International Journal of
Food Science & Technology

Original article

Sensory and bio-chemical preservation of ricotta cheese using natural products

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(Received 10 March 2014; Accepted in revised form 19 May 2014)

Summary The objective of this study was to evaluate the antimicrobial activity and to study their preserving effect on quality and sensory parameters on flavoured ricotta cheese of Argentinean oregano essential oils (EOs). Criollo essential oil was the most effective agent controlling foodborne bacteria, with MIC values of 1.90 E-04 mg mL^{-1} for *S. aureus* and *Salmonella* sp. Ricotta with Cordobes essential oil exhibited the lowest percentage of lactic acid at the end of the storage (0.246%). After 30 days of storage, ricotta flavoured with Cordobes and Criollo EOs had the lowest total count (5.63×10^3 and 7.80×10^3 cfu g^{-1}, respectively). The Pearson's correlation analyses ($P \leq 0.05$) showed that sour flavour was negatively related to cheese and casein flavours ($r = -0.46$ and -0.52, respectively). The inclusion of oregano EOs into ricotta cheese improves quality parameters but affects its sensory attributes.

Keywords Antimicrobial, cheese, foodborne, preserving, ricotta, sensory.

Introduction

An increasing number of consumers prefer minimally processed foods. The food industry shows interest for new food preservation techniques to replace the old ones (intense heat treatments, salting, drying and chemical preservation) by new ones due to the increased consumers' demand for tasty, nutritious, natural, and easy-to-handle food products and the evidence of toxicity of synthetic antimicrobials. Food legislation has restricted the use of some synthetic antimicrobials based on a possible toxicity effect (Burt, 2004). Food preservation is continuously fighting via protective cultures and other antimicrobials against micro-organisms that can spoil the food or make it unsafe (Appendini & Hotchkiss, 2002). Growing awareness and concern about quality and safety of cheese led to the development of new methods for cheese preservation.

Some spices are known to provide distinctive aroma and flavour to food and for having compounds such as essential oils (EOs) that convey antimicrobial and antioxidant properties to foods. Additionally, these EOs are classified by USDA as Generally Recognized as Safe (GRAS) in food preparations. However, people are not familiar with applications of EOs as a natural preserver in food systems (Asensio *et al*., 2013; Olmedo *et al*., 2013; Quiroga *et al*., 2013).

Many bacteria are considered unacceptable in food products. The gram-positive rod, *Bacillus cereus*, has been related to raw and processed meat, vegetables, rice and dairy products. *Staphylococcus aureus* is a major human pathogen that causes a wide spectrum of infections considered the third most important cause of disease in the world (Normanno *et al*., 2007). *Escherichia coli* lives in human and animal intestinal tract, and some strains can cause severe diarrhoeas such as enteropathogenic, enterotoxigenic, enteroinvasive, enteroadherent, enteroaggregatives and enterohemorragic strains (Doyle *et al*., 1997).

There is an increasing interest in the food industry to use EOs as natural substances with antioxidant, antibacterial and antifungal activities. Essential oils with compounds like eugenol, citral, pinene, thymol, cinnamic acid and carvacrol are characterised by a prominent antimicrobial activity (Konning *et al*., 2004). Oregano EO has in its composition thymol, carvacrol, pinene among others (Asensio *et al*., 2013). Some researchers have found antimicrobial activity in *Origanum vulgare* EOs on gram-positive and gram-negative

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doi:10.1111/ijfs.12604

bacteria (Baydar *et al*., 2004). Oregano EOs have also demonstrated antioxidant activity in food systems like peanut products (Olmedo *et al*., 2012) and olive oil (Asensio *et al*., 2011, 2012, 2013).

Ricotta is a fresh dairy product that has soft, grainy, thick, lightly sour taste and presents a white colour. The protein content is high but has little fat content (14%). For that reason, this product is recommended for people who must make low-calorie diets for children or adults who are overweight or obese. It is also rich in calcium, almost as much as milk, and vitamins B1, B2 and folic acid, and is also highly recommended for elderly people as an excellent supply of calcium (Nollet & Toldrá, 2010; ANMAT, 2013). This product is obtained in the early steps of cheese preparation and has high water activity. Because of that, the shelf life of ricotta cheese (RC) is a continue concern for the food industry.

An experiment in which oregano essential oil is added in a food product with high water activity and lipid content should contribute with new information about the antimicrobial and antioxidant properties. The aim of this study was to evaluate the antimicrobial activity in foodborne isolated micro-organism and lactic acid bacteria using oregano EOs obtained from different Argentinean varieties and also, to evaluate their preserving effect in the chemical and sensory properties of RC.

Material and methods

Leaves and flowers of *Origanum vulgare* L. ssp. *Vulgare*, common name: Compacto (COM); *Origanum vulgare* ssp. *hirtum* (Link), common name: Cordobes (COR); *Origanum vulgare* ssp. *hirtum* (Link), common name: Criollo (CRIO); and *Origanum x majoricum* Cambess, common name: Mendocino (MEN) were used in this study. Plants were collected from the Experimental Area of the Agricultural Science College, National University of Cordoba on May (crop 2011).

The activity of the oregano essential oil was tested against gram-positive and gram-negative bacteria. The gram-positive bacteria were *Staphylococcus aureus* (milk), *Bacillus cereus* (oregano leaves), *Streptococcus termophillus* (cheese) and *Lactobacillus helveticus* (cheese). The gram-negative bacteria were *E. coli* (water), *Salmonella* sp. (ice cream) and *Pseudomonas aeruginosa* (cedron leaves). The micro-organisms were isolated and characterised in the Laboratory of Food Microbiology of the Microbiology and Immunology Department of the National University of Rio Cuarto (Argentina).

Ricotta cheese (La Serenísima, General Rodriguez, Buenos Aires, Argentina) purchased from a local supermarket.

Essential oil extraction and GC-MS analysis

Leaves and flowers were hydrodistilled for 2 h in a Clevenger-type apparatus. A Perkin-Elmer® Clarus 600 GC-MS (Shelton, CT, USA) coupled with an ion trap mass detector and equipped with a capillary column DB-5 (30 m long, 0.25 mm i.d. and 0.25-mm coating thickness) was used for the separation of the oregano essential oil components. The analysis, identification and quantification of the different peaks of oregano EOs were performed according to Asensio *et al*. (2011).

Antimicrobial assays

Micro-organism cell concentration necessary to cause reduction of resazurin (Riedel–de Haën; Sigma-Aldrich, St. Louis, MO, USA) within 2 h was determined. Ten microliter of resazurin solution (0.1 g L^{-1} in sterile distilled water) were added and incubated for 2 h at 37 °C (Gallucci *et al*., 2011).

Minimum inhibitory concentration

The minimum inhibitory concentration (MIC) of EOs was determined by the Broth Microdilution Method (Oliva *et al*., 2010). Sterile 96-well micro-titre tray was set up as follows: column 1–10, 170 μL inoculums plus 20 μL EOs dilution; column 11, 170 μL inoculums plus 20 μL diluents (DMSO) (positive control = pink); column 12, assay medium (MHB) plus 20 μL diluents (DMSO) (negative control = blue). After 3-h incubation, 10 μL resazurin solution was added to every well. After a second incubation (2 h at 37 °C), wells were assessed visually for colour change. The highest dilution remaining blue was considered as MIC.

Minimum bactericidal concentration

Minimum inhibitory concentration dilution (100 μL) and previous dilutions were inoculated in Mueller-Hinton Agar (MHA) and incubated at 37 °C for 24 h. The Minimum bactericidal concentration (MBC) was considered as the last dilution that did not show cellular growth (Oliva *et al*., 2010).

Storage study of RC: physico-chemical, microbiological and sensory analyses

Oregano EOs (0.05% w/w) of the varieties COM, COR, CRIO and MEN were added to ricotta and mixed with a hand mixer during 5 min. A control sample (C) was prepared identically without the addition of the essential oil. The ricotta samples flavoured with oregano.

EO (RC-COM; RC-COR; RC-CRIO; RC-MEN) (30 g) and control sample (RC-C) were stored at 23 °C ± 2.0 °C in Ziploc ® brand bags with zip. Samples were analysed every 10 days during 30 days.

pH and total titratable acidity determination

The pH values of flavoured ricotta samples were determined using a digital pH meter (Accumet Basic AB15; Fisher Scientific Inc., Waltham, MA, USA). Initially, samples were homogenised with an Ultrataurux for 60 s in distilled water (1:9 ratio) prior to pH determination (Amirdivani & Salihin Baba, 2011). Total titratable acidity (TTA) was determined according to the procedures describe by AOAC (2007). The amount of acid produced during fermentation was calculated as follows:

$$\text{Percentage of Lactic Acid (\%)} = \text{V (0.1 N NaOH)} \times 0.1 \times 0.009 \times 100$$

where V is volume of Na OH required to neutralise the acid.

Microbiological count

Mesophilic micro-organisms were enumerated by serial dilutions and viable plate counts on TSA. Ricotta cheese (10 g) was diluted in 90 mL peptone water (0.1%) in sterile sample bags. Serial 10-fold dilutions were made in 0.1% peptone water solution before spread plating on TSA plates. Plates were incubated for 24 h at 37 °C (Gomes *et al.*, 2011).

Sensory analysis

Sensory evaluation was performed in the Instituto de Ciencia y Tecnología de los Alimentos (ICTA), Universidad Nacional de Cordoba (Cordoba, Argentina). A total of seven trained panellists (five female and two male, aged between 26 and 50) participated in the descriptive analysis. They had 7 years of experience in sensory analysis and were members of the sensory panel of ICTA. The panellists were selected and trained according to Meilgaard *et al.* (2010). At first, the panel leader determined (i) each panellists capability to detect sensory properties (fragrance, flavour, and oral texture); (ii) the ability to verbally communicate the observations regarding the product; (iii) people available for all sessions; (iv) the capacity of abstract reasoning; (v) people interested in full participation; (vi) people with age from 25 to 56 years old; (vii) people without food allergies; (viii) nonsmokers; and (ix) people who usually consume dairy products. To evaluate the point (i), a screening test was performed on the selected panellists according to Grosso & Resurrección (2002).

The panellist training lasted 36 h (2 h per day) spread over 6 weeks. Terminology was developed using commercial samples. On the first 2 week of training, panellist was given a review of concepts of sensory analysis and they were asked to taste standard solutions. After that, panellists worked together to develop the language to describe perceivable product attributes in RC. Panellists also identified references to be used to describe each appearance, flavour and textural attribute. The mean intensity rating was calculated and used as attribute in intensity rating for that particular reference. On the 3rd and 4th week of training, panellists reviewed descriptors, definitions and reference standards to describe ricotta samples. Panellists tasted each reference and provided a rating. The panel was calibrated by obtaining an average panel rating with a standard deviation within ten points. On the 5th and 6th week of training, panellists reviewed the definitions, descriptors, and intensities of standard references, and also worked on calibration enhance. Then, the list of definitions and warm-up and reference intensity ratings were finalised. Flavour descriptors were illustrated by physical references, whereas those for texture were provided with definitions (Bárcenasa *et al.*, 2007). A hybrid descriptive analysis method consisting of the Quantitative Descriptive Analysis (QDA; Tragon Corp., Redwood City, CA, USA; Cadena *et al.*, 2012; Albenzio *et al.*, 2013; Inglett *et al.*, 2014) and the Spectrum TM (Sensory Spectrum, Inc., Chatham, NJ, USA; Meilgaard *et al.*, 2010; Olmedo *et al.*, 2012) was performed. This hybrid method use part of the procedures from QDA and Spectrum methods. In this hybrid method, statistical analysis was used to determine appropriate terms, procedures and panellists. Panellists evaluated products one at a time and did not discuss data and terminology after each session. Results were also statistically analysed. A list of definitions and a sheet with warm-up and reference intensity ratings (Table 1) were developed by the panel during the training for this specific product. A 100-mm unstructured line scale was used for intensity evaluation. Cheese samples were transported in portable refrigerators (4 ± 1 °C) and equilibrated at room temperature (20 ± 2 °C) for 1 h before the sensory analysis sessions. The samples were evaluated in booths under fluorescent light at room temperature. 10 g of the product sample was placed into plastic cups cover with a clock glass coded with three-digit random numbers (IDF, 1997; Olmedo *et al.*, 2013).

Statistical analysis

Three replications of the experiment were carried out. The data were analysed using the InfoStat software, version 2012p (Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba). Analysis of variance (ANOVA, $\alpha = 0.05$) and LSD Fisher's multiple range test were performed to find significant differences among means. Pearson coefficients were estimated to establish correlations between analysed sensory variables. Principal component analysis (PCA; Souza *et al.*,

Table 1 Attributes list, definitions and intensity ratings of reference sample and 'warm-up' used during the sensory evaluation of ricotta samples

Attributes[a]	Reference	Reference intensity[b]	Warm-up intensity[b]
Positive attributes			
Appearance			
Colour	Whole cream[c]	58	40
Glossiness	White bean[d]	30	16
Surface moisture	Slice of Granny smith apple[e]	15	14
Aroma			
Cheese taste	Whole cream[c]	65	50
Cooked milk/milky	Hot milk[f]	80	50
Oregano flavour	'Mendocino' oregano essential oil[g]	100	42
Taste			
Sweet	2.0 g 100 mL^{-1} sucrose solution	20	18
	5.0 g 100 mL^{-1} sucrose solution	50	
	10.0 g 100 mL^{-1} sucrose solution	100	
Salty	0.2 g 100 mL^{-1} NaCl solution	25	5
	0.35 g 100 mL^{-1} NaCl solution	50	
	0.5 g 100 mL^{-1} NaCl solution	85	
Bitter	0.05 g 100 mL^{-1} caffeine solution	20	3
	0.08 g 100 mL^{-1} caffeine solution	50	
	0.15 g 100 mL^{-1} caffeine solution	100	
Sour	0.05 g 100 mL^{-1} citric acid solution	20	10
	0.08 g 100 mL^{-1} citric acid solution	50	
	0.15 g 100 mL^{-1} citric acid solution	100	
Negative attributes			
Aroma			
Rancid	Rancid milk cream	5	0
Fermented	Vineger solution 20 mL 100 mL^{-1} water	80	10

[a]Attributes listed in order of appearance by panellists.
[b]Intensity ratings based on a 10 cm line unstructured scale.
[c]Whole milk cream 'La Serenísima', Argentina.
[d]White beans 'Grandiet', Córdoba, Argentina.
[e]Granny Smith apple.
[f]Whole milk 'La Serenísima', Argentina heated at 80 °C.
[g]'Mendocino' oregano essential oil, harvest 2010 Agronomy College Experimental Station. (Universidad Nacional de Córdoba, Argentina).

2007; Cruz *et al.*, 2012) was performed on the correlation matrix of the standardised (normalised) data from descriptive analysis. Associations between different treatments and sensory parameters of RC were explored by PCA.

Results and discussion

Oregano essential oil compositions

Components with higher concentration than 0.1% are listed in Table 2. The major components were thymol (19.98%), 3-carene (14.31%), and terpinene-4-acetate (23.90%) in Mendocino EO; terpinolene (23.09%), carvomenthol (21.65%), and thymol (13.01%) in Compacto EO; carvacrol (24.54%), 4-terpineol (20.09%), and o-cimene (14.60%) in Cordobes EO; and carvacrol (30.19%), 4-terpineol (16.54%), and o-cymol (8.72%) in Criollo EO. The components that constitute an EO are those that confer its biological properties (Gallucci *et al.*, 2009). The position that has the hydroxyl group in the ring exerted an influence upon the component effectiveness as carvacrol and thymol showed differences in the activity against gram-negative and gram-positive bacteria (Veldhuizen *et al.*, 2006).

Essential oil compositions of *Origanum vulgare* ssp. *vulgar*e and ssp. *virens* rich in acyclic compounds and sesquiterpenoids had previously been reported (Essen *et al.*, 2007) as in this study. The volatile terpenes as carvacrol, *p*-cymene and thymol were probably responsible for the antimicrobial activity. Carvacrol presents in Cordobes (24.54% w/w) and Criollo (30.19%) EOs, probably acts as a transmembrane carrier of monovalent cations by exchanging its hydroxyl proton for another ion, it showed the best antimicrobial activity for *S. aureus* (Gallucci *et al.*, 2009). Cymene present in Cordobes EO does not have this property but probably acted synergistically with carvacrol by expanding

Table 2 Terpenoid components of essential oils from oregano varieties Compacto, Cordobes, Criollo, and Mendocino according to their elution order in the GC-MS analysis

		Relative percentages (%)			
	Compounds	Mendocino	Compacto	Cordobes	Criollo
5.89	ρ-xilene	0.73 b	0 a	0 a	0 a
6.19	α-thujene	0.31c	0.43 b	0 a	0 a
6.38	α-pinene	0 a	0.53 b	0.8 c	3.8 d
6.78	Camphene	0 a	0.14 b	0 a	0 a
7.39	sabinene	0.61 c	0 a	0 a	0.31 b
7.83	Mycrene	0 a	0 a	0 a	0.56 b
8.03	pseudocumol	0.49 b	0 a	0 a	0 a
8.26	β-Phellandrene	0 a	2.88 b	0 a	0 a
8.62	α-terpinene	2.57 a	0 a	3.08 b	2.99 b
8.84	*p*-cymene	0 a	8.64 b	14.6 c	0 a
8.96	*o*-cymol	1.74 b	0 a	0 a	8.72 c
9.06	limonene	0 a	0.62 b	0 a	0 a
9.10	1,8 cineole	0 a	0.41 b	4.26 c	0 a
9.24	β-Ocimene	0 a	0 a	0 a	4.09 b
9.92	γ-terpinene	11.85 c	9.89 c	8.51 b	7.3 a
10.87	Terpinolene	0.69 a	23.09 a	0.92 a	0.85 a
11.19	Sabinene hydrate trans	23.9 b	0 a	0 a	0 a
12.44	Pinene hydrate trans	0 a	0 a	0 a	0.52 a
13.4	borneol	0 a	0.7104 b	3.77 d	3.3 c
13.78	Terpinen 4 ol	8.48 a	21.65 b	20.09 b	16.45 c
14.2	α-terpineol	2.39 a	2.16 a	5.07 c	4.61 b
8.81	γ-terpineol	0 a	0 a	0.55 c	0.24 b
15.34	Trans-pipertol	0 a	0.86 b	0 a	0 a
15.62	thymol methyl ether	0.99 b	6.38 c	0.59 a	0.41 a
15.88	β-pinene	0 a	0 a	1.43 b	0 a
16.43	3-carene	14.31 b	0.07 a	0 a	0 a
16.78	bergamol	0 a	0 a	0 a	1.23 b
17.45	thymol	19.98 c	13.01 b	0 a	0.6 a
17.78	Carvacrol	3.86 b	0 a	24.54 c	30.19 d
21.65	caryophyllene	1.4 a	1.04 a	3.83 b	4.35 c
22.10	Farnesene epoxide	0 a	0.19 b	0 a	0.2 b
22.69	γ-murolene	0 a	0 a	0.61 b	0 a
23.51	δ germacrene	0 a	0.08 a	0 a	0.88 b
23.35	γ-gurjunene	0.79 c	0 a	0.43 b	0 a
22.41	Naphtalene	0 a	1.44 b	0 a	0 a
21.95	γ-elemene	0 a	0.72 b	0 a	0.98 c
24.27	β-bisabolene	0 a	0 a	0 a	0.82 b
26.30	spathulenol	0 a	0.86 b	1.82 c	1.85 c
26.44	himachalene epoxide	0 a	0.19 b	0 a	0 a
26.5	caryophyllene oxide	0 a	0 a	1.3 b	1.39 b

Different letters in the column for every dependent variable means that there are significant differences between oregano essential oils ($\alpha = 0.05$).

the membrane. Terpineol found in Cordobes and Criollo EOs has a hydroxyl group but it does not possess high antimicrobial activity, probably because of the absence of a system of delocalized electrons (double bonds) and, consequently, the inability of the hydroxyl group to release its proton (Ultee *et al*., 2002).

Differences in the chemical composition of the EOs could be attributed to genetic, and soil and weather conditions during the crop year (Torres *et al*., 2010). Oreganos' varieties Compacto, Cordobes, Criollo and Mendocino were grown and harvested in the same experimental station at the same time. Differences in chemical composition could be attributed mainly to genotypic characteristics. Mendocino was botanically identified as a hybrid *Origanum* × *majoricum*; Compacto is a different specie *Origanum vulgare* ssp. *vulgare*; and Criollo and Cordobés are both clones which belong to the same specie *Origanum vulgare* ssp. *hirtum* but with phenotypic differences (Torres *et al*., 2010).

Table 3 Minimum inhibitory concentration of essential oils (mg mL^{-1}) obtained from oregano varieties (Compacto, Cordobes, Criollo and Mendocino)

Micro-organism	Essential oils (mg mL^{-1}) Mendocino (0.0486–9.49 E-05)	Compacto (0.0486–2.37 E-05)	Cordobes (0.0486–1.18 E-05)	Criollo (0.0486–1.18 E-05)
S. aureus	3.80E-04^{c,2}	7.59E-04^{c,3}	7.59E-04^{c,3}	1.90E-04^{c,1}
S. termophyllus	3.80E-04^{c,2}	>2.37E-05^{a,1}	1.91E-05^{a,1}	>1.18E-05^{a,1}
B. cereus	1.90E-04^{b,3}	9.49E-05^{b,2}	7.59E-04^{c,4}	>1.18E-05^{a,1}
L. helveticus	>9.49E-05^{a,1}	>2.37E-05^{a,1}	>1.18E-05^{b,1}	>1.18E-05^{a,1}
Salmonella sp.	2.43E-02^{d,4}	1.52E-03^{d,3}	7.59E-04^{c,2}	1.90E-04^{c,1}
E. coli	7.59E-04^{c,2}	7.59E-04^{c,2}	7.59E-04^{c,2}	9.49E-05^{b,1}

Different letters in the column means that there are significant differences between micro-organism for each essential oil and different numbers in the row means that there are significant differences between essential oils (α = 0.05) for each micro-organism.

Antimicrobial activity

Minimum inhibitory concentration

All oregano EOs presented antimicrobial activity. Criollo EO was very effective showing MIC values lower than 1.18 E-05 mg EO mL^{-1} for *L. helveticus*, *S. termophyllus* and *B. cereus* (Table 3). This EO also had good activity against *S. aureus* (MIC: 1.90 E-04 mg mL^{-1}), *Salmonella* sp. (MIC: 1.90 E-04 mg mL^{-1}) and *E. coli* (MIC: 9.49 E-05 mg mL^{-1}) representing significant differences respect to other EOs. The gram-positive bacteria were strongly inhibited by oreganos' EOs, showing more sensitivity than the gram-negative bacteria *E. coli* and *Salmonella*.

According to the results observed, not only gram-positive bacteria but also gram-negative bacteria demonstrate marked sensitivity. Gram-negative bacteria are known to be more resistant. Their outer membrane of gram-negative bacteria shows a very low permeability, but it has been demonstrated that highly lipophilic compounds penetrate easily through the outer membrane of several bacteria (Oliva *et al*., 2010). In this study, *E. coli* and *Salmonella* sp. showed high sensitivity to oreganos' EOs, especially to Criollo and Cordobes EOs (Table 3). A MIC of 0.6 mg mL^{-1} was found for oregano *Majorana* EO against *E. coli* when they used the diffusion method in agar medium (Ezzeddine *et al*., 2001). Conversely, activity of *Marjoram* EO against *E. coli* was reported at concentrations higher than 0.1 mg mL^{-1} *in vitro* assays (Baratta *et al*., 2000). The variability in the antimicrobial activity might be due to differences in the basic test method, type of emulsifier used and incubation time.

Minimum bactericidal concentration

Cordobes, Criollo and Mendocino EOs exhibited bactericidal activity while Compacto EO was only bacteriostatic. Criollo EO had values of bactericidal activity against *E. coli* and *B. cereus* (6.80 E-03 mg mL^{-1}), whereas Mendocino EO was bactericidal against *L. helveticus* (MBC value: 1.52 E-03 mg mL^{-1}). The minimum bactericidal concentration for Cordobes EO against *S. termophillus* was 1.52 E-03 mg mL^{-1} (Table 4).

Some studies have concluded that whole EOs have a greater antibacterial activity than the major components mixed (Mann & Markham, 1998; Gallucci *et al*., 2009). The minor components may have a synergistic effect. The two structurally similar major components, carvacrol and thymol, were found to give an additive effect when tested against *S. aureus* and *P. aeruginosa* (Gallucci *et al*., 2009). This study reported that Criollo and Cordobes EOs were more active *in vitro* antimicrobial activity than the other tested EOs, probably because of the presence of carvacrol and cymene/cymol, and the synergistic action taking place among these terpenes.

Storage study: chemical, microbiological and sensory analysis

Physicochemical indicators

Changes in chemical indicators of deterioration are shown in Figs 1 and 2. The content of lactic acid has been reported as an indicator related to the degree of fermentation (AOAC, 2007; Rodríguez-Alonso *et al*., 2011). Lactic acid in RC samples presented significant increases ($P < 0.001$) throughout storage for all treatments. RC-C sample exhibited the highest lactic acid content at the end of the storage (0.266%) followed by RC-MEN (0.260%). These samples also had higher rates ($\beta1$) of increase (lactic acid percentage/day) in TTA (RC-C $\beta1 = 0.0051$, $r = 0.94$; RC-MEN $\beta1 = 0.0048$, $r = 0.97$). On the contrary, RC-COR samples had the lowest percentage of TTA at the end of the storage (0.246%) and the lowest rate ($\beta1 = 0.0044$, $r = 0.99$). Similar values were observed in the RC-CRIO samples, but the slope of increase was higher ($\beta1 = 0.0045$, $r = 0.97$). Organic acids like lactic acid,

Table 4 Minimum bactericidal concentration of essential oils (mg mL^{-1}) obtained from essential oil of oregano varieties (Compacto, Cordobes, Criollo, and Mendocino)

	Essential oils (mg mL^{-1})			
Micro-organism	**Mendocino**	**Compacto**	**Cordobés**	**Criollo**
S. aureus	NB	NB	NB	NB
S. termophyllus	NB	NB	1.52E-03	
B. cereus	NB	NB	NB	6.08E-03
L. helveticus	1.52E-03	NB	NB	NB
Salmonella sp.	NB	NB	NB	NB
E. coli	NB	NB	NB	6.08E-03

NB, Not bactericidal activity against the micro-organism.

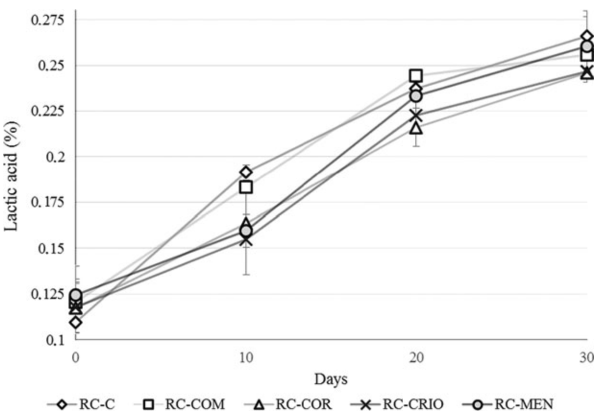

Figure 1 Lactic acid content (%) of ricotta cheese (RC) samples with and without the addition of oregano essential oil during 30 days of storage. Treatments: samples with the addition of essential oil of the oregano varieties Compacto (COM), Cordobes (COR), Criollo (CRIO), and Mendocino (MEN) and without the addition of essential oil (C).

citric acid, formic acid, acetic acid and butyric acid have been reported to be linearly related with the accumulation of TTA (Rodríguez-Alonso *et al.*, 2011). In the present study, different contents of lactic acid in RC samples could be attributed to the different account of micro-organism during fermentation. At time 0, pH of RC samples ranged from 4.37 to 4.44 without significant differences between samples ($\alpha = 0.05$). These values may indicate intensive curd washing during cheese making and are consistent with medium acidic cheese (Rodríguez-Alonso *et al.*, 2011). After ten storage days, a slight decrease in ricotta pH values was detected. RC-C showed a pH of 3.44 whereas RC-CRIO exhibited pH of 3.56. Similar or higher pH values were observed from day 10 to 30. This effect may be attributed to proteolysis, and it is associated to the formation of amines and ammonium. There was no clear correlation between pH and titratable acidity (Garabal *et al.*, 2010).

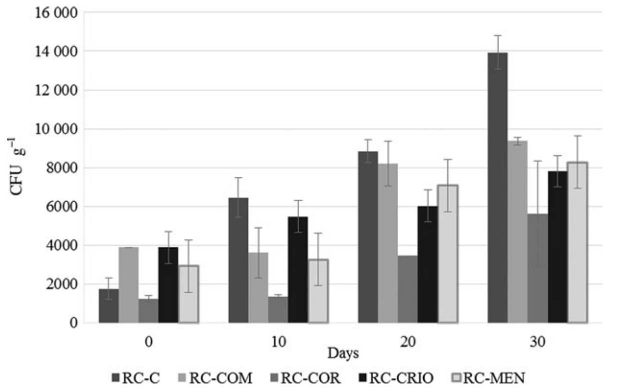

Figure 2 Change in total mesophilic micro-organisms count in ricotta cheese (RC) samples with and without the addition of oregano essential oil during 30 days of storage. Treatments: samples with the addition of essential oil of the oregano varieties Compacto (COM), Cordobes (COR), Criollo (CRIO), and Mendocino (MEN) and without the addition of essential oil (C).

Microbiological count

A total count increase of mesophilic micro-organism were observed over the storage for all treatments (Fig. 2). Significant differences ($\alpha = 0.05$) were observed between samples. After 30 days of storage, RC-COR and RC-CRIO had the lowest cfu g^{-1} (5.63×10^3 and 7.80×10^3 cfu g^{-1}, respectively) whereas RC-C the highest. A previous study reported that cheese samples develop sour taste during ripening for 180 days because of lactose fermentation produced by micro-organism activity (Galán *et al.*, 2008). In the present study, acidity and mesophilic micro-organism count increased in all treatments during storage, whereas pH decreased. Similar results were reported by Olmedo *et al.* (2013). In other study, cheese samples treated with cinnamon, clove grape seed and pomegranate plant extracts had lower food-borne bacterial counts (*S. aureus, S. enterica*, and *L. monocytogenes*) than the corresponding control (Shan *et al.*, 2011). To achieve adequate shelf life, food products require low initial microbial load (controlled or inhibited during development).

Based on the results of the present study, the total count of viable mesophilic in RC-C stored at 23 °C, increased from 1.76×10^3 to 1.39×10^5. The Argentine Food Code provides for dairy products a limit of 1×10^5 as provisionally acceptable quality (ANMAT, 2013). The remaining RC samples supplemented with oregano essential oil would be accepted. The decrease in the efficacy of antimicrobials derived from plants in the transition from broth to food is unprecedented and emphasises the danger in extrapolating the practical value of the observations from studies in liquid medium, where growing conditions are ideal and optimise the antimicrobial effect occurred (Nychas *et al.*, 2003). In this study, the addition of oregano EOs to RC after processing and before packaging lessened total mesophilic micro-organism count during storage at 23 °C. For that reason, this EOs can be considered as potential natural preserving compound for this food product".

Sensory analysis

Twelve sensory attributes showed significant differences ($\alpha = 0.05$) between treatments (Table 5). The intensity bitterness in samples flavoured with oregano EOs (COM, COR, CRIO, and MEN) showed significantly higher values than RC-C sample. RC-C had higher intensities in milky and cheese flavours, which can be attributed to the absence of oregano EO that can increase bitterness and sourness intensities. Sweetness intensity was also lower in samples supplemented with oregano EO.

Appearance attributes, glossiness and surface moisture were related during storage (Fig. 3). An increase of moisture on the surface, increased the amount of light reflected. RC-COM showed a considerable increase in the intensity of surface moisture which was almost twice its magnitude compared with the beginning of the storage (15–28.4 points). On the other hand, the sample RC-COR registered almost no change (16.17–17).

In this study, sourness increased in all treatments throughout the storage. The lowest value was detected at the end of storage was for RC-COR samples. This result is related to the chemical indicator lactic acid, in which this sample also had the lowest value. The sweetness intensity ratings decreased significantly ($\alpha = 0.05$) in all treatments. At the beginning and at the end of storage, the samples with higher intensity of this attribute were RC-C, RC-COR, and RC-COM. No significant differences were found between them. An increase in sourness and a decrease in sweetness intensity could be related to an increase in fermented flavour. This effect is probably due to the fermentation of lactose mediated by hetero-fermentative micro-organisms that increase the content of organic acids such as acetic acid and the degree of peptide hydrolysis (Ong & Shah, 2009; Murtaza *et al*., 2012). Fermented and sourness are closely related (Olmedo *et al*., 2013). Fermented attribute significantly increased in all samples during storage ($\alpha = 0.05$). The highest intensities for this attributes were observed in RC-COM, RC-CRIO, and RC-MEN (19.17, 18.57, and 19.83, respectively).

Sensory attributes that significantly change during storage were included in the PCA. The biplot obtained from the two principal components (CP) in the PCA is presented in the Fig. 4. The two principal components explained 77% variability of RC samples during 30 days of storage. CP 1 represented 48.8% variability. The sensory attributes milky and sweetness were placed on the right side of the CP1 biplot. The RC samples that showed higher values for these variables were also placed to the right side of the biplot (RC-C and RC-MEN). Surface moisture, glossiness, oregano flavour, fermented and sourness were placed on the left side of the plot. Those variables were positively related among them. A strong association was observed among oregano flavour, fermented, and sourness and also among surfaced moisture, glossiness, and sweetness. RC-CRIO was the closest treatment to fermented, sourness and oregano flavour, probably due to the presence of a strong essential oil, whereas RC-C was not associated to any of these attributes but closely related to milky. The dispersion of the points showed great variability

Table 5 Intensities of sensory attributes of ricotta samples with and without the addition of oregano essential oils (varieties: Compacto, Cordobes, Criollo and Mendocino) evaluated by descriptive analysis at day 0 of storage

Attributes	RC-C[a] $\bar{\chi}^b \pm$ SD	RC-COM[a] $\bar{\chi}^b \pm$ SD	RC-COR[a] $\bar{\chi}^b \pm$ SD	RC-CRIO[a] $\bar{\chi}^b \pm$ SD	RC-MEN[a] $\bar{\chi}^b \pm$ SD
Glossiness[c]	15.43 ± 0.98 b	16.00 ± 2.00 b	17.86 ± 3.48 a	16.00 ± 0.48 b	14.86 ± 1.57 b
Surface moisture[c]	14.14 ± 0.71 b	15.35 ± 0.45 a	16.17 ± 0.48 a	15.00 ± 0.71 b	14.57 ± 0.45 b
Colour[c]	40.00 ± 0.00 b	40.14 ± 0.38 b	40.57 ± 1.13 a	40.57 ± 0.79 a	40.57 ± 0.31 a
Fermented[c]	9.57 ± 0.45 b	9.71 ± 0.76 b	9.86 ± 0.38 b	13.86 ± 0.48 a	10.57 ± 0.45 b
Cheese taste[c]	50.14 ± 0.37 a	44.57 ± 1.05 c	45.24 ± 1.13 c	42.73 ± 1.13 c	47.14 ± 1.05 b
Milky[c]	49.71 ± 1.23 a	44.29 ± 1.23 b	46.00 ± 1.23 b	45.29 ± 1.23 b	47.71 ± 1.24 a
Sourness[c]	10.14 ± 0.38 b	10.43 ± 2.99 a	11.00 ± 1.29 a	9.43 ± 3.51 b	10.29 ± 0.49 b
Bitterness[c]	3.57 ± 0.98 b	10.43 ± 0.91 a	6.29 ± 0.97 b	7.57 ± 0.91 b	4.86 ± 1.89 b
Sweetness[c]	18.14 ± 0.89 a	17.00 ± 0.63 a	17.29 ± 0.65 a	15.34 ± 0.65 b	16.57 ± 0.63 a
Saltiness[c]	5.00 ± 0.00 a	4.86 ± 0.38 b	5.00 ± 0.00 a	5.14 ± 0.38 a	5.00 ± 0.00 a
Oregano flavour[c]	0.00 ± 0.00 b	40.17 ± 1.30 a	40.14 ± 1.20 a	38.29 ± 1.42 a	38.8 ± 11.88 a
Rancid[c]	0.00 ± 0.00 b	0.57 ± 0.42 a	0.43 ± 0.43 a	0.43 ± 0.44 a	0.43 ± 0.40 a

[a]Ricotta cheese (RC) samples with the addition of oregano EO: Compacto (COM), Cordobes (COR), Criollo (CRIO), and Mendocino (MEN) and without addition of EO (C).

[b]Media and standard deviation (n = 3).

[c]Different letters in a row means that there are significant differences between ricotta samples ($\alpha = 0.05$, LSD Fisher).

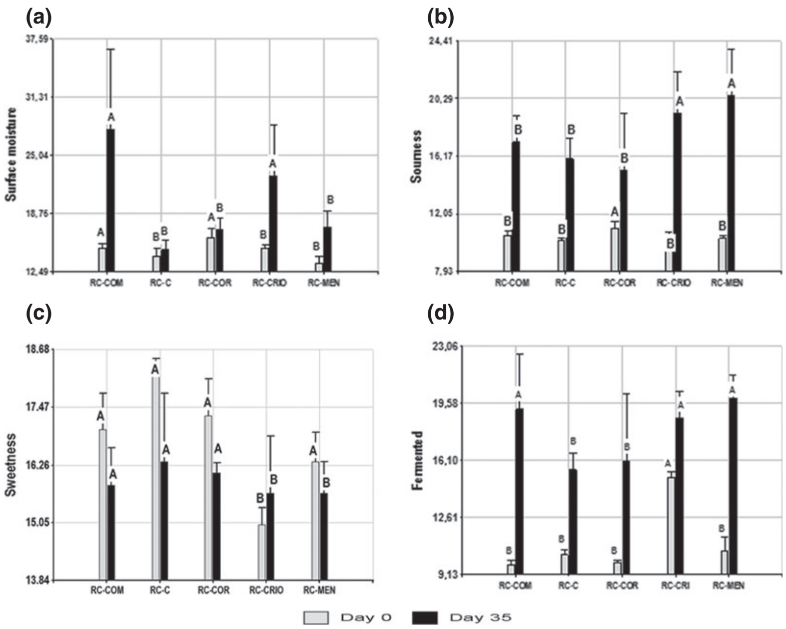

Figure 3 Statistically significant differences (α = 0.05; Test LSD) in sensory attributes of ricotta cheese (RC) samples with and without the addition of oregano essential oils during 30 days of storage evaluating the following sensory attributes: (a) surface moisture, (b) sourness, (c) sweetness and (d) fermented. Treatments: samples with the addition of essential oil of the oregano varieties Compacto (COM), Cordobes (COR), Criollo (CRIO), and Mendocino (MEN) and without the addition of essential oil (C). Different letters for each period in each dependent variable means that there are significant differences between oregano essential oils (α = 0.05).

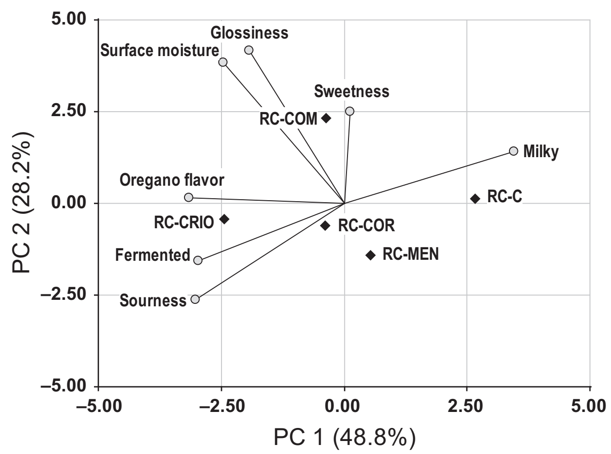

Figure 4 Biplot from the first and second principal components of PCA. Euclidean distance variables: sweetness, milky, oregano flavour, fermented, sourness, glossiness and surface moisture. Treatments: ricotta cheese control (RC-C), ricotta cheese added with oregano essential oils (RC-COM; RC-COR; RC-CRIO and RC-MEN).

among samples. The ricotta samples with EOs were placed on the left side of the plot (except RC-MEN).

The Pearson's correlation coefficients ($P \leq 0.05$) showed that sour attribute was negatively related to attributes cheese and casein flavours (r = −0.46 and −052, respectively) and positively related to fermented flavour (r = 0.64). Additionally, glossiness was positively related to surface moisture (r = 0.58). Fermented and rancid flavours were negatively related to milky flavour (r = −0.45). The lactic acid content was also positively correlated with the attributes acid (r = 0.33) and fermented (r = 0.30). All negative attributes associated to quality deterioration of ricotta (fermented, sour, surface moisture, gloss and lactic acid) are interrelated. In other study, it was also observed that the hydrolysis of proteins is highly correlated with increases in the bitter taste (Ong & Shah, 2009).

The relation between EO chemical composition and sensory profile is still not clearly determined. The study of Mosciano (1998) revealed the sensory effect of some components of EOs like γ-terpinene that has been reported with herbaceous and citrus notes, terpinen-4-ol that has been described with citrus, minty, coniferous and woody notes, and the alcohol α-terpineol has been mentioned with floral notes and that these components, present in oregano essential oil, can have variation in the chemical composition in different lot of the same essential oil. Further sensory studies should perform on RC flavoured with oregano essential oil and also include the relation between the chemical composition of essential oil and the sensory profile of the individual compound and the whole oregano EO to understand the possible effect in this food product. Also, consumer test of the RC with the addition of oregano essential oil should be performed to know how the acceptance is affected in the final product.

Conclusion

Essential oils of oregano species farmed in Cordoba province, Argentina possess high antimicrobial activity (in vitro) against *S. aureus*, *B. cereus*, *E. coli* and *Salmonella* sp. The RC flavoured with oregano essential oil improves their physicochemical characteristics and lessens its microbiological count, especially RC supplemented with oregano essential oil obtained from variety Cordobes. However, the results of this study show that the addition of oregano EOs to RC affect some sensory attributes. For that reason, consumer test should be performed in further studies to evaluate the impact of the oregano EOs inclusion in this food product.

Taking into account the possible sensory property effect, the use of oregano EOs in dairy products constitutes an alternative as preserving method that can meet certain food manufacturers' expectation and consumers' demands. Therefore, oregano essential oil can be used in RC as a natural preserving agent to prevent spoilage increasing its shelf life.

Acknowledgments

Thanks to CONICET y SECYT who supported this study.

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