



## Oregano essential oil in panela-type cheese: its effects on physicochemical, texture and sensory parameters



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### Abstract:

Plant essential oils are increasingly used in the food industry for their antimicrobial, antioxidant and sensory properties. The effects of added oregano essential oil (OEO) in panela cheese (QP) production was evaluated on cheese physicochemical, textural and sensory properties during 15 day's storage. Two addition levels were used, resulting in three treatments: control with no OEO (QP1); 0.05 g OEO/ L milk (QP2); and 0.10 g OEO/L milk (QP3). In all treatments, cheese pH was highest ( $P<0.05$ ) on d 1 and lowest on d 15, although

it remained higher overall in QP1. Weight and weight loss did not differ between treatments. Color parameters differed minimally between treatments and over time, although increased OEO content pushed  $b^*$  values towards yellow. Addition of OEO lowered cheese hardness and shear force values. Based on sensory parameters, consumer acceptance was highest ( $P < 0.05$ ) for the control treatment. Addition of OEO generally did not affect cheese physicochemical, textural and sensory characteristics during storage. It did slightly lower hardness and shear force values, and decreased consumer acceptance. If used at adequate levels, oregano essential oil can improve cheese performance during storage without substantially affecting quality parameters.

**Key words:** Essential oil, Quality, Color, Milk, Attributes.

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Cheese and other milk derivatives constitute an important protein source in human diets. Myriad cheeses are produced in Mexico, panela-type fresh cheese being a well-known type. Production of panela cheese in Mexico has increased in recent years. National production in 2018 was 51,340 tons worth \$2.96 million Mexican pesos, and in the first half of 2019 production was 25,850 tons worth \$1.53 million pesos<sup>(1)</sup>. Panela cheese is popular in Mexico, white in color and inverted truncated-conical in shape<sup>(2)</sup>, with physical and sensory characteristics similar to those of Greek feta cheese<sup>(3)</sup>.

Cheeses, especially fresh types, experience biochemical processes such as lipolysis and proteolysis, and high water activity, making them susceptible to oxidation and microbiological deterioration, and reducing their shelf life<sup>(4)</sup>. Fresh cheeses are considered to be a highly perishable food and it is recommended they be stored at temperatures below 5 °C<sup>(5)</sup>. Addition of carbonates, citrates, gums, sorbates and propionates to fresh cheeses can increase their shelf life<sup>(6)</sup>, by reducing water activity, regulating acidity, and thus stabilizing and better preserving the cheese. However, consumers increasingly demand cheeses free of preservatives and chemicals with potential health consequences, driving a search for natural additives to promote conservation, such as essential oils (EO) from aromatic plants. Oregano essential oil (OEO) has antioxidant and antimicrobial properties that can retard oxidation in food, maintaining its physicochemical properties, lengthening its shelf life, and thus improving consumer acceptance<sup>(4)</sup>. Very few studies have been done to date on the use of EO as preservative agents in cheese or dairy products<sup>(7)</sup>. Both OEO and rosemary EO are reported to reduce lipid oxidation and fermentation in cheeses prepared with a cream base<sup>(7)</sup>. Addition

of OEO to organic cottage cheese is also reported to lower the rate of quality parameter deterioration during storage, suggesting its use as a natural preservative in perishable foods<sup>(4)</sup>.

The present study objective was to evaluate the effects of oregano (*Lippia berlandieri* Schauer) essential oil (OEO) on the physicochemical, textural and sensory properties of panela cheese during 15 day's storage at 4 °C.

The effects of addition of two OEO concentrations (0.05 and 0.10 g/L milk) to the milk used to produce panela cheese on physicochemical and sensory characteristics were evaluated at 1, 4, 8 and 15 d after production. The completely randomized design involved three treatments: QP1= control (no OEO); QP2= 0.05 g OEO/L milk; and QP3= 0.10 g OEO/L milk. Each treatment involved two replicates per treatment and period (1, 4, 8 and 15 d; 24 total cheeses), and the studied variables were evaluated four times per replicate (n= 8 periods/treatment).

A total of 3.5 L commercial pasteurized milk (Comercializadora de Lácteos y Derivados, S.A. de C.V.) were used per cheese. Milk composition (g/100 ml) was 3.12 g protein, 3.32 g fat, 4.80 g carbohydrates, 0.046 g Na and 0.116 g Ca. The OEO was sourced from Natural Solutions SMI (Jiménez, Chihuahua, Mexico). Composition of the OEO was quantified by gas chromatography (PerkinElmer Clarus 600 and SQ8 GC/MS; PerkinElmer Inc., Waltham, MA, USA) according to Vazquez and Dunford<sup>(8)</sup>. Its main constituents were carvacrol (60.0 %), cymene (16.1 %), terpinene (5.4 %) and thymol (3.4 %). Before addition to the milk the OEO was emulsified with Tween20 at a 50:50 ratio (OEO:emulsifier). This ratio was established based on preliminary tests in which OEO was mixed with Tween20 (polyoxyethylene [20] sorbitan monolaurate), stirred manually for 3 min and stored at 26 °C for 10 days. An emulsion was considered unstable when a creamy, oily layer or oil drops were observed. Using FAO guidelines (01.6.1, Note 38 – based on mixture to be skimmed)<sup>(9)</sup>, a maximum limit of 80 mg Tween 20/kg unripened cheese was employed.

Panela cheese production was done following an established protocol<sup>(10)</sup>. Milk temperature was adjusted to 34 °C, and CaCl<sub>2</sub> (15 g/100 L milk; dissolved in purified water) slowly added under constant stirring. In the QP2 and QP3 treatments, the indicated amount of emulsified OEO was then added by slow mixing for 2 min. Rennet (CUAMIXM.R., CHR HANSEN de México, S.A. de C.V., Mexico City; 15 mL/100 L milk; diluted in purified water) was slowly added to the milk under constant stirring for 1 min and allowed to settle for 40 min to set the milk. The resulting curd was cut into cubes (1 cm<sup>3</sup>), rested for 5 min and slowly stirred while raising its temperature to 38 °C for 2 min. After resting for 5 min, the cheese mass was obtained by partial draining (2/3 whey removed). Salt (600 g NaCl/100 L milk) was added under constant, slow stirring. The cheese was placed into cylindrical plastic self-pressed molds and turned every 30 min, twice per side. Finished cheeses were weighed, vacuum packed, labelled (replicate/treatment/period) and refrigerated at 4 °C until evaluation.

Five physicochemical variables were evaluated: weight (W); weight loss (% , WL); pH; titratable acidity (TA); and color. The cheeses were weighed when finished (day 0) and after 1, 4, 8 and 15 day's storage; the results were used to calculate WL  $[(W_{\text{initial}} - W_{\text{final}}) / W_{\text{initial}}] * 100$ . The pH was measured with a puncture electrode (Orion 3 star ThermoFisher Scientific, Pittsburgh, USA) at four points in 50 g cheese for each replicate in each treatment. Titratable acidity (TA) was measured using an established method<sup>(11)</sup>, with modifications in sample preparation: 1 g cheese was totally macerated in 10 g distilled water, 9 g mixture removed, three drops phenolphthalein added to it and this titrated with 0.1 M NaOH. Titratable acidity (TA) was calculated with the formula  $\text{TA (g lactic acid/100 g product)} = (V \times 0.9) / m$ ; where V is volume (ml) of 0.1 M sodium hydroxide; m is sample mass (grams), and 0.9 is the lactic acid conversion factor. Five color parameters (luminosity [L\*], red tendency [a\*], yellow tendency [b\*], Hue angle and Chroma [Chro; saturation]) were measured with a CR-400 colorimeter (Konica Minolta®, Tokyo, Japan), based on the CIE Lab system<sup>(12)</sup>. Measurement of each variable was done eight times per treatment per period.

On d 1, 8 and 15, a texture analysis device (TA.XT.Plus , Stable M Micro Systems, Surrey, England) was used to measure shear force (SF) and run a texture profile analysis (TPA) for four cheese samples/replicate/period (i.e. n = 8 samples/treatment/period). For SF measurement, a 3 mm inverted triangle Warner-Bratzler blade was used to cut standardized rectangular samples (1 cm wide x 1 cm high x 3.5 cm long). Test conditions were a pre-test speed = 1.0 mm sec<sup>-1</sup>, a test speed = 2.0 mm sec<sup>-1</sup> and a post-test speed = 10.0 mm s<sup>-1</sup>. The SF was considered the maximum point of the resulting curve. In the TPA, standardized samples (1.5 cm high x 2.5 cm diameter) at 8 °C were compressed to 50 % of their height in a cylindrical piston (75 mm diameter). Test conditions were a pre-test speed = 2 mm sec<sup>-1</sup>, a test speed = 2 mm sec<sup>-1</sup> and a post-test speed = 5 mm sec<sup>-1</sup>, with 5 s between cycles. Using parameters defined by Bourne<sup>(13)</sup>, and implemented by Lobato-Calleros *et al*<sup>(14)</sup> and Salinas-Valdés *et al*<sup>(15)</sup>, deformation curves were produced from two compression cycles and used to calculate hardness (Newton; N), stickiness (g sec<sup>-1</sup>), elasticity (mm), cohesiveness (dimensionless), rubberiness (g), chewiness (g mm<sup>-1</sup>) and resistance (dimensionless).

Using twenty semi-trained panelists (n = 20 per period) who were habitual cheese consumers, an affective attribute-based sensory evaluation was done on days 1, 4, 8 and 15. Each consumer was given four random 1 cm<sup>3</sup> cheese cubes at 8 °C, placed in transparent plastic cups coded with three random digits. The evaluated attributes were white color, odor, flavor, softness and overall acceptability. A 5-point hedonic scale was used in the evaluation: 5 = I like it a lot; 4 = I like it; 3 = I neither like it nor dislike it; 2 = I dislike it; and 1 = I very much dislike it<sup>(16,17)</sup>.

The data produced with the completely random experimental design was analyzed with the GLM procedure of SAS<sup>(18)</sup> using the statistical model:

$$y_{ij} = \mu + T_i + \delta_j + (T\delta)_{ij} + \epsilon_{ij};$$

Where:

$y_{ijk}$  = physicochemical, texture and sensory variables evaluated over time;

$\mu$  = general mean;

$T_i$  = effect of the  $i$ -th treatment (QP1, QP2 and QP3);

$\delta_j$  = effect of the  $j$ -th evaluation day (1, 4, 8 and 15 days);

$(T\delta)_{ij}$  = effect of the interaction between the  $i$ -th treatment and the  $j$ -th day;

$\epsilon_{ijk}$  = random error normally distributed with mean and variance [ $\epsilon_{ij} \sim N(\mu, \sigma^2)$ ].

In the analysis of variance, a probability less than 0.05 ( $P < 0.05$ ) was considered significant, rejecting the null hypothesis ( $H_0$ ; equality of treatments, days and their interaction;  $\alpha = 0.05$ ). When the fixed factors and/or their interaction produced an effect, the means were compared with the adjust = Tukey instruction<sup>(18)</sup>.

The parameters whey loss, pH and TA are used to evaluate the quality of cheeses in storage. In the present results (Table 1), the treatments/time (days) interaction affected cheese weight, pH and TA ( $P < 0.05$ ). In QP2, weight was highest on day 1 but in QP3 it was highest on d 15. Values for pH were highest ( $P < 0.05$ ) on d 1, decreased gradually to d 8 and then notably by d 15 ( $P < 0.05$ ). Weight loss did not differ ( $P > 0.05$ ) between treatments and days. Water release during commercial cheese production is known as syneresis, and depends on factors such as pH, temperature, salt, milk composition and pre-treatments<sup>(19)</sup>. When panela-type cheese loses water (i.e. whey) during storage at 4 °C it is also considered syneresis; additives can affect syneresis during storage. In the present results, weight at 15 d was lowest in QP1 and WL tended to increase over time ( $\delta_j$ ;  $P = 0.0592$ ) but not between treatments ( $T_i$ ;  $P = 0.2553$ ). Apparently, addition of OEO did not modify syneresis during storage (WL) in the studied cheeses.

**Table 1:** Weight, pH and titratable acid in panela cheese produced with milk containing added oregano essential oil during 15 day's storage.

Treatments (T <sub>i</sub> ) <sup>1</sup> /Days (δ <sub>j</sub> )	Variables <sup>2</sup>			
	Weight (g)	WL	pH	TA
1 day				
QP1	183.50 <sup>b;AB</sup>	5.39	6.41 <sup>A</sup>	0.016 <sup>a;BC</sup>
QP2	223.00 <sup>a;A</sup>	3.88	6.42 <sup>A</sup>	0.014 <sup>a;C</sup>
QP3	199.50 <sup>b;AB</sup>	5.91	6.42 <sup>A</sup>	0.016 <sup>a;BC</sup>
4 days				
QP1	204.00 <sup>a;AB</sup>	5.78	6.32 <sup>B</sup>	0.019 <sup>a;B</sup>
QP2	202.00 <sup>a;AB</sup>	5.40	6.31 <sup>B</sup>	0.018 <sup>a;B</sup>
QP3	184.50 <sup>a;AB</sup>	5.87	6.36 <sup>AB</sup>	0.014 <sup>a;C</sup>
8 days				
QP1	179.50 <sup>a;AB</sup>	6.03	6.28 <sup>B</sup>	0.028 <sup>a;B</sup>
QP2	196.00 <sup>a;AB</sup>	5.55	6.29 <sup>B</sup>	0.025 <sup>a;B</sup>
QP3	177.00 <sup>a;AB</sup>	5.60	6.31 <sup>B</sup>	0.029 <sup>a;B</sup>
15 days				
QP1	173.00 <sup>a;B</sup>	7.80	5.68 <sup>C</sup>	0.044 <sup>a;A</sup>
QP2	177.00 <sup>a;AB</sup>	6.81	5.73 <sup>C</sup>	0.040 <sup>a;A</sup>
QP3	182.00 <sup>a;AB</sup>	5.70	5.66 <sup>C</sup>	0.029 <sup>b;B</sup>
SME	6.14	0.68	0.01	0.002
<i>P</i> -value				
T <sub>i</sub>	0.0094	0.2553	0.2858	0.0109
δ <sub>j</sub>	0.0013	0.0592	< 0.0001	< 0.0001
(Tδ) <sub>ij</sub>	0.0383	0.3793	0.0124	0.0025

<sup>1</sup> QP1= control (no OEO); QP2= 0.05 g OEO/L milk; QP3= 0.10 g OEO/L milk. SME= standard mean error; T<sub>i</sub>= effect of i-th treatment; δ<sub>j</sub>= effect of j-th evaluation day (1, 4, 8 and 15 days); (Tδ)<sub>ij</sub>= effect of i-th treatment/ j-th evaluation day interaction.

<sup>2</sup> WL= weight loss; TA= titratable acid (g lactic acid/100 g product).

<sup>a-b</sup> Means with different lowercase superscripts in the same column, between treatments and/or evaluation days are significantly different ( $P < 0.05$ ).

<sup>A-C</sup> Means with different uppercase superscripts in the same column, for all treatments and evaluation days, are significantly different ( $P < 0.05$ ).

All treatments had more acidic pH on day 15 than on d 1 ( $P < 0.05$ ), and, consequently, TA was also highest ( $P < 0.05$ ) on d 15. Among the treatments, TA on d 15 was lowest ( $P < 0.05$ ) in QP3 and highest ( $P < 0.05$ ) in QP1. In the center of panela-type cheese pH is reported to range from 6.4 on d 1 to 5.94 on d 15<sup>(2)</sup>; the latter value is similar to those observed in the treatments on d 15. The decreases in pH observed here are probably due to production of lactic acid by bacteria, which contribute to cheese aroma and texture<sup>(3)</sup>. Titratable acidity (TA) in all three treatments was low on d 1 and 4 (no difference;  $P > 0.05$ ) and increased up to d 15. This coincides with a study in which fresh cheeses containing *Lactobacillus*

*acidophilus* had increasingly higher TA at 1, 7, 14 and 21 d, which is attributed to the natural and continuous production of lactic acid and organic acids<sup>(20)</sup>. The one exception in the present study was QP3, in which TA did not change from day 8 to d 15. This treatment had the highest OEO content (0.10 g OEO/L milk), which may have inhibited lactic acid bacteria growth after d 8. Carvacrol and thymol are known bioactive components of OEO and act to disintegrate the outer membrane of bacteria, increasing cytoplasmic membrane permeability and leading to cell death<sup>(21)</sup>. Essential oils like OEO commonly exhibit greater inhibitory activity against Gram-positive bacteria than Gram-negatives because the latter have a lipopolysaccharide barrier on their outer membrane<sup>(21)</sup>.

Addition of OEO to the milk used to produce the studied panela-type cheeses had no effect ( $P>0.05$ ) on their color over time ( $(T\delta)_{ij}$ )(Table 2). However, treatment did affect ( $P<0.05$ ) luminosity ( $L^*$ ) on d 1, with QP1 having the lowest ( $P<0.05$ ) value and QP2 the highest ( $P<0.05$ ). From d 4 onwards  $L^*$  values did not differ between treatments. In contrast, time ( $(T\delta)_{ij}$ ) did affect  $a^*$ ,  $b^*$ , Hue and Chroma ( $P<0.001$ ). As expected,  $a^*$  values were near zero in all treatments throughout the storage period because panela-type cheese is white, therefore manifesting no tendency to green or red. Increases in  $b^*$  values in all the treatments suggest that, in conjunction with ripening, the pigments present in OEO, such as phenolic monoterpenes (carvacrol)<sup>(22)</sup>, pushed  $b^*$  values towards yellow. Consequently, the saturation index and tonality values varied somewhat by d 15 ( $b^*>a^*$ ).

**Table 2:** Color evaluation in panela cheeses produced with milk containing added oregano essential oil during 15 day's storage.

Treatments ( $T_i$ ) <sup>1</sup> /Days ( $\delta_j$ )	Color Variables <sup>2</sup>				
	L*	a*	b*	Hue	Chroma
1 day					
QP1	99.61 <sup>b</sup>	-0.41	9.78	91.24	9.78
QP2	100.00 <sup>a</sup>	-0.31	9.28	92.15	9.28
QP3	99.99 <sup>ab</sup>	-0.34	9.36	92.23	9.34
4 days					
QP1	99.48 <sup>a</sup>	0.06	9.51	89.58	9.51
QP2	99.83 <sup>a</sup>	-0.06	9.19	90.44	9.20
QP3	99.79 <sup>a</sup>	0.01	9.23	89.84	9.24
8 days					
QP1	99.88 <sup>a</sup>	-0.14	10.23	90.81	10.23
QP2	100.00 <sup>a</sup>	-0.23	10.11	91.55	10.11
QP3	100.00 <sup>a</sup>	-0.23	10.48	91.49	10.48
15 days					
QP1	99.54 <sup>a</sup>	0.03	9.84	89.65	9.84
QP2	99.98 <sup>a</sup>	-0.04	10.05	90.08	10.05
QP3	99.88 <sup>a</sup>	0.26	9.86	88.25	9.88
SME	0.13	0.10	0.17	0.71	0.17
<i>P</i> -value					
$T_i$	0.0008	0.4944	0.3255	0.2965	0.3340
$\delta_j$	0.0844	< 0.0001	< 0.0001	< 0.0001	< 0.0001
$(T\delta)_{ij}$	0.9110	0.5553	0.2466	0.6935	0.2377

<sup>1</sup> QP1= control (no OEO); QP2= 0.05 g OEO/L milk; QP3= 0.10 g OEO/L milk. SME= standard mean error;  $T_i$ = effect of *i*-th treatment;  $\delta_j$ = effect of *j*-th evaluation day (1, 4, 8 and 15 days);  $(T\delta)_{ij}$ = effect of *i*-th treatment/ *j*-th evaluation day interaction.

<sup>2</sup> L\*= luminosity; a\*= red color tendency; b\*= yellow color tendency; Hue: Hue angle; Chroma= saturation index.

<sup>a-b</sup> Means with different lowercase superscripts in the same column, between treatments and/or evaluation days are significantly different ( $P < 0.05$ ).

Texture analysis identified the treatment/evaluation days interaction as affecting cohesiveness and resistance ( $(T\delta)_{ij}$ ;  $P < 0.05$ ) (Table 3). Values for both these variables were highest ( $P < 0.05$ ) in QP2 on d 1 and lowest ( $P < 0.05$ ) in QP1 on d 8. The treatments affected ( $T_i$ ;  $P < 0.05$ ) SF, hardness, rubberiness and chewiness. Both SF and hardness values were highest in QP1 and lowest in QP2 on days 1 and 8. By d 15, the lowest SF ( $P < 0.05$ ) was in QP2 and the lowest ( $P < 0.05$ ) hardness in QP3. Values for rubberiness and chewiness were consistently higher ( $P < 0.05$ ) over time in QP1 than in QP2 and QP3, which did not differ ( $P > 0.05$ ). This coincides with a study of a fresh cheese in which, at d 3, hardness, elasticity



and cohesiveness values were similar to those of QP1 on d 1<sup>(23)</sup>. Reduction in formulation fat content is the suggested cause, since it reduces interruption of protein areas in the cheese structure, which is reflected in higher hardness values<sup>(23)</sup>. In cheeses, a larger protein fraction has been associated with higher firmness and SF values, which occurs because fat globules occluded in the casein matrix occupy interstitial space, effectively extending the protein network and undermining hardness<sup>(2,3)</sup>. This could explain the lower hardness in QP2 and QP3 on d 8 and 15: OEO may have occupied interstices in the protein network and thus maintained cheese softness during storage. Reductions in hardness and cohesiveness have also been reported in panela-type cheese containing additives such as canola oil and whey protein<sup>(24)</sup>. High hardness and chewiness values in fresh cheeses over time has been observed in a previous study<sup>(2)</sup>. The present results suggest that addition of OEO (QP2 and QP3) reduced texture values over time compared to the control (QP1). Proteins are known to contribute to hardness in cheeses because they constitute the continuous solid phase, but proteolysis and lipolysis can also promote hardness; during storage, natural additives with antioxidant and antimicrobial properties, such as OEO, can mitigate these processes, slowing degradation and preserving product texture<sup>(15)</sup>.

**Table 3:** Texture analysis of panela cheese made with milk containing added oregano essential oil during 15 day's storage

Treatment ( $T_i$ ) <sup>1</sup> /Days ( $\delta_j$ )	SF ( $kg_f$ ) <sup>2</sup>	Hardness (N)	Stickiness ( $g\ s^{-1}$ )	Elasticity (mm)	Cohesiveness	Gumminess (g)	Chewiness ( $g\ mm^{-1}$ )	Resistance
1 day								
QP1	0.8109 <sup>a</sup>	5.48 <sup>a</sup>	-7.99	0.86	0.71 <sup>a;A</sup>	3.91 <sup>a</sup>	3.40 <sup>a</sup>	0.33 <sup>a;AB</sup>
QP2	0.6891 <sup>b</sup>	4.23 <sup>c</sup>	-6.12	0.87	0.75 <sup>a;A</sup>	3.20 <sup>b</sup>	2.81 <sup>b</sup>	0.38 <sup>a;A</sup>
QP3	0.7389 <sup>b</sup>	5.14 <sup>b</sup>	-2.13	0.86	0.73 <sup>a;A</sup>	3.76 <sup>b</sup>	3.24 <sup>b</sup>	0.36 <sup>a;AB</sup>
8 days								
QP1	0.8700 <sup>a</sup>	9.57 <sup>a</sup>	-11.71	0.85	0.65 <sup>a;B</sup>	6.16 <sup>a</sup>	5.29 <sup>a</sup>	0.27 <sup>b;C</sup>
QP2	0.7779 <sup>ab</sup>	7.50 <sup>c</sup>	-9.59	0.78	0.68 <sup>a;AB</sup>	4.92 <sup>b</sup>	4.09 <sup>b</sup>	0.31 <sup>a;B</sup>
QP3	0.8905 <sup>a</sup>	8.98 <sup>b</sup>	-9.51	0.85	0.66 <sup>a;B</sup>	5.94 <sup>b</sup>	5.09 <sup>b</sup>	0.29 <sup>a;BC</sup>
15 days								
QP1	0.8674 <sup>a</sup>	9.20 <sup>a</sup>	-7.78	0.85	0.70 <sup>a;A</sup>	6.41 <sup>a</sup>	5.48 <sup>a</sup>	0.32 <sup>a;B</sup>
QP2	0.8131 <sup>ab</sup>	8.33 <sup>b</sup>	-11.01	0.86	0.65 <sup>a;B</sup>	5.40 <sup>b</sup>	4.67 <sup>b</sup>	0.28 <sup>b;BC</sup>
QP3	0.8191 <sup>ab</sup>	7.98 <sup>c</sup>	-12.05	0.86	0.68 <sup>a;B</sup>	5.44 <sup>b</sup>	4.72 <sup>b</sup>	0.31 <sup>a;B</sup>
SME	0.0295	0.48	1.78	0.024	0.012	0.28	0.26	0.013
<i>P</i> -value								
$T_i$	0.0018	0.0038	0.6939	0.5144	0.7314	0.0004	0.0009	0.2148
$\delta_j$	0.0002	< 0.0001	0.0014	0.1840	< 0.0001	< 0.0001	< 0.0001	< 0.0001
( $T\delta$ ) <sub>ij</sub>	0.3163	0.4020	0.0786	0.2415	0.0032	0.4002	0.4047	0.0334

<sup>1</sup> QP1= control (no OEO); QP2= 0.05 g OEO/L milk; QP3= 0.10 g OEO/L milk. SME= standard mean error;  $T_i$ = effect of i-th treatment;  $\delta_j$ = effect of j-th evaluation day (1, 4, 8 and 15 days); ( $T\delta$ )<sub>ij</sub>= effect of i-th treatment/ j-th evaluation day interaction.

<sup>2</sup> SF: shear force ( $kg_f$ ).

<sup>a-c</sup> Means with different lowercase superscripts in the same column, between treatments and/or evaluation days are significantly different ( $P<0.05$ ).

<sup>A-C</sup> Means with different uppercase superscripts in the same column, for all treatments and evaluation days, are significantly different ( $P<0.05$ ).

Odor, taste, softness and overall acceptability varied slightly between the treatments each day, with QP1 differing ( $P<0.05$ ) most frequently from QP2 and QP3 (Table 4). In contrast, white color did not differ ( $P>0.05$ ) between treatments or days, or in response to the treatment/day interaction. Overall, QP1 was the most accepted ( $P<0.05$ ) treatment and QP2 the least ( $P<0.05$ ). Indeed, QP2 and QP3 had lower acceptance values in all the sensory attributes except white color, suggesting that OEO content may have negatively affected these attributes. Its unpleasant taste and strong odor have limited use of OEO as a food preservative even though it does improve food safety and shelf life<sup>(25)</sup>. Many essential oils have an intense aroma and their use in high concentrations to compensate for interactions with food components can negatively affect food sensory attributes<sup>(21)</sup>.

**Table 4:** Sensory attributes of panela-type cheese made with milk containing added oregano essential oil during 15 day's storage

Treatment (T <sub>i</sub> ) <sup>1</sup> /Days (δ <sub>j</sub> )	Sensory attributes <sup>2</sup>				
	White color	Oregano odor	Flavor	Softness	Overall acceptance
1 day					
QP1	4.60	4.25 <sup>a</sup>	4.40 <sup>a</sup>	4.45 <sup>a</sup>	4.32 <sup>a</sup>
QP2	4.60	3.60 <sup>b</sup>	3.00 <sup>b</sup>	4.05 <sup>b</sup>	3.30 <sup>b</sup>
QP3	4.50	3.90 <sup>ab</sup>	3.50 <sup>b</sup>	4.30 <sup>ab</sup>	3.50 <sup>b</sup>
4 days					
QP1	4.40	4.10 <sup>a</sup>	4.35 <sup>a</sup>	4.50 <sup>a</sup>	4.35 <sup>a</sup>
QP2	4.50	3.70 <sup>a</sup>	2.80 <sup>b</sup>	4.00 <sup>b</sup>	3.40 <sup>b</sup>
QP3	4.50	4.05 <sup>a</sup>	3.45 <sup>b</sup>	4.35 <sup>ab</sup>	3.80 <sup>b</sup>
8 days					
QP1	4.70	4.25 <sup>a</sup>	4.70 <sup>a</sup>	4.55 <sup>a</sup>	4.60 <sup>a</sup>
QP2	4.60	3.60 <sup>b</sup>	2.85 <sup>b</sup>	4.25 <sup>a</sup>	3.10 <sup>b</sup>
QP3	4.70	3.85 <sup>ab</sup>	3.30 <sup>b</sup>	4.35 <sup>a</sup>	3.45 <sup>b</sup>
15 days					
QP1	4.60	4.30 <sup>a</sup>	4.35 <sup>a</sup>	4.45 <sup>a</sup>	4.35 <sup>a</sup>
QP2	4.35	3.65 <sup>a</sup>	2.90 <sup>b</sup>	3.90 <sup>a</sup>	3.05 <sup>b</sup>
QP3	4.45	3.75 <sup>a</sup>	3.40 <sup>b</sup>	3.90 <sup>a</sup>	3.70 <sup>ab</sup>
SME	0.14	0.19	0.23	0.19	0.21
<i>P</i> -value					
T <sub>i</sub>	0.8154	0.0001	< 0.0001	0.0055	< 0.0001
δ <sub>j</sub>	0.2381	0.9695	0.9387	0.2793	0.7909
(Tδ) <sub>ij</sub>	0.8935	0.9108	0.9178	0.9163	0.6979

<sup>1</sup> QP1= control (no OEO); QP2= 0.05 g OEO/L milk; QP3= 0.10 g OEO/L milk. SME= standard mean error; T<sub>i</sub>= effect of i-th treatment; δ<sub>j</sub>= effect of j-th evaluation day (1, 4, 8 and 15 days); (Tδ)<sub>ij</sub>= effect of i-th treatment/ j-th evaluation day interaction.

<sup>2</sup> 5-point hedonic scale: 5= I like it a lot; 4= I like it; 3= I neither like nor dislike it; 2= I dislike it; 1= I very much dislike it.

<sup>a-b</sup> Means with different lowercase superscripts in the same column, between treatments and/or evaluation days are significantly different ( $P<0.05$ ).

Use of 0.05 g OEO/L milk in the production of panela-type cheese did not substantially affect physicochemical, textural and sensory characteristics during storage in comparison to a control. In terms of softness, the cheeses produced with 0.1 g OEO/L milk exhibited the overall lowest shear force and hardness values. Sensory acceptance was most consistent for the cheese containing no OEO, although acceptance of that containing 0.1 g OEO/L milk increased slightly after 15 day's storage. The present results show that natural alternatives such as oregano essential oil can be used in fresh cheeses to improve storage performance as long as levels are controlled to avoid quality parameter deterioration.

### Conflicts of interest

The authors declare no conflict of interest.

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