Universitat Autònoma de Barcelona

Facultat de Veterinària

Departament de Ciencia Animal i dels Aliments.

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"Secondary lipid oxidation and microbial stability assessment in a modified lipid profile fresh cheese with essential oils"

Carolina Emperatríz Carrión López

Directora

Dra. Nàtalia Nicolau i Villellas

Tutor

Dr. Antonio José Trujillo Mesa

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List of abbreviations

CFU	Colony forming unit
GC-MS	Gas Chromatograohi-Mass Spectrometry
G	Grams
Kg	Kilo grams
Log	Logarithms
MDA	Malondialdehyde
ng	Nanograms
PCA	Plate Count Agar
rpm	Revolutions per minute
µg	Micrograms

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Abstract

The food industry has a high interest in developing healthier dairy products. The substitution of milk fat by vegetable oil emulsions in fresh cheese could improve the saturated-unsaturated fat balance profile. The aims of this study were to analyze the antioxidant and antimicrobial effects of oregano and cinnamon essential oils added to a modified lipid profile of fresh cheese during storage period (1, 5, 10 and 15 days) at 4ºC. MDA and hexanal products of lipid oxidation were analyzed by HPLC and gas chromatography- mass spectroscopy (GC-MS) equipment, respectively. The microbiological parameters were monitored coliforms and psychrotrophs bacteria. Results showed that cheeses treated with essential oils had significant antioxidant activity and cinnamon essential oil present a high antioxidant efficacy when was compared to oregano essential oil. The essential oils used in this study did not present antimicrobial effect on fresh cheeses.

Keywords: Essential oils, fresh cheese, hexanal, MDA

Resumen

La industria alimentaria tiene un alto interés en desarrollar productos lácteos con un perfil lipídico más saludables. La substitución de la grasa de la leche por aceites vegetales podría mejorar el perfil de grasa saturadas/insaturadas de estos productos. El objetivo de este estudio se enfoca en analizar el efecto antioxidante y antimicrobiano de los aceites esenciales de orégano y canela, en un queso fresco con el perfil lipídico modificado. Las muestras de queso se analizaron a través del tiempo los días 1, 5, 10 y 15 y se almacenaron a 4ºC. MDA y hexanal se analizaron por medio del HPLC y el cromatógrafo de gases-espectrometría de masas (GC-MS), respectivamente. Los análisis microbiológicos se monitorearon a través de bacterias coliformes y psycricrotrofos. Los resultados mostraron que los aceites esenciales utilizados en este estudio poseen una actividad antioxidante significativa, pero que el aceite esencial de canela presentó una alta eficacia antioxidante en comparación con el aceite esencial de orégano. Los aceites esenciales no presentaron actividad antimicrobiana en los quesos frescos.

Palabras claves: Aceites esenciales, hexanal, MDA, queso fresco.

Introduction

Approximately 30% of global deaths are caused by cardiovascular diseases (World Health Organization, 2007). Some factors that promote the risk of development of these diseases may have a genetic origin or could be determined by environmental factors such as diet. Nowadays, the intake of saturated fats is considered a cause of increase of cardiovascular diseases. That is why it is necessary to reduce this health problem through the improvement of saturated-unsaturated fat balance of food (Lobato *et al.*, 2006). However, fat considerably affects the acceptability of food on the level of consumers' sensorial perception, and its reduction may cause a significant change in the mechanical characteristics, flavor, and odor of a product (Lobato *et al.*, 2001).

Therefore, the use of vegetable oils could be profitable since some researchers have found that vegetable oils are cholesterol free and have a higher level of unsaturated fatty acids (Yu and Hammond, 2000). The composition of fatty acids in some vegetable oils, such as canola, sunflower and soybean oil, makes them appropriate replacers for a variety of processed products low in cholesterol and thus contribute to a healthier saturated-unsaturated fatty acid balance (Yu and Hammond, 2000). The n-3 and n-6 families are among the essential fatty acids deriving from vegetable oils and fish sources and are fundamental for prevention of cardiovascular diseases. However, they cannot be synthesized alone in human organism and should be supplied in diet. (Lee *et al.*, 2016).

The food industry has a high interest in developing healthier dairy products, modifying dairy products for the benefit of human health. The emulsified vegetable oils in milk are a good alternative to milk fat in elaboration of dairy products with a healthier saturated-unsaturated fat balance (Yu and Hammond, 2000). Cheese is the most diverse group of dairy products, and in the market it is easy to find different types of cheese with certain modifications, for example, the low fat and low salt cheeses, or cheeses containing functional ingredients like probiotics, minerals, and vitamins (Gutiérrez *et al.*, 2013).

Fresh cheeses are commonly made from pasteurized cow milk, moreover are ready to be consumed after their production (Silva *et al.*, 2015). The contamination after processing by microorganisms may occur, producing cheese spoilage and reduction of shelf life, which could become a potential risk for consumers' health (Baruzzi *et al.*, 2012).

Coliforms are the most common indicators of sanitary conditions in manufactured products because these Gram-negative bacterias show the efficiency of pasteurization treatment and other good manufacture practices applied in the process. Hence, there is a great necessity to extend cheese shelf life and keep it fresh for an extended period, since it contains a high amount of water and becomes one of the most perishable dairy products (Khoshgozaran *et al.,* 2012).

The factors such as exposure to light, packaging, and management in retail stores constitute other causes of quality loss of fresh cheese. Those aspects also influence the lipid oxidation on cheese. The formation of toxic compounds such as malondialdehyde (MDA) and hexanal result from lipid oxidation and could be harmful to human health because of their impact on protein chains and deoxyribonucleic acid (DNA) (Andersen *et al.*, 2008).

Malondialdehyde is a three carbon dialdehyde with a carbonyl group at the C-1 and C-3 position in the chain and is proven to be a mutagenic substance for humans (Giera *et al.*, 2012). MDA is originated from linolenate and arachidonic acid and is the most abundant aldehyde from lipid peroxidation in foods (Gorelik *et al.*, 2005)

Hexanal is another product of lipid oxidation that could increase during storage, which is produced from linoleic acid as a secondary oxidation volatile compound. Besides, hexanal in food is related to oxidative off-flavors and thus can be detected by consumers at sensorial level, wich can reduce their acceptability respect to oxidized food products (Frankel *et al.*, 1989).

Because of consumers' concern about the safe use of chemical and artificial preservatives, the interest in developing natural food preservatives with the purpose of keeping food safe is increasingly growing. Natural antioxidants, such as some essential oils, could benefit the protection of lipids from oxidative degradation (Shan *et al.*, 2005). However, these essential oils generally have a lower antioxidant activity compared to the more used synthetic antioxidants (Olmedo *et al.*, 2009). On the other hand, when higher concentrations of essential oils are added to food products, the flavor magnitude may cause adverse effects on the sensory profile and consumers acceptability (Olmedo *et al.*, 2009).

A previous research work carried out in CIRTTA indicates that the application of natural essential oils on food could provide an antioxidant and antimicrobial effect. As some other authors reported, they have a high content of phenolic compounds such as phenolic acids, flavonoids and aromatic compounds (Shan *et al.*, 2005), being effective sources of phytochemicals (Lambert *et al.*, 2001). Some essential oils such as cinnamon and oregano were reported to have the high level of phenolic compounds that have antimicrobial activity (Tayel *et al.*, 2015). In particular, eugenol and cinnamaldehyde have the potential to be antimicrobials and are the main bioactive compounds of cinnamon essential oil (Tzortzakis, 2009).

This research work aims at finding a solution to reduce the amount of saturated fats in cheese fat profile by making a new type of fresh cheese. Replacing milk fat with a vegetable oil emulsion of sodium caseinate and soybean oil will lead to an improved fat balance in the new fresh cheese.

The objectives of this study were to evaluate the antioxidant and antimicrobial effect of specific essential oils (oregano and cinnamon) on the secondary lipid oxidation products (MDA and hexanal) and the microbial load (coliforms and psychrotrophs) in a cheese of lipid profile modified with soybean oil. The monitoring of these factors was carried out during the cheese shelf life (15 days at 4ºC).

Material and Methods

Preparation of protein dispersion

Sodium caseinate (88% of protein) (Zeus Quimica, Barcelona, Spain) dispersion containing 5%w/w of protein was prepared using distilled water, by agitation with a magnetic stirrer. Then, it was stored overnight at 4ºC in order to let protein hydrate the day before cheese production.

Preparation of emulsion

Three different emulsions were prepared based on sodium caseinate, soybean oil and distilled water. Essential oils of oregano and cinnamon were added into two of them, leaving the third one as control emulsion.

After hydration, the emulsions were prepared with the protein dispersion, soybean oil (Dialma S.A.U., Palma de Mallorca, Spain) (40%w/w) and essential oils of oregano and cinnamon (1mg/mL). All ingredients were mixed with a turmix homogenizer for 5 min at 500 rpm. Finally, the emulsion was homogenized at a pilot plant scale (Universitat Autònoma de Barcelona) in the processing plant at 250 bar.

Cheese making

The three types of fresh cheese were produced: oregano cheese, cinnamon cheese and control cheese. The manufacturing procedure for processed fresh cheese was carried out in the pilot plant of Veterinary Faculty in the Universitat Autònoma de Barcelona (UAB). Cow's raw milk was pasteurized (72ºC 15 s) and skimmed. Then, 20 Kg of skimmed milk was distributed into the three previously disinfected experimental cheese vats (control, oregano, and cinnamon). The milk placed in each vat was added with 1.25 Kg of emulsion, and a gentle stirring was applied until a complete mixture. 10 mL of calcium chloride commercial solution and 10 mL of rennet (Laboratorios Arroyo S.A., Santander, Spain.) were added to the milk when it reached the temperature of 32ºC. After that, it was mixed for 1 min and coagulated for 30 min. Curd was manually cut and molded into baskets, and then consecutive turning processes in 3 times were applied

to cheeses in order to remove the remaining whey. The cheese samples were stored at 4ºC for 15 days.

Malondialdehyde analysis

Lipid oxidation was assessed by the evaluation of malondialdehyde formed during storage; procedures reported by Papastergeadis (2012) were used for evaluation, including some modifications.

After the 1, 5 and 10 day storage in a cold room at 4 ºC, samples were measured in duplicate. The cheese samples were handily homogenized into a plastic zip bag to obtain a representative sample. A small portion of 5 g (± 0.05) of sample was added with 15 mL of TCA (7,5%) (Sigma-Aldrich, Madrid, Spain) into a 50 mL falcon tube and vortexed for 1 minute at 18000rpm. The mixture was filtered through Whatman 1 filter paper (GE Healthcare, Barcelona, Spain) and 1 mL of clear filtrated was measured in an assay tube, with 3mL of 40mM thiobarbituric acid (TBA) (Sigma-Aldrich, Madrid, Spain). The solutions were vortexed for 15 seconds and heated in a boiling water bath, for 40 minutes until they gained a pink tone. After that, the solutions were cooled down with previously refrigerated water and 1 mL of Methanol of HPLC grade (Sigma-Aldrich, Madrid, Spain) was added to stop the reaction. The solution was vortexed and filtrated through PVDF filters in chromatography vials.

A calibration curve was prepared using control cheese sample to avoid matrix effect, starting from different dilutions of tetraetoxypropane (TEP) (Sigma-Aldrich, Madrid, Spain), with concentrations ranging from 0.0075 to 5 μM.

Hexanal analysis

The samples were measured 1, 5 and 10 days after storage at cooling conditions (4ºC). In order to perform the analysis, 1 g of each cheese sample (control, oregano, and cinnamon) was weighted in duplicate into a dark vial. Then 4 mL of buffer solution pH 2.0 was added to each vial with 5 μL of Butylated Hydroxy Anisole (BHA) (Sigma-Aldrich, Madrid, Spain), in a methanol solution of HPLC grade (Sigma-Aldrich,

Madrid, Spain). After vortexing for 1 minute, cheese samples were subjected to solid-phase microextraction (SPME) using a 75 µm CAR-PDMS fiber (Fused Silica 23Ga) for 10 min at 75ºC. An external calibration curve was prepared at different concentrations of hexanal, ranging from 65 to 163 ng.

Microbiological analysis

Microbiological analyses were performed on all samples (control, oregano, and cinnamon) in duplicate after 1, 5, 10 and 15 days of storage at 4ºC. Cheese samples were handly homogenized into the package to achieve a representative sample. In order to proceed with the analysis, 10 grams of each sample was homogenized for 5 min at 200 rpm with 90 mL of buffered peptone water (Oxoid, Basingstoke, UK) in the stomacher (Lab-blender 300, Seward Medical, London, UK). A range of decimal dilutions was prepared (up to 10^{-4} in coliforms and 10^{-7} in psychrotrophic bacteria). The colonies count were prepared adding 1 milliliter of each dilution in a sterile Petri plate and poured with coliform brilliance agar (Oxoid, Basingstoke, UK) and for psychotrophic bacteria was used Plate Count Agar (PCA, Oxoid, Basingstoke, UK) .

Coliforms bacteria were incubated at 37º C for 24h, while psychrotrophs bacteria were determined after the 5 day incubation at 20ºC.

Results were expressed as a logarithm of colonies forming units per gram of cheese (Log CFU/g).

Instrumental analysis

HPLC determination of MDA

For MDA analysis on fresh cheese samples, HPLC equipment was used with a pump (Perkin Elmer Serie 200) connected to an automatic injector (Waters 717) and a fluorescence detector (Perkin Elmer series 200) (SAF0111), equipped with an informatic system of data collection (Turbochrom 6.2). The column was ODS-2, 5 um (4.6 x 200 mm) (Scharlab), and the equipment operated isocratically with an HPLC

mobile phase pumped at 1.0 ml/min and consisting of 50 mM KH2PO4 buffer solution, methanol, and acetonitrile in the proportion 72:17:11 (v/v). The injection volume was 20 µl, and fluorescence detector wavelengths were set at 525 nm (excitation) and 540 nm (emission). Results were expressed as micrograms of MDA per grams of sample (µg of MDA/g)

Solid Phase Microextraction (SPME) Gas Chromatography / Mass Spectrometry (GC/MS) Analysis

Hexanal analysis was performed in fresh cheese sample by SPME-GC/MS analysis system (Model: HP 6890 Series II, Agilent, Santa Clara, CA, USA) coupled to a Hewlett-Packard quadrupole mass spectrometer 5973, equipped with an electronic source and a CTC Analytics, Autosampler (Ref.:57343-U) injector configured for SPME experiments. A TRB-WAX (Aligent technologies) column with 60m length, 0.25 mm internal diameter and 0.25 µm film thickness was used. The fiber was desorbed in the gas chromatograph GC injector. Results were expressed as nanograms of hexanal per grams of sample (ng/g)

Statistical analysis

All experiments were carried out independently in triplicate. The sample measures were analyzed in duplicate. Results were processed by analysis of variance (ANOVA) of Statistical Analysis System (SAS) University Edition 2017. A Duncan's multiple range tests were used to determine significance between means, and evaluations were based on a significance level of $p<0.05$.

Results and discussion

Modified lipid profile fresh cheeses were made at Planta Pilot Tecnologia dels Aliments (UAB, Barcelona, Spain). Figure 1 shows the flow chart process of the emulsions preparation.

Preliminary studies have been carried out within the framework of this research project to determine appropriate ratios of protein percentage, water and soybean oil used in this research . Also, the methodology was established and optimized to elaborate three emulsions in CIRTTA facilities.

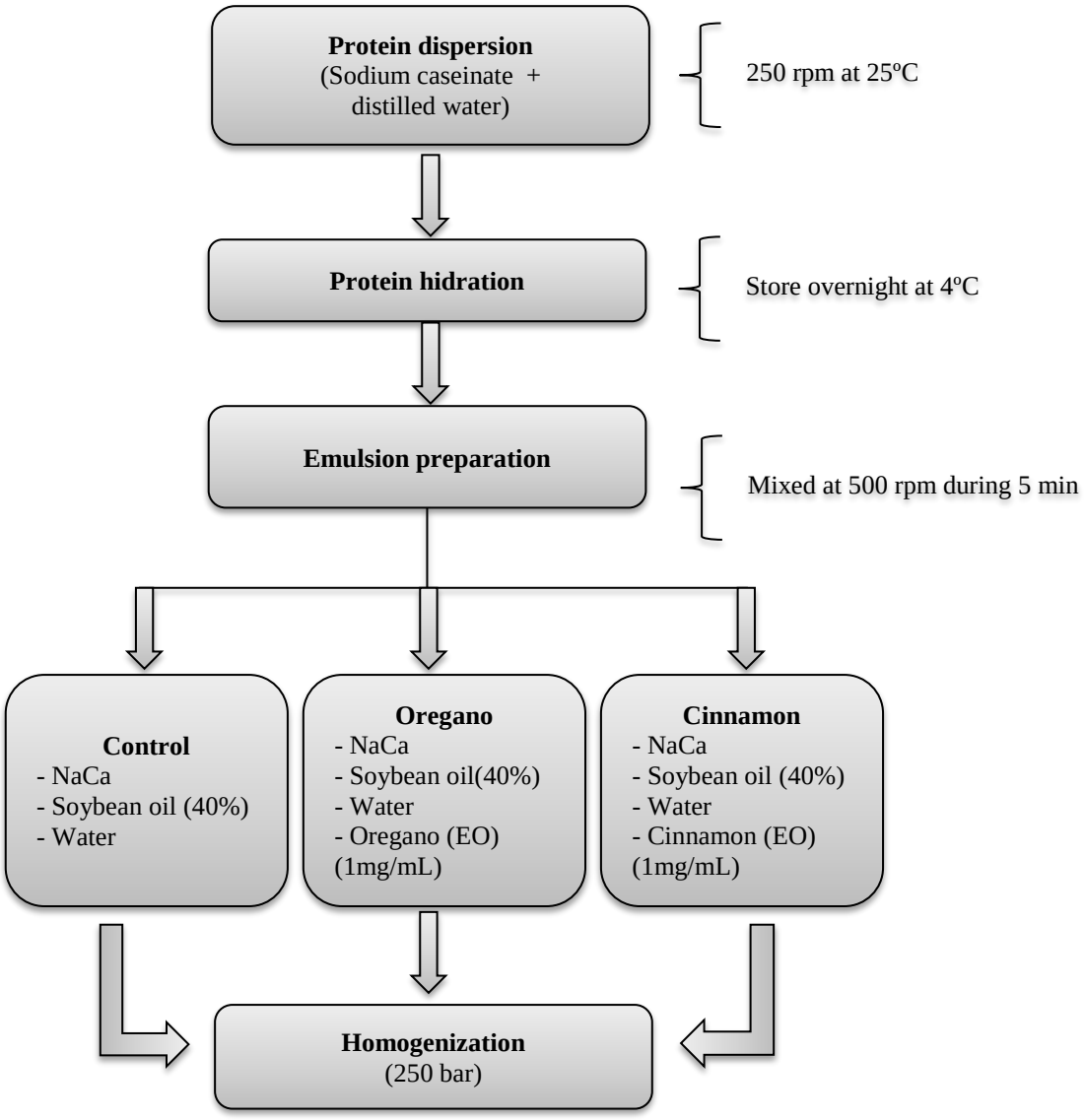

Figure 1. Flow chart process of emulsions preparation.

Figure 2 shows the optimized process of cheese making. In this study, to accomplish with the elaboration of this new type of fresh cheese, the conditions of the process have been adapted to the pilot plant (UAB). Amounts of calcium chloride and rennet were modified from the traditional fresh cheese making conditions being necessary to increase the addition of these ingredients concentration. Since milk added with vegetal soybean oil did not coagulate at the first experimental trial, other experiments were carried out to determine the optimum calcium chloride and rennet amount and finally 2 times amount of these ingredients were added to the milk vat.

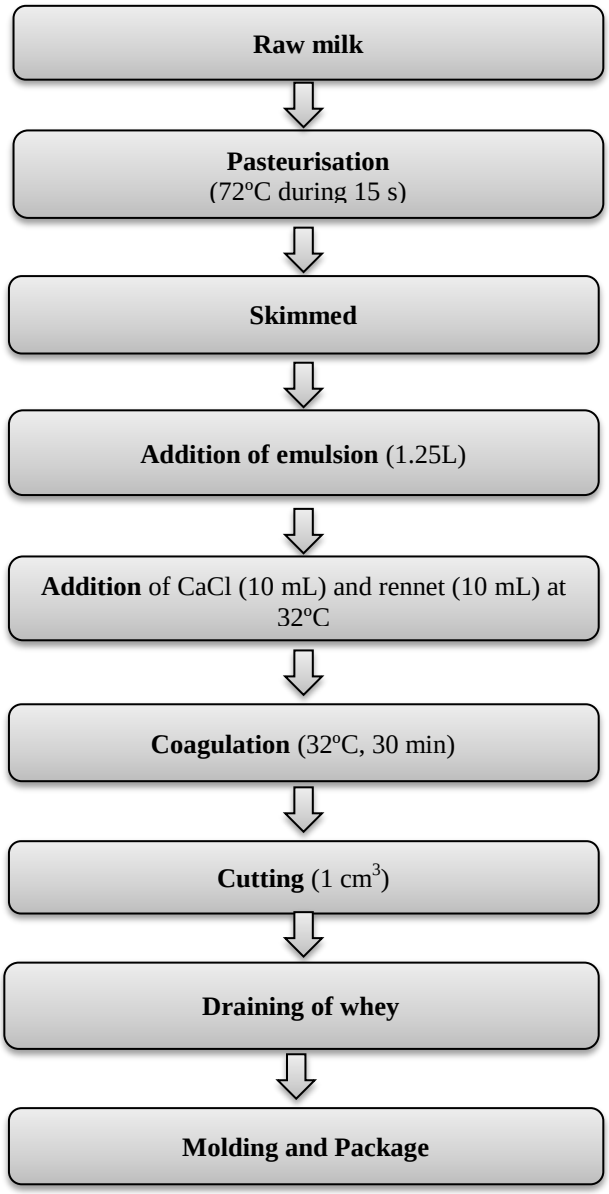

Figure 2. Flow chart process of modified lipid profile cheese based on 20 Kg of milk.

Microbiological Analysis

In this research work, the microbial quality of cheeses was monitored by measuring the growth of total coliforms and psychrotrophs as selected microorganisms of fresh cheese.

Table 1 shows the results obtained from microbiological analysis of coliform counts performed on cheese samples during 15 days of storage at 4ºC. Differences between coliform counts of cheese samples were determined to be significant ($p < 0.05$) during storage period (1, 5, 10 and 15 days) and between cheese treatments (control, oregano and cinnamon).

Cheese samples demonstrated significant differences ($p < 0.05$) through storage period, on day 1, 5, 10 and 15. Regardless of essential oil application, each sample presented an increasing trend in coliforms count during the storage period.

Table 1. Mean values and standard deviation of coliforms count (Log CFU/g) in cheese samples (control, oregano, and cinnamon) analyzed on days 1, 5, 10 and 15.

	Storage time (days)			
Sample	**1**	**5**	**10**	**15**
Control	$1.18 \pm 0.80^{XY,c}$	$1.83 \pm 0.13^{Y,b}$	$2.32 \pm 0.73^{Y,ab}$	$2.93 \pm 0.31^{Y,a}$
Oregano	$0.97 \pm 1.12^{Y,d}$	$1.53 \pm 0.66^{Z,c}$	$2.12 \pm 0.94^{Z,b}$	$2.86 \pm 0.59^{Y,a}$
Cinnamon	$1.85 \pm 0.98^{X,c}$	$2.12 \pm 0.46^{X,c}$	$3.00 \pm 1.01^{X,b}$	$3.54 \pm 0.66^{X,a}$

Mean ± standard deviation
a-d Different letters in the same row indicate differences between days of storages ($P < 0.05$).
X-Z Different letters at the same column indicate differences between samples of cheese ($P < 0.05$).

Control, oregano and cinnamon cheeses showed an increase of 1.75 Log CFU/g, 1.89 Log CFU/g and 1.85 Log CFU/g, respectively, for 15 days. On the 15th day of storage, the control sample did not show differences compared to oregano cheeses. The increment of coliform bacteria over time could take place because of the favorable conditions that fresh cheese provides to develop those bacterial group, such as the high water content of cheese (Ledenbach and Marshall, 2009). A research work based on

antimicrobial properties and potential applications of essential oils in different foods had similar results. The Gram-negative bacteria was resistant to natural antimicrobials due to its lipopolysaccharide outer membrane. The membrane inhibited the diffusion of hydrophobic compounds and did not interact with essential oils (Burt, 2004).

However, Gammariello *et al.* (2010) obtained different results regarding coliforms. Those authors assessed the antimicrobial activity of essential oils analyzing total coliforms in a kind of fresh cheese called Fior Di Latte cheese. However, coliform counts were reduced over time and their shelf life was prolonged. This was caused because of great concentrations of a combination of citrus essential oils (2250 and 6000 ppm) was added to the cheese sample.

According to a research work conducted by Ma *et al.* (2016), eugenol, a major antimicrobial compound of cinnamon oil, has an effect on inhibiting the growth of Gram-negative bacteria at high concentrations, but it does not inhibit the growth of Gram-positive bacteria. This fact differs from the results obtained in our research work, where cinnamon essential oil seems to have no antimicrobial effect on Gram-negative bacteria coliforms. This could be explained by the casein effect, as milk components like casein could create a protective effect on the bacterial cell structure. The protective effect could be caused by the stronger interaction of casein with the cell surface of Gram-positive instead of Gram-negative bacteria (Ma *et al.*, 2016).

Table 2 shows the results of psychrotrophs analysis in control, oregano and cinnamon cheeses, stored during 15 days at 4 ºC. Significant differences ($p < 0.05$) between storage time (1, 5, 10 and 15 days) and cheese samples (control, oregano and cinnamon) have been observed.

Each cheese treatment showed statistical differences ($p < 0.05$) between storage times regardless of the type of cheese; the psychrotrophs count grew up over time. The psychrotrophs increased by 2.35 Log CFU/g in control cheese over 15 days, 3.03 Log CFU/g in oregano cheese and 2.58 Log CFU/g in cinnamon cheese. As it was expected, psychotrophic bacteria grew over time because optimum conditions of temperature were amended during storage (4ºC) for their development. Also, high water content in cheese benefited this bacterial group growth. Similar results have been shown in other studies despite the fact they were carried out in a different food matrix. For example, some other authors who analyzed the combined effect of oregano extract and packaging on

microbiological quality of chilled chicken carcasses, observed that psychrotrophs count increased with storage time period and decreased with the addition of a higher concentration of oregano extract to the sample (Khaled *et al.*, 2016).

Table 2. Mean values and standard deviation of psychrotrophs count (Log CFU/g) in cheese samples (control, oregano, and cinnamon) analyzed at days 1, 5, 10 and 15 of storage.

	Storage time (days)			
Sample	**1**	**5**	**10**	**15**
Control	$4.03 \pm 0.13^{Y,c}$	$5.06 \pm 0.57^{Y,b}$	$5.33 \pm 0.28^{Z,b}$	$6.38 \pm 0.12^{Y,a}$
Oregano	$3.81 \pm 0.11^{Z,d}$	$5.17 \pm 0.60^{X,c}$	$5.96 \pm 0.18^{Y,b}$	$6.84 \pm 0.33^{X,a}$
Cinnamon	$4.30 \pm 0.67^{X,d}$	$5.35 \pm 0.52^{X,c}$	$6.15 \pm 0.28^{X,b}$	$6.88 \pm 0.34^{X,a}$

Mean ± standard deviation
a-d Different letters in the same row indicate differences between days of storages ($P < 0.05$).
X-Z Different letters at the same column indicate differences between samples of cheese ($P < 0.05$).

Another research work, based on low-fat cheese added with oregano essential oil and mandarin fiber, revealed that using a higher concentration of a 2% essential oil, achieved an antimicrobial effect on the cheese. Instead, the levels below 1.5% did not have the capacity to inhibit psychrotrophic bacteria (Artiga *et al.*, 2017).

In this study, cinnamon essential oil did not seem to have an antimicrobial effect to inhibit psychrotrophs on the cheese matrix. Similar results were obtained in other research works, where cinnamon essential oil reduced its antimicrobial capacity when it was exposed to a dairy base, compared to when it was placed in a laboratory medium. The reason is that milk matrix required higher concentrations of essential oil to improve the bactericidal effect. Another reason that could explain this fact is that the components of food may interact with essential oils and could affect the capacity to reduce microorganisms, or that microorganisms could be hiding behind those food components (Tayel *et al.*, 2015). Other authors inferred that some organic materials existing in food could create a barrier between antibacterial activity and essential oil (Smith *et al.*, 2001).

Lipid oxidation analysis

In this research work, the lipid secondary oxidation products were quantified by the assessment of MDA and hexanal aldehydes in fresh cheese samples.

Table 3 shows the evolution of MDA concentration (µg/g) of control, oregano, and cinnamon cheeses, stored at days 1, 5 and 10 at 4ºC. Differences between MDA levels of cheese samples were determined to be significant ($p < 0.05$) during the storage period (1, 5, 10 and 15 days) and in between the cheese treatments.

Table 3. Mean values and standard deviation of MDA content (µg/g) in cheese samples (control, oregano, and cinnamon) on days 1, 5, 10 and 15 of storage.

	Storage time (days)		
Sample	**1**	**5**	**10**
Control	$0.24 \pm 0.18^{XY,a}$	$0.35 \pm 0.29^{X,a}$	$0.15 \pm 0.20^{X,a}$
Oregano	$0.05 \pm 0.19^{Y,b}$	$0.34 \pm 0.31^{X,a}$	$0.12 \pm 0.27^{X,ab}$
Cinnamon	$0.80 \pm 1.05^{X,a}$	$0.14 \pm 0.14^{Y,b}$	$0.02 \pm 0.12^{Y,b}$

Mean ± standard deviation
a-c Different letters in the same row indicate differences between days of storages ($P < 0.05$).
X-Z Different letters at the same column indicate differences between samples of cheese ($P < 0.05$).

At the beginning of storage, all samples were statistically different throughout the cheese treatments. At day 1, oregano cheese had the lowest concentration of MDA (0.05 ± 0.19 µg/g) compared to all other samples analyzed. At day 5, the cinnamon cheese had the lowest levels of MDA (0.14 ± 0.14 µg/g) compared to control and oregano cheese samples. No major differences were observed between control, oregano, and cinnamon cheese at day 10 of storage. Table 3 shows that cinnamon cheese had an important reduction of 0.78 µg/g MDA concentration over the days. According to Keshvari *et al.* (2013), similar results were obtained, when the preventive effect of cinnamon essential oil on lipid oxidation of vegetable oil was analyzed. The reason for this significant reduction was because cinnamon essential oil reduced the number of free radicals which were not anymore available to the chain reaction of lipid peroxidation.

Kulisic *et al.* (2004) investigated different methods to test the antioxidant activity of oregano essential oil. They pointed that antioxidant effect depends on several factors

like the method applied in the testing of antioxidant activity, the concentration, and natural properties of the essential oil.

Table 4 shows the evolution during the storage period of hexanal concentration for control, oregano, and cinnamon cheeses, stored on days 1, 5 and 10, at 4ºC. Treatments presented significant differences ($p < 0.05$) between storage period and cheese samples.

Table 4. Mean values and standard deviation of hexanal content (ng/g) in cheese samples (control, oregano, and cinnamon) at days 1, 5, 10 and 15 of storage.

	Storage time (days)		
Sample	**1**	**5**	**10**
Control	$54.34 \pm 24.82^{X,a}$	$36.83 \pm 27.43^{X,b}$	$23.84 \pm 26.82^{X,c}$
Oregano	$31.06 \pm 10.80^{Y,a}$	$16.73 \pm 7.70^{Y,b}$	$6.49 \pm 5.75^{Y,c}$
Cinnamon	$26.43 \pm 7.48^{Z,a}$	$15.09 \pm 7.55^{Y,b}$	$6.41 \pm 6.34^{Y,c}$

Mean ± standard deviation
a-d Different letters in the same row indicate differences between days of storages ($P < 0.05$).
X-Z Different letters at the same column indicate differences between samples of cheese ($P < 0.05$).

The hexanal concentration of all cheeses decreased linearly during the storage period. Control cheese presented the highest concentration over the storage compared to the rest of the samples. Control cheese reduced by 30.5 ng/g during 10 days of storage, oregano cheese by 24.54 ng/g and cinnamon cheese by 20.02 ng/g. On day 1 of storage, cinnamon cheeses had the lowest concentration of hexanal compared to the rest of cheese samples. On day 5 and 10 of storage, oregano and cinnamon cheeses had similar results but showed significant differences when compared to control cheese.

Similar results were obtained by Marques *et al.* (2013), who investigated the use of oregano as an antioxidant to reduce hexanal formation in pre-cooked chicken. They found that the addition of oregano essential oil decreased hexanal levels in samples through storage period. This research revealed that the addition of oregano essential oil had a direct effect on the hexanal development during refrigerated storage. It was found in other studies based on dairy beverage formulation that oregano essential oil was added as an antioxidant and also inhibited hexanal production (Boroski *et al.*, 2012).

Conclusions

Taking together the results obtained in this research work, some conclusions could be drawn.

Vegetal emulsions based on sodium caseinate, as a protein carrier ingredient, soybean oil, as Ω-6 and Ω-3 carrier ingredient, and essential oils like oregano and cinnamon, are easily elaborated and could be an appropriate delivery system for bioactive compounds.

A dairy product based on cow milk and a vegetal emulsion containing soybean oil riched in Ω-6 and Ω-3 fatty acids, like a fresh cheese-like and added in essential oils, could be a suitable product to improve the saturated-unsaturated fat balance in dairy products.

The use of oregano essential oil at concentrations of 1mg/mL in the emulsions had a slight or near-null antimicrobial effect when they were added to fresh cheese. A similar tendency was observed in cinnamon added fresh cheese samples.

MDA concentrations decreased in all samples over time, specially in oregano and cinnamon cheeses which arrived to last steps of storage with lowest values of MDA. However, further studies on this compound are recommended since the behavior of this chemical compound is unstable and fluctuating.

The results of the present study demonstrate that oregano and cinnamon essential oils added in fresh cheese have an antioxidant effect, since they reduce hexanal levels over storage period. Cinnamon essential oil showed to be more efficient as an antioxidant when compared to oregano essential oil.

Based on these results, essential oils like oregano and cinnamon could be suggested as antibacterial agents, as long as highest concentrations are used but taking into account sensory changes in cheeses organoleptic profile. Essential oils used in this research work could be also suggested as natural antioxidants, specially regarding to hexanal formation in secondary lipid oxidation processes.

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