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Quality preservation of organic cottage cheese using oregano essential oils

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ABSTRACT

Organic cottage cheese was flavoured with four different Argentinean oregano essential oils (EOs) (Compacto, Cordobes, Criollo, and Mendocino) and thymol. Chemical indicators of lipid oxidation, oxidation, and changes in the fatty acid and organic acid profiles were determined during 30 days under thermal storage. The samples flavoured with Cordobes EO and thymol presented lower conjugated dienes (15.94 and 15.53, respectively), whereas the control sample the maximum value (17.54). Values of unsaturated fatty acids decreased significantly ($p < 0.05$) in cottage cheese samples because of oxidation deterioration. Samples flavoured with Compacto, Cordobes, and Criollo EOs showed lower saturated/unsaturated fatty acid ratios than the control (1.67, 1.62, and 1.68, respectively). Samples flavoured with Cordobes and Compacto EOs significantly reduced the production of organic acids during storage. The addition of oregano essential oil in organic cottage cheese decrease the deterioration process of quality parameter during storage prolonging its shelf-life.

1. Introduction

Cheese is a food that is consumed throughout the world obtained from curded milk by removal of whey and by curd ripening in the presence of special microflora" (Belitz, Grosch, & Schieberle, 2009). Processed cheese may normally be considered a stable product with a reasonable shelf life. However, its shelf-life may be shortened considerably during storage for an extended period (Kristensen et al., 2001). The quality of food products inevitably changes during storage due to exposure to heat, enzymes, transition metal ions, oxygen, and light, and these quality changes eventually cause degradation or formation of active flavour compounds. Food constituents, mainly lipids, always react with surrounding oxygen and cause rancidity in foods (Huvaere et al., 2011).

Dairy products, like other water–oil emulsions, can suffer hydrolytic and oxidative rancidity (Shan, Cai, Brooks, & Corke, 2011). There is a release of volatile fatty acids (from C4 to C10) and their subsequent conversion to other acids and/or ethyl esters by microbial lipases. Oxidation of lipids results in the formation of hydroperoxides which can easily react with fatty acids, leading to the formation of secondary oxidation products, essentially aldehydes. (Boroski et al., 2012). Organic acids are important flavour compounds, intermediates, and metabolites of a variety of biochemical processes formed as a result of carbohydrate catabolism and hydrolysis of milk fat, bacterial growth, or addition of acidulates during cheese preparation. Their quantitative determination is important for nutritional reasons and as an indicator of bacterial activity (Garde, Ávila, Gaya, Arias, & Nuñez, 2012; Murtaza et al., 2012).

Growing awareness and concern about the quality and safety of cheese have led to the development of improved methods of cheese preservation. Alternative preservation techniques using naturally derived ingredients are being investigated. Spices and herbs,

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known for providing distinctive aroma and flavour to food, are widely used and classified as generally recognised as safe (GRAS). In particular, plant essential oils (EOs) are attracting interest as potential preserving agents and have a wide acceptance from consumers. EOs are volatile, natural, and complex compounds which are characterised by a strong odour (de Oliveira et al., 2012). These naturally occurring products may extend the shelf-life of cheese by reducing or eliminating survival of pathogenic bacteria and enhancing overall quality through inhibition of oxidative rancidity (Bandyopadhyay, Chakraborty, & Raychaudhuri, 2008). Only few studies have looked at the addition of EO as antioxidants to minimise oxidative reactions in dairy products (Blair, 2012; Boroski et al., 2012; Giroux, Houde, & Britten, 2010; Olmedo, Nepote, & Grosso, 2013).

Considering that the oregano EO is a natural product with proved antioxidant activity and preserving food properties (Asensio, Nepote, & Grosso, 2012, 2013; Olmedo, Asensio, Nepote, Mestrallet, & Grosso, 2009; Olmedo et al., 2013) and that this EO can be produced in organic condition, the oregano EO could be used as a natural preserving agent in organic food products with short shelf-life. The objective of this research was to evaluate changes in fatty and organic acids profiles, and in quality parameters of organic cottage cheese added with Argentinean oregano EOs during an accelerated deterioration test.

2. Materials and methods

2.1. Essential oil extraction and gas chromatography analysis

Leaves and flowers of *Origanum vulgare* spp. *vulgare* (Compacto), *O. vulgare* ssp *hirtum* (clone Cordobes), *O. vulgare* ssp *hirtum* (clone Criollo), and *Origanum x majoricum* (Mendocino) were provided for the Facultad Ciencias Agropecuarias, Universidad Nacional de Cordoba, Cordoba, Argentina. Plants were farmed in Capilla de los Remedios (Cordoba, Argentina) and harvested in May (2012).

Samples of leaves and flowers were hydro-distilled for 2 h in a Clevenger-type apparatus for obtaining oregano essential oil (EO). Chemical composition was analysed by GC/MS/FID using a capillary HP-5 column on Agilent 6890 gas chromatograph (Wilmington, DE, USA) coupled to a mass spectrometer and flame ionization detectors (Juliani & Simon, 2008).

2.2. Antioxidant activity. FRAP method

This assay measures the ability of antioxidants to reduce Fe^{+3} and was used to assess the ascorbic acid equivalent antioxidant activity (AEAC). In a reaction tube, 10 μL of essential oil, or blank (water) and 990 μL FRAP reagent, consisting of ferric chloride and TPTZ (2,4,6-Tri(2-pyridyl)-s-triazine) (Across Organics) in acetate buffer (pH 3.6), were placed. The absorbance was measured after 5 min at 593 nm. A calibration curve with serial dilutions of ascorbic acid was done in order to determined AEAC (Juliani, Koroch, & Simon, 2009).

2.3. Storage study and chemical analysis of stored samples

Commercial organic cottage cheese (Organic Valley, La Farge, WI, USA) was purchased from local supermarket. Essential oils of the four oregano varieties and thymol (SIGMA® St. Louis, MO) were added at 0.05 (g/100 g) to cottage cheese and mixed with a cooker emulsifier during 8 min. Control sample was treated identically to samples but without the essential oil addition. Cottage cheese was transferred to 50 mL plastic jar and stored at 40 °C for 30 days until analyses.

The following organic cottage cheeses (CC) samples were prepared:

- CC without any addition (CC-C).
- CC with the addition of oregano EO variety Compacto (CC-COM).
- CC with the addition of oregano EO variety Mendocino (CC-MEN).
- CC with the addition of oregano EO variety Cordobes (CC-COR).
- CC with the addition of oregano EO variety Criollo (CC-CRI).
- CC with the addition of the monoterpene thymol (CC-THY).

Hydroperoxide value (HV), conjugated dienes (CD), pH, total titratable acidity (TTA), changes in the fatty acid profile and organic acids were the quality parameter measured every 10 days.

2.3.1. Lipid hydroperoxides

For measuring the presence of lipid hydroperoxides, cheese sample preparation was performed according to Dalsgaard et al. (2011). One gram of cream cheese was dispersed in 5 mL demineralized water and mixed by Ultraturax for 45 s. Lipid hydroperoxides were extracted into a 10 mL methanol: chloroform (1:1, mL/mL) mixture. Samples were centrifuged for 10 min at 1000 × *g*. Then, 1 mL chloroform phase was transferred into a chloroform-rinsed glasses and mixed with 1 mL iron (II)/thiocyanate mixture [50 mL of 32.7 mmol/L $BaCl_2$ was slowly added to 50 mL of 36 mmol/L $FeSO_4$ under continuous magnetic stirring, 2 mL of 10 mol/L HCl was added, and the solution was filtered to remove precipitated $(Ba)_3(PO4)_2$. Five hundred μL of this solution and 500 μL of 3.94 mol/L NH_4SCN were added to 49 mL methanol: chloroform (1:1 mL/mL)] according to the IDF standard (74A:1991) modified by Østdal, Andersen, & Nielsen (2000). Absorbance was measured at 500 nm with 700 nm used as background subtraction. The quantification was based on external standards using a calibration curve made with concentrations of 0.5, 1.0, 2.5, 5.0, 7.5, 10.0, and 20.0 μg/mL iron (III).

2.3.2. Lipid extraction

The sample (20 g cheese) was transferred to a beaker. Then, chloroform–methanol (3:2 mL/mL; 50 mL) were added. The mixture and lipids were extracted according to Kristensen et al. (2001).

2.3.3. Conjugated dienes

The extracted lipids were dissolved in 6 mL n-hexane. The conjugated dienes absorbencies were measured at 232 nm. The results were reported as the sample extinction coefficient E (1%, 1 cm) (COI, 2001).

2.3.4. Fatty acid methyl esters (FAME)

FAME were prepared from extracted lipids by transmethylation using a 30 g/L solution of sulphuric acid in methanol as described by (Grosso, Nepote, & Guzmán, 2000). The fatty acid methyl esters of total lipids were analysed in an Agilent GC System 6890 Series, Mass Selective Detector, Agilent 5973 Network, equipped with a flame ionization detector. An EconoWax (30 m, 0.25 mm internal diameter, 0.25 μm phase thickness) capillary column was used. The column temperature increased from 100 to 200 °C (10 °C/min) and then, from 200 to 250 °C (5 °C/min). The carrier gas was nitrogen (1 mL/min flow rate). The separated fatty acid methyl esters were identified by comparing their retention times with those of authentic samples purchased from Sigma Chemical Co (St Louis, MO, USA). Quantitative fatty acid analysis was performed using heptadecanoic acid methyl ester (Sigma Chemical Co.) as internal standard.

2.3.5. pH and total titratable acidity (TTA)

Cottage cheese samples were initially homogenized with an Ultrataurux for 60 s in water (1:9 ratio) prior to pH determination. The pH of homogenized cheese sample (10 gr) was read using a digital pH meter (Accumet Basic AB15, Fisher Scientific) (Amirdivani & Salihin Baba, 2011). TTA was determined by titration using 0.1 (mol equi/L) NaOH under continuous stirring until the acid content is neutralized with the consequent development of a consistent pink colour (AOAC, 2007). The amount of acid produced during fermentation was calculated as follows:

$$Lactic\ Acid\left(\frac{g}{kg}\right) = V{*}0.1{*}0.009$$

where V is volume of NaOH required to neutralize the acid, and 0.1 (mol equi/L) the normality of NaOH used.

2.3.6. Organic acids

Lactic, acetic, citric, pyruvic, formic, and propionic contents in cheese samples were determined using high-performance liquid chromatography (HPLC) as described by Murtaza et al. (2012). Cheese sample (7 g) was added to 40 mL buffer-acetonitrile mobile phase, extracted for 1 h, agitated on a shaker and centrifuged. The supernatant was filtered twice through a 0.45-μm pore size syringe membrane filter (Whatman™) and injected to HPLC (Waters 2695). A reverse-phase Skim-Pack C18 (LC) column (Shimadzu Corporation) was used. Operating conditions were: mobile phase: aqueous 0.5 (mL/100 mL) $(NH_4)_2HPO_4$ (0.038 mol/L) − 0.2 (mL/100 mL) acetonitrile (0.049 mol/L) adjusted to pH 2.24 with H_3PO_4; flow rate 0.5 mL/min; and room temperature. The UV detector was set at 214 nm for detection and peak quantification. HPLC-grade standards and solvents were used (Sigma Chemical Co., St. Louis, MO).

2.4. Statistical analysis

The experiment was carried out in three replications. The data was analysed using InfoStat software, version 2012p (Facultad de Ciencias Agropecuarias, Universidad Nacional de Cordoba). Analysis of variance (ANOVA, $\alpha = 0.05$) and LSD Fisher multiple range test were performed to figure out significant differences among means. The linear regression equations of the variables measured during the storage study of cottage cheese were obtained.

3. Results and discussion

3.1. EO composition and antioxidant activity

Only those compounds present in an amount higher than 1 g/100 g are listed in Table 1. (Dambolena et al., 2010) reported that the principal components of Argentinean oregano EOs were the monoterpenes trans-sabinene hydrate and thymol, with less amounts of terpinene, limonene, cis-hydrate sabinene, terpinen-4-ol, and carvacrol. The four species analysed in this study had high amounts of trans-sabinene hydrate (17.9–28.12 g/100 g), thymol (12.09–18.58 g/100 g), γ-terpinene (7.09–9.8 g/100 g), terpineno-4-ol (6.18–9.52 g/100 g), orto-cymene (5.6–7.78 g/100 g), and sabinene (3.57–4.49 g/100 g). The EO of variety Mendocino had the highest amount of trans-sabinene hydrate (28.12 g/100 g) followed by Compacto EO (27.2 g/100 g). EO of Cordobes and Criollo showed the lowest amounts of trans-sabinene hydrate (22.94 g/100 g and 17.9 g/100 g, respectively) and the highest content of thymol (18.58 g/100 g and 17.14 g/100 g, respectively). On the other hand, Mendocino EO presented the lowest amount (12.09 g/100 g) of this compound. Criollo EO had the highest concentration of terpinen-4-ol (9.52 g/100 g) whereas Compacto EO showed the highest content of γ-terpinene (9.8 g/100 g).

Table 1
Chemical composition of essential oils analysed by GC-MS and antioxidant capacity value expressed as AEAC (Ascorbic acid antioxidant capacity mM Asc.Ac/mgEO).

RI	Compound[a]	Oregano essential oil			
		Compacto[b]	Cordobes[b]	Criollo[b]	Mendocino[b]
977	Sabinene	4.48c	3.9b	3.57a	4.49c
980	β-pinene	1.84c	1.36b	5.91d	1.02a
992	Myrcene	1.83c	1.79b	1.66a	1.66a
1020	δ-terpinene	2.91b	2.62a	3.31d	3.01c
1028	orto-cymene	5.6b	5.13a	6.29c	7.78d
1033	β-phelandrene	1.82a	1.85a	1.93b	1.95b
1040	cis ocimene	1.4a	2.76d	2.45c	1.62b
1063	γ-terpinene	9.8d	7.09a	8.04c	7.53b
1071	cis sabinene hidrate	1.83a	3.3c	2.98b	3.44d
1090	Terpinolene	0.95b	0.82a	1.14d	0.97c
1100	trans sabinene hidrate	27.23c	22.94b	17.9a	28.12d
1170	borneol	0.53b	1.47c	1.83d	0.42a
1181	Terpinen-4-ol	7.76c	6.18a	9.52d	6.61b
1192	α terpineol	1.45a	2.33c	2.57d	2b
1237	Thymol methyl ether	3.66c	0.27a	0.29a	1.08b
1247	carvacrol methyl ether	1.47b	1.3a	1.31a	1.65c
1258	Linalool acetate	0a	1.06b	1.07b	1.81c
1292	Thymol	14.39b	18.58d	17.14c	12.09a
1425	Caryophyllene	1.00a	2.81c	2.85c	2.16abb
1486	Germacrene D	1.49d	1.23c	1.01b	0.3a
1500	Bicyclogermacrene	1.4a	1.71c	1.37a	1.54b
FRAP[d]	AEAC[c]	0.13776b	0.15486c	0.18474d	0.07293a

[a] Only those compounds presents in an amount higher than 1 g/100 g are presented in the table.
[b] Different letter in the row means that there are significant differences at $\alpha = 0.05$ ($n = 3$, LSD Fisher).
[c] AEAC (Ascorbic acid antioxidant capacity mmol/L Asc.Ac/mgEO).
[d] Ferric Reducing Power test values.

The most active oxygenated monoterpenes are thymol and carvacrol. Monoterpenes hydrocarbons are known to have less or no antioxidant activity, but three monocyclic components terpinolene, α-terpinene, and γ-terpinene, and sabinene, a bicyclic monoterpene have shown high activity. In addition, the oxygenated sesquiterpenes as caryophyllene oxide and germacrene D have shown antioxidant activity comparable to that of the oxygenated monoterpenes (Ruberto & Baratta, 2000). These last two compounds were present in Compacto, Cordobes, and Criollo EOs in concentrations greater than 1%. In a compound mix like EOs, it is very difficult to find out the relationship between content and antioxidant activity of each component due to the synergistic action which occurs among the present components typical of natural extracts.

3.2. Antioxidant activity by FRAP method

The four EOs evaluated in this study had the ability to reduce the ferric di-TPTZ complex used in FRAP assay. The reducing power assay is an effective means for evaluating the ability of antioxidants to donate electrons. The ferric reducing activity is mainly influenced by the size of the conjugated double bond (CDB) system and hydroxyl functions of phenolic compounds (Müller, Theile, & Böhm, 2010). Samples with a higher content of monoterpenes which have CDB as o-cimene, cymene, α-terpinene, and thymol showed this characteristic. Criollo and Cordobes EOs presented higher AEAC (0.18474 mM/mg and 0.15486 mM/mg, respectively). These results could be attributed mainly to the high content of thymol in both EOs and, also, to the presence of o-cymene and terpinene in their chemical composition. On the other hand, Mendocino EO was the sample which showed the lowest value (0.072 mM/mg) making is the sample that presented a lower thymol concentration.

3.3. Storage study

3.3.1. Lipid hydroperoxides

The hydroperoxide value (HPV) of organic cottage cheese samples added with oregano EO over 30 days of storage at 40 °C is shown in Fig. 1a. The accumulation of hydroperoxides was affected by the storage time ($p<0.001$) and antioxidant addition ($p<0.013$). In general, the hydroperoxide value increased significantly with storage time. Even the mean of each sample observed for HPV was different to the control, over the three periods evaluated. At storage day 10 and 20, significant differences were only detected between the control and the rest of the treatments ($p<0.0082$). At storage day 30, all flavoured organic cottage cheese samples differed significantly ($p<0.0136$) in their HPV. The highest hydroperoxide value was observed in control sample (3.93 $\mu eqO_2/kg$) and the lowest value was detected in the sample with thymol addition (2.46 $\mu eqO_2/kg$), followed by the sample with Criollo EO (2.66 $\mu eqO_2/kg$). What is more, it was also observed that CC-COR, CC-CRIO, CC-MEN, and CC-THY samples did not differ significantly between each other at storage day 30. The lipid peroxidation reaction in organic cottage cheese decreased in this treatment order CC-C – CC-COM – CC-COR – CC-CRIO – CC-MEN – CC-THY. According to this order, it is evident that thymol acted as the strongest antioxidant compound. Bandyopadhyay et al. (2008) reported that natural extracts from mint, ginger, and beet and their combinations have stronger antioxidant activity than the synthetic antioxidants BHA and BHT in dairy desert sandesh. This effect emphasises the importance of using antioxidants in controlling lipid oxidation in dairy products. In cheese containing green tea extract, hydroperoxides were not detected (Huvaere et al., 2011). The prevalence of lipids in dairy compounds, even in low-fat derivatives, remark the importance of researching their oxidation products and how to prevent them.

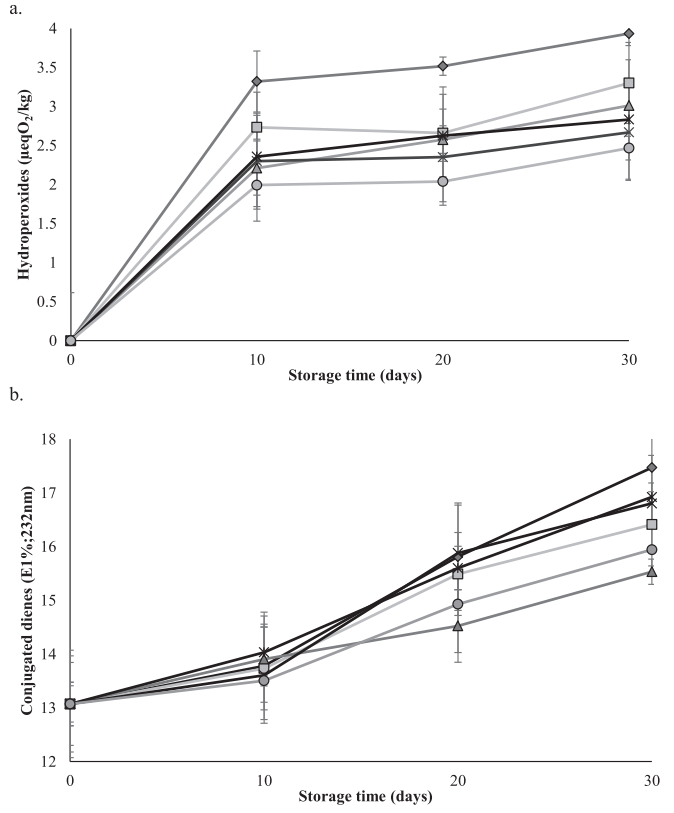

Fig. 1. (a) Hydroperoxides and (b) conjugates dienes in organic cottage cheese samples flavoured with oregano essential oils stored at 40 °C during 30 days ($n = 3$). CC-C (cottage cheese without any addition), CC-COM (cottage cheese with the addition of Compacto EO), CC-COR (cottage cheese with the addition of Cordobes EO), CC-CRIO (cottage cheese with the addition of Criollo EO), CC-MEN (cottage cheese with the addition of Mendocino EO), CC-THY (cottage cheese with the addition of thymol).

3.3.2. Conjugated dienes

Spectrophotometrical detection of all organic cottage cheese samples showed that accumulation of conjugated dienes was significant ($p < 0.001$) from the first day to the end of the storage at 40 °C (Fig. 1b). Until storage day 20, cottage cheese samples including the control sample did not differ significantly. At storage day 30, CC-COR and CC-THY presented lower conjugated dienes (15.94 and 15.53, respectively; $p<0.001$), whereas CC-C showed the highest value (17.54). In several studies, good absorptivity results in dairy products were reported with respect to lipid peroxidation, because milk itself is a good antioxidant (Bandyopadhyay et al., 2008; Chen, Lindmark-Mansson, Gorton, & Akesson, 2003).

3.3.3. Fatty acids' profile during storage

In order to prevent or retard oxidative deterioration, EOs were tested in this study as free radical quenchers, reducing compounds, singlet oxygen scavengers, and pro-oxidant metal suppressors. At storage day 0, the following fatty acids were found: 0.06 g/100 g caproic, 0.13 g/100 g caprylic, 1.10 g/100 g capric, 6.42 g/100 g lauric, 1.09 g/100 g lauroleic, 6.65 g/100 g myristi, 1 g/100 g myristoleic, 1.05 g/100 g pentadecaoinc, 26.7 g/100 g palmitic, 1.59 g/100 g palmitoleic, 0.97 g/100 g hexadecadienoinc, 0.77 g/100 g heptadecanoic, 15.6 g/100 g estearic, 29.2 g/100 g oleic, 1.05 g/100 g elaidic, 2.13 g/100 g linoleic, 1.08 g/100 g linoelaidic, 1.27 g/100 g linolenic, and 1.96 g/100 g arachidic acids. The fatty acid composition of flavoured organic cottage cheese experimented significant changes during 40 days of storage at 40 °C (Table 2). During oxidation, numerous chemical reactions can occur including decomposition of tri-acyl-glycerides (TAGs); polymerisation of TAGs into non-saponificable matters; and formation of volatile compounds (Warner, 2002). Major fatty acids in cottage cheese were oleic, palmitic, and stearic acids. As thermal oxidation time increased during storage, the relative percentage of saturated fatty acids such as lauric, myristic, and stearic acids increased. Simultaneously, unsaturated fatty acids including lauroleic, myristoleic, hexadecadienoic, elaidic, octadecadienoic, and linolenic acids decreased continuously. The amounts of lauric and stearic acids increased up to 11 g/100 g and 16 g/100 g in CC-C and CC-MEN samples, respectively. CC-COR showed a lower increase in these two fatty acids (up to 8.02 g/100 g and 15.57 g/100 g, respectively). No significant differences were detected in the oleic acid amount between samples, despite its concentration did vary during storage. During lipid oxidation, the double bond oxidation process in unsaturated fatty acids makes to increase the proportion of saturated with respect to the proportion of unsaturated fatty acids (Kim, Yeo, Kim, Kim, & Lee, 2013). Significant differences between samples at storage day 40 were found in almost all unsaturated fatty acids. Linear regression equations showed R^2 values higher than 0.55 in all samples for these fatty acids. Ruminant dairy products are the major dietary sources of conjugated linoleic acid (CLA) known for its health benefits (Prandini, Sigolo, & Piva, 2011). In this study, CLA concentration decreased significantly under thermal storage. CC-C samples exhibited 1.59 g/100 g CLA after 40 days of storage, while CC-COR samples showed 1.89 g/100 g. The presence of essential oil prevented CLA from lipid oxidation and kept its content higher than in the control sample until storage day 40. The linolenic acid decreased to 0.57 g/100 g in CC-C and to 1.23 g/100 g in CC-COM. In general, control samples of organic cottage cheese presented higher negative slope than the other samples when the

Table 2
Fatty acids composition of organic cottage cheese samples flavoured with oregano essential oils at storage days zero and 40.

Fatty acid	12:0[a]	12:01[a]	14:01[a]	16:02[a]	17:00[a]	18:0[a]	Oleic[a]	Elaidic[a]	18:2 T[a]	18:3[a]	S/U[a]
Day 0	6.42 ± 0.79	1.09 ± 0.106	0.97 ± 0.068	0.97 ± 0.016	0.77 ± 0.03	15.6 ± 0.37	29.2 ± 0.66	1.05 ± 0.05	3.21 ± 0.25	1.27 ± 0.14	1.53
	CC-C[b]										
Day 40	11.00 ± 0.17b	0.88 ± 0.1ab	0.86 ± 0.04b	0.80 ± 0.18ab	0.94 ± 0.02b	16.27 ± 0.5ab	27.44 ± 0.51a	0.92 ± 0.1a	2.11 ± 0.64ab	0.57 ± 0.05a	1.83b
β0	5.75	1.26	1.43	1.07	0.69	15.19	30.11	1.11	3.49	1.32	
β1	0.155	−0.0106	−0.0148	−0.0069	0.0063	0.0311	−0.0597	−0.0046	−0.0349	−0.0196	
R^2	0.6769[c]	0.8307[c]	0.5518[c]	0.9571[c]	0.9746[c]	0.2836	0.9175[c]	0.8622[c]	0.9692[c]	0.983[c]	
	CC-Com[b]										
Day 40	10.9 ± 0.09b	0.84 ± 0.01a	0.62 ± 0.02a	0.91 ± 0.07c	0.93 ± 0.02b	16.17 ± 0.01ab	28.79 ± 0.55a	1.02 ± 0.01b	2.7 ± 0.01b	1.23 ± 0.03c	1.66a
β0	4.46	1.01	0.94	1.06	0.77	16.23	29.53	1.10	3.30	1.51	
β1	0.1959	−0.0052	−0.0082	−0.0037	0.0043	−0.0076	−0.0161	−0.0021	−0.0123	−0.0042	
R^2	0.6354[c]	0.7848[c]	0.9856[c]	0.5065	0.7991[c]	0.0617[c]	0.8025[c]	0.7639[c]	0.6734[c]	0.8852[c]	
	CC-Cor[b]										
Day 40	8.02 ± 0.27a	0.97 ± 0.08b	0.85 ± 0.01b	0.89 ± 0.05bc	0.81 ± 0.03a	15.57 ± 0.35a	28.8 ± 0.97a	0.99 ± 0.03ab	2.18 ± 0.37ab	1.16 ± 0.16c	1.62a
β0	6.04	1.18	0.98	1.02	0.75	15.67	30.45	1.09	3.29	1.37	
β1	0.0465	−0.0055	−0.0033	−0.0035	0.0017	−0.0039	−0.0347	−0.0028	−0.0414	−0.0057	
R^2	0.8671[c]	0.9838[c]	0.9955[c]	0.9449[c]	0.8148[c]	0.141	0.5844[c]	0.7482[c]	0.9457[c]	0.9246[c]	
	CC-Crio[b]										
Day 40	8.9 ± 0.02a	0.93 ± 0.07ab	0.81 ± 0.08b	0.85 ± 0.02bc	1.02 ± 0.12b	16.16 ± 0.42ab	28.86 ± 0.67a	0.92 ± 0.06a	1.86 ± 0.72a	0.96 ± 0.05b	1.67a
β0	7.23	1.19	1.02	1.03	0.75	15.43	30.34	1.10	4.16	1.42	
β1	0.0422	−0.0058	−0.0054	−0.0046	0.0054	0.0155	−0.05	−0.0045	−0.061	−0.016	
R^2	0.9587[c]	0.8601[c]	0.9367[c]	0.994[c]	0.6757[c]	0.2582	0.6381[c]	0.9759[c]	0.6543[c]	0.6348[c]	
	CC-Men[b]										
Day 40	11.45 ± 0.26b	0.86 ± 0.01ab	0.77 ± 0.07b	0.77 ± 0.02a	1.01 ± 0.04b	16.39 ± 0.21b	26.62 ± 0.56a	0.97 ± 0.07ab	1.92 ± 0.07ab	0.99 ± 0.01b	1.93c
β0	4.89	1.14	1.05	1.04	0.77	14.54	32.06	1.10	3.28	1.61	
β1	0.1709	−0.0068	−0.0068	−0.0063	0.006	0.0278	−0.133	−0.0031	−0.0368	−0.0155	
R^2	0.9151[c]	0.545	0.9922[c]	0.9276[c]	0.9093[c]	0.322	0.9286[c]	0.7659[c]	0.9283[c]	0.9988[c]	
	CC-Thy[b]										
Day 40	8.13 ± 0.44a	0.93 ± 0.04ab	0.99 ± 0.04c	0.88 ± 0.08bc	1.03 ± 0.05b	15.69 ± 0.13ab	26.44 ± 1.1a	0.95 ± 0.01ab	2.09 ± 0.87ab	0.99 ± 0.16b	1.76ab
β0	6.55	1.39	1.08	1.01	0.75	15.35	29.9	1.08	3.37	1.24	
β1	0.0425	−0.011	−0.0049	−0.0033	0.0078	0.0068	−0.0745	−0.0036	−0.0295	−0.0047	
R^2	0.8676[c]	0.9675[c]	0.7872[c]	0.987[c]	0.7881[c]	0.0949	0.738[c]	0.7505[c]	0.8518[c]	0.4655	

[a] Different letter in the row means that there are significant differences at $\alpha = 0.05$ ($n = 3$, LSD Fisher).
[b] CC-C (cottage cheese without any addition), CC-COM (cottage cheese with the addition of Compacto EO), CC-COR (cottage cheese with the addition of Cordobes EO), CC-CRIO (cottage cheese with the addition of Criollo EO), CC-MEN (cottage cheese with the addition of Mendocino EO), CC-THY (cottage cheese with the addition of thymol).
[c] Corresponding to R^2 values with p value <0.05.

concentration of polyunsaturated fatty acids were compared among them.

Unsaturated fatty acids decomposed faster than saturated fatty acids and the content of saturated fatty acids seem to increase in the unit of the relative percentage. For that reason, it is possible to use the ratios of fatty acids as parameters for determining the degree of oxidation (Kim et al., 2013). Changes in the amounts of saturated and unsaturated fatty acids in organic cottage cheese and their ratio are shown in Fig. 2. Values of unsaturated fatty acids decreased significantly ($p < 0.05$) for oxidation during storage. Meanwhile, saturated fatty acids increased significantly ($p < 0.05$). The S/U ratio increased with storage time in all treatments. Organic cottage cheese flavoured with Mendocino EO and control samples showed a higher S/U ratio (1.94 and 1.88, respectively). On the other hand, samples flavoured with Compacto, Cordobes, and Criollo EOs presented a lower S/U ratio (1.67, 1.62, and 1.68, respectively). The fatty acids ratio changes differently during oxidation depending on the type of EO and fatty acid composition. It was previously reported that the saturated/unsaturated ratio continuously increased as oxidation time increased from 5 h to 13 h at 180 °C in an oxidation study of soybean oil and lard (Kim et al., 2013).

3.3.4. pH

A slight decrease of pH in dairy product during storage is attributed to the production of different organic acids (Ayyash & Shah, 2011). The initial pH values in the cottage cheese samples varied between 4.35 (CC-C) and 4.44 (CC-COM). During storage, significant differences were observed between CC-C and the other treatments at storage days 0 (p<0.15), 10 (p<0.0025), and 20 (p<0.0108). At storage days 10 and 20, CC-COR and CC-THY presented the highest pH value in both periods. At storage day 30, the pH ranged from 3.64 to 3.73. Changes of pH may be attributed not only to proteolysis and formation of amines and ammonium during ripening, but also to metabolic activity of microbiological contamination (Garabal, Rodríguez-Alonso, Franco, & Centeno, 2010; Garde et al., 2012).

3.3.5. Total titratable acidity

At storage day 0, the titratable acidity was around 0.04 g lactic acid/kg organic cottage cheese. After 30 days of storage, acidity values increased significantly (Fig. 3). Significant differences were also detected among oregano-flavoured cottage cheese (p<0.05). CC-C showed the highest value (0.30 g/kg) whereas CC-COM and CC-COR (0.24 g/kg and 0.26 g/kg, respectively) exhibited the lowest values (p<0.05). The increase in TTA as an indicator of proteolysis may also explain the changes in pH values in cheese (Rodríguez-Alonso, Centeno, & Garabal, 2011). CC-C showed a higher decrease in pH and increase in acidity value with respect to the treatments with oregano EOs. Therefore, these results indicate that the addition of oregano EO and thymol acts as a natural preserving agent in this product.

3.3.6. Organic acids (OA)

Organic acids contribute to the flavour and aroma of most cheese varieties. Short chain and water-soluble organic acids may appear in cheese as the result of milk fat hydrolysis during lipolysis (acetic and butyric acids), normal ovine biochemical metabolism (citric, orotic, and uric acids), or bacterial metabolism (lactic, acetic, pyruvic, propionic, formic, and butyric acids). Thus, quantitative determination of organic acids may serve to monitor bacterial growth and metabolism (Garde et al., 2012). Concentrations of organic acids in organic cottage cheese samples are shown in Fig. 4.

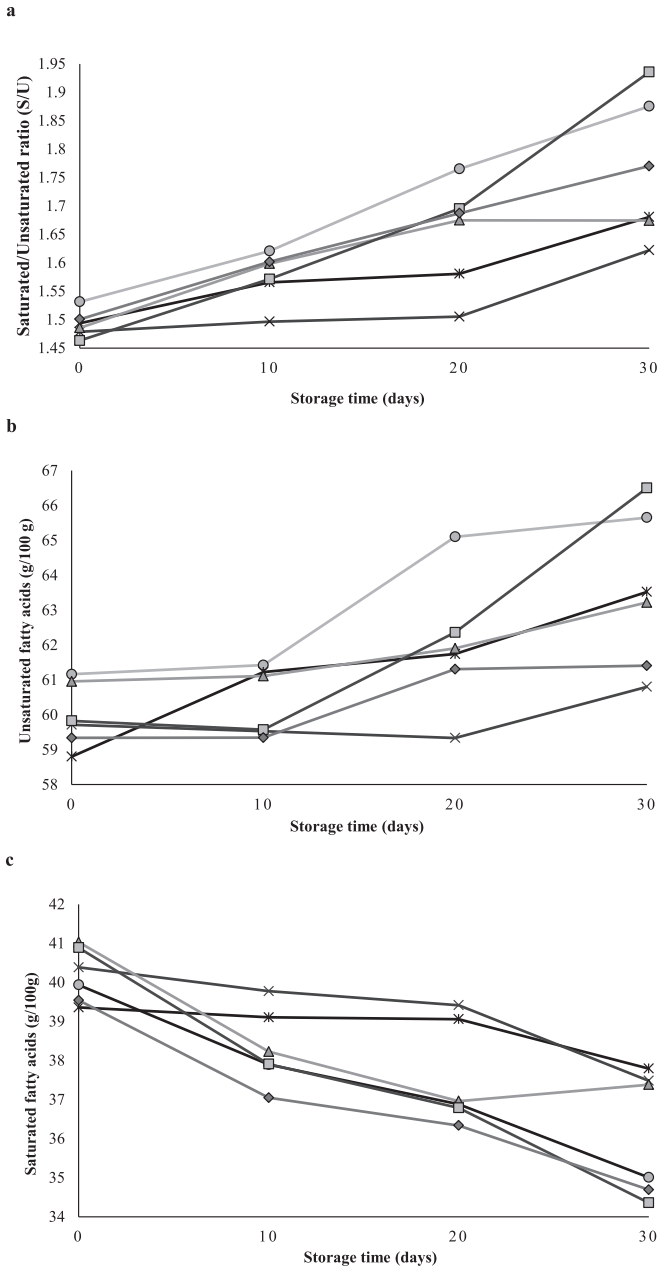

Fig. 2. Saturated/unsaturated ratio (a), and saturated (b) and unsaturated (c) fatty acid percentages in organic cottage cheese samples flavoured with oregano essential oils stored at 40 °C during 30 days ($n = 3$). CC-C (cottage cheese without any addition), CC-COM (cottage cheese with the addition of Compacto EO), CC-COR (cottage cheese with the addition of Cordobes EO), CC-CRIO (cottage cheese with the addition of Criollo EO), CC-MEN (cottage cheese with the addition of Mendocino EO), CC-THY (cottage cheese with the addition of thymol).

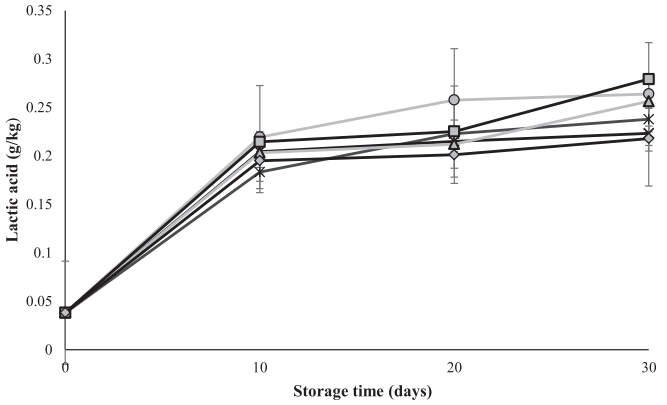

Fig. 3. Lactic acid content in samples of organic cottage cheese flavoured with oregano essential oils stored at 40 °C during 30 days ($n = 3$). CC-C (cottage cheese without any addition), CC-COM (cottage cheese with the addition of Compacto EO), CC-COR (cottage cheese with the addition of Cordobes EO), CC-CRIO (cottage cheese with the addition of Criollo EO), CC-MEN (cottage cheese with the addition of Mendocino EO), CC-THY (cottage cheese with the addition of thymol).

After 30 days of storage, significant differences were found in the organic acid contents for each period.

Lactic acid (Fig. 4a), a major product of the fermentation of lactose, reached levels between 55.45 mg/kg in CC-C and 43.6 mg/kg in CC-COR at the end of storage. This last treatment (Cordobes) was not only significantly different to the control cheese, but also to other flavoured treatments. Normally, the concentration of lactic acid was considerable higher than other organic acids (Mato Rodriguez, Ritvanen, Joutsjoki, Rekonen, & Alatossava, 2011; Murtaza et al., 2012).

Citric and pyruvic acids are intermediary metabolites which show irregular changes during cheese ripening. Citric acid may be formed by fermentation of glucose with the aid of certain moulds. Moreover, citrate can be used as a substrate by the starter *lactococci* to produce pyruvic and acetic acids (Upreti, McKay, & Metzger, 2006). In the present study, citric acid (Fig. 4b) was detected in higher amounts after 10 days of storage. No significant differences between treatments were detected for this organic acid at the end of the storage.

Pyruvate may be produced by mesophilic lactic acid bacteria through the glycolytic pathway, and may act as a substrate of several metabolic reactions. On the other hand, these bacteria can fix CO_2 using pyruvate carboxylase and convert pyruvate to oxaloacetate (Upreti et al., 2006). The concentrations of pyruvic acid varied between 7.41 mg/kg and 7.46 mg/kg during storage. A change in this organic acid content for each sample was observed. At the end of storage, pyruvic acid content increased in some samples but in others presented a slight decrease. In general, significant differences in this organic acid between treatments and periods during storage were not observed. Similar behaviour in the pyruvic acid results were reported by Mato Rodriguez et al. (2011). This molecule is an intermediate in sugar metabolism, and acts as substrate for different metabolic reactions. For that reason, pyruvic acid is continuously synthetised and consumed by the cell.

High contents of formic acid (Fig. 4c) may be attributed to pyruvate metabolism (Rodríguez-Alonso et al., 2011). The formic acid content increased significantly through storage. The CC-C sample presented the highest concentration (5.64 mg/kg) whereas CC-THY showed the lowest value (4.33 mg/kg) at the last storage day.

Perceptible amounts of acetic acid could transfer to cheese a sour off-flavour (Garabal et al., 2010). Acetic acid may also be produced by non-starter *lactobacilli* from lactose or from amino acids (Garde et al., 2012). Significant differences between treatments were detected after 10 storage days (Fig. 4d), where only the control sample exhibited the highest value (10.27 mg/kg). At storage day 30, CC-C still showed the highest concentration of acetic acid (10.89 mg/kg). This result indicates a higher degree of heterofermentative metabolism in these cheese samples. What is more, CC-COM, CC-COR, and CC-CRIO samples exhibited the lowest content of acetic acid after 30 days of storage (Fig. 4d).

The formation of propionate in the organic cottage cheese samples during storage is shown in Fig. 4e. In all samples, the propionic acid content was higher at the end of storage than at the beginning of storage. CC-COM and CC-COR samples had a lower concentration of this organic acid (1.23 mg/kg and 1.24 mg/kg, respectively). CC-MEN exhibited the highest concentration of this

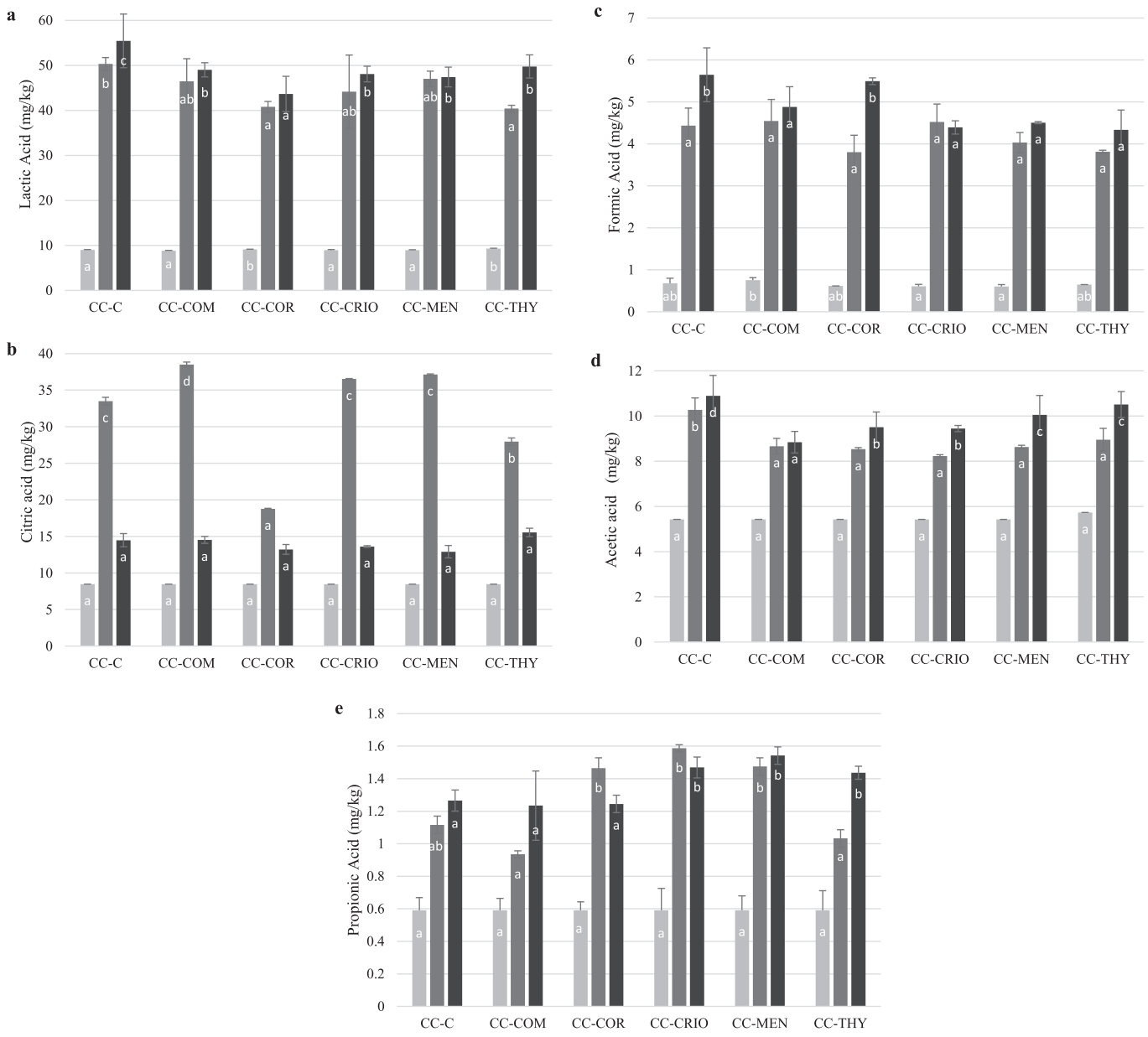

Fig. 4. Lactic (a), Citric (b), Formic (c), Acetic (d), and Propionic (e) acid concentrations in organic cottage cheese flavoured with oregano essential oils at 0 (▒), 10 (▒), and 30 (■) storage days stored at 40 °C ($n = 3$). CC-C (cottage cheese without any addition), CC-COM (cottage cheese with the addition of Compacto EO), CC-COR (cottage cheese with the addition of Cordobes EO), CC-CRIO (cottage cheese with the addition of Criollo EO), CC-MEN (cottage cheese with the addition of Mendocino EO), CC-THY (cottage cheese with the addition of thymol). Different letter in the bar for the same period of time means that there are significant differences between treatments at $\alpha = 0.05$ ($n = 3$, LSD Fisher).

organic acid at the end of the storage (1.54 mg/kg) which indicates no preservative effect of this EO on organic cottage cheese.

Organic acid production is the result of several microbiological, biochemical, and metabolic processes including glycolysis, lipolysis, and proteolysis during cheese making and ripening. Concentrations of organic acids increase with progression in cheese ripening (Ong & Shah, 2009), since cheese, being a biochemically dynamic product, undergoes significant changes during ripening which are the consequence of numerous metabolic processes. Elevated temperature accelerates the process of ripening and consequently the production of all organic acids is increased (Murtaza et al., 2012). Salt addition lessens the microbial and enzyme activities, and ultimately affects the biochemical changes which occur during cheese ripening.

The results of this study showed that the addition of oregano EOs, especially those coming from Cordobes and Compacto, significantly decrease the production of organic acids during storage in organic cottage cheese. This reduction in the production of organic acids could be a consequence of the decrease in microbial and enzyme activities affected by oregano EO.

The addition of the oregano essential oil was after the ripening process of the cottage cheese preparation. Therefore, the EO do not affect the ripening of the product but this compound helps to preserve this product, decreasing for one side, the formation of organic acids like lactic, formic, and acetic acids, mainly, and for other side, the descend of the pH in samples with oregano EO; where these both aspect, pH and acid compound concentration are related between them. Also, the samples supplemented with oregano EO show lower rating of lipid oxidation indicators like hydroperoxide values and conjugated dienes and less deterioration process of fatty acids like linolenic, elaidic, and linoleic acids,

among others. All these results indicate that oregano essential oil acts a preserving agent for this cheese product. However, a sensory study of this product should be carry on so as to determine how consumer acceptance is affected for the addition of oregano essential oil.

4. Conclusion

Organic cottage cheese flavoured with oregano EO shows a lower degree of chemical deterioration during storage. Moreover, this organic product with the addition of Cordobes and Criollo EOs preserves longer polyunsaturated fatty acids against oxidation reactions. The addition of Cordobés and Compacto oregano EOs in organic cheese could be an alternative to reduce the production of organic acids during storage, which could be associated with a reduction in microbial activity. The inclusion of this kind of natural compound as a food preserving agent would satisfy the current food manufacturers and consumers' demands for healthier food. Oregano EO can extend the shelf-life of sensitive food products which normally have a short time for logistic distribution. In addition, this natural GRAS compound, oregano EO, can be farmed and processed in organic conditions and, as a consequence, it could act as an organic preserving agent useful for organic food products.

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