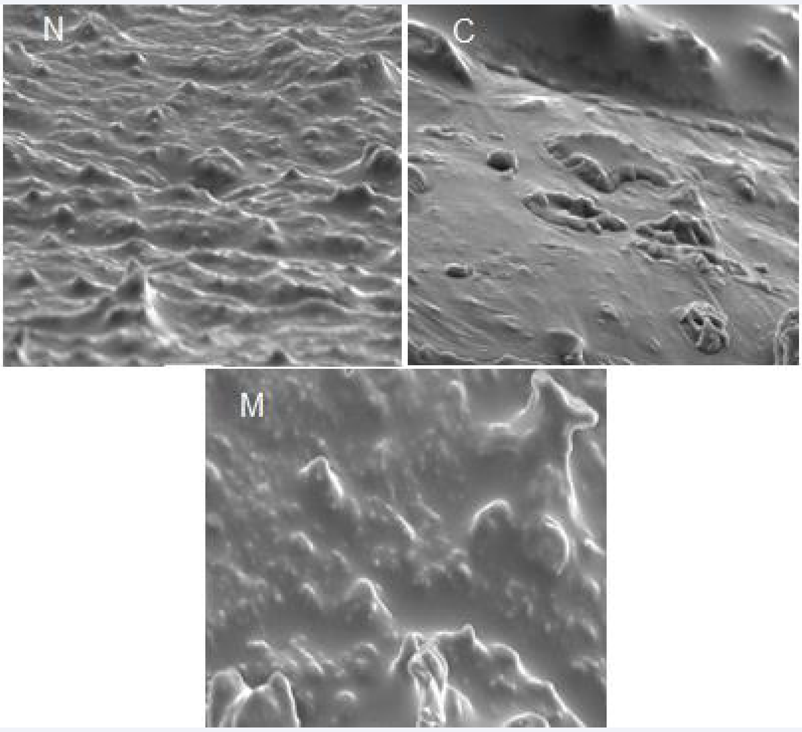

Figure 3 Micrographs from three manufactured cheeses (370 X), N: natural, C: citric, and M: malic acid cheeses.

proteinic and less rough surface is shown. and malic acid cheese shows an intermediate appearance with lower corpuscles in comparison with natural cheese, but more corpuscles and rough surface in comparison with citric acid cheese. In general, natural cheese surface looks more “uniform” than the other two cheese surfaces, corresponding to citric and malic acids.

For texture determinations, also clear differences were recorded by the texture meter, exhibiting 23.67 N for compression force, and 3.20 N for penetration force in natural one, whereas for the other cheeses, 15.22, 10.54 and 8.31 N on compression test, and 3.11, 2.54 and 2.51 N in penetration were obtained correspondingly, in malic, citric, and acetic cheeses. A more compact proteinic matrix may be visualized in natural, and lower solids interactions may be observed for the acidic cheeses, mainly measured in the compression test, with 23.7 N for natural vs. 8.3-15.2 N for the other three cheeses. In contrast, the

Table 6: Sensory Parameters* in Three Manufactured Oaxaca Cheeses.

Cheese	Acidity	Flavor	Color	Stringiness	Texture	Overall Acceptance
Citric	7.25 ± 1.32[a]	7.20 ± 1.41[a]	7.20 ± 1.38[a]	6.95 ± 1.75[a]	6.37 ± 2.03[a]	7.33 ± 1.34[a]
Acetic	7.25 ± 1.45[a]	7.29 ± 1.45[a]	7.25 ± 1.70[a]	6.62 ± 1.83[a]	6.12 ± 2.15	6.75 ± 1.70[a]
Malic	7.58 ± 1.21[a]	7.50 ± 1.38[a]	7.29 ± 1.39[a]	6.25 ± 1.84[a]	6.83 ± 1.63[a]	7.37±1.37[a]

*Mean and standard deviation

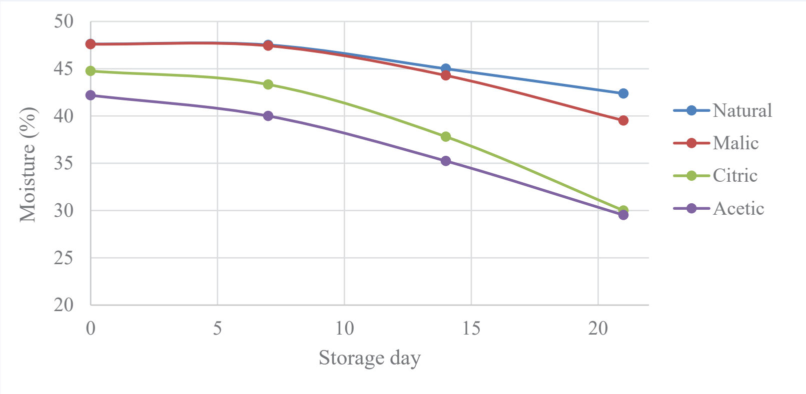

Figure 4 Moisture of manufactured Oaxaca cheeses, through 21 days of storage.

penetration test showed similar forces, 3.2 N in natural vs. 2-51-3.11 N in acidic cheeses. Then, significant effect was computed for manufacturing process on the compression parameter for the four manufactured cheeses. In comparison with the hardness reported by Caro et al. [29], the compression force (8.3-23.7 N) from our manufactured Oaxaca cheeses is comparable with 14.9 N for that hardness.

Finally at this stage of the work, a sensory assessment of the acidic manufactured cheeses was conducted with a non-trained panel, constituted by students and workers at the labs of the University. Six sensory parameters were evaluated, and the obtained responses are included in Table 6.

Knowing that the natural cheese is very well accepted, with high scores (> 7.5), good scores were given, to the other three cheeses, to all the assessed parameters, with a range of 6.12 to 7.58; in which texture and stringiness were the parameters with lower evaluation(< 7.0). The other four parameters received a score higher to 7.20, with exception of acetic cheese in overall acceptance (6.75), and then a good general acceptance for our manufactured cheeses was recorded. No one of the sensory attributes exhibited significant difference in the three acidic cheeses.

Stability Analysis

As the last part of this research, the manufactured cheeses were stored through three weeks at refrigeration conditions (5 ± 1°C), in which several of the same physicochemical analysis was completed, doing the corresponded observations. The results of this stage are exposed and briefly commented next.

With respect to the measured physicochemical analysis, moisture recorded a decreasing trend as consequence of the matrix contraction and the syneresis phenomena, by protein hydrolysis, such is cited by McSweeney [31]. Natural and malic cheeses exhibited a lower loss (Figure 4), in contrast to the other two acidic cheeses, that showed a higher loss, since the second week. Even though, the moisture decreasing did not show a significant effect of the storage time.

And related with moisture content, the water activity showed a light decreasing (Figure 5), being more detectable in natural cheese; without significant difference in general, for the four cheeses, through the storage. Although natural and malic cheeses exhibited significant difference between the day 0 and day 21. The relative stability of this a_w parameter in the three acid cheeses, mainly citric and acetic cheeses, may be considered as an advantage for this dairy product. The natural one, decreased from 0.98 to 0.938, whereas malic acid changed from 0.963 to 0.942, citric cheese varied from 0.947 to 0.933 and acetic cheese changed from 0.931 to 0.925; being the last that showed less decreasing, all decreases related with the loss of water through storage.

For acidity and pH, an expected contrary effect was observed. A light increasing trend in acidity (0.70 to 0.95) related with a more observable decreasing in pH (5.6 to 4.8) was recorded, a change that was more notable in acidic cheeses. A significant difference was recorded for the four cheeses through the storage time, being attributed to the developed metabolic changes, proteolytic and lipolytic processes, among others. In those changes (Figure 6), lactic acid (natural), and malic cheese exhibited the lowest augment (0.70 to 0.75), whereas citric and acetic ones showed more increasing (0.72 to 0.95).

For pH (Figure 7), it was recorded a decreasing from 5.64 to 4.75, being more notable in malic (5.64 to 5.0) and acetic (5.39 to

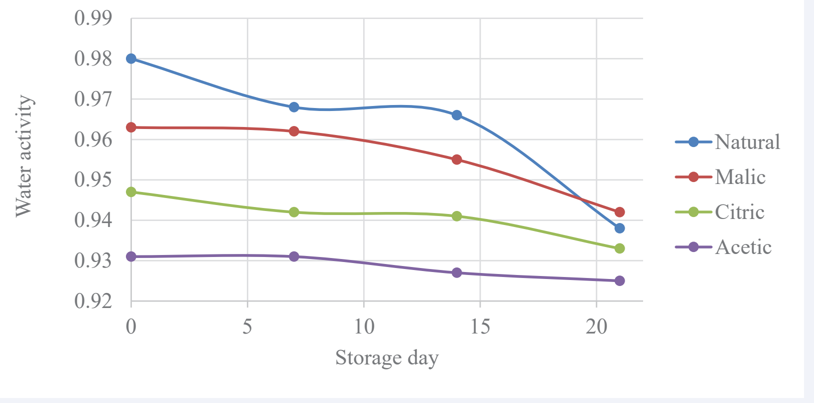

Figure 5 Water activity of manufactured Oaxaca cheeses, through 21 days of storage.

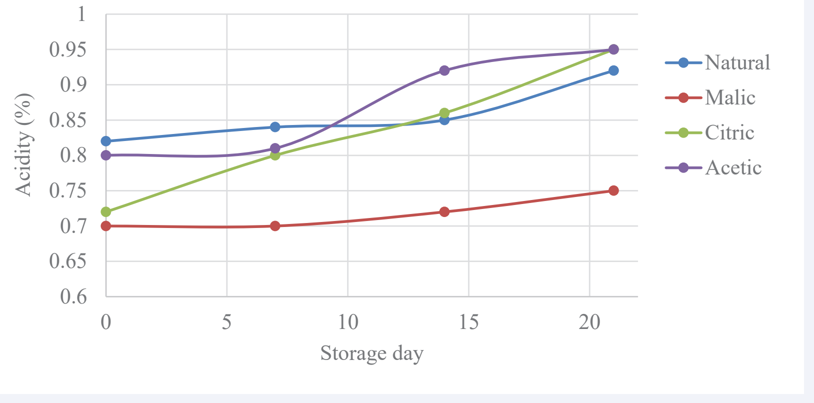

Figure 6 Acidity of manufactured Oaxaca cheeses, through 21 days of storage.

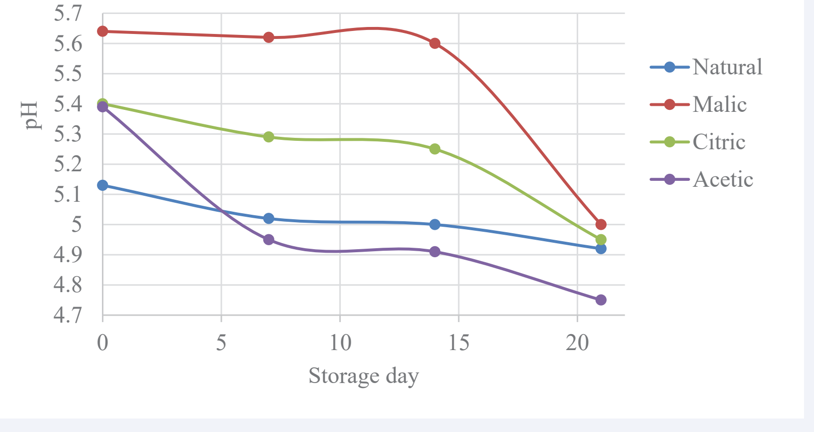

Figure 7 pH of manufactured Oaxaca cheeses, through 21 days of storage.

4.75) cheeses, followed by the citric one (5.4 to 4.95), and natural cheese that showed the lowest decrease (5.13 to 4.92). This difference between natural and acidic cheeses is related to lactic acid formation; it is also an important factor to be considered by the manufactures, in order to select the specific acid.

For the color parameters, the next trends and observations were completed. The luminosity showed higher values for natural and citric cheeses in comparison with malic and acetic cheeses; exhibiting a light decreasing, in general, after the three weeks of storage; being more observable in natural (76 to 52) than in acidic cheeses. It was a significant effect of the storage time on this color parameter. Although the luminosity of two of them are low, this relative stability, lower decreasing in acidic cheeses, 70 to 65 for citric, 48 to 47 for acetic and 54 to 45 for malic one, may be an advantage for these manufactured items.

Redness (a*) of the four cheeses showed small changes, it was less notable in acetic cheese. Natural cheese was -5.1 fresh and -5.3 after storage; citric cheese had - 4.1 fresh and - 4.2 after three weeks; malic cheese without change - 3.1 fresh and after storage, and acetic cheese recorded - 5.0 fresh and - 6.9 after storing. And, contrary to the two previous parameters, yellowness (b*) showed an increasing trend, with 26.5 to 28.2 for natural, 14.4 to 17.7 for citric, 10.3 to 12.0 for malic and 14.2 to 18.0 for acetic cheese; b* parameter was also influenced significantly by the storage time. Then, as consequence of these variations in color parameters, a general whiteness loss and a yellow increasing of the four cheeses, was observed through storage.

In the rheological determinations, in general the viscous moduli decreased; whereas the elastic moduli did not follow a general trend with storage, both responses of the moduli may be related to the syneresis phenomena, in which the loss of water and structural changes, contributed to the elastic and viscous behaviors of the four cheeses. As an interesting physical phenomenon, the natural cheese showed a decreasing in the elastic modulus (Table 5), whereas the acidic cheeses exhibited a contrary response, an increasing in this parameter (Table 5), as consequence of a kind of crust on the surface, being around the double of the elasticity (G´) recorded as fresh items. This difference in the storage modulus of our manufactured cheeses could be an important characteristic to be considered also by the manufacturers.

Finally, in addition to the mentioned parameters, the measured textural forces, compressive and penetrative, had a notable decreasing trend in the four manufactured cheeses, attributed to the proteinic matrix that lost structural consistency. There was a significant effect of the storage time on texture, measured through these two parameters; the evolution of both forces may be observed in Figures 8a and 8b. The penetration force decreased from 23.7 to 11.9 N, for natural, 15 to 5.4 N for malic, 10.6 to 5.4 N for citric, and 8.1 to 2.3 N for acetic cheeses, as fresh and as stored items, respectively. On the other hand, the penetration force also exhibited a decreasing trend, with higher forces in natural and malic cheeses than in citric and acetic cheeses. These punctual forces decreased through storage from 3.2 to 1.6 N in natural, 3.1 to 1.4 N in malic, around a half in both cheeses; and from 2.6 to 1.1 N in citric and 2.5 to 1.0 in acetic, practically the same change in these two cheeses.

CONCLUSION

Oaxaca cheese was manufactured conventionally and using three organic acids, finding different properties and characteristics. Some commercial items offered a context of characteristics that were used as a comparison and framework basis. Additionally, a stability study was developed for the four manufactured cheeses.

This work allowed determining the properties of each manufactured cheese, natural and acidic. Also, the physicochemical properties of the four cheeses were recorded, observing the changes and differences though three weeks of storage; thus, any interested person, may to know, and to apply, the knowledge that has been expressed in this work, in order to manufacture a cheese with desired characteristics.

In general, the manufactured cheeses presented analog properties to commercial items, as expected. And depending of what aspect or property could be taken into account, for a specific

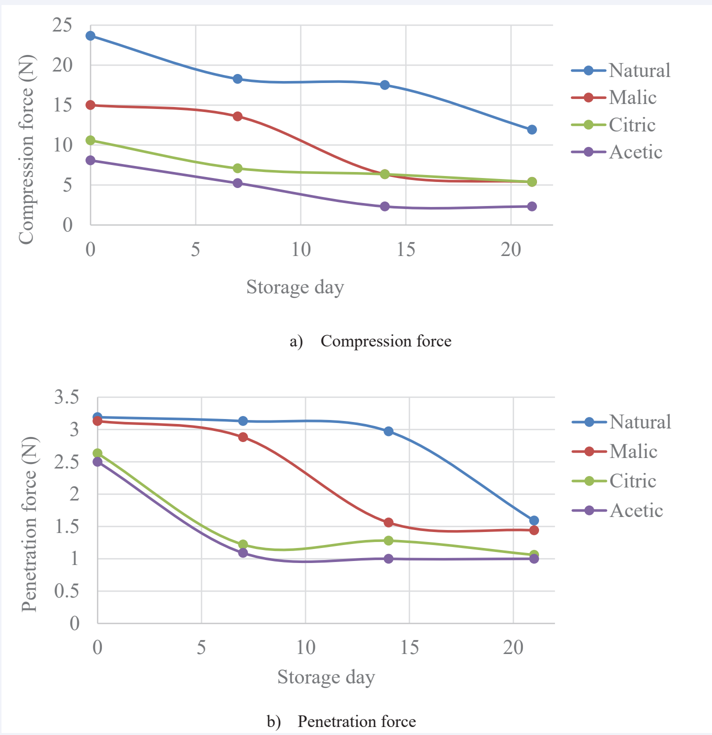

Figure 8 Textural forces for manufactured Oaxaca cheeses, through 21 days of storage, a) compression force, b) penetration force.

manufacturing process, the three acidic cheeses are more or less similar to the natural one. We can say that malic and citric acid are preferred, based on general acceptation by the non-trained panel.

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