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Preservation of sensory and chemical properties in flavoured cheese prepared with cream cheese base using oregano and rosemary essential oils

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A B S T R A C T

The purpose of this study was to evaluate the effect of oregano and rosemary essential oils on the oxidative and fermentative stabilities of flavoured cheese prepared with cream cheese base. The studied samples were cream cheese (CC) and cream cheese with the addition of oregano (CO) and rosemary (CR) essential oils which were evaluated for peroxide (PV) and anisidine (AV) values, descriptive analysis and fermentation parameters as stability indicators during storage. The samples CO and CR showed higher stability during storage. On day 35, CO and CR exhibited lower PV (11.70 and 12.32 meq O_2/kg, respectively) than CC. Also, rancid flavour intensities were much higher in CC during storage showing ratings of 26.27 with respect to the ratings of 20.22 in CO and 20.67 in CR detected on storage day 35. Furthermore, the samples with essential oils treatments showed lower acidity and total viable counts (TVCs) and higher pH than CC. On storage day 35, CO samples had the highest pH (4.68), and the lowest acidity (1.24 mg lactic acid/100 g) and TVC (2.35 CFU/g). Oregano and rosemary essential oils demonstrated a protective effect against lipid oxidation and fermentation in flavoured cheese prepared with cream cheese base.

1. Introduction

Cream cheese contains more than 60 g/100 g lipids of dry matter and about 65–80 g/100 g humidity according to CODEX STAN 275-1973. These lipids contain about 32–37 g/100 g unsaturated fatty acids such as palmitoleic (16:1), oleic (18:1), linoleic (18:2) and linolenic (18:3) acids (Fox & McSweeney, 1998). On the one hand, lipolysis is an important biochemical event occurring during cheese ripening and has been studied quite extensively in cheese varieties where lipolysis reaches high levels and is a major pathway for flavour generation (Collins, McSweeny, & Wilkinson, 2003). During the degradation and oxidation process, free fatty acids produce volatile compounds responsible for characteristic flavours in cheese when they undergo oxidative or hydrolytic degradation. On the other hand, polyunsaturated fats are susceptible to oxidation processes and develop rancidity, leading to the formation of various unsaturated aldehydes during the deterioration process (Boskou & Elmadfa, 2011). These kinds of aldehydes have a strong flavour, resulting in a defect related to rancidity (Fox, Guinee, Cogan, & McSweeny, 2000). As result of the oxidation process and presence of rancid flavours, the sensory quality of cheese products decreases making them unacceptable to consumers. In addition, the formation of secondary oxidation compounds has harmful effects on human health (O'Brien, 2009). As consequence of lipid deterioration and growth of microbes, cream cheese has, usually, a short shelf life which is around of 30 days at 4–6 °C (refrigerator temperature).

In order to avoid lipid oxidation and to prolong the shelf-life of a food product, the addition of antioxidant is an alternative used for the food industry. However, synthetic antioxidants are questioned for safety reasons, but natural antioxidants are considered safe and are accepted by consumers (Sachetti et al., 2005). The essential oils obtained form aromatic plants are natural products. Many essential oils have demonstrated antioxidant properties; specifically, the essential oils of oregano, rosemary, laurel and other plants have been studied as potential natural antioxidants (Asensio, Nepote, & Grosso, 2011; Kulisic, Radonic, Katalinic, & Milos, 2004; Olmedo, Asensio, Nepote, Mestrallet, & Grosso, 2009; Olmedo, Nepote, & Grosso, 2012; Olmedo, Nepote, Mestrallet, & Grosso, 2008). However, there have been few studies researching the use of essential

Abbreviations: FC-CCB, flavoured cheese prepared with cream cheese base; CC, cream cheese; CO, cream cheese with addition of oregano; CR, cream cheese with addition of rosemary; PV, peroxide value; AV, anisidine value; FRSA, free radical scavenging activity; TVC, total viable count; PCA, principal component analysis; EOA, essential oil aroma.

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oils in cheese or dairy products as preserving agent. For example, Smith-Palmer, Stewart, and Fyfe (2001) evaluated bay, clove, cinnamon and thyme as antimicrobial compounds in soft cheese. Govaris, Botsoglou, Sergelidis, and Chatzopoulou (2011) reported on the antibacterial activity of oregano and thyme essential oils added to slices of cheese inoculated with *Escherichia coli* O157:H7 and *Listeria monocytogenes*. Gandomi et al. (2011) found that *Zataria multiflora* essential oils had an inhibiting effect on *Aspergillus niger* and aflatoxin production in cheese. Tornambé et al. (2008) observed that essential oils decreased volatile compounds such as 2-butanol, propanol and 3-heptanone caused by oxidation processes.

The objective of this study was to evaluate the preservative effect of oregano and rosemary essential oils on the oxidative and fermentative stabilities of flavoured cheese prepared with cream cheese base.

2. Materials and methods

2.1. Materials

Plastic containers of cream cheese, Mendicrim (Nestle, Argentina) were purchased from a local market. According to the declared composition, this product complies with the CODEX STAN 275-1973 for cream cheese denomination and was the cheese base used for sample preparation. This commercial cheese product is prepared by the manufacturer using combined method carried out by enzymatic (rennin) and acid precipitation. The moisture content of this product is 71 g/100 g, fat content 69 g/100 g of dry extract, and the two major fatty acids in fat are palmitic (29.89 g/100 g) and oleic (26.47 g/100 g) acids analysed as methyl esters by gas chromatography according to Agboola and Radovanovic-Tesic (2002).

Leaves of oregano (*Origanum vulgare*) and rosemary (*Rosmarinus officinalis*) (crop 2011) were provided by the Facultad de Ciencias Agropecuarias – Universidad Nacional de Cordoba (Cordoba, Argentina). The essential oil was obtained by hydrodistillation using Clevenger type apparatus. 50 g of plant material (oregano or rosemary) was placed in the extraction chamber (1 L) and distillated for 1 h at 100 °C. In this extraction process, the volatile compounds (essential oils) were removed by steam water from the plant material. The essential oils were condensate and collected in a glass recipient and were separated from the water because due to the different polarity and density. The essential oil extracted was stored in glass vial at −18 °C in darkness with the addition of sodium sulfate to eliminate residual moisture (Olmedo et al., 2012). This extraction process allowed obtaining volatile compounds with low boiling point (essential oils), especially terpenoids.

2.2. Essential oils analysis

A Perkin–Elmer® Clarus 600 GC–MS (Shelton, Conneticut, USA) coupled with an ion trap mass detector and equipped with a capillary column Elite-ms5, methylpolysiloxane (5% phenyl, 30 m, 0.25 mm i.d. and 0.25 mm coating thickness) was used. The chromatographic conditions were 40 °C initial temperature for 3 min; rate 1 of 10 °C/min until to reach 100 °C; rate 2 of 15 °C/min until to reach 245 °C. The injector temperature was 250 °C. Direct liquid injection (1 μL) of the essential oil using a 5 μL Hamilton syringe was performed for analysis. The carrier gas (helium) had 0.9 mL/min flow. Ionization was obtained by electron impact at 70 eV and mass spectral data were acquired in the scan mode in the m/z range 35–450. The identification compounds were performed by comparing their retention time and mass spectra with NIST library. Authentic standards (SIGMA® St. Louis, MO, USA) were also used to help identification process. Terpenoid component quantification was performed according to Dambolena et al. (2010).

2.3. Free-radical scavenging activity (test DPPH-FRSA) and total phenolic content

The oregano and rosemary essential oils were tested for free-radical scavenging activity and total phenolic content. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma–Aldrich, St Louis, MO) test was carried out according to Choi, Song, Ukeda, and Sawamura (2000). The phenol content was analysed according to Dambolena et al. (2010) using Folin–Cicolteau reagent (Anedra, San Fernando, Buenos Aires, Argentina).

2.4. Sample preparation

Flavoured cheeses prepared on cream cheese base (FC-CCB) with the addition of oregano and rosemary essential oils were made adding 0.2 g essential oil per 100 g fresh cream cheese (equivalent to add 0.058 g/100 g on dry weight of cream cheese). The commercial cream cheese used was Mendricrim manufactured by Nestle (Argentina). The added essential oil was mixed with sterilized spatula under laminar flow cabinet for 5 min until the product (FC-CCB) was homogenised. After the inclusion of essential oils, the sample was repacked and sealed in the same original plastic container. The prepared samples were cream cheese (CC), and FC-CCB with addition of oregano (CO) and rosemary (CR) essential oils.

2.5. Storage conditions and samplings

The samples were stored at 5 ± 1 °C (refrigerator temperature). The temperature inside of refrigerator (Whirlpool 307 T, Rio Claro, Brasil) was checked every day using a mercury thermometer that recorded minimum and maximum temperatures. The experiment was run in 3 repetitions. Samples were removed from storage for chemical, micro and sensory evaluation at 0, 7, 14, 21, 28 and 35 storage days.

2.6. Analyses on cheese samples

2.6.1. Parameter of chemical oxidation

The cream cheese fat was extracted and used for measuring chemical indicators of lipid oxidation as peroxide and *p*-anisidine values. The peroxide value (PV) was determined according to the AOAC (2000) and the *p*-anisidine value (AV) was evaluated following the IUPAC method (IUPAC, 1987).

2.6.2. Parameter related to fermentation of cream cheese and FC-CCB

Chemical and microbiological parameters were evaluated for the study of fermentative deterioration in cream cheese and FC-CCB samples. For chemical parameter related to fermentation process, acidity (expressed as mg lactic acid) and pH were measured according to AOAC (2000). For microbiological fermentation, aerobic total count was determined in 1 g cream cheese sample. Serial dilutions (1/10) were performed with 0.1 g/100 g peptone–water (Laboratorio Britania, Buenos Aires, Argentina). 1 mL from appropriate dilution was put in petri dish and put on 5 mL dilution of agar medium (Laboratorio Britania, Buenos Aires, Argentina) for total count. Petri dishes were incubated for 48 h at 35 °C. The result was expressed as colony forming units (CFU).

2.6.3. Sensory descriptive analysis on cheese samples and FC-CCB

A total of 12 trained panellists (9 female and 3 male) participated in the descriptive analysis of cream cheese and FC-CCB samples. The panellists were very experienced. They had 11 years working on sensory analysis of food products but they had never evaluated cheese samples before. Panellists were trained and calibrated in 8 training sessions that lasted 2 h. A hybrid descriptive analysis method consisting of the Quantitative Descriptive Analysis (Tragon Corp., Redwood City, Calif., USA) and the Spectrum TM (Sensory Spectrum, Inc., Chatham, NJ, USA) were used in this study according to the procedure reported by Grosso and Resurreccion (2002). A 150-mm unstructured line scale was used for intensity evaluation. A list of definitions and a sheet with warm-up and reference intensity ratings (Table 1) were developed during the training.

Samples were evaluated in booths under fluorescent light at room temperature. 10 g of the product sample were placed into plastic cups with lids coded with 3-digit random numbers. Four or five samples plus a warm-up sample were evaluated per evaluation session. Two test sessions were carried out per day, one in the morning (five samples) and other in the afternoon (four samples). Samples were tested using a randomized complete block design. Intensity rating lists of warm-up and references were posted in the booths for test sessions. Data were registered on paper ballots.

2.6.4. Colour determination

The colour of cheese samples was determined using a colourimeter Minolta CR 410 (Hachioji, Tokyo, Japan). The readings were made using illuminant set to D65, and colour space *L*, *a*, *b*. The samples (1 cm cheese thickness) were placed in 8 cm petri dish for colour readings.

2.7. Statistical analysis

All experiments were carried out in three replications. Data were analysed using the InfoStat software, version 2011p (Facultad de Ciencias Agropecuarias, Universidad Nacional de Cordoba). The evaluation was arranged into blocks according to the panellist. Block-to-block variability was taken into account to increase the sensitivity of the study (Meilgard, Civille, & Carr, 2006). Means and standard deviations were calculated for each attribute. Analysis of variance ($\alpha = 0.05$) and the LSD Fisher's Multiple Range test were performed to find significant differences among means. The linear regression equations of chemical, micro and sensory variables evaluated during storage were calculated. The regression analyses were validated by the determination coefficient (R^2), analysis of variance ($\alpha = 0.5$), and assumptions of analysis (independence errors, normality of errors, zero mean, and constant variance). The regression model validations were probed by graphical analysis of residuals (graphics Q–Q plots and residuals versus predictors). Principal component analysis was performed with the purpose to explore associations between variables, and treatments (Nepote, Olmedo, Mestrallet, & Grosso, 2009).

Table 1
Attribute definitions and intensity ratings of standard references and warm-up sample used during sensory descriptive analysis of cream cheese and FC-CCB samples with addition of oregano and rosemary essential oils.

Attribute[a]	Definition[b]	Reference	Reference intensity[c]	Warm-up intensity[c,d]
Appearance				
1- Colour	The intensity or the strength of yellow colour from white to yellow.	Colour OWZ 1P[e]	30	14
Aromatics				
2- Oregano essential oil aroma	The aromatic associated with essential oil of oregano.	0.1 g/100 g oregano essential oil in sunflower oil[f]	35	0
3- Rosemary essential oil aroma	The aromatic associated with essential oil of rosemary.	0.1 g/100 g oregano essential oil in sunflower oil[g]	55	0
4- Cream cheese typical flavour	The aromatic associated with cream cheese	Cream cheese[d]	63	63
5- Rancid	The aromatic associated with the autooxidative deterioration of fats and oils.	Rancid cream milk[h]	36	15
6- Fermented flavour	The aromatic associated with wet-fermented and acid odours.	Vinegar 5 mL/100 mL[i]	17	17
Tastes				
7- Sweetness	Taste on the tongue associated with sucrose solutions.	2.0 g/100 mL sucrose solution	20	7
		5.0 g/100 mL sucrose solution	50	
		10.0 g/100 mL sucrose solution	100	
8- Saltiness	Taste on the tongue associated with sodium chloride solutions.	0.2 g/100 mL NaCl solution	25	15
		0.35 g/100 mL NaCl solution	50	
		0.5 g/100 mL NaCl solution	85	
9- Sourness	Taste on the tongue associated with acid agents such as citric acid solutions.	0.05 g/100 mL citric acid solution	20	33
		0.08 g/100 mL citric acid solution	50	
		0.15 g/100 mL citric acid solution	100	
10- Bitterness	Taste on the tongue associated with bitter solutions such as caffeine.	0.05 g/100 mL caffeine solution	20	18
		0.08 g/100 mL caffeine solution	50	
		0.15 g/100 mL caffeine solution	100	

[a] Attributes listed in order as perceived by panellists.
[b] Attributes definitions.
[c] Intensity ratings based on 150 mm unstructured line scales.
[d] Cream cheese "Mendicrim", Nestle, Buenos Aires, Argentina.
[e] Board colour paint "ALBA" (Buenos Aires, Argentina).
[f] 0.1 g/100 g oregano essential oil in refined sunflower oil (Natura, AGD, General Cabrera, Argentina).
[g] 0.1 g/100 g rosemary essential oil in refined sunflower oil (Natura, AGD, General Cabrera, Argentina).
[h] Cream milk 48 h at 60 °C.
[i] Vinegar wine at 5 mL/100 mL in distillate water.

3. Results and discussion

3.1. Essential oil analysis

The constituent compounds of oregano and rosemary essential oils were analysed by GC–MS; the results are shown in Table 2. The major components of oregano essential oil were terpineol (E) beta (55.5 g/100 g), terpinen-4-ol (15.9 g/100 g) and thymol (12.9 g/100 g). The major components in rosemary essential oil were 35.70 g/100 g camphor, 26.20 g/100 g verbenone and 15.80 g/100 g β-caryophyllene.

Other studies of oregano essential oils have reported different percentages of the major compounds described in this study. Kulisic et al. (2004) reported 35.0 g/100 g thymol, 32.0 g/100 g carvacrol and 10.5 g/100 g γ-terpinene. Tomaino et al. (2005) reported 48.9 g/100 g carvacrol, 11.7 g/100 g *p*-cimene and 5.03 g/100 g thymol. Sujah (2006) observed important variations in the concentration of carvacrol (between 0 and 12 mg/kg), gamma-terpinene (0–13 mg/kg), linalyl-acetate (0–50 mg/kg), myrcene (0–50 mg/kg) and terpinen-4-ol (0–220 mg/kg). In rosemary essential oil, Sacchetti et al. (2005) reported 21.8 g/100 g verbenone, 14.6 g/100 g camphor, 12.3 g/100 g bornyl acetate and 10.4 g/100 g borneol.

Suhaj (2006) observed variations in the concentrations of camphene (0–145 mg/kg) and terpineol (0–40 mg/kg).

3.2. Free radical scavenging activity (DPPH-FRSA) and total phenol content

The antioxidant capacity of a determined compound is related to its ability to act as an H-donor. FRSA is a useful indicator for evaluating this capacity. Moreover, phenols are molecules with a high potential for hydrogen transference to other molecules. For those reasons, FRSA and phenol content indicate indirectly the potential antioxidant property that could have a compound (Roginsky & Lissi, 2005). The values obtained for DPPH-FRSA and total phenol content of oregano and rosemary essential oils are shown in Table 3. The FRSA of the studied essential oils exhibited a high inhibition percentage towards the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). Oregano essential oil had a higher value (59.97%) than rosemary essential oil (48.23%). Kosar, Dorman, and Hiltuen (2005) reported lower FRSA in oregano (33%) and rosemary (20%) essential oils with respect to the results observed in this study.

Oregano essential oil (18.27%) had higher values than rosemary essential oil (14.01%) in terms of total phenol content. Similar results regarding phenol content were observed by Dambolena et al. (2010). The studied essential oils showed a high phenol content in their composition. The FRSA value is proportional to the total phenol content, and these results provide evidence that these essential oils could have high antioxidant activity.

Table 2
Major components of oregano and rosemary essential oils analysed by GC–MS.

Oregano essential oil		Rosemary essential oil	
Components	g/100 g[a] ± SD	Components	g/100 g[a] ± SD
Gamma terpinene	1.1 ± 0.1	Linalool	4.1 ± 0.2
Pinene hydrate (trans)	0.5 ± 0.1	Camphor	35.7 ± 0.4
Terpineol (E) beta	55.5 ± 0.4	Terpinen-4-ol	5.4 ± 0.4
Terpineol cis beta	3.4 ± 0.3	Alpha terpineol	7.2 ± 0.2
Borneol	2.1 ± 0.1	Verbenone	26.2 ± 0.4
Terpinen-4-ol	15.9 ± 0.4	Beta caryophyllene	15.8 ± 0.3
Linalyl acetate	5.6 ± 0.2	Alpha humulene	1.4 ± 0.2
Thymol	12.9 ± 0.3	Epi-alpha-cadinol	0.8 ± 0.2
Carvacrol	0.9 ± 0.2		
Total	97.9	Total	96.6

[a] Means and standard deviations.

Table 3
Total phenolic content (expressed as mg gallic acid/g dry weight) and free-radical scavenging activity (FRSA) of oregano and rosemary essential oils.

	Oregano essential oil[a]	Rosemary essential oil[a]
FRSA (%)	59.97 ± 2.53b	48.23 ± 1.00a
Phenol content (mg/g dry weight)	18.27 ± 0.12b	14.01 ± 0.36a

[a] Means ± Standard deviation followed by different letters in each file indicate significant differences (ANOVA *y* test LSD, $\alpha = 0.05$).

3.3. Sensory descriptive analysis and colour measurements in cream cheese and FC-CCB samples

Ten sensory attributes were analysed for a descriptive analysis. The intensity ratings of these attributes analysed in the fresh product (storage day 0) are presented in Table 4. In general, cream cheese and FC-CCB samples (CC, CO and CR) showed similar intensities in most sensory attributes except for the attributes oregano and rosemary essential oil aromas, cream cheese typical flavour, bitterness, saltiness and sourness. As expected, the intensity rating of essential oil aroma was higher in CR (52.43 on a 150 mm line scale) than in CO (38.19) and was absent in CC.

The colour of cheese samples was also measured by colourimeter (Table 4). The *L* values were 87.225 in CC, 87.221 in CO, and 87.218 in CR. Significant differences were not detected between samples.

Moreover, creamy flavour is an essential property to many dairy products and is related positively to product preference (Richardson-Harman et al., 2000). Consumers seem to consider a product creamy when it has a high fat content, dairy flavour, and viscous, greasy and mouth coating textures. Studies on the perception of fat in milk suggested a descriptor terms “total fattiness” used to describe the overall sensory properties of fat in milk

Table 4
Means and standard deviations of sensory attribute intensity ratings from descriptive analysis and colour measurements by colourimeter in cream cheese and FC-CCB samples at storage day 0 (zero).

Variable sensory attributes[c]	Cream cheese and FC-CCB[a]		
	CC[b]	CO[b]	CR[b]
Colour	14.06 ± 0.10a	14.14 ± 0.38a	14.19 ± 0.16a
Oregano essential oil aroma	n.d.	38.19 ± 0.97	n.d.
Rosemary essential oil aroma	n.d.	n.d.	57.06 ± 1.45
Rancid	15.12 ± 0.09b	13.90 ± 0.16a	13.81 ± 0.19a
Fermented flavour	17.09 ± 0.15b	15.52 ± 0.16a	15.86 ± 0.38a
Cream cheese typical flavour	63.06 ± 0.22c	59.29 ± 0.38b	58.71 ± 0.25a
Sweetness	7.05 ± 0.60a	6.05 ± 0.65a	6.24 ± 0.92a
Saltiness	15.11 ± 0.14a	16.24 ± 0.16b	16.48 ± 0.07b
Bitterness	33.11 ± 0.12a	33.97 ± 0.52b	34.05 ± 0.66b
Sourness	18.03 ± 0.16a	20.14 ± 0.52b	20.90 ± 0.54b
Colour by colourimeter (*L*)	87.225 ± 0.16a	87.221 ± 0.13a	87.218 ± 0.10a
a	−4.65 ± 0.00a	−4.65 ± 0.00a	−4.65 ± 0.00a
b	14.06 ± 0.008a	14.05 ± 0.007a	14.05 ± 0.005a

[a] CC: cream cheese control, CO: FC-CCB added with oregano essential oil, and CR: FC-CCB with rosemary essential oil.
[b] Means ± standard deviation followed by different letters in each file indicate significant differences (ANOVA *y* test LSD, $\alpha = 0.05$).
[c] Unstructured lineal scale 0–150 mm.

(Johansen, Laugesen, Janhoj, Ipsen, & Frost, 2008). The intensity rating of cream cheese typical flavour was higher in CC with respect to the samples added with essential oils. It is likely that the essential oils had the effect of masking the intensity rating of cream cheese typical flavour in CO and CR. In addition, essential oil supplementation probably increased bitterness and sourness intensity ratings in the CR and CO samples in comparison with CC at storage day 0.

3.4. Quality parameter changes in cream cheese and FC-CCB samples during storage

3.4.1. Chemical changes

Changes in the chemical indicators of lipid oxidation (PV and AV) in cream cheese and FC-CCB samples during storage are shown in Fig. 1. These chemical indicators increased with storage time in all cheese samples. The samples did not show significant differences ($\alpha = 0.05$) between them before day 7 in terms of PV and AV (Fig. 1). From day 7–35, all treatments were significantly different in both chemical indicators. CC had the highest peroxide and *p*-anisidine values during storage, whereas CO had the lowest values. These results indicate that the cheese samples prepared with the addition of oregano and rosemary essential oils had better resistance to lipid oxidation than CC. In previous studies, oregano essential oil showed similar antioxidant activity to BHT and increased the shelf-life of fried salted peanuts (Olmedo et al., 2008, 2009). Chemical and sensory parameters of cheese quality change during storage. Chemical indicators of quality like peroxide and *p*-anisidine values increase with storage time. In previous studies, it was observed that oxidation indicators in fat milk increase during storage (Pettersen, Eie, & Nilsson, 2005; Smet et al., 2008; Wold, Veberg, Lundby, Nilsen, & Moan, 2006).

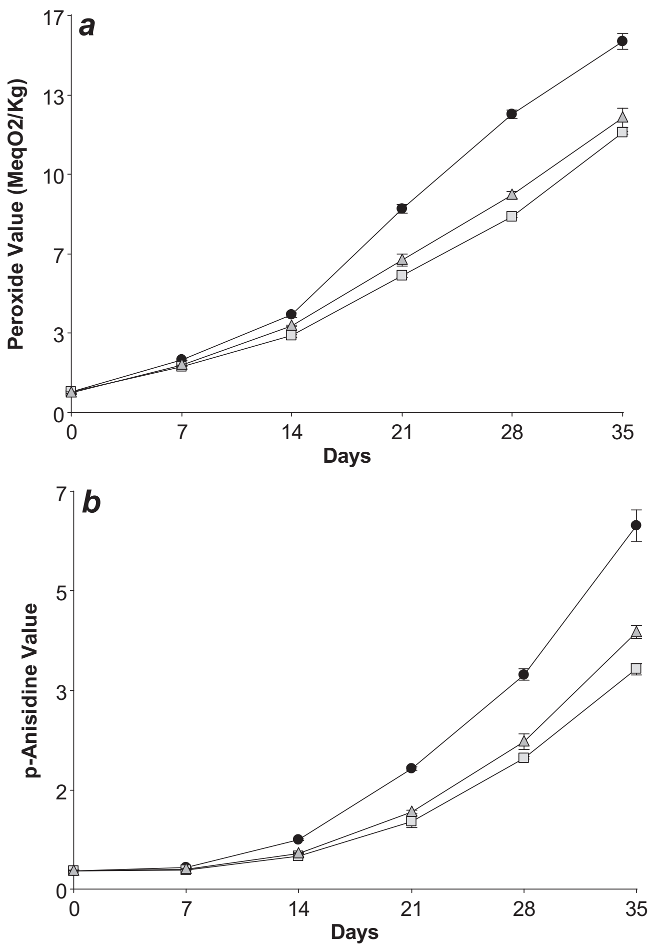

Fig. 1. (a) Peroxide value and (b) *p*-anisidine value in cream cheese (CC) and FC-CCB with addition of oregano (CO) and rosemary (CR) essential oils during storage.

3.4.2. Sensory changes

The intensity ratings of the attributes that changed during storage were rosemary and oregano essential oil aromas and rancid, fermented and cream cheese typical flavours. The intensity ratings of rosemary and oregano essential oil aromas and cream cheese typical flavour (Fig. 2) decreased in the cream cheese and FC-CCB samples during storage.

The decrease in essential oil aroma was greater in CR than CO after 35 days of storage. On the other hand, the cream cheese typical flavour attribute in CC was higher than in CR and CO at the beginning of storage. After 21 days, the decrease of cream cheese typical flavour intensity was higher in CC. The treatments with essential oils did not show significant differences between them. Creamy cheese flavour is a sensory attribute related to ripening and the lipid content in cream cheese. The decrease in the intensity of this attribute occurred because of the formation of carboxylic acid from free fatty acid, suggesting a partial fermentative origin (Di Cagno et al., 2003).

On the contrary, intensity ratings of rancid and fermented flavours increased during storage in all cheese treatments (Fig. 2). These attributes are considered negative and are associated with reduced shelf-life of the product. The presence of straight-chain acids with odd numbers of carbon atoms, such as pentanoic, heptanoic and nonanoic acids, may suggest a partial fermentative origin in the product (Moio & Addeo, 1998). Acetic acid is probably originated from the degradation of lactose by heterofermentative lactic bacteria such as *Lb. fermentum* and *Leuconostoc* spp. (Di Cagno et al., 2003). This kind of modification in cream cheese should increase the values of fermentation indicators. The intensity ratings of fermented flavour increased in all treatments during storage (Fig. 2). The CC sample received higher intensity ratings with respect to samples with the addition of essential oils. CO and CR were not significantly different during storage. Pettersen et al. (2005) observed an increase in acidulous flavour in cream cheese during storage and reported that cream cheese packed in PET/PE 70 mm (polyethylene terephthalate + polyethylene) in the dark at 6 °C led to increased acidulous flavour intensity from 5.59 to 6.50 (on a 10 mm scale) during 6 months of storage. Those authors also reported that cream cheese stored in PET/PE 25 mm under the same conditions showed values from 3.83 to 5.17 after 6 months of storage. Acidulous and fermented flavours are closely related because both sensory attributes are associated with fermentation products.

Oxidized odour and flavour are produced by volatile compounds derived from the lipid oxidation process. CC exhibited a higher rancid intensity rating with respect to CO and CR. Samples supplemented with essential oils (CR and CO) did not show significant differences during storage. Wold et al. (2006) reported that the intensity ratings of oxidized, paint and sunlight flavours increase in Jarlsberg cheese exposed to white fluorescent light during storage. In the present study, oregano and rosemary essential oils had the effect of protecting FC-CCB against an increase in this negative attribute.

3.5. Fermentative deterioration of cream cheese and FC-CCB samples during storage

Acidity (expressed as mg lactic acid), pH and total viable counts (TVCs) of microorganisms were used as indicators of fermentation.

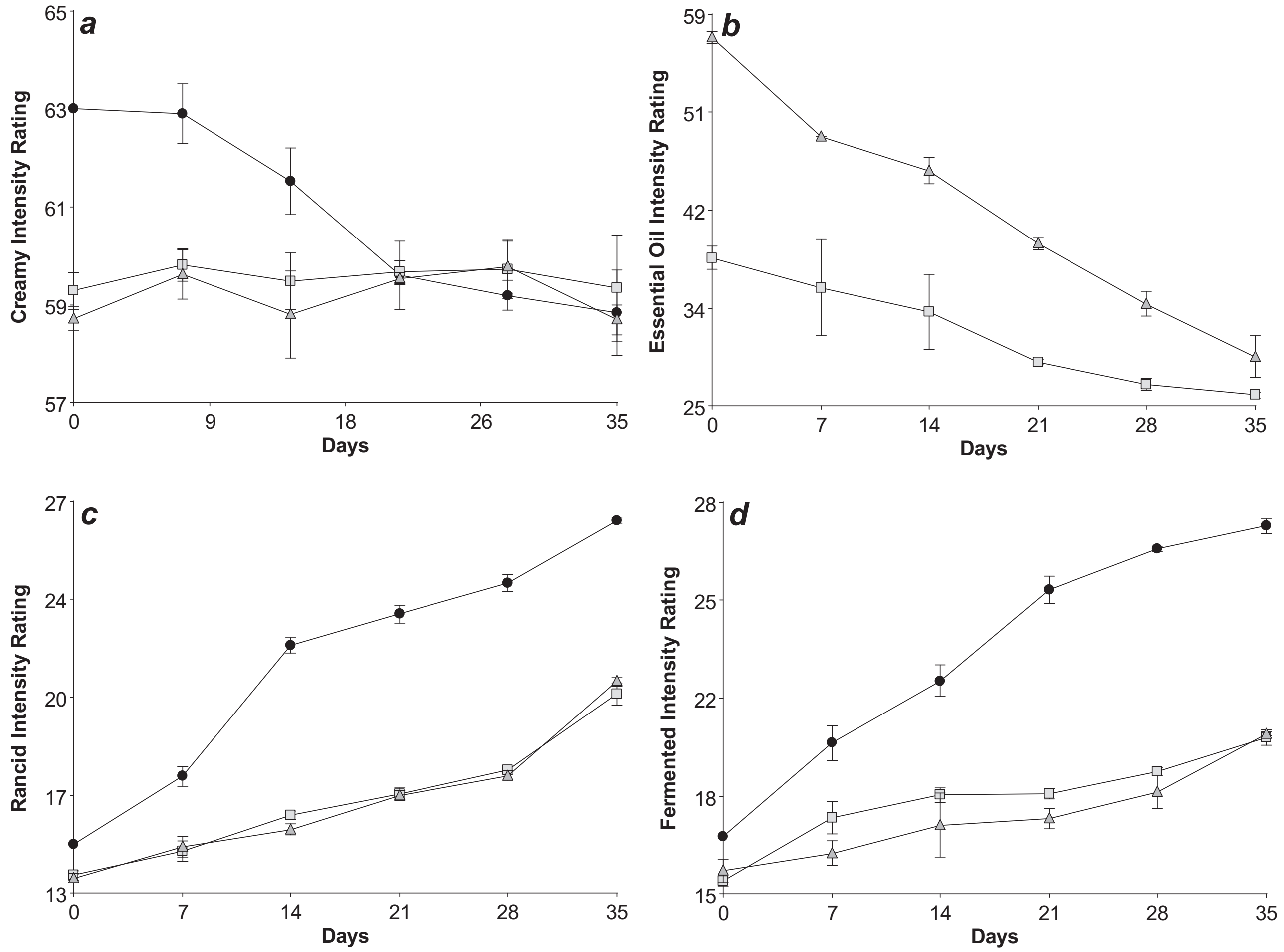

Fig. 2. Intensity ratings of sensory attributes: (a) cream cheese typical flavour, (b) essential oil aroma, (c) rancid flavour and (d) fermented flavour evaluated in cream cheese (CC), and FC-CCB samples with addition of oregano (CO) and rosemary (CR) essential oils during storage.

TVC was expressed in millions of cells (10^6) for easier interpretation of the results. The TVC results increased in all treatments during storage (Fig. 3). Significant differences were observed after day 21 of storage. The behaviour of TVC was similar to acidity. CC had the highest TVC values, whereas CO had the lowest ones.

The pH values decreased in all cheese samples (Fig. 3). Significant differences were observed after 21 days of storage. CC showed lower pH values than CO and CR during storage. CO kept the highest pH values. Acidity increased in all cheese samples. Significant differences were observed after storage day 7. CO and CR showed lower acidity values than CC during storage. The lowest acidity values were observed in CO.

Regarding fermentative deterioration, Galán, Prados, Pino, Tejeda, and Fernandez-Salguero (2008) reported that cheese made with sheep milk develops an increased sour taste during ripening for 180 days because of lactose fermentation produced by microorganism activity. In the present study, acidity and TVC increased in all treatments during storage, whereas pH decreased. The increase in TVC in cream cheese and FC-CCB samples was probably responsible for a fermentation process producing lactic acid that, simultaneously, increased the acidity and decreased the pH of the product. Cardarelli, Saad, Gibson, and Vulevic (2007) observed a diminution in glucose equivalents from 2.500 g l^{-1} to 0.500 g l^{-1} in 8 h of in vitro fermentation. The authors also observed that the microorganism counts of the fermentative bacterial group increased during the experiment, and those bacteria were responsible for fermenting lactose to lactic acid. In this study, the results in terms of the fermentation parameters clearly demonstrated that oregano and rosemary essential oils inhibited the fermentation process in FC-CCB and that these essential oils are good natural preserving agents for this product.

Essential oils were used in previous research as an additive to decrease lypolysis effect (Ayar, 2002). That author reported that essential oils of sirmo (wild *Allium* sp.), thyme (wild *Thymus* sp.), and mint (wild *Mentha* sp.) added individually or in combinations to white cheese stored at 5 °C decrease lipolysis rate. The degree of lipolysis was analysed by the increase of free fatty acid percentage. In that research, it was observed that the highest lipolysis rate occurs in the control sample while white cheeses added with thyme essential oil presents the lowest rate of lipolysis. However, Sirmo and mint essential oils do not affect significantly the lipolysis of white cheese. In the present study, the addition of oregano and rosemary also show an effect decreasing acidity and pH. Probably, some components like thymol and carvacrol present in oregano and thyme essential oils could be responsible for the anti-lipolysis activity. In addition, Quiroga et al. (2013) reported that oregano essential oils obtained from (*Origanum vulgarae* L.) rich in thymol and carvacrol show a remarkable anti-lipase activity tested on lipase enzymes (commercial lipases from *Candida antactica* and *Pseudomonas fluorescens*). Therefore, some essential oils like oregano that is rich in thymol and carvacrol decrease lipolysis for a double effect inhibiting microorganism growth and lipase activity.

Devegliere, Vermeiren, and Debevere (2004) described a "new form of preservation" in his review, such that natural antimicrobial

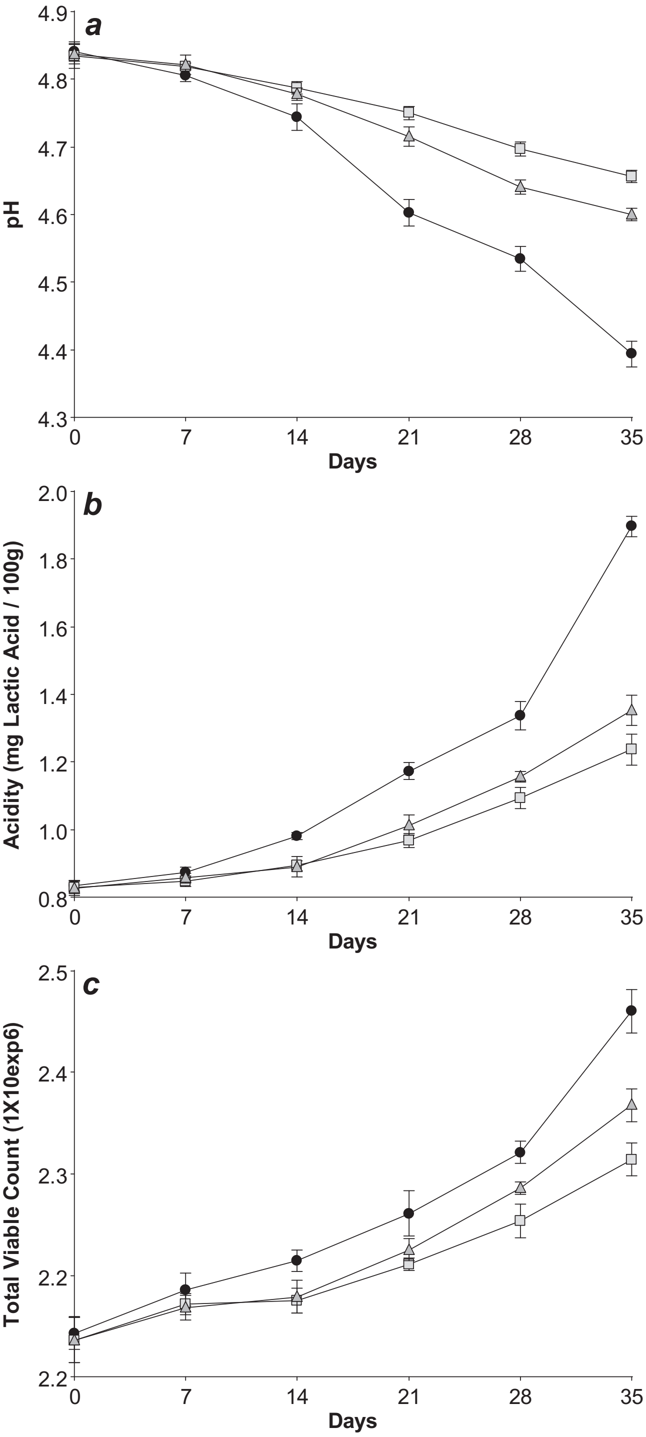

Fig. 3. Fermentation indicators: (a) pH value, (b) acidity value and (c) total viable count in cream cheese (CC) and FC-CCB samples with addition of oregano (CO) and rosemary (CR) essential oils during storage.

compounds like essential oils have a new opportunity in food processing because they have a very close relationship with green labelling and a natural image, but they have some drawbacks: "often expensive, interaction with food ingredients, low water solubility and interference with sensory properties". The present research found a remarkable effect of essential oils against oxidative and fermentative deterioration in FC-CCB. In addition, it was observed that essential oil had good solubility in the product.

3.6. Regression and principal component analyses

The linear regression equations between the chemical, sensory and fermentative variables (dependent variables) and storage time (independent variable) in cream cheese and FC-CCB samples are presented in Table 5. In general, the regression models for these variables showed adjusted R^2 values higher than 0.82. The variables were well-adjusted by a simple linear regression model. Therefore, these equations can be used to predict the chemical, sensory and fermentative changes in cream cheese and FC-CCB samples during storage. In CC, higher slopes ($\beta 1$) of the linear regression equation were detected in the dependent variables associated to product deterioration like rancid flavour, fermented flavour, peroxide value, anisidine value, acidity, and TVC with respect to the slopes of CO and CR regression equation due to a significant increase during storage. Moreover, the regression slopes of cream cheese typical flavour and pH variables showed higher negative values evidencing a significant decrease in CC with respect to CO and CR during storage. This regression analysis also demonstrated the preserving effect of the essential oil addition in the cheese samples. Asensio et al. (2011) reported that oregano essential oil added to olive oil showed an antioxidant effect protecting it against primary lipid oxidation. Those authors describe that lipid oxidation indicators like peroxide, conjugated dienes, and anisidine value increase during storage time. They found significant differences between slopes of the regression equations. In that study, linear regression

Table 5
Regression coefficients and adjusted R^2 for the dependent variables: Sensory attributes, peroxide and anisidine values, acidity and pH in cream cheese and FC-CCB samples during storage.

Indicators	Dependent variable	Samples[c]	Regression[a]			
			$\beta 0$	$\beta 1$[b]	ANOVA	R^2
Sensory	Essence	CO	38.0093	−0.36	B	0.83
		CR	55.7433	−0.7603	A	0.98
	Rancid	CC	15.3746	0.317	B	0.94
		CO	13.6216	0.1663	A	0.94
		CR	13.43	0.1752	A	0.90
	Fermented	CC	17.7757	0.302	B	0.96
		CO	16.2014	0.1159	A	0.88
		CR	15.6163	0.1188	A	0.85
	Cream cheese typical flavour	CC	63.2618	−0.1383	A	0.86
		CO	59.5371	−0.0007	B	0.84
		CR	59.1119	0.0044	B	0.82
Chemical	Peroxide	CC	0.0049	0.4437	C	0.97
		CO	−0.1295	0.308	A	0.96
		CR	−0.1343	0.3332	B	0.97
	p-Anisidine	CC	−0.8721	0.1725	C	0.87
		CO	−0.4521	0.1002	A	0.84
		CR	−0.5542	0.1174	B	0.84
Fermented	pH	CC	4.9203	−0.0139	A	0.96
		CO	4.8863	−0.0056	C	0.95
		CR	4.8978	−0.0078	B	0.96
	Acidity	CC	0.6894	0.0282	C	0.86
		CO	0.7744	0.0116	A	0.89
		CR	0.7594	0.0149	B	0.89
	TVC	CC	2.1705	0.0078	B	0.90
		CO	2.1778	0.0045	A	0.90
		CR	2.1676	0.0059	A	0.91

[a] Regression equations: $Y = \beta_0 + \beta_1 X$; where Y = dependent variable (peroxide and *p*-anisidine values, oregano and rosemary essential oils aromas, rancid and fermented flavours, cream cheese typical flavour, pH, acidity and TVC); β_0 = a constant that it is equal the value of Y when the value of $X = 0$; β_1 = coefficient of X; X = independent variable (time); R^2: adjusted determination coefficient.
[b] ANOVA and LSD Fisher test: The slopes (β_1) of each variable and sample followed with the same letters in the column are not significantly different at $\alpha = 0.05$.
[c] CC: cream cheese; CO: FC-CCB added with oregano essential oil; CR: FC-CCB added with rosemary essential oil.

equations were used as a prediction model to estimate the deterioration effect and shelf-life of olive oil with the addition of oregano essential oil as antioxidant compound in comparison to natural olive oil. Moreover Quiroga, Riveros, Zygadlo, Grosso, and Nepote (2011) reported regression equations that represent quite well the effect of time on variables related to chemical oxidation parameter like peroxide and anisidine values measured in canola oil. Those authors also found that the slope of peroxide value variable shows significant differences between treatments being higher in control sample. The results reported in that study evidenced the antioxidant activity of oregano essential oils added to canola oil.

Using the equation for acidity (Table 5) and considering a value of 2 mg lactic acid 100 g^{-1} as the value limit for shelf-life in cream cheese and FC-CCB samples, it is possible to estimate the shelf-life of this product. Using the equation, the estimated shelf-life is 47 days for CC, 83 days for CR and 106 days for CO. These results demonstrate, once again, that oregano and rosemary essential oils extend the shelf-life of FC-CCB based on acidity parameters.

The principal component analysis (PCA) is presented in Fig. 4. The first two principal components explained 100% of the variability. What is more, the PC-1 axis is the most important principal component explaining 96.3% variability of the experiment. This statistical result could be attributed to the effect of storage time. As consequence, the vectors of each variable studied in this experiment had a high magnitude in the PC-1. Clearly, the treatments were separated on this axis. CC it was placed on the PC-1 positive side and was associated with variables related to oxidative and fermentative deterioration which were variables that increased during storage. The treatments with the addition of oregano and rosemary essential oils (CO and CR) were found on the negative side of PC-1 and were related to variables like essential oil aroma and pH. These results of this principal component analysis also demonstrated that the addition of essential oils in FC-CCB has the effect of inhibiting oxidative and fermentative deterioration. Other authors researched antioxidant activity of oregano essential oil in other food products and also performed principal component analysis. Asensio et al. (2011) reported antioxidant effect of oregano essential oil added to olive oil. They found that the two principal components explain the 93.2% of the variability of results obtained during storage study. In that research, lipid oxidation indicators like conjugated and *p*-anisidine value were associated to temperature and exposure light treatments while darkness treatments were placed in opposite side of the plot. Moreover, Quiroga et al. (2011) reported antioxidant activity of essential oils of oregano species tested in canola oil. Those authors found that the first two PCs of the PCA explain 88.8% of total variability in the oregano essential oil samples. Oxidation indicators like peroxide and anisidine values obtained from stored canola oil samples were positively associated between them and were negatively associated with the contents of some components of the oregano essential oil like thymol and carvacrol which are named as responsible for the antioxidant activity in the oregano EO.

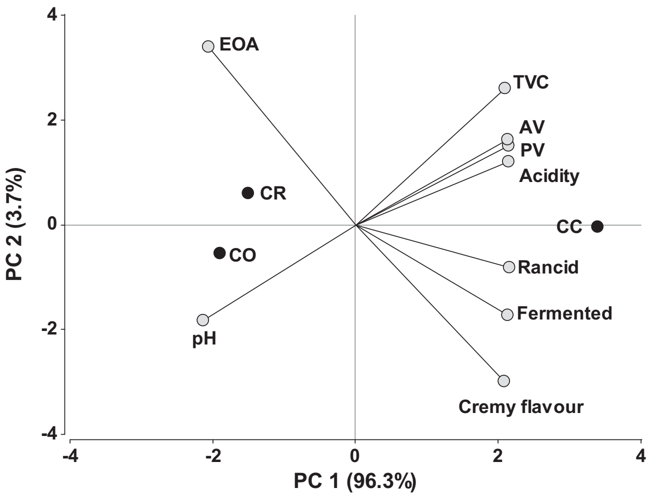

Fig. 4. Biplot of principal component analysis. Dependent variables: essential oil aroma (EOA); rancid, fermented and cream cheese typical flavours; total viable count (TVC); anisidine value (AV); peroxide value (PV); pH and acidity. Samples: cream cheese (CC), FC-CCB with addition of oregano (CO) and rosemary (CR) essential oils.

Finally, it is important to be considered the concept expressed for the definition of cream cheese in the CODEX STAN. Cream cheese is a soft, spreadable, unripened and rindless cheese in conformity with the Standard for Unripened Cheeses Including Fresh Cheeses (CODEX STAN 221-2001) and the General Standard for Cheese (CODEX STAN 283-1978). The cheese has a near white through to light yellow colour. The texture is spreadable and smooth to slightly flaky and without holes, and the cheese spreads and mixes readily with other foods. This product has the following permitted ingredients:

- Starter cultures of harmless lactic acid and/or flavour producing bacteria and cultures of other harmless micro-organisms.
- Rennet or other safe and suitable coagulating enzymes.
- Sodium chloride and potassium chloride as salt substitutes.
- Potable water.
- Safe and suitable processing aids.
- Gelatine and starches: These substances can be used in the same function as stabilizers, provided they are added only in amounts functionally necessary as governed by Good Manufacturing Practice taking into account any use of the stabilizers/thickeners listed in Section 4 (CODEX STAN 275-1973).
- Vinegar.

The CODEX STAN also mentions food additives for cream cheese (Section 4 – CODEX STAN 275-1973). Only, those food additives classes indicated as justified may be used for cream cheese categories with functions specified: colours, bleaching agents, acidity regulators, stabilizers, thickeners, emulsifiers, antioxidants, preservatives, foaming agents, and anticaking agents. The following antioxidants mentioned are allowed: ascorbic acid, sodium ascorbate, calcium ascorbate, ascorbyl palmitate, ascorbyl stearate, tocopherol concentrate, and tocopherol. Ascorbic acid and tocopherols are natural antioxidants. Essential oils are also natural antioxidants but they are not listed in the CODEX STAN. Therefore, the addition of essential oils to cream cheese moves this product out of standard definition. Then, cream cheese with the addition of essential oils is a non standard product made on cream cheese base. For that reason, cream cheese with the addition of essential oils in this study was called flavoured cheese prepared with cream cheese base (FC-CCB).

4. Conclusion

The addition of oregano and rosemary essential oils improves the oxidative and fermentative stability of flavoured cheese prepared with cream cheese base, prevents lipid oxidation and the development of rancid and fermented flavours. As consequence, these essential oils prolong the shelf-life of this product. The food industry should consider the addition of essential oils as an alternative natural antioxidant agent useful for the preservation of quality parameters in this kind of product. Considering the strong aroma and flavour of essential oils like oregano or rosemary, the next step in this research is to determine the impact of the inclusion of these essential oils in this cheese product on consumer acceptability.

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