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EVALUATION OF WHEY- PROTEIN- ISOLATE EDIBLE FILMS CONTAINING OREGANO (*ORIGANUM VULGARE*) ESSENTIAL OIL TO IMPROVE SHELF LIFE OF CHEESES DURING REFRIGERATED STORAGE

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The Department of Nutrition and Food Sciences

by
Cristhiam Eugenia Gurdian Curran
B.S., Zamorano University, Honduras, 2011
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ABSTRACT

Oregano essential oil (OEO) is recognized for antimicrobial and antioxidant properties. Incorporation of OEO into an edible film prepared with whey protein isolate (WPI) may improve the shelf-life of queso blanco (cheese). The overall goal of the proposed study was to develop and evaluate the effectiveness of antimicrobial and antioxidant WPI edible films (WPIFs) with OEO to improve the shelf life of queso blanco wrapped in the WPIFs during one month of refrigerated storage. Production of potential antimicrobial and antioxidant WPIFs with OEO (WPIOF) was possible. Flaxseed oil (FO) was incorporated into cheese by homogenization (HFQ), which caused significant susceptibility to oxidation when compared to the control cheese (QU). However, it did not have major effects on any other physic-chemical characteristics such as color and texture. Unwrapped HFQ oxidation rate significantly increased during 1-mo of storage, but wrapping with WPIOF was effective in extending the shelf life of omega 3 queso blanco (WOHFQ) by preventing the growth of yeast and molds and retarding oxidative reactions. This study demonstrated that WPIOF can be used to improve refrigerated shelf-life of queso-blanco and queso blanco containing FO.

CHAPTER 1. INTRODUCTION

Among the new tendencies in food packaging, as the demand for more natural and biodegradable products and materials increases, protective coatings as suitable packaging materials have become an interesting issue for food industry as their potential increase in the shelf life of many products has been widely reported (Chien, Sheu, & Yang, 2007; Del-Valle, Hernández-Muñoz, Guarda, & Galotto, 2005; Guilbert, Gontard, & Gorris, 1996; J. Lee, Park, Lee, & Choi, 2003; Nadarajah, Prinyawiwatkul, No, Sathivel, & Xu, 2006; Park, 1999; Peressini, Bravin, Lapasin, Rizzotti, & Sensidoni, 2003; Ponce, Roura, del Valle, & Moreira, 2008; Rojas-Graü, Raybaudi-Massilia, Soliva-Fortuny, Avena-Bustillos, McHugh, & Martín-Belloso, 2007; Shaw, Monahan, O'riordan, & O'sullivan, 2002; Yaman & Bayoındırlı, 2002). Most of the current materials employed in packaging industries are produced from fossil fuels and are non-degradable. Hence, food packaging materials represent an important contribution to environmental pollution (Bech-Larsen, 1996; Derraik, 2002). Efforts to reduce environmental pollution and increase shelf life of foods have increased exploration of new bio-based packaging materials such as edible and biodegradable films from renewable resources. Still, their poor barrier properties and poor mechanical properties limit their usage in food. Hence, sometimes they are blended with synthetic polymers or chemically modified (Petersen, Væggemose Nielsen, Bertelsen, Lawther, Olsen, Nilsson, & Mortensen, 1999b; Sorrentino, Gorrasi, & Vittoria, 2007). Biologically based packaging means that raw materials originated from agricultural resources are contained in the package, meaning that they are

produced from renewable, biological materials and include both, edible films and coatings along with primary and secondary packaging materials(Petersen et al., 1999b). The use of natural oils in order to improve mechanical and antimicrobial properties of biodegradable films and WVP is well documented (Alma, Mavi, Yildirim, Digrak, & Hirata, 2003; Debeaufort & Voilley, 2009; Du, Avena Bustillos, Hua, & McHugh, 2011; Helander, Alakomi, Latva-Kala, Mattila-Sandholm, Pol, Smid, Gorris, & von Wright, 1998; Kulisic, Radonic, Katalinic, & Milos, 2004; Lambert, Skandamis, Coote, & Nychas, 2001; Oussalah, Caillet, Salmiéri, Saucier, & Lacroix, 2004; Teixeira, Marques, Ramos, Serrano, Matos, Neng, Nogueira, Saraiva, & Nunes, 2013; Viuda-Martos, Ruiz-Navajas, Fernández-López, & Pérez-Álvarez, 2007; Zivanovic, Chi, & Draughon, 2005) and can be considered as an antimicrobial packaging technique, which can help to extend shelf life of products and prevent product deterioration. Incorporation of antimicrobials into the packaging matrix can prevent surface growth in foods where spoilage microorganisms and contamination mainly occur. Therefore, antimicrobials added to the food can be potentially reduced and release rate to the food can be controlled. The most popular antimicrobial compounds are both synthetic (benzoic acid and benzoates) and natural in origin. Presently, consumer's demand for natural products is one of the most investigated novel tendencies. This has forced the food industry to develop and investigate ways in which natural additives could be added to foods. Consequently, there has been an increase in the use of essential oils (EOs) as a new approach to replace conventional synthetic additives (Royo, Fernández‐Pan, & Maté, 2010).

The majority of film's studies are based on the reduction of bacteria that were inoculated onto media or foods before they were exposed to the film. Little examination of antimicrobial activity on native bacteria present in foods has been done. This is an important evaluation when determining antimicrobial effect of films because such microorganisms are involved in the product spoilage. It has been showed that when some antimicrobials are combined a synergistic effect occurs. Hence, research is focused in finding cost effective and active alternatives (C. A. Campos, L. N. Gerschenson, & S. K. Flores, 2011; Joerger, 2007).

Oregano essential oil (OEO) is a well-known source of natural antimicrobials including antifungal and antiviral agents. Carvacrol, which is a monoterpenoid-phenol, is the most important antimicrobial agent present in OEO (Dadalioglu & Evrendilek, 2004; Lagouri, Blekas, Tsimidou, Kokkini, & Boskou, 1993; Milos, Mastelic, & Jerkovic, 2000; Teixeira et al., 2013). Plant derived essential oils (EO's) are used as flavoring and food preservation agents. The functionality of this EO meets the demand for natural and minimally processed food products being a potential substitute of synthetic antimicrobials.

Green tea extract (GTE) is rich in polyphenolic compounds, especially catechins (flavan-3-ols), which are potent antioxidants. Numerous studies have demonstrated that it contains anticarcinogenic, antimicrobial, cardiovascular and cerebrovascular protective effects, anti-obesity, and anti-hypercholestolemic effects. Moreover, it also has anti-browning, antidiscoloration, and deodorizing

properties. The functional properties of GTE make it an ideal ingredient for suppressing lipid oxidation and improving foods' nutritional value.

Nowadays food industries are strongly focused in the enhancement of microbial safety and shelf-life of foods through natural ingredients avoiding artificial additives as the consumers' demand for minimally processed products increases. Edible films are a type of packaging made from edible materials that have barrier properties to external conditions (i.e. moisture, oils, gases, and vapors). Whey-protein-isolate (WPI) edible films are an attractive alternative for packaging because they can protect and incorporate functional compounds present in essential oils such as antioxidants, and antimicrobials. In addition, edible films are readily biodegradable, contributing to the reduction of environment pollution.

Queso blanco, a Latin-American unripened cheese with variant preparation faces low shelf-life due to the absence of packaging and rapid development of yeast and molds in some countries. As the Latino population continues to grow in the US, more native products are imported and commercialized in the US. The flavor and other sensorial characteristics of this cheese depend on the particular microflora, ingredients, composition of milk, and steps in its manufacture. Fat being an important component in cheeses influences besides the organoleptic characteristics, the nutritional profile, and shelf-life. Milk fat is mainly composed of saturated fats which have been proved to have detrimental effects in human health. Still, some other nutrients present in milk like rumenic acid (RA, cis-9 trans-11 C18:2), make it an ideal food. Hence, efforts have been conducted in increasing rumenic acid in milk by modifications in ruminants' diet.

Flaxseed oil, which has been historically used for a wide variety of diseases, is a rich source of the essential fatty acids linoleic and alpha-linolenic (ALA). Since ALA and linoleic acid are considered essential fatty acids, they can only be obtained when present in diet as human body cannot synthesize them. In addition, ALA is a bioprecursor of the omega-3 eicosapentaenoic fatty acid (EPA) and docosahexaenoic fatty acid (DHA), which are important in body functionality. In fact, DHA has been considered as a building block of tissue in brain and retina and is closely related to formation of neural transmitters like phosphatidylserine (vital for brain function). EPA and DHA are converted to prostaglandins, which regulate cell activity and cardiovascular function (Gorjão, Azevedo-Martins, Rodrigues, Abdulkader, Arcisio-Miranda, Procopio, & Curi, 2009; Kidd, 2007; Sargent, Bell, McEvoy, Tocher, & Estevez, 1999). Omega-3 fatty acids have acted like antioxidant agents by suppressing oxygen free radicals in cells, which further allowed a positive effect in clinical studies in the prevention of tumor growth (Basch, Bent, Collins, Dacey, Hammerness, Harrison, Smith, Szapary, Ulbricht, & Vora, 2006).

The development of WPI- edible films containing OEO is a novel packaging approach for queso blanco that will contribute to extend its shelf life during its storage at refrigerated conditions. Furthermore, providing queso blanco with omega-3 (ALA) and omega-6 (linoleic acid) fatty acids present in flaxseed oil may improve the nutritional value of queso blanco and by wrapping with edible films having antioxidant and/or antimicrobial properties a longer shelf-life could be achieved. The overall goal of this study was to produce a Whey-Protein-Isolate

edible film containing OEO and apply it as a wrapping material on queso blanco to extend its shelf-life. Accordingly, this project was divided into three studies namely: (1) production and characterization of whey protein isolate (WPI) edible films containing oregano essential oil (OEO) and/or green tea extract (GTE); (2) development of queso blanco containing omega-3; and (3) application of edible films containing OEO to extend the shelf life of queso blanco containing omega-3. Bare queso blanco (cheese) was used as a control and analyses were conducted over time for 1 month. Triplicate experiments were conducted and data were statistically analyzed (α=0.05).

CHAPTER 2. LITERATURE REVIEW

2.1 Food packaging definitions and importance

Packaging is defined as a socio-scientific discipline that allows the delivery of safe goods to the final consumer. The Packaging Institute International defines packaging as the action of enclosing products, items, or packages in a wrapped pouch, bag, box, cup, tray, can, tube, bottle or other container to perform one or more of the following functions: containment, protection, preservation, communication, utility, and performance. A product may be contained in one or more packages levels. A primary package, which is in direct contact with the product, provides the initial and commonly the major protective barrier. A secondary package is the one containing one or several primary packages sometimes designed for the display or the primary package. A tertiary package is made up of secondary packages; and finally a quaternary package is used to facilitate and transport tertiary packages. From the definition of packaging functions defined by the *Codex Alimentarius* Commission in 1985 “Food is packaged to preserve its quality and freshness, add appeal to consumers and to facilitate storage and distribution”, four primary functions of packaging have been recognized: containment, protection, convenience, and communication which are all interrelated. Containment is the most obvious function, but all products must be contained to allow their movement and avoid their content to be released to the environment (pollution and loss of product). Protection is recognized as the primary function of packaging as it protects the product from the surrounding conditions (water, moisture vapor, gases, odors, micro-organisms, dust, shocks,

vibrations and compressive forces). Convenience function of packaging is a response to the changes in life styles (less time to prepare foods, more time spent at work). Ready to eat foods have a greater demand and besides the preparation time (cooked or reheated in short time), two main advantages of convenience are identified: apportionment of food so that a considerable size is provided to the consumers and shape of the package that makes the product easy to manage (open, pour, hold). Communication basically means the package promotes the product's commercialization, it is closely related to a marketing function. In conjunction with the brand, this communication function allows consumer to easy-recognize the product they are looking for during shopping. Furthermore, the UPC (Universal Product Code) and nutritional facts are part of this communication function allowing easy and rapid checkout and inventory control (Robertson, 2012).

On the other hand, every package has to deal and perform adequately in three environments. The physical environment includes everything that could physically damage the product (drops, falls, bumps, vibrations, compressions). Ambient environment is the ambient surrounding the package (gases, water, water vapor, light, temperature, microorganisms, macroorganisms, dust, dirt). Human environment refers to the interaction between people and the package. It is necessary to study human behavior and capabilities (vision, strength, weakness) so that a resistant package can overcome most common abuses (Robertson, 2012).

Today packaging is an essential component of products. It contains, enhances, and protects the product from the very beginning of the productive chain up to the final consumer. Packaging contribution to the Gross National Product (GNP) is 2% in developed countries and half of all packaging is designated to food packaging. Food beverage and packaging represents 55-66% of the $130 billion value of packaging in the U.S (Brody, 2008). Food packaging and processing spent in packaging materials is about the 15% of the total variable costs (Esse, 2002).

2.2 Oregano (*Origanum vulgare*)

Oregano (*Origanum vulgare)* is an aromatic herb of the Lamiaceae family, and widely cultivated in Asia, Europe, and northern Africa (Teixeira et al., 2013). *O. Vulgare* is used in many medicine applications such as respiratory disorders, dyspepsia, rheumatoid arthritis, scrofulosis, and urinary tract disorders. It is also used as a condiment in gastronomy and numerous studies have reported its preservative properties in food. Most of the studies of oregano essential oils are focused on its chemical composition, antioxidant, and antimicrobial properties (Alma et al., 2003; Kulisic et al., 2004; Lambert et al., 2001; Royo et al., 2010).

2.3 Antimicrobial mechanisms of essential oils

Plant derived essential oils (EOs) are odorous, volatile products of an aromatic plant's secondary metabolism and are capable of reaching microbial pathogens through the liquid and the gas phase as they have high vapor pressures. This bioactivity in vapor phase allows their use as possible fumigants during storage for the protection of foods. Moreover, EOs have been recognized as antimicrobial

agents and can be used to control food spoilage and foodborne pathogenic bacteria. Their use as flavoring agents and natural agents for food preservation provides a novel way to improve the safety and shelf life of ready-to-eat foods. The antimicrobial activity of EOs is attributed to terpenoid and phenolic compounds, which exhibit antibacterial and/or antifungal properties in pure form (Du et al., 2011). Monoterpenes, due to their lipophilic nature, disrupt the integrity of the microbial cytoplasmic membrane, which consequently loses its high impermeability for protons and bigger ions. Microbial membrane disruption occurs when the lipophilic compounds accumulate in the lipid bilayer depending on its partition coefficient. Therefore, membrane barrier, enzymatic, and energy transducer functions are compromised (C. Campos, L. Gerschenson, & S. Flores, 2011). Several studies demonstrated that oregano essential oil (OEO) had an antibacterial effect against *Brochothrix thermosphacta, Escherichia Coli, Listeria innocua, Listeria monocytogenes, Pseudomonas putida, Salmonella typhimurium, Shewanella putrefaciens* (Teixeira et al., 2013), *L. innocua, Staphylococcus aureus, Salmonella enteriditis (Royo et al., 2010)*, *E. Coli* O157:H7 (C. Campos et al., 2011), total viable count, *Pseudomonas* spp, and lactic acid bacteria (Zinoviadou, Koutsoumanis, & Biliaderis, 2009). Several authors also report antifungal activity of OEO. In one study OEO and thyme EO were applied as a fumigants against the mycelia and spores of *Aspergillus flavus, Aspergillus nige* , and *Aspergillus ochraceus,* as well as against natural microflora of wheat grains. OEO exhibited the strongest fungicidal activity and a reduction in the percent of infested grain was achieved. The study proved that

oregano and thyme essential oils could be used as potential fumigants and substitutes of preservative-chemicals against fungi attacking stored grain (Paster, Menasherov, Ravid, & Juven, 1995). In another study, the antifungal potential of EO of oregano *(Origanum vulgare), thyme (Thymus vulgaris), and clove (Syzygium aromaticum)* was determined using *Aspergillus niger* and *Aspergillus flavus* as target microorganisms. This study showed that OEO had the highest inhibition of mold growth, followed by clove and thyme (Viuda-Martos et al., 2007).

Previous studies have demonstrated that EOs are more effective against Gram-positive bacteria rather than against Gram-negative bacteria. This could be explained by the composition of the cellular membrane of Gram-negative bacteria. The hydrophilic surface present in the cellular membrane of gram-negative bacteria allows the presence of lipopolysaccharides, which is a barrier difficult to penetrate for the EO. Nevertheless, not all studies demonstrate that Gram-positive bacteria have a higher susceptibility to EO (Royo et al., 2010). Indeed, a study reported that the hydrophobic constituents of essential oils were capable of reaching the periplasm of Gram-negative bacteria through the porin proteins of the outer membrane (Helander et al., 1998).

2.4 Essential oils and lipid oxidation

OEO has also been used for diminishing lipid oxidation (Alma et al., 2003; Gómez-Estaca, Bravo, Gómez-Guillén, Alemán, & Montero, 2009a; Kulisic et al., 2004); attributing this antioxidant activity to phenolic compounds (carvacrol, carvacrol methyl ether, and thymol methyl ether).

2.5 Chemical composition of oregano essential oil

Table 1 shows the chemical composition of OEO.

Table 1. Composition (area %) of *Origanum vulgare L.* essential oil

No.	Compound	RI[a]	Area%	
			In total oil	In fraction
Hydrocarbons CH fraction (CH)				
1	α- Thujene	1031	1.4	5.2
2	β- pinene	1102	-	0.7
3	Myrcene	1149	-	6.1
4	α- Terpinene	1161	3.6	10.4
5	ϒ- Terpinene	1231	10.5	31
6	ρ- Cymene	1247	9.1	22.1
7	Terpinolene	1262	-	0.9
8	Alloocimene[b]	1351	-	0.3
9	α- Copaene	1466	-	0.4
10	β- Burbonene	1496	-	0.3
11	*trans*- caryophyllene	1578	2.4	9.1
12	Aromadendrene	1583	-	0.4
13	α- Humulene	1638	-	1.5
14	Ledene	1644	-	0.3
15	β- Bisabolene	1694	1.4	2
16	δ- Cadinene	1729	0.5	3.8
17	α- Muurolene	1735	-	0.2
				Total 94.7
Oxygen containing compounds fraction (CHO)				
18	1-Octen-3-ol	1411	1	0.8
19	Borneol	1653	1	1
20	Thymol	2115	35	47.3
21	Carvacrol	2140	32	46.4
			Total 97.9	Total 95.5
Phenolic fraction				
1	Thymol	2115	58.9	
2	Carvacrol	2140	41.1	
				Total 00.0

Source: (Kulisic et al., 2004)
[a] Retention indices relative to C_8-C_{22} alkanes on polar HP-20M column.
[b] Correct isomer is not identified.

The study of Kulisic et al. (2004), reported antioxidant activity of OEO due to more polar constituents, being the oxygen containing fraction more effective than

the phenolic fraction or its pure constituents (thymol and carvacrol).The major compounds in OEO are phenolic monoterpenes thymol (35.0%) and carvacrol (32.0%) in total oil without fractionation. OEO antimicrobial, medicinal, antioxidant, and other properties are mainly attributed to its high phenolic composition. Several studies conduct chemical profiles analyses obtaining differences among species. Nevertheless, all the ones reported in literature have proved beneficial effects for human applications.

2.6 Essential oils applied to packing technologies

There are different methods to apply EOs to foods in order allow them to perform functional properties (i.e. directly on food using a spray, as an active compound in active antimicrobial packaging, and in edible food coating-formulations). To control the release rate and coverage of the EO and avoid degradation of the active compounds present in the oils, the later method is the most convenient. With this method, the active compounds will be in contact with the food surface where microbial proliferation mainly occurs.

2.7 Edible films with essential oils

Several works have reported successful applications of OEO in edible food coatings as carriers of food additives. Royo *et al*. (2010) demonstrated OEO efficacy when it was incorporated to whey protein isolate (WPI) and cellulose-based filter paper. Sage EO was also studied and incorporated in both matrices but it did not exhibit antimicrobial activity. A study based on the antibacterial properties of carvacrol, oregano, and cinnamaldehyde against antibiotic-resistant *Bacillus cereus*, *Campylobacter jejuni, E. coli*, *S. enterica*, and *S. aureus*

suggested their incorporation of the into film formulations to reduce pathogens in foods (Friedman, Buick, & Elliott, 2004). Zivanovic, *et al, (Zivanovic et al., 2005)* reported in the study of antimicrobial and physicochemical properties of chitosan films and chitosan films enriched with essential oils (EO) in vitro and on processed meat that similar antibacterial activity of EO could be achieved when applying alone or incorporated in the films. A greater efficacy was observed for oregano EO followed by coriander, basil, and anise. Antibacterial activity increased in the chitosan films incorporated with oregano EO when compared to the chitosan films against *Listeria monocytogenes* and *Escherichia coli*. Furthermore, addition of oregano EO into the chitosan films reduced WVP, puncture and tensile strength, but increased elasticity of the films. This study concluded that the films had antimicrobials effect and could be used as active biodegradable films.

2.8 Green tea production and consumption

“Green tea” is the product obtained after manufacture of fresh tea leaves by high-temperature steaming or drying avoiding phenolic compounds’ oxidation, which include flavonols. Most of the commercially available tea comes from the leaf and bud of the plant and “about 2.5 million metric tons of dried tea are manufactured annually”; 20 % is designated for green tea, which has a higher demand in Asian countries (i.e. Japan, China, Korea, and India); around 78% is used in the manufacture of black tea which is mainly demanded by Western countries and some Asian countries; finally about 2% is for oolong tea whose target market is southeastern China. *Camellia sinensis* var *sinensis* is the most common variety

from which green tea is produced (Cabrera, Artacho, & Giménez, 2006). The green tea process initiates with a heating process to inactivate polyphenol oxidase (to avoid conversion of flavonols into dark polyphenolic compounds present in black tea). Then the rolling step is followed where leaves are cut and twisted. Different forms of green tea can be achieved depending on the variant produced. Figure 1 shows tea-processing steps for black and green tea.

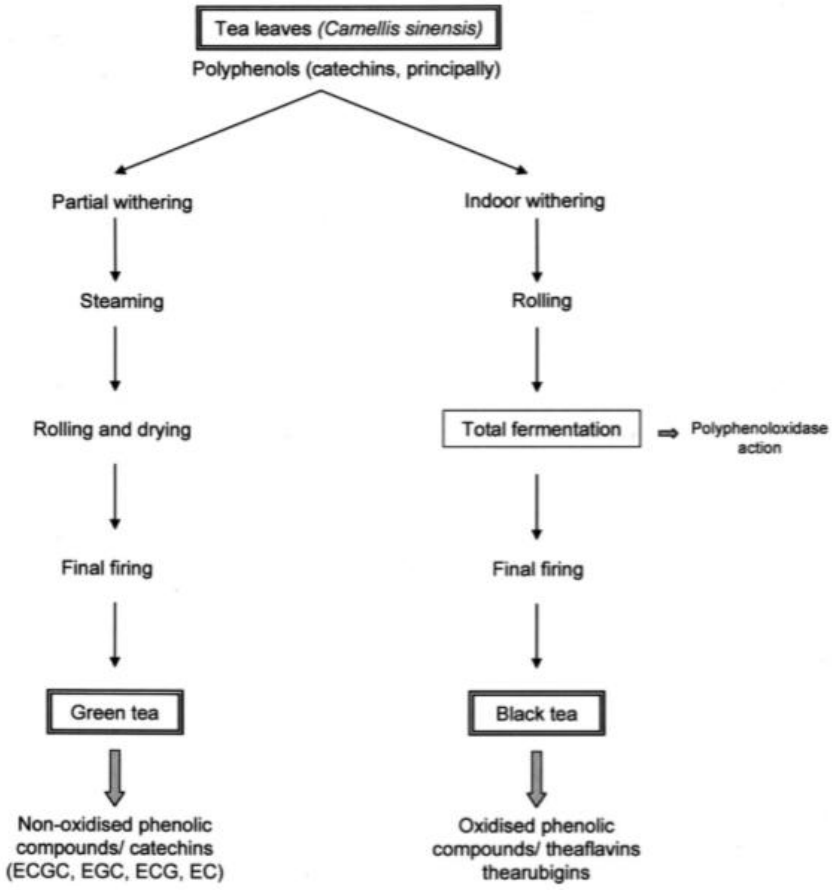


Figure 1. Black and green tea processing steps.
Source: (Cabrera et al., 2006)

2.9 Green tea health benefits

Green tea has become a popular beverage worldwide because of its special aroma and health benefits (Hamilton-Miller, 1995). In fact, “tea is the most consumed drink in the world after water” (Cabrera et al., 2006). The per capita consumption of tea was estimated as 120mL/day (McKay & Blumberg, 2002), from which approximately 20-22% is green tea (Wu, Wei, & Wilson, 2009). Green tea contains several polyphenolic compounds, which have proved to be antioxidant agents in that they prevent oxidant-induced damage to cells. Recently, the applications and chemopreventive (protective against cancer) properties of antioxidants present in fruits, vegetables, and beverages of human consumption have been widely studied (Aeschbach, Löliger, Scott, Murcia, Butler, Halliwell, & Aruoma, 1994; Armitage, Hettiarachchy, & Monsoor, 2002; De Martino, De Feo, Fratianni, & Nazzaro, 2009; Shahidi, Janitha, & Wanasundara, 1992; Tsimidou, Papavergou, & Boskou, 1995; Wanasundara & Shahidi, 1998) as they play an important role in initiation and promotion stages of multistage carcinogenesis. Several laboratory studies reported inhibitory effects of green tea polyphenols against tumor formation and growth in skin, lung, forestomach, esophagus, duodenum and small intestine, colon, liver, pancreas, and mammary glands (Mukhtar, Katiyar, & Agarwal, 1994). The chinese medicine has employed green tea for headaches, body aches, and pains, digestion, depression, detoxification, as an energizer and, broadly to prolong life. Most of the green tea human health benefits have been attributed to xanthic bases (caffeine and

theophylline), essential oils (being green tea the type of tea with the highest content), and phenolic compounds (Cabrera et al., 2006).

2.10 Green tea composition

Figure 2 shows the main epicatechins found and studied in green tea. Green tea leaves have a polyphenol content of up to 36% (dry weight basis) with catechins as the major fraction which includes (-)epicatechin (EC), (-) epicatechin gallate (ECG), (-)epigallocatechin (EGC), (-)epigallocatechin gallate (EGCG), (+)catechin and (+)gallocatechin (CC) (Ho, Ferraro, Chen, Rosen, & Huang, 1994; Huang, Ho, Wang, Ferraro, Finnegan-Olive, Lou, Mitchell, Laskin, Newmark, & Yang, 1992; Shahidi et al., 1992). The antioxidant activity of natural extracts has been extensively studied recently due to the novel tendency of the food industry to use natural bioactive compounds which have nutraceutical properties (Wambura, Yang, & Mwakatage, 2011).

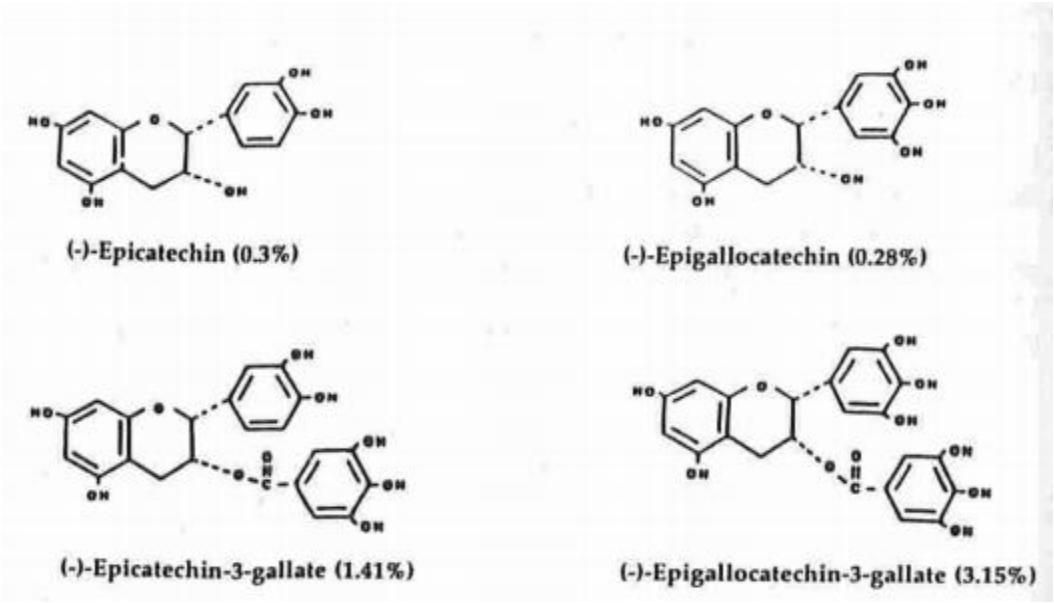


Figure 2. Major epicatechin derivatives present in green tea.
Source: Mukhtar et al. (1994)

In a study where roasted peanuts were coated with carboxymethylcellulose (CMC) incorporating rosemary extract (RROSE), tea extract (RTEA), and α-tocopherol (RTOCO) and a sonication process was applied to compare oxidation with uncoated peanuts (R), tea and rosemary extracts had better antioxidative effects on peanuts during storage than synthetic α- tocopherol. Roasted-coated and roasted-sonicated treatments with CMC incorporated with the natural extracts exhibited higher oxidative stability during storage when compared to the uncoated treatment (Wambura et al., 2011). Another study revealed Longjing tea (unfermented green tea) water extracts' strong inhibitory effect on DNA synthesis in human epidermoid carcinoma cells. In this study it was also found that green tea polyphenols and EGCG significantly inhibited tumor cell DNA synthesis and peroxyl-radical generation. The strong antioxidative and antiproliferative activities of Longjing tea water extracts and EGCG was attributed to the contents of polyphenols in tea. In addition, EGCG demonstrated significant inhibitory action on the growth of immortalized mouse embryo fibroblast cells and murine sarcoma cells (Lin, Juan, Chen, Liang, & Lin, 1996).

2.11 Green tea antioxidant activity

Green tea antioxidant activity is mainly attributed to its polyphenolic content (catechins and gallic acid, especially) carotenoids, tocopherols, ascorbic acid (vitamin C), minerals (Cr, Mn, Se, and ZN) and some phytochemical compounds are also present. These compounds may increase green tea polyphenols' (GTP) antioxidant activity. GTP has antioxidant activity *in vitro* by scavenging reactive oxygen and nitrogen species and chelating redox-active transition metal ions;

GTP is able to chelate metal ions like iron and copper to prevent their involvement in Fenton and Haber-Weiss reactions (McKay & Blumberg, 2002). Their antioxidant activity could also be addressed by 1) inhibition of the redox-sensitive transcription factors; 2) inhibiton of pro-oxidant enzymes (e.g. nitric oxide synthase, lypoxygenases, cyclooxygenases, and xanthine oxidase); and 3) induction of antioxidant enzymes such as glutathione-S-transferases and superoxide dismutases (Cabrera et al., 2006).

2.12 Green tea antimicrobial activity

In addition, GTE antimicrobial activity has also been widely reported. In a study of edible *Gelidium corneum*- gelatin (GCG) films containing grapefruit seed extract (GFSE) or GTE the quality of pork loins packed with the films during storage was assessed. Mechanical behavior (i.e. tensile strength) and WVP were improved in films with natural extracts. The films' antimicrobial activity was tested against *Escherichia coli* O157:H7 and *Listeria monocytogenes;* both extracts reduced the populations of both bacteria during storage when compared to the control (Hong, Lim, & Song, 2009). It has been reported that extracts of tea had inhibitory and bactericidal activity against *S. aureus, S. epidermidis, S. typhi, S typhimurium, S. enteriditis, S. flexneri, S. dysenteriae, and Vibrio spp.,* including *Vibrio cholera* (Masako Toda, Okubo, Hiyoshi, & Shimamura, 1989). In fact, the equivalent concentration of tea found in a cup inhibited methicillin-resistant *S. aureus (M Toda, Okubo, Hara, & Shimamura, 1991).* In another study, aqueous extracts of green tea were effective in inhibiting cariogenic streptococci.

Antibacterial activity against mouth flora has also been reported (Tsunoda, Yamazaki, & Mukai, 1991).

On the other hand, GTE has also been studied for its capacity of interfering with the sympathoadrenal system thus, contributing to weight control. In one study, GTE treatment significantly increased the 24-h energy expenditure and significantly decreased the 24-h respiratory quotient without changes in urinary nitrogen. When compared to the treatment with equivalent-caffeine-content, the later one had no effect on EE and RQ or on urinary nitrogen or catecholamines. Green tea demonstrated to have thermogenic properties promoting fat oxidation not only addressed by its caffeine content per se. GTE may be used as a functional food affecting beneficially one or more target functions in the body (Cabrera et al., 2006). In this case it demonstrated body composition control through sympathetic activation of thermogenesis, fat oxidation, or both (Dulloo, Duret, Rohrer, Girardier, Mensi, Fathi, Chantre, & Vandermander, 1999). Another study also reported that GTE standardized at 25% catechins inhibited digestive lipases and could potentially reduce fat digestion in humans (Juhel, Armand, Pafumi, Rosier, Vandermander, & Lairon, 2000).

2.13 Antioxidant - food- packaging studies

Also, there is an increasing tendency for incorporation of natural antioxidants into packaging materials to improve food quality and shelf life of food products especially for those mainly affected by oxidation. Butilated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are the most frequently used synthetic antioxidants in active packaging, but their proven toxicology brings significant

concern regarding health issues. Research has been done on the incorporation of naturals antioxidants into films and coatings in different foods susceptible to oxidation (Alma et al., 2003; Armitage et al., 2002; Gómez-Guillén, Ihl, Bifani, Silva, & Montero, 2007; Oussalah et al., 2004; Pereira, Losada, Maroto, & Cruz, 2010; Ponce et al., 2008; Siripatrawan & Harte, 2010)

2.14 Queso blanco commercial description

The Commercial Item Description (CID) for queso blanco approved by the United States Department of Agriculture (USDA) states that queso blanco is a Latin-style cheese elaborated by means of acid set or rennet –set (enzyme) process. It has a creamy white appearance and is made of pasteurized cow's milk. It has a slightly acidic flavor, is not melted when heated and can be sliced. It has a firm-curdy texture and salty taste. It is mainly used in Mexican foods and with fruits as a dessert. It must not contain any impurities, mold, soft spots or any kind of condition that has a detrimental effect in its quality**.** Color must be creamy-white uniform, bright and with no gloss. Queso blanco elaboration should not exceed 30 days prior to delivery and the shelf life when refrigerated between 0.6 to 3.3ºC (33 to 38ºF) of acid-set produced cheese shall be 1 year whereas for rennet-set produced cheese when refrigerated at the same temperature shall be 90 days (Haytowitz, Lemar, Pehrsson, Exler, Patterson, Thomas, Nickle, Williams, Showell, & Khan, 2011).

2.15 Queso blanco production

Queso blanco, which is Spanish for "white cheese" has several variations during its preparation. In general, its texture is firm, and it has a bland, mildly sweet

flavor (Carroll, 2010). Its simple preparation and organoleptic properties makes it a popular product for the dairy market although standardized, commercial manufacturing procedures for queso blanco are not defined. Composition (mainly fat, protein, salt, and moisture) among different types of queso blanco varies between countries and even between processors within a country. Moisture and salt contents are the most important variables regarding microbial growth and shelf life stability of cheese. Queso blanco has a very short shelf life and reported yields vary depending on composition of the milk, moisture content of the obtained cheese, and degree of recovery of fat and casein by the curd. Although higher moisture cheeses are recommended to achieve better economic profits, the significant relationship between microbial growth and moisture content of the cheese suggest a deeper evaluation of formulations to determine shelf life and protect consumers. During storage, small amounts of fats are hydrolyzed to butyric, caproic, and caprylic short chain fatty acids, which are responsible for the development of an aromatic flavor (Parnell-Clunies, Irvine, & Bullock, 1985a). Since queso blanco is an unripened cheese, fat plays an important role in imparting texture and nutritional characteristics to the cheese. In the study of (Arispe & Westhoff, 1984), it was observed that in lower moisture cheeses, the fat content (wet basis) increased as a consequence of moisture loss during storage; in addition, time (0, 2, 5, and 8 days) and refrigeration temperature (3 and 15 °C) during storage significantly influenced pH in cheeses.

2.16 Edible films and coatings production and applications

Edible films and coatings are thin and continuous layers of edible material employed in food components as barriers to mass transfer (moisture, gases, and solutes). Edible coatings are directly applied to the product by immersion on a liquid film forming solution or by molten compounds. Coatings are applied with brushes, by atomization with sprays, dipping, or fluidizing. On the contrary, edible films are separated structures, formed by casting or extrusion (Debeaufort & Voilley, 2009; Sorrentino et al., 2007). Both, edible films and coatings are well known barrier materials against moisture, oxygen, carbon dioxide, lipids, and flavor compounds (Tara Habig McHugh & John M Krochta, 1994a). Edible films are capable of regulating water vapor transfer in foods, which allows a longer shelf life and extended quality. Moisture is an important factor to be controlled in order to extend quality, keep freshness and crispness, and avoid microbial growth, shrinkage, loss of firmness and wilting(Yoshida & Antunes, 2004). Moreover, they represent an alternative to synthetic packaging materials contributing thus to recyclability and reduction of packaging waste (Debeaufort & Voilley, 2009; Maté & Krochta, 1996; Tara Habig McHugh & John M Krochta, 1994b).

2.17 Edible film structure

Film elaboration needs at least one component that provides a structural matrix of sufficient cohesiveness, but the functional properties are mainly determined by the nature of additives incorporated (C. Campos et al., 2011). Recent studies have performed the production of composite films by adding polysaccharides,

proteins, and lipids to the matrix in order to obtain better functionality. In addition, synergistic effect can be obtained by combining components and thus, their individual features. Still, mechanical and barrier properties depend not only on the characteristics of the polymer constituents but also on their compatibility. It is necessary to understand the mechanisms that control moisture transfer in films for designing films with barrier properties (Debeaufort & Voilley, 2009). Hence, equilibrium properties and kinetics of water transport through the film are crucial. Sorption isotherms have been extensively studied in films as they characterize the relationship between water content and its activity at constant temperature (Galus & Lenart, 2013).

2.18 Whey protein isolate

Whey protein, being a byproduct of the dairy industry can be incorporated in edible films imparting nutritional and functional properties. Whey protein fractionated composition is β- lactoglobulin (β-Lg, 57%), α-lactoalbumin (α-La, 19%), bovine serum albumin (BSA, 7%), immunoglobulins (Igs, 13%), and specific polypeptides (PP, 4%) (Maté & Krochta, 1996). Whey protein isolate (WPI) is obtained by ultra-filtration and ion-exchange to get a highly purified whey protein product (90-95% protein), being β-Lg the major fraction. Several functional properties have been reported for WPI such as, emulsifying, foaming, gelling, and barrier agent (Fairley, Monahan, German, & Krochta, 1996; Maté & Krochta, 1994; Tara Habig McHugh & John M Krochta, 1994b).

2.19 Edible films use in antimicrobial food packaging

Edible films matrix can be polysaccharides, proteins, lipids, and their blends (Debeaufort & Voilley, 2009). These materials are capable of carrying additives such as antimicrobials, antioxidants, nutraceuticals, and flavoring agents (C. A. Campos et al., 2011). Substantial efforts have been made in the past 10 years to develop and test antimicrobial films to improve food safety and shelf life of products (Nadarajah et al., 2006). A review reported that nisin was the most used antimicrobial (either by itself or in conjunction with other antimicrobials), followed by food acids and salts, chitosan, plant extracts, lysozyme and lactoperoxidase. Nisin's popularity as an antimicrobial incorporated to films is related to its allowance as a food additive and its demonstrated effect against *L. monocytogenes*. Still, inhibition of coliforms, *E. coli* or *S. enterica* was low to non-existent. Chitosan's low cost and antimicrobial activity against Gram-negative and Gram-positive bacteria and yeast and molds make it an attractive additive for incorporation in films, but variability in molecular weight and degree of deacetylation influence its antimicrobial activity. Films produced with plant extracts have been also developed (Joerger, 2007) in response to the demand for natural ingredients; spice powders, essential oils, and extracts have been incorporated in different matrices (e.g. chitosan, alginate, and proteins). In one study, casein emulsion coatings blended with turmeric extended carrots' shelf life exhibiting $\log_{10}$ reductions in total aerobic bacteria, coliforms, and yeast and mold counts of 8.3, 6.2, and 4.7 respectively (Jagannath, Nanjappa, Gupta, & Bawa, 2006). The majority of film's studies are based on the reduction of bacteria

that were inoculated onto media or foods before they were exposed to the film. Little examination of antimicrobial activity on native bacteria present in foods has been done. This is an important evaluation when determining antimicrobial effect of films because such microorganisms are involved in product spoilage. It has been showed that when some antimicrobials are combined a synergistic effect occurs. Hence, research is focused in finding cost effective and active alternatives (Joerger, 2007).

2.20 Mechanical properties and gas permeability of edible films

Protein based edible films are excellent gas and moderate moisture barriers. Nevertheless, they produce brittle films and plasticizers are needed to impart flexibility. Glycerol, sorbitol, and polyethylene glycol are common plasticizers with glycerol being most commonly used in formulation of edible films. Glycerol addition reduces tensile strength and increases elongation in all edible films. The effectiveness of plasticizers in WPI edible film (in terms of extensibility) is as follows: sorbitol> glycerol >> sodium dodecyl sulfate (SDS). But as extensibility is increased, WVP is also increased in the same order. Plasticizers mechanism of action is through the disruption of hydrogen bonds between adjacent proteins strands, thus, mobility is increased (Fairley et al., 1996; Yoshida & Antunes, 2004). Gas and WVP are affected with the addition of plasticizers, as the mobility in the chain increases, diffusion coefficients, gas and WVP also increase. A study on wheat gluten films suggested that the mechanism of action of plasticizers was due to modification of moisture uptake and glass transition temperature of films (Gontard, Guilbert, & Cuq, 1993). Extensive studies have been conducted aimed

to reduce WVP in films (Chambi & Grosso, 2011; Yoshida & Antunes, 2004). In one study, incorporation of lipids in WPI edible film was analyzed and resulted in reduced WVP and protein solubility but also lowered mechanical properties (Tara Habig McHugh & John M Krochta, 1994b; Yoshida & Antunes, 2004). Film structure is partially determined by intermolecular disulfide bonds allowing different permeabilities and mechanical properties depending on the protein fraction used in the film (Maté & Krochta, 1996).

2.21 Flaxseed oil

Flaxseed (*Linum* usitatissimum), has been historically used for oil extraction. Recently, flaxseed oil (FO) health benefits have considerably increased research interest for its incorporation in human and animal diets (Herchi, Arraez-Roman, Trabelsi, Bouali, Boukhchina, Kallel, Segura-Carretero, & Fernandez-Gutierrez, 2014). FO is a rich source of α -linolenic acid (18:3-n or ALA), which is a precursor of docosahexaenoic acid (22:6 n-3 or DHA), responsible for appropriate development of infant's brain and retina. This has led to the use of flaxseed oil as a dietary supplement for lactating women expecting increments of omega 3 fatty acids in milk (Francois, Connor, Bolewicz, & Connor, 2003). Similarly, it has also been used in ruminants' diet to increase the level of omega 3 in ruminant milk (Benchaar, Petit, Berthiaume, Whyte, & Chouinard, 2006; Chilliard, Ferlay, Mansbridge, & Doreau, 2000). Although FO has not been declared as a GRASS (generally recognized as safe) ingredient by the FDA (Food and Drug Administration), its presence in food at a maximum of 12% (w/w basis) is allowed (Basch et al., 2006). It is important to remark that FO differs in

its chemical composition from flaxseed in that FO only contains ALA fraction present in flaxseed but not fiber or lignin components. Hence, FO may have lipid-lowering properties but not the laxative and other nutraceutical properties attributed to flaxseed. Similarly, flaxseed adverse effects, possible contraindications, and interactions differ between the two. One study reported that FO, rich in ω-3 fatty acids decreased colon tumor growth in rats whereas corn oil rich in ω-6 fatty acids actually had the opposite effect (Dwivedi, Natarajan, & Matthees, 2005). In addition, ALA which is present in FO has decreased systolic and diastolic blood pressure in a previous study suggesting a pathway from which ALA has cardio-protective properties. Other nutraceutical applications of FO include but are not limited to: anti-inflammatory, by competitive inhibition of arachidonic acid (AA) by eicosapentaenoic acid (EPA) as AA that leads to production of pro-inflammatory eicosanoids (James, Gibson, & Cleland, 2000), antioxidant, and lipid and cholesterol-lowering properties (Basch et al., 2006).

2.22 References

Aeschbach, R., Löliger, J., Scott, B., Murcia, A., Butler, J., Halliwell, B., & Aruoma, O. (1994). Antioxidant actions of thymol, carvacrol, 6-gingerol, zingerone and hydroxytyrosol. *Food and Chemical Toxicology, 32*(1), 31-36.

Alma, M. H., Mavi, A., Yildirim, A., Digrak, M., & Hirata, T. (2003). Screening chemical composition and in vitro antioxidant and antimicrobial activities of the essential oils from Origanum syriacum L. growing in Turkey. *Biological and Pharmaceutical Bulletin, 26*(12), 1725-1729.

Arispe, I., & Westhoff, D. (1984). Manufacture and quality of Venezuelan white cheese. *Journal of Food Science, 49*(4), 1005-1010.

Armitage, D., Hettiarachchy, N., & Monsoor, M. (2002). Natural antioxidants as a component of an egg albumen film in the reduction of lipid oxidation in cooked and uncooked poultry. *Journal of food science, 67*(2), 631-634.

Basch, E., Bent, S., Collins, J., Dacey, C., Hammerness, P., Harrison, M., . . . Vora, M. (2006). Flax and flaxseed oil (*Linum usitatissimum*): a review by the Natural Standard Research Collaboration. *Journal of the Society for Integrative Oncology, 5*(3), 92-105.

Bech-Larsen, T. (1996). Danish consumers' attitudes to the functional and environmental characteristics of food packaging. *Journal of Consumer Policy, 19*(3), 339-363.

Benchaar, C., Petit, H., Berthiaume, R., Whyte, T., & Chouinard, P. (2006). Effects of addition of essential oils and monensin premix on digestion, ruminal fermentation, milk production, and milk composition in dairy cows. *Journal of dairy science, 89*(11), 4352-4364.

Brody, A. L. (2008). Packaging by the numbers. *Food technology, 2008*.

Cabrera, C., Artacho, R., & Giménez, R. (2006). Beneficial effects of green tea—a review. *Journal of the American College of Nutrition, 25*(2), 79-99.

Campos, C., Gerschenson, L., & Flores, S. (2011). Development of Edible Films and Coatings with Antimicrobial Activity. *Food and Bioprocess Technology, 4*(6), 849-875. doi: 10.1007/s11947-010-0434-1

Campos, C. A., Gerschenson, L. N., & Flores, S. K. (2011). Development of edible films and coatings with antimicrobial activity. *Food and Bioprocess Technology, 4*(6), 849-875.

Carroll, R. (2010). *Home Cheese Making: Recipes for 75 Homemade Cheeses*: Storey Publishing, LLC.

Chambi, H. N. M., & Grosso, C. R. F. (2011). Mechanical and water vapor permeability properties of biodegradables films based on methylcellulose, glucomannan, pectin and gelatin. *Food Science and Technology (Campinas), 31*(3), 739-746.

Chien, P.-J., Sheu, F., & Yang, F.-H. (2007). Effects of edible chitosan coating on quality and shelf life of sliced mango fruit. *Journal of Food Engineering, 78*(1), 225-229.

Chilliard, Y., Ferlay, A., Mansbridge, R. M., & Doreau, M. (2000). *Ruminant milk fat plasticity: nutritional control of saturated, polyunsaturated, trans and conjugated fatty acids.* Paper presented at the Annales de Zootechnie.

Dadalioglu, I., & Evrendilek, G. A. (2004). Chemical compositions and antibacterial effects of essential oils of Turkish oregano (*Origanum minutiflorum*), bay laurel (*Laurus nobilis*), Spanish lavender (*Lavandula stoechas L*.), and fennel (*Foeniculum vulgare*) on common foodborne pathogens. *Journal of agricultural and food chemistry, 52*(26), 8255-8260.

De Martino, L., De Feo, V., Fratianni, F., & Nazzaro, F. (2009). Chemistry, antioxidant, antibacterial and antifungal activities of volatile oils and their components. *Natural product communications, 4*(12), 1741-1750.

Debeaufort, F., & Voilley, A. (2009). Lipid-based edible films and coatings *Edible Films and Coatings for Food Applications* (pp. 135-168): Springer.

Del-Valle, V., Hernández-Muñoz, P., Guarda, A., & Galotto, M. (2005). Development of a cactus-mucilage edible coating (Opuntia ficus indica) and its application to extend strawberry (Fragaria ananassa) shelf-life. *Food Chemistry, 91*(4), 751-756.

Derraik, J. G. (2002). The pollution of the marine environment by plastic debris: a review. *Marine pollution bulletin, 44*(9), 842-852.

Du, W.-X., Avena Bustillos, R., Hua, S. S., & McHugh, T. (2011). Antimicrobial volatile essential oils in edible films for food safety. *Science against microbial pathogens: Communicating current research and technological advances, 2*, 1124-1134.

Dulloo, A. G., Duret, C., Rohrer, D., Girardier, L., Mensi, N., Fathi, M., . . . Vandermander, J. (1999). Efficacy of a green tea extract rich in catechin polyphenols and caffeine in increasing 24-h energy expenditure and fat oxidation in humans. *The American journal of clinical nutrition, 70*(6), 1040-1045.

Dwivedi, C., Natarajan, K., & Matthees, D. P. (2005). Chemopreventive effects of dietary flaxseed oil on colon tumor development. *Nutrition and cancer, 51*(1), 52-58.

Esse, R. (2002). Flexible packaging end-use market analysis. *Linthicum, Md.: Flexible Packaging Assn*.

Fairley, P., Monahan, F. J., German, J. B., & Krochta, J. M. (1996). Mechanical properties and water vapor permeability of edible films from whey protein isolate and sodium dodecyl sulfate. *Journal of Agricultural and Food Chemistry, 44*(2), 438-443.

Francois, C. A., Connor, S. L., Bolewicz, L. C., & Connor, W. E. (2003). Supplementing lactating women with flaxseed oil does not increase docosahexaenoic acid in their milk. *The American journal of clinical nutrition, 77*(1), 226-233.

Friedman, M., Buick, R., & Elliott, C. T. (2004). Antibacterial activities of naturally occurring compounds against antibiotic-resistant *Bacillus cereus* vegetative cells and spores, *Escherichia coli*, and *Staphylococcus aureus*. *Journal of Food Protection®, 67*(8), 1774-1778.

Galus, S., & Lenart, A. (2013). Development and characterization of composite edible films based on sodium alginate and pectin. *Journal of Food Engineering, 115*(4), 459-465.

Gómez-Estaca, J., Bravo, L., Gómez-Guillén, M., Alemán, A., & Montero, P. (2009). Antioxidant properties of tuna-skin and bovine-hide gelatin films induced by the addition of oregano and rosemary extracts. *Food Chemistry, 112*(1), 18-25.

Gómez-Guillén, M., Ihl, M., Bifani, V., Silva, A., & Montero, P. (2007). Edible films made from tuna-fish gelatin with antioxidant extracts of two different murta ecotypes leaves (*Ugni molinae Turcz*). *Food Hydrocolloids, 21*(7), 1133-1143.

Gontard, N., Guilbert, S., & Cuq, J. L. (1993). Water and glycerol as plasticizers affect mechanical and water vapor barrier properties of an edible wheat gluten film. *Journal of food science, 58*(1), 206-211.

Gorjão, R., Azevedo-Martins, A. K., Rodrigues, H. G., Abdulkader, F., Arcisio-Miranda, M., Procopio, J., & Curi, R. (2009). Comparative effects of DHA and EPA on cell function. *Pharmacology & therapeutics, 122*(1), 56-64.

Guilbert, S., Gontard, N., & Gorris, L. G. (1996). Prolongation of the shelf-life of perishable food products using biodegradable films and coatings. *LWT-Food Science and Technology, 29*(1), 10-17.

Hamilton-Miller, J. (1995). Antimicrobial properties of tea (*Camellia sinensis L*.). *Antimicrobial agents and chemotherapy, 39*(11), 2375.

Haytowitz, D., Lemar, L., Pehrsson, P., Exler, J., Patterson, K., Thomas, R., . . . Khan, M. (2011). USDA National Nutrient Database for Standard Reference, Release 24.

Helander, I. M., Alakomi, H.-L., Latva-Kala, K., Mattila-Sandholm, T., Pol, I., Smid, E. J., . . . von Wright, A. (1998). Characterization of the action of selected essential oil components on Gram-negative bacteria. *Journal of agricultural and food chemistry, 46*(9), 3590-3595.

Herchi, W., Arraez-Roman, D., Trabelsi, H., Bouali, I., Boukhchina, S., Kallel, H., . . . Fernandez-Gutierrez, A. (2014). Phenolic compounds in flaxseed: a review of their properties and analytical methods. An overview of the last decade. *J Oleo Sci, 63*, 7-14.

Ho, C.-T., Ferraro, T., Chen, Q., Rosen, R. T., & Huang, M.-T. (1994). *Phytochemicals in teas and rosemary and their cancer-preventive properties.* Paper presented at the ACS Symposium series.

Hong, Y. H., Lim, G. O., & Song, K. (2009). Physical properties of Gelidium corneum–gelatin blend films containing grapefruit seed extract or green tea extract and its application in the packaging of pork loins. *Journal of food science, 74*(1), C6-C10.

Huang, M.-T., Ho, C.-T., Wang, Z. Y., Ferraro, T., Finnegan-Olive, T., Lou, Y.-R., . . . Yang, C. S. (1992). Inhibitory effect of topical application of a green tea polyphenol fraction on tumor initiation and promotion in mouse skin. *Carcinogenesis, 13*(6), 947-954.

Jagannath, J. H., Nanjappa, C., Gupta, D. D., & Bawa, A. S. (2006). Studies on the stability of an edible film and its use for the preservation of carrot (*Daucus carota*). *International journal of food science & technology, 41*(5), 498-506.

James, M. J., Gibson, R. A., & Cleland, L. G. (2000). Dietary polyunsaturated fatty acids and inflammatory mediator production. *The American journal of clinical nutrition, 71*(1), 343s-348s.

Joerger, R. D. (2007). Antimicrobial films for food applications: a quantitative analysis of their effectiveness. *Packaging Technology and Science, 20*(4), 231-273.

Juhel, C., Armand, M., Pafumi, Y., Rosier, C., Vandermander, J., & Lairon, D. (2000). Green tea extract (AR25®) inhibits lipolysis of triglycerides in gastric and duodenal medium in vitro. *The Journal of nutritional biochemistry, 11*(1), 45-51.

Kidd, P. M. (2007). Omega-3 DHA and EPA for cognition, behavior, and mood: clinical findings and structural-functional synergies with cell membrane phospholipids. *Alternative medicine review, 12*(3), 207.

Kulisic, T., Radonic, A., Katalinic, V., & Milos, M. (2004). Use of different methods for testing antioxidative activity of oregano essential oil. *Food chemistry, 85*(4), 633-640.

Lagouri, V., Blekas, G., Tsimidou, M., Kokkini, S., & Boskou, D. (1993). Composition and antioxidant activity of essential oils from oregano plants grown wild in Greece. *Zeitschrift für Lebensmittel-Untersuchung und Forschung, 197*(1), 20-23.

Lambert, R., Skandamis, P. N., Coote, P. J., & Nychas, G. J. (2001). A study of the minimum inhibitory concentration and mode of action of oregano

essential oil, thymol and carvacrol. *Journal of applied microbiology, 91*(3), 453-462.

Lee, J., Park, H., Lee, C., & Choi, W. (2003). Extending shelf-life of minimally processed apples with edible coatings and antibrowning agents. *LWT-Food Science and Technology, 36*(3), 323-329.

Lin, Y.-L., Juan, I.-M., Chen, Y.-L., Liang, Y.-C., & Lin, J.-K. (1996). Composition of polyphenols in fresh tea leaves and associations of their oxygen-radical-absorbing capacity with antiproliferative actions in fibroblast cells. *Journal of Agricultural and Food Chemistry, 44*(6), 1387-1394.

Maté, J. I., & Krochta, J. M. (1994). β-Lactoglobulin Separation from Whey Protein Isolate on a Large Scale. *Journal of food science, 59*(5), 1111-1114.

Maté, J. I., & Krochta, J. M. (1996). Comparison of oxygen and water vapor permeabilities of whey protein isolate and β-lactoglobulin edible films. *Journal of agricultural and food chemistry, 44*(10), 3001-3004.

McHugh, T. H., & Krochta, J. M. (1994a). Sorbitol-vs glycerol-plasticized whey protein edible films: integrated oxygen permeability and tensile property evaluation. *Journal of Agricultural and Food Chemistry, 42*(4), 841-845.

McHugh, T. H., & Krochta, J. M. (1994b). Water vapor permeability properties of edible whey protein-lipid emulsion films. *Journal of the American Oil Chemists' Society, 71*(3), 307-312.

McKay, D. L., & Blumberg, J. B. (2002). The role of tea in human health: an update. *Journal of the American College of Nutrition, 21*(1), 1-13.

Milos, M., Mastelic, J., & Jerkovic, I. (2000). Chemical composition and antioxidant effect of glycosidically bound volatile compounds from oregano (*Origanum vulgare L. ssp. hirtum*). *Food Chemistry, 71*(1), 79-83.

Mukhtar, H., Katiyar, S. K., & Agarwal, R. (1994). Green tea and skin--anticarcinogenic effects. *Journal of investigative dermatology, 102*(1), 3-7.

Nadarajah, K., Prinyawiwatkul, W., No, H. K., Sathivel, S., & Xu, Z. (2006). Sorption behavior of crawfish chitosan films as affected by chitosan extraction processes and solvent types. *Journal of Food Science, 71*(2), E33-E39.

Oussalah, M., Caillet, S., Salmiéri, S., Saucier, L., & Lacroix, M. (2004). Antimicrobial and antioxidant effects of milk protein-based film containing essential oils for the preservation of whole beef muscle. *Journal of Agricultural and Food Chemistry, 52*(18), 5598-5605.

Park, H. J. (1999). Development of advanced edible coatings for fruits. *Trends in Food Science & Technology, 10*(8), 254-260.

Parnell-Clunies, E. M., Irvine, D., & Bullock, D. (1985). Composition and yield studies for Queso Blanco made in pilot plant and commercial trials with dilute acidulant solutions. *Journal of Dairy Science, 68*(11), 3095-3103.

Paster, N., Menasherov, M., Ravid, U., & Juven, B. (1995). Antifungal activity of oregano and thyme essential oils applied as fumigants against fungi attacking stored grain. *Journal of Food Protection®, 58*(1), 81-85.

Pereira, D., Losada, P. P., Maroto, J., & Cruz, J. (2010). Evaluation of the effectiveness of a new active packaging film containing natural antioxidants (from barley husks) that retard lipid damage in frozen Atlantic salmon (*Salmo salar L.*). *Food Research International, 43*(5), 1277-1282.

Peressini, D., Bravin, B., Lapasin, R., Rizzotti, C., & Sensidoni, A. (2003). Starch–methylcellulose based edible films: rheological properties of film-forming dispersions. *Journal of Food Engineering, 59*(1), 25-32.

Petersen, K., Væggemose Nielsen, P., Bertelsen, G., Lawther, M., Olsen, M. B., Nilsson, N. H., & Mortensen, G. (1999). Potential of biobased materials for food packaging. *Trends in Food Science & Technology, 10*(2), 52-68.

Ponce, A. G., Roura, S. I., del Valle, C. E., & Moreira, M. R. (2008). Antimicrobial and antioxidant activities of edible coatings enriched with natural plant extracts: *in vitro* and *in vivo* studies. *Postharvest biology and technology, 49*(2), 294-300.

Robertson, G. L. (2012). *Food packaging: principles and practice*: CRC press.

Rojas-Graü, M. A., Raybaudi-Massilia, R. M., Soliva-Fortuny, R. C., Avena-Bustillos, R. J., McHugh, T. H., & Martín-Belloso, O. (2007). Apple puree-alginate edible coating as carrier of antimicrobial agents to prolong shelf-life of fresh-cut apples. *Postharvest Biology and Technology, 45*(2), 254-264.

Royo, M., Fernández-Pan, I., & Maté, J. I. (2010). Antimicrobial effectiveness of oregano and sage essential oils incorporated into whey protein films or cellulose-based filter paper. *Journal of the Science of Food and Agriculture, 90*(9), 1513-1519.

Sargent, J., Bell, G., McEvoy, L., Tocher, D., & Estevez, A. (1999). Recent developments in the essential fatty acid nutrition of fish. *Aquaculture, 177*(1), 191-199.

Shahidi, F., Janitha, P., & Wanasundara, P. (1992). Phenolic antioxidants. *Critical Reviews in Food Science & Nutrition, 32*(1), 67-103.

Shaw, N., Monahan, F., O'riordan, E., & O'sullivan, M. (2002). Physical properties of WPI films plasticized with glycerol, xylitol, or sorbitol. *Journal of Food Science, 67*(1), 164-167.

Siripatrawan, U., & Harte, B. R. (2010). Physical properties and antioxidant activity of an active film from chitosan incorporated with green tea extract. *Food Hydrocolloids, 24*(8), 770-775.

Sorrentino, A., Gorrasi, G., & Vittoria, V. (2007). Potential perspectives of bio-nanocomposites for food packaging applications. *Trends in Food Science & Technology, 18*(2), 84-95.

Teixeira, B., Marques, A., Ramos, C., Serrano, C., Matos, O., Neng, N. R., . . . Nunes, M. L. (2013). Chemical composition and bioactivity of different oregano (Origanum vulgare) extracts and essential oil. *Journal of the Science of Food and Agriculture*.

Toda, M., Okubo, S., Hara, Y., & Shimamura, T. (1991). Antibacterial and bactericidal activities of tea extracts and catechins against methicillin resistant *Staphylococcus aureus*. *Nihon saikingaku zasshi. Japanese journal of bacteriology, 46*(5), 839-845.

Toda, M., Okubo, S., Hiyoshi, R., & Shimamura, T. (1989). The bactericidal activity of tea and coffee. *Letters in applied microbiology, 8*(4), 123-125.

Tsimidou, M., Papavergou, E., & Boskou, D. (1995). Evaluation of oregano antioxidant activity in mackerel oil. *Food research international, 28*(4), 431-433.

Tsunoda, T., Yamazaki, T., & Mukai, I. (1991). Oral preparations containing catechins for periodontitis and halitosis control. *Japanese patent*.

Viuda-Martos, M., Ruiz-Navajas, Y., Fernández-López, J., & Pérez-Álvarez, J. (2007). Antifungal activities of thyme, clove and oregano essential oils. *Journal of Food Safety, 27*(1), 91-101.

Wambura, P., Yang, W., & Mwakatage, N. R. (2011). Effects of sonication and edible coating containing rosemary and tea extracts on reduction of peanut lipid oxidative rancidity. *Food and Bioprocess Technology, 4*(1), 107-115.

Wanasundara, U. N., & Shahidi, F. (1998). Antioxidant and pro-oxidant activity of green tea extracts in marine oils. *Food Chemistry, 63*(3), 335-342.

Wu, C., Wei, G., & Wilson, M. (2009). Tea as a functional food for oral health. *Food constituents and oral health: current status and future prospects*, 396-417.

Yaman, Ö., & Bayoındırlı, L. (2002). Effects of an edible coating and cold storage on shelf-life and quality of cherries. *LWT-Food science and Technology, 35*(2), 146-150.

Yoshida, C., & Antunes, A. (2004). Characterization of whey protein emulsion films. *Brazilian Journal of Chemical Engineering, 21*(2), 247-252.

Zinoviadou, K. G., Koutsoumanis, K. P., & Biliaderis, C. G. (2009). Physico-chemical properties of whey protein isolate films containing oregano oil and their antimicrobial action against spoilage flora of fresh beef. *Meat Science, 82*(3), 338-345.

Zivanovic, S., Chi, S., & Draughon, A. F. (2005). Antimicrobial activity of chitosan films enriched with essential oils. *Journal of food science, 70*(1), M45-M51.

CHAPTER 3. PRODUCTION AND CHARACTERIZATION OF WHEY PROTEIN ISOLATE EDIBLE FILMS CONTAINING OREGANO (*ORIGANUM VULGARE*) ESSENTIAL OIL AND/OR GREEN TEA (*CAMELLIA SINENSIS*) EXTRACT

3.1 Introduction

As an increase in the demand for natural and biodegradable products have occurred recently, protective coatings and edible films have gained attention as potential packaging that can increase the shelf life of food-products (Chien et al., 2007; Del-Valle et al., 2005; Guilbert et al., 1996; Peressini et al., 2003; Rojas-Graü et al., 2007; Yaman & Bayoındırlı, 2002). As conventional packaging materials have a negative impact on the environment (Bech-Larsen, 1996; Derraik, 2002), efforts in developing bio-based, degradable packaging materials is encouraged for recent generations. On the other hand, natural oils are similarly added to products in order to improve flavor and impart antimicrobial, antioxidant, and nutraceuticals properties. Moreover, essential oils (EO's) have also been applied to packaging during their development creating active packaging. It is desired that the compounds are in contact with the target region of the food (mainly surface where spoilage microorganism grow) (Royo et al., 2010).

Oregano essential oil (OEO) has proven to be an efficient antimicrobial mainly due to its carvacrol content, which acts by destroying the cytoplasmic membranes of microbes causing changes in their permeability and barrier-function causing a loss of energy until the cell dies (Seydim & Sarikus, 2006). Green tea, which is rich in polyphenolic compounds, has antioxidant properties (Cabrera et al., 2006; Hong et al., 2009; Siripatrawan & Harte, 2010). Moreover, anticarcinogenic, antimicrobial, and several other health benefits have also been attributed to green tea (Dulloo et al., 1999; Huang et al., 1992; Juhel et al., 2000; Mukhtar et al., 1994). Green Tea Extract (GTE) has also

been extensively reported with nutraceutical properties including antioxidant (Henning, Niu, Lee, Thames, Minutti, Wang, Go, & Heber, 2004), antibacterial (Sakanaka, Kim, Taniguchi, & Yamamoto, 1989), antiobesity (Chantre & Lairon, 2002), cholesterol-lowering and cardio-protective (Nagao, Hase, & Tokimitsu, 2007). These functional properties of plant EO's make the ideal ingredients that can be incorporated into a whey-protein-isolate (WPI) edible film. Yet, addition of essential oils into films can change its barrier properties like Water Vapor Permeability (WVP) and tensile properties for which a characterization is needed and may cause changes in the food regarding its shelf-life stability and organoleptic characteristics (C. Campos et al., 2011; Chambi & Grosso, 2011; Debeaufort & Voilley, 2009; Fang, Tung, Britt, Yada, & Dalgleish, 2002; Fernández-Pan, Royo, & Ignacio Maté, 2012; Gounga, Xu, & Wang, 2007; Guilbert et al., 1996; Joerger, 2007; Maté & Krochta, 1996; Parris & Coffin, 1997; Shaw et al., 2002; Sobral, Menegalli, Hubinger, & Roques, 2001; Wang, Auty, & Kerry, 2010; Yoshida & Antunes, 2004; Zinoviadou et al., 2009; Zivanovic et al., 2005).

The objective of this project was to produce and characterize WPI- edible films containing OEO and/or GTE.

3.2 Materials and methods

3.2.1 Elaboration of edible films

Whey protein isolate (WPI) powder (BiPRO Davisco Foods International, Inc. Eden Prairie, MN), green tea extract (GTE) alcohol free supplement (The Vitamin Shoppe, Baton Rouge, LA), and oregano essential oil (OEO) dietary supplement (Global Healing Center ND Operations, LTD. Houston, TX) were purchased to prepare edible film solutions. The method of Royo et al. (2010) was used to elaborate WPI edible films. A

whey-protein-isolate-film-solution (WPIS) was prepared by dissolving 10 g of WPI-powder in 100mL of deionized water with 5 g of glycerol (plasticizer) and stirring until dissolved. This solution was heated to 90 ºC for 30 min while stirring. After heating, filtration with cheese cloth was performed to avoid lumps. Film solutions, WPIS with GTE (WPIGS), WPIS with GTE plus OEO (WPIGOS), and WPIS with OEO (WPIOS), were separately prepared by adding 3g GTE, 1.5g GTE plus 1.5g OEO, and 3g OEO to the 100mL WPIS, respectively followed by homogenization at 28,979 x *g* with an homogenizer (UltraShear M, Omni International). WPIGS, WPIGOS, and WPIOS were filtered with cheese cloth to eliminate any insoluble solids present in the GTE or OEO. After that, WPIS, WPIGS, WPIGOS, and WPIOS were submitted to vacuum conditions in a rota-vapor distillation unit to remove bubbles. Aliquots of 8.15 g of WPIS, WPIGS, WPIGOS, and WPIOS were poured into polyethylene Petri dishes of 8.5 cm diameter and dried at 21 °C for 46 h to produce edible films WPIF (WPI edible film), WPIGF (WPI edible film with GTE), WPIGOF (WPI edible film with GTE and OEO), and WPIOF (WPI edible film with OEO), respectively. Peeling of films was performed using a spatula and were stored in an incubator at 25°C and 50% RH (REVCO Kenner, LA) until analyses were performed.

3.2.2 Thickness and density of edible films

Thickness of WPIF, WPIGF, WPIGOF, and WPIOF were measured using a Mitutoyo digital thickness gage (Quick Mini 25, Alfa Mirage Co. Ltd, Osaka, Japan). Measurements were performed at five different random positions around each film and by triplicates of each film were prepared. Mean values were calculated. Films' densities were determined from film weight and volume using an analytical balance (Sartorius ED

224s Data Weighting Systems, Inc., Elk Grove IL, USA). Volumes of films were determined from the areas and thicknesses of films.

3.2.3 Whiteness index, yellowness index, total color difference, opacity, and light transmittance of edible films

The method of *Ekthamasut and Akesowan (2001)* was used to evaluate color of WPIF, WPIGF, WPIGOF, and WPIOF using a Scan XE colorimeter (Hunter Associates Laboratory Inc., Reston, VA). The colorimeter was standardized using white and black tiles. Films were placed on the white standard plate (L= 93.54, a=-0.87, and b=0.73). Values for L* (lightness), a* (redness), and b* (yellowness) were measured. Whiteness index (WI), yellowness index (YI), and total color difference (∆E) were calculated using the equations 1, 2, and 3, respectively:

$$WI = 100 - [(100 - L)^2 + a^2 + b^2]^{0.5} \quad (1)$$

$$YI = \left(142.86\, x\, \frac{b}{L}\right) \quad (2)$$

$$\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{0.5} \quad (3)$$

Where $\Delta L = L_{standard} - L_{sample}$; $\Delta a = a_{standard} - a_{sample}$; $\Delta b = b_{standard} - b_{sample}$.

Measurements were performed by triplicate on each film (WPIF, WPIGF, WPIGOF, and WPIOF).

Opacity was determined according to the method of *Siripatrawan and Harte (2010)* using an UV spectrophotometer (Thermo Scientific GENESYS 20, Thermo Fisher Scientific, Inc., Madison, WI, USA) with absorbance at 600 nm. First, an empty cuvette was used as a blank. Then, triplicates of each type of film were cut into rectangular shape and placed directly into a cuvette. The opacity of films was calculated according to equation 4:

$$T = \frac{Abs_{600}}{x} \qquad (4)$$

Where T = transparency; Abs= value of absorbance at 600 nm; and x= film thickness (mm). By means of this equation greater values of T represent lower transparency and more opacity.

Light transmittance of edible films was determined according to the method described by Song, Zhou, Fu, Chen, and Wu (2013). Films were cut into rectangles of 5 cm x 2 cm (length, width) and placed into cuvettes. Films' transmittance was determined by triplicate at wavelengths from 350 to 800 nm using a spectrophotometer (Thermo Scientific GENESYS 20, Thermo Fisher Scientific, Inc., Madison, WI, USA) in order to test barrier effect of films against UV and visible light, which have been recognized as prooxidant agents (Damodaran, Parkin, & Fennema, 2007). An empty cuvette was used as a blank.

3.2.4 Moisture content, water absorption, water solubility, and water activity of edible films

Moisture content of WPIF, WPIGF, WPIGOF, and WPIOF was determined using a vacuum-oven (NATIONAL APPLIANCE COMPANY, Model 5851, IL, USA). Films were dried at 60 °C for 24 h. Moisture content (wet basis) of films was calculated according to equation 5.

$$MC_{wb} = \left[\frac{(Ms+d)-(Mds+d)}{Ms}\right][100] \qquad (5)$$

Where MC_{wb}= moisture content wet basis (%); Ms= mass of moist film (g), d= mass of aluminum dish (g); and Mds= mass of dry solids of film (g).

Water absorption of WPIF, WPIGF, WPIGOF, and WPIOF was determined according to the method of Song et al. (2013) using distilled water at room temperature. Film

samples were cut into squares of 25 mm x 25 mm and weighed (w_1, g). Then, films squares were immersed in distilled water at 25 °C for 2 min. Wet film squares were then wiped with a filter paper (Whatman 2) to remove the excess of water and re-weighed (w_2, g). The percentage of water absorbed was calculated according to equation 6:

$$WA = \left[\frac{(w_2 - w_1)}{w_1}\right] [100] \quad (6)$$

Where WA = water absorption (%); w_1 =weight of film before water immersion (g); and w_2= weight of wet film (g).

Water solubility of WPIF, WPIGF, WPIGOF, and WPIOF was determined according to the method of Gounga et al. (2007). Film samples were weighed into aluminum crucibles and dried at 105 °C in a forced-air-convection oven (VWR Scientific 1330FM Sheldon Manufacturing Inc, Cornelius, OR, USA) for 24 h. Moisture content wet basis was determined by expressing moisture loss as a percentage of initial film weight. Dried film samples (0.2 g) were placed into 100 mL beakers and immersed into 40 mL of distilled water at 25 °C for 24 h with initial agitation of 10 min with a magnetic bar stirrer. After 24 h beakers were poured into pre-weighed No. 1 Whatman filter paper and placed into pre-weighed aluminum crucibles and dried at 105 °C for 24 h to determine the weight of undissolved film. Film solubility in water was calculated by determining the film dry matter solubilized after 24 h immersion in distilled water and dividing by the initial dry film weight and multiplying by 100. The weight of dissolved film was calculated as the difference between initial dry matter film and the undissolved dry matter.

Water activity of WPIF, WPIGF, WPIGOF, and WPIOF was measured by triplicate on each film using a water activity meter (Decagon devices, Inc. Pullman, WA). Films were cut from the center into squares of 25 mm x 25 mm and placed in 15 ml-sample cups.

3.2.5 Water vapor permeability of edible films

Water vapor permeability (WVP) of WPIF, WPIGF, WPIGOF, and WPIOF was determined using the ASTM gravimetric standard method E96-95 (Standard, 1995). Films were separately placed on the top of test cells containing anhydrous calcium sulfate (W.A Hammond Drierite Co. Ltd. Xenia, OH, USA) as a desiccant. Test cells were placed in a chamber with controlled temperature and relative humidity (32 °C and 50% RH, respectively). Test cells with desiccant were weighed at 0 h and every 2 h for 27 hours using an analytical balance (Pioneer ™, Ohaus Corp. Pine Brook, NJ USA). Acquired weight was used to calculate the water vapor transmission (WVT), Permeance (P), and WVP according to equations 7, 8, and 9, respectively. Five replicates and a dummy specimen (no desiccant added) of each film were used to determine the average value of WVP.

$$WVT = \frac{G}{tA} = (\frac{G}{t})/A \qquad (7)$$

Where WVT= rate of water vapor transmission (g/hm^2); G= weight change (from the straight line, g); t = time (h); A = test area (cup mouth area, m^2); and G/t = slope of the straight line (g/h).

$$P = \frac{WVT}{\Delta p} = WVT/S(R_1 - R_2) \qquad (8)$$

Where P= Permeance (g/Pa s m^2); WVT= rate of water vapor transmission (g/hm^2); Δp = vapor pressure difference (4.771 x 10^3 Pa); S = Saturation vapor pressure at test temperature (4.771 x 10^3 Pa); R_1 = relative humidity at the source expressed as a fraction (test chamber); and R_2 = relative humidity at the vapor sink expressed as a fraction.

$$WVP = Px \qquad (9)$$

Where P= Permeance (g/Pa s m^2) and x= thickness (μm).

3.2.6 Mechanical properties of edible films

WPIF, WPIGF, WPIGOF, and WPIOF were preconditioned and tested under controlled conditions (23 °C and 50% relative humidity) following the ASTM standard method for tensile properties of thin plastic sheeting in order to avoid variations in films' properties due to extrinsic factors. Films were cut into strips of 20 mm wide and 70 mm long. Thickness of each film sample was taken as the average of five measurements made at random positions on the equilibrated samples using a Mitutoyo digital thickness gage (Quick Mini 25, Alfa Mirage Co. Ltd, Osaka, Japan). Both ends of the strips were placed between stainless steel grips mounted with double-sided adhesive tape closing the grips until strips were tighten allowing accurate alignment in the jaws of the Instron grips. Final film area exposed between the grips was 34 x 20 mm (length x width). An Instron Universal testing instrument Model (Instron Co. Canton, MA, USA) was used to determine the tensile properties of film according to the static weighting, constant-rate-of-grip separation standard testing method D882 (Standard, 2002). The Instron instrument was programmed to tensile mode and the film samples were clamped into the screw-action grips of the tensile clamps. A 2 kN load cell was use and initial grip separation was set at 34 mm and crosshead speed at 15 $mm.min^{-1}$. At least 15 strips of each film type were tested and percent elongation at break (EB), nominal tensile strength (TS), and elastic modulus (EM) were calculated as described in the ASTM D882 (Standard, 2002). EB was expressed as a percentage and calculated by dividing the extension of the film at the moment of rupture (mm) by the initial gage length of the film (34 mm) and multiplying by 100. TS was expressed in Mega Pascals (MPa) and

calculated by dividing the maximum load (N) by the original cross-sectional area (m^2) of the film. EM was expresses in MPa and calculated from the tensile stress-strain curve by the computer software.

3.2.7 Microstructure of edible films

Scanning electron microscopy (JSM-6610LV Scanning-Electron Microscope) was used to examine the microstructure and cross-sectional area of WPIF, WPIGF, WPIGOF, and WPIOF. The method of Tara Habig McHugh and John M Krochta (1994b) was followed for edible films preparation. Films were first fractured under liquid nitrogen. Then, films were sectioned and mounted on aluminum stubs with double-stick tape and silver paint. Sputter coating with gold-palladium alloy was done at 20 mA.

3.2.8 Statistical Analysis

The collected data were analyzed using Statistical Analysis System (SAS, Version 9.2, SAS Institute Inc., Cary, NC., USA). Triplicate experiments were conducted and Tukey's studentized range mean separation test was used to detect statistical differences (α=0.05).

3.3 Results and discussion

3.3.1 Thickness and density of edible films

Thickness and density of WPIF, WPIGF, WPIGOF, and WPIOF are shown in Table 2. These results suggest that incorporation of EO´s to a WPI film matrix increases thickness and decreases density of the films. Still, significant ($p<0.05$) increase in thickness was observed only for WPIGF and WPIGOF. Although a negative correlation ($R=-0.90$) was observed between thickness and density of films, only WPIGOF was significantly ($p<0.05$) less dense than the other films, which may have resulted from

reduced polymer interactions in films when GTE and OEO were added and is in accordance with the mechanical behavior and observed microstructure of WPIGOF. Similar results were found by Siripatrawan and Harte (2010) in their study performed on active chitosan films incorporated with GTE. In the study, only GTE concentrations of 5, 10, and 20% were significantly more dense than the control; in our study, GTE and OEO concentrations are 3% (w/v) alone and when combined 1.5% and 1.5% (w/v), respectively.

Table 2. Thickness and density of edible films

Treatment	Thickness[1]	Density[2]
WPIF	186.67 ± 10.07^{B}	1.10 ± 0.06^{A}
WPIGF	232.00 ± 2.00^{A}	1.04 ± 0.02^{A}
WPIGOF	250.67 ± 17.01^{A}	0.89 ± 0.05^{B}
WPIOF	201.33 ± 6.11^{B}	1.06 ± 0.03^{A}

Means with different letters (AB) within columns are significantly different (P<0.05).
WPIF= whey-protein-isolate-film, WPIGF= WPIF with green tea extract (GTE), WPIGOF= WPIF with GTE plus oregano essential oil (OEO), WPIOF= WPIF with OEO.
[1]= (μm), [2]= (g/cm^3).

3.3.2 Whiteness index, yellowness index, total color difference, opacity, and transmittance of edible films

The effects of GTE and/or OEO addition on color and opacity of WPIF, WPIGF, WPIGOF, and WPIOF are presented in Table 3. According to these results, incorporation of GTE resulted in a significant ($p<0.05$) decrease of whiteness index (WI) and significant ($p<0.05$) increase in yellowness index (YI) for both treatments, WPIGF and WPIGOF when compared to WPIF and WPIOF; whereas WI of WPIOF was not significantly ($p>0.05$) different when compared to WPIF. Similarly, although YI values for WPIOF were higher than those for WPIF, WPIOF was not significantly ($p>0.05$) different on YI when compared to WPIF. Total color difference (ΔE) showed that when compared

to a white standard dish, WPIF and WPIOF are not significantly different ($p>0.05$) but WPIGF and WPIGOF are ($p<0.05$). Regarding opacity of films, WPIF was transparent and not significantly ($p>0.05$) different from WPIGF probably because the level of addition of GTE was low (3% w/v). However, when comparing WPIF and WPIGF with WPIGOF and WPIOF, WPIGOF and WPIOF were significantly ($p<0.05$) more opaque.

Table 3.Whiteness index, yellowness index, total color difference, and opacity of edible films

Treatment	WI[1]	YI[2]	ΔE[3]	Opacity
WPIF	90.29 ± 0.12^{A}	1.83 ± 0.10^{B}	3.16 ± 0.13^{B}	0.21 ± 0.06^{B}
WPIGF	61.07 ± 8.08^{B}	54.09 ± 16.27^{A}	34.26 ± 8.15^{A}	0.47 ± 0.21^{B}
WPIGOF	57.48 ± 6.62^{B}	63.87 ± 13.38^{A}	38.43 ± 6.66^{A}	1.50 ± 0.47^{A}
WPIOF	81.37 ± 0.56^{A}	20.83 ± 0.73^{B}	13.89 ± 0.49^{B}	1.90 ± 0.24^{A}

Means with different letters (AB) within columns are significantly different ($P<0.05$).
WPIF= whey-protein-isolate-film, WPIGF= WPIF with green tea extract (GTE), WPIGOF= WPIF with GTE plus oregano essential oil (OEO), WPIOF= WPIF with OEO.
WI[1]= Whiteness index, YI[2]= Yellowness index, ΔE[3]= Total color difference.

This means that addition of OEO imparted greater opacity to WPI edible films thereby conferring a greater barrier to light-induced oxidation. Another study testing GTE at different concentrations (2, 5, 10, and 20 % w/v) to chitosan films also reported a decrease in L value (lightness), increase in a values (tendency towards redness), and b values (tendency towards yellowness), and increase in opacity as GTE concentration increased.(Siripatrawan & Harte, 2010) Furthermore, previous studies have also reported improved light barrier properties of films when adding antioxidant compounds (Gómez-Estaca et al., 2009a; Gómez-Estaca, Montero, Fernández-Martín, Alemán, & Gómez-Guillén, 2009b; Gómez-Guillén et al., 2007; Hong et al., 2009). Greater film opacity containing GTE and/or OEO is directly attributed to the presence of polyphenols

and to possible polyphenol-protein interactions (Gómez-Estaca et al., 2009b; Gómez-Guillén et al., 2007). Figure 3 shows the results of light transmittance measurements versus wavelength of edible films. WPIGOF showed the least transmittance at all the wavelength tested indicating less passing of light whereas WPIF having no oil incorporated showed the greatest light transmittance. Films containing OEO showed less light transmittance (WPIOF) than those containing GTE (WPIGF) at all the wavelengths tested except at 750 nm, at which transmittances were similar. These results indicated that as GTE and/or OEO were added, films became less transparent and homogeneous. These results are in accordance with Table 3, where opacity values of WPIGOF and WPIOF were significantly ($p<0.05$) greater than those for WPIF and WPIGF. Similar results were found by Gómez-Guillén et al. (2007) who reported improved light barrier properties (especially against UV light, which induces oxidation) in edible films when incorporating antioxidant extracts.

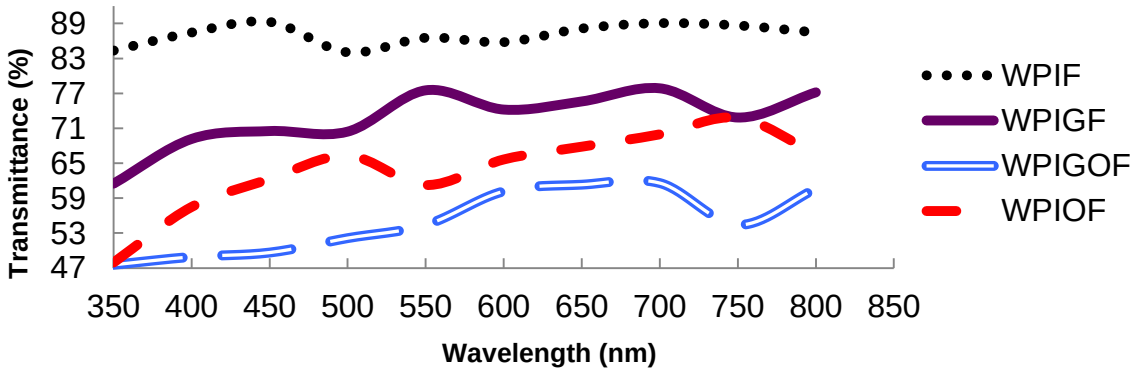


Figure 3. Changes in transmittance at wavelengths ranging from 350 to 800 nm of edible films. WPIF= whey-protein-isolate-film, WPIGF= WPIF with green tea extract (GTE), WPIGOF= WPIF with GTE plus oregano essential oil (OEO), WPIOF= WPIF with OEO.

Other studies have also reported UV-light barrier properties for WPI films (Fang et al., 2002; Gounga et al., 2007) and applicability as see-through coating and/or packaging

material to prevent changes in color and lipid oxidation (Gounga et al., 2007).

3.3.3 Moisture content, water absorption, water solubility, and water activity of edible films

Percentage moisture content wet basis (MC), percentage of water absorption (WA), percentage water solubility (WS), and water activity (a_w) of WPIF, WPIGF, WPIGOF, and WPIOF are shown in Table 4. WPIGOF had the highest ($p<0.05$) MC. Similarly, WPIOF MC was significantly ($p<0.05$) higher than WPIGF and WPIF, which exhibited the lowest MC ($p<0.05$).

Table 4. Moisture content, water absorption, water solubility, and water activity of edible films

Treatment	MC[1]	WA[2]	WS[3]	a_w[4]
WPIF	15.68± 0.61^{C}	139.29 ± 11.45^{C}	36.03 ± 0.78^{A}	0.69 ± 0.02^{A}
WPIGF	15.86± 0.78^{C}	178.06± 16.65^{B}	38.55 ± 1.47^{A}	0.58 ± 0.01^{B}
WPIGOF	27.70± 0.58^{A}	260.04 ± 0.50^{A}	36.75 ± 1.47^{A}	0.53 ± 0.01^{C}
WPIOF	23.94± 0.88^{B}	144.62 ± 4.55^{C}	35.98 ± 0.86^{A}	0.53 ± 0.01^{C}

Means with different letters (AB) within columns are significantly different (P<0.05).
WPIF= whey-protein-isolate-film, WPIGF= WPIF with green tea extract (GTE), WPIGOF= WPIF with GTE plus oregano essential oil (OEO), WPIOF= WPIF with OEO
MC[1] = Percentage of moisture content wet basis, WA[2]= Percentage of water absorption, WS[3]= Percentage water solubility, a_w[4]= Water activity.

These may be attributed to the high content of aromatic compounds present in GTE and OEO rather than to high water content (as GTE and OEO are not hydrophilic or hygroscopic). OEO contained more termolabile compounds than GTE, which were evaporated at the conditions set for the analysis (60 °C/ vacuum) in a greater amount giving a lower weight at the end point of the analysis which was accounted as water loss. Regarding water absorption (WA) of films, WPIGOF exhibited the highest WA ($p<0.05$) followed by WPIGF. On the contrary, WPIOF and WPIF exhibited the lowest WA ($p<0.05$); this could be ascribed to a change in the structure of whey protein molecules when heated and subsequently denatured, which caused greater exposure of

hydrophobic groups. It has been previously documented that during film formation, S-S bonds are formed through SH/S-S interchange reactions which leads to the appearance of a matrix that entraps water, which was observed when analyzing the microstructure of films in section 3.3.6 (Gounga et al., 2007; McHugh, Avena-Bustillos, & Krochta, 1993; Tara Habig McHugh & John M. Krochta, 1994; Pérez-Gago, Nadaud, & Krochta, 1999). The previous theory does not apply for WPIGF and WPIGOF in which addition of GTE and GTE plus OEO, respectively, resulted in an increased WA. This phenomenon could be explained by possible interference/interaction of GTE with intermolecular disulfide bonds, which are formed during protein denaturation conferring to some extent hydrophilic characteristics even when OEO was also present. Another additional explanation could be that addition of GTE contributed to more hydrogen bonds rather than to hydrophobic interactions. Films' WS was not significantly ($p > 0.05$) different and was relatively low as the films maintained their integrity during the 24 h immersion treatment. This indicated that the network was highly stable and only small-non-protein particles were solubilized. These results indicate that addition of GTE and OEO didn't affect the low solubility of WPIF, which has been attributed to disulfide bonds formed during the heat denaturation of the protein (Gounga et al., 2007). On the contrary, some studies have reported decreased protein solubility by the addition of fatty acids that caused structural changes at the protein-lipid interface (Yoshida & Antunes, 2004). Low water solubility is desirable if the film will be used for high moisture-content foods or will be in contact with water. Low water affinity is required for dry foods that will be rehydrated or dissolved in water (Guilbert & Biquet, 1989). On the other hand, WPIGOF and WPIOF presented the lowest ($p < 0.05$) a_w suggesting that both could potentially

represent a stronger barrier than WPIGF and WPIF for microbial development. Addition of OEO to WPI edible films decreased a_w in a greater scale than GTE. However, addition of GTE also reduced ($p<0.05$) a_w when compared to WPIF.

3.3.4 Water vapor permeability of edible films

Water vapor permeability (WVP) was measured on WPIF, WPIGF, WPIGOF, and WPIOF to test their barrier properties against water vapor. WVP studies are performed on food packages because water decreases shelf life of foods (Karel, 1980; Maltini, Torreggiani, Venir, & Bertolo, 2003) by changing the texture, creating a profitable environment for microbial growth, and by acting like a solvent in chemical-deteriorative reactions (e.g. enzymatic reactions, oxidative reactions). The composition of the films, temperature, and ambient relative humidity affect the permeability of edible films (Debeaufort & Voilley, 2009; Parris & Coffin, 1997). Addition of oils and fats has been widely documented in edible films in order to increase hydrophobicity and decrease WVP (Alyanak, 2004; Anker, Berntsen, Hermansson, & Stading, 2002; Tara Habig McHugh & John M Krochta, 1994b). WVP of WPI edible films containing EO's is shown in Table 5. According to these results, WPIF and WPIGF had the lowest WVP ($p<0.05$) when compared with the other edible films. WPIGOF exhibited the highest ($p<0.05$) WVP, followed by WPIOF. Although literature reports improved WVP properties through addition of EO's, addition of EO's in this study (especially when combining OEO with GTE) resulted in higher WVP. One study about physicochemical and antimicrobial properties of whey protein isolate films containing oregano oil reported no significant differences in WVP of edible films at either of the levels used (Zinoviadou et al., 2009) and their WVP for the film without OEO was higher than the one obtained in this study,

which is attributed to the higher content of sorbitol as plasticizer; lower values have been reported when lower plasticizer (glycerol) content has been used which are similar to the ones obtained in this study (Gounga et al., 2007; Shellhammer & Krochta, 1997).

Table 5. Water vapor permeability of films

Treatment	WVP[1]
WPIF	$3.29 \times 10^{-10} \pm 3.37 \times 10^{-11C}$
WPIGF	$4.20 \times 10^{-10} \pm 4.70 \times 10^{-11BC}$
WPIGOF	$6.22 \times 10^{-10} \pm 6.56 \times 10^{-11A}$
WPIOF	$4.43 \times 10^{-10} \pm 5.12 \times 10^{-11B}$

Means with different letters (AB) within column are significantly different (P<0.05).
WVP[1]=Water vapor permeability (g.m/ Pa.s.m^2)
WPIF= whey-protein-isolate-film, WPIGF= WPIF with green tea extract (GTE), WPIGOF= WPIF with GTE plus oregano essential oil (OEO), WPIOF= WPIF with OEO

Plasticizer in film forming matrix reduces inter-chain attractive forces and hence, energy of activation for diffusion (Gounga et al., 2007; Zinoviadou et al., 2009). On the other hand, different test conditions (relative humidity and temperature), which have to be reported with WVP values, vary between experiments and results cannot be directly compared (Zinoviadou et al., 2009). Another fact that accounts for higher WVP in films containing OEO and/or GTE in this study is the level of EO's added to the film (3% w/v alone or when combined 1.5% w/v each), which may be too low to decrease WVP as well as the type of lipid (Tara Habig McHugh & John M Krochta, 1994b; Shellhammer & Krochta, 1997) and particle size of the emulsion (Pérez-Gago & Krochta, 2001). The increase of WVP of films with EO's can also be attributed to their greater thickness (Gounga et al., 2007). Several authors have reported that greater thickness of films increased WVP (Bravin, Peressini, & Sensidoni, 2006). Higher thickness can cause film swelling (Banker, Gore, & Swarbrick, 1966; Barrer, 1941), increased water vapor partial

pressure due to stagnant air layer formation, and consequently reduced resistance to WVT (McHugh et al., 1993).

3.3.5 Mechanical properties of edible films

Table 6 shows nominal tensile strength (TS), percentage elongation at break (EB), and elastic modulus (EM) of WPIF, WPIGF, WPIGOF, and WPIOF. According to these results, WPIF presented highest ($p < 0.05$) TS and EM but lowest ($p < 0.05$) EB. On the contrary, WPIGF, WPIGOF, and WPIOF presented lower ($p < 0.05$) TS but highest ($p < 0.05$) EB. WPIF and WPIOF had similar EM while WPIGF and WPIGOF exhibited lower ($p < 0.05$) EM.

Table 6. Mechanical properties of films

Treatment	TS[1]	EB[2]	EM[3]
WPIF	5.69 ± 0.93^{A}	69.01 ± 18.55^{B}	117.46 ± 31.16^{A}
WPIGF	2.33 ± 0.32^{C}	100.25 ± 15.06^{A}	10.66 ± 5.84^{C}
WPIGOF	2.73 ± 0.30^{C}	116.33 ± 24.96^{A}	30.49 ± 17.64^{B}
WPIOF	4.99 ± 0.77^{B}	98.33 ± 14.81^{A}	120.60 ± 19.49^{A}

Means with different letters (AB) within columns are significantly different ($P<0.05$).
TS[1] = Tensile strength (MPa), EB[2] = Elongation at break (%), [3] EM = Elastic modulus (MPa).
WPIF= whey-protein-isolate-film, WPIGF= WPIF with green tea extract (GTE), WPIGOF= WPIF with GTE plus oregano essential oil (OEO), WPIOF= WPIF with OEO

These results indicate that incorporation of GTE and/or OEO resulted in lower TS yielding weak films. It has been previously documented that higher TS is observed at protein phase than at lipid phase (Yoshida & Antunes, 2004). Carboxyl groups present in the structure of OEO and GTE can affect the protein network structure by reducing the polymer interactions and consequently reducing TS. In contrast to previous studies, addition of OEO and GTE resulted in increased EB probably due to the homogenization performed at 28,979 x *g* for 3 min for WPIGF, WPIGOF, and WPIOF, which produced more homogeneous films (Tara Habig McHugh & John M Krochta, 1994a). Similarly,

decreased EM was observed only when GTE was present but not when OEO was present. The decrease in TS and increase in EB for WPIGF, WPIGOF, and WPIOF (in lower scale) can be explained by a reduction in the number of intermolecular cross-link interactions; EO's could have bound water molecules competing with the active sites in the polymer network decreasing intermolecular interactions and increasing intermolecular distances (Shaw et al., 2002). Also, EM, which indicates the degree of films' stiffness, is strictly related to the number of crosslinks. Then, differences between films in EM can be explained by the ability of glycerol when combined with the EO's to trap water molecules and interfere with the protein-protein interactions (Shaw et al., 2002).

3.3.6 Microstructure of edible films

Scanning electron microscopy (SEM) was used to observe the microstructure, surface area, and cross-sectional area of WPIF, WPIGF, WPIGOF, and WPIOF. This analysis showed that WPIF had some pores and uneven surface with clots (Figure 4a). WPIGF, WPIGOF, and WPIOF (Figures 4b, 5a, and 5b) presented less pores and clots when compared to WPIF.

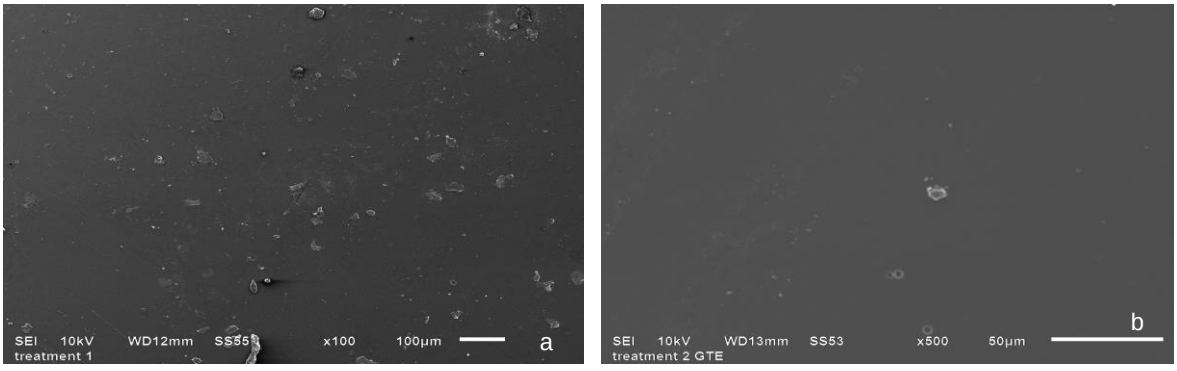


Figure 4. Surface area scanning electron micrographs of: (a) WPIF (whey-protein-isolate-film); and (b) WPIGF (whey-protein-isolate film with green tea extract).

This may be attributed to the homogenization process performed at 28,979 x *g* for 3 min after addition of GTE (green tea extract), GTE and OEO (oregano essential oil), and OEO, respectively to the film forming solution.

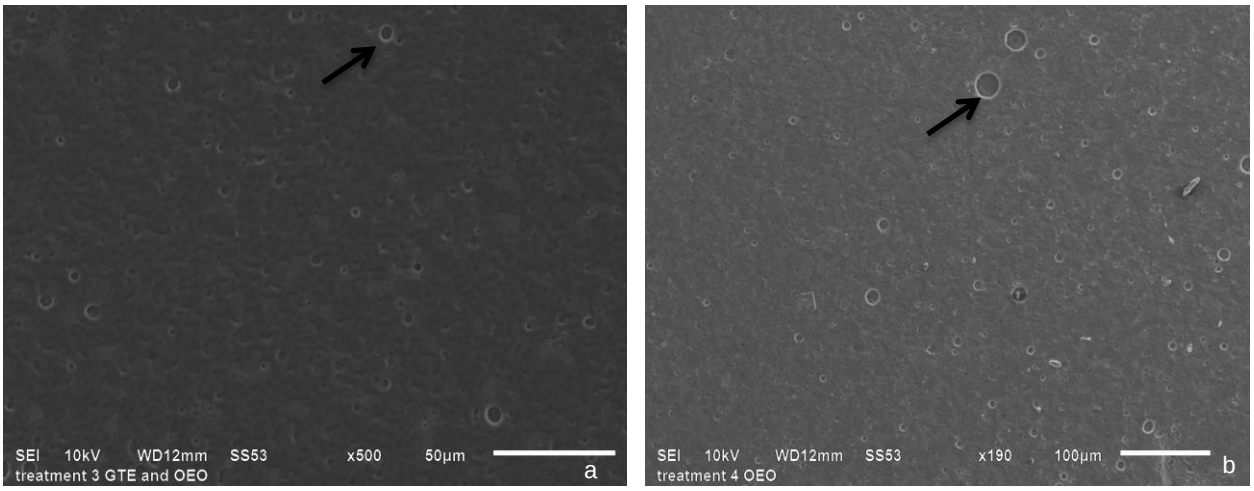


Figure 5. Surface area scanning electron micrographs of: (a) WPIGOF (whey-protein-isolate-film with green tea extract and oregano essential oil); and (b) WPIOF (whey-protein-isolate film with oregano essential oil).

Cross-sectional area of all WPI edible films provided a sponge-like structure (Figures 6a, 6b, 7a, and 7b) in which WPI forms a continuous network (Fang et al., 2002; Gounga et al., 2007) being the predominant phase (Wang et al., 2010) and for WPIGF (Figure 6b), WPIGOF (Figure 7a), and WPIOF (Figure 7b) an additional layer of dispersed droplets of GTE, GTE and OEO, and OEO, respectively appears on the top of the predominant phase (indicated by black arrows). Some spherical particles were observed within the protein matrix surface (Figures. 5a, 5b, 7a, and 7b), which is attributed to the presence of OEO droplets (indicated by black arrows). In general, all of the films had different microstructure, which may affect their barrier properties. Additives such as essential oils can interfere with gelation and aggregation properties of proteins, which are key factors in the structuring of biological materials (Wang et al., 2010) conferring smoother film surfaces.

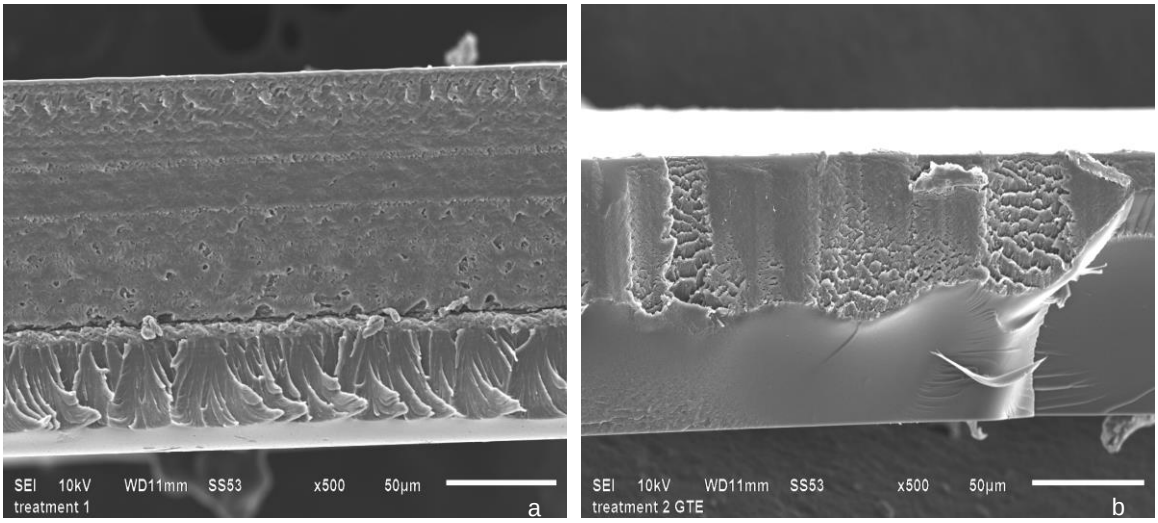


Figure 6. Cross-sectional area scanning electron micrographs of: (a) WPIF (whey-protein-isolate film); and (b) WPIGF (whey-protein-isolate film with green tea extract).

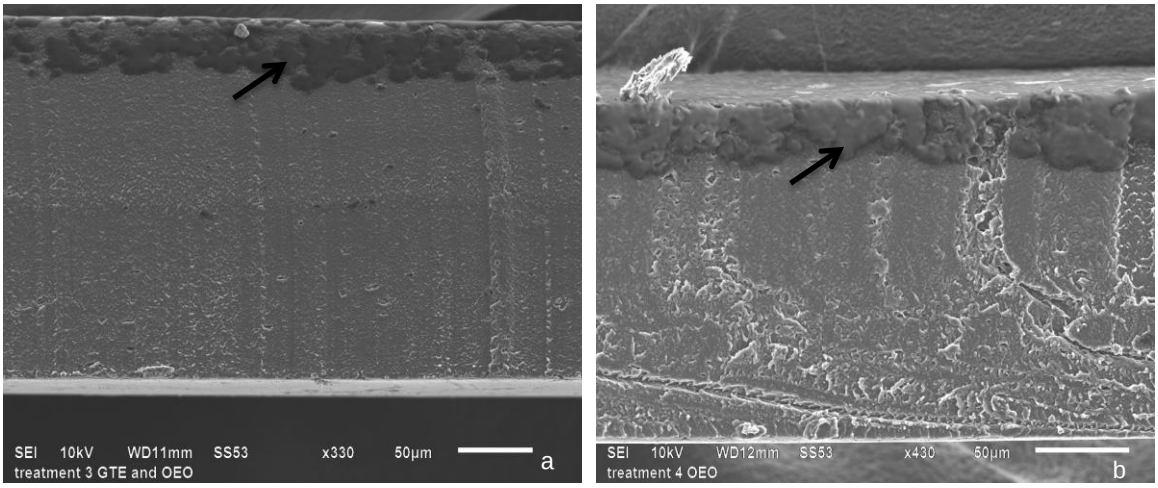


Figure 7. Cross-sectional area scanning electron micrographs of: (a) WPIGOF (whey-protein-isolate film with green tea extract and oregano essential oil); and (b) WPIOF (whey-protein-isolate film with oregano essential oil).

3.4 Conclusions

Higher film thicknesses and lower densities were obtained when incorporating both GTE and OEO to WPI (WPIGOF), which also resulted in significantly ($p<0.05$) higher WVP for WPIGOF when compared to WPIF, WPIGF, and WPIOF. GTE addition to WPIF (WPIGF, WPIGOF) resulted in significantly ($p<0.05$) lower WI and higher YI and ΔE but did not significantly ($p>0.05$) affect opacity. On the contrary, addition of OEO to WPIF

(WPIOF) resulted in non-significant ($p>0.05$) differences on WI, YI, and ΔE but significantly ($p<0.05$) increased opacity (WPIOF, WPIGOF) suggesting better barrier properties against UV and visible light. WPIGOF and WPIOF presented lower a_w while WPIF had the highest a_w. WPIOF exhibited higher tensile strength and elastic modulus than WPIGF and WPIGOF and behaved similar to WPIF. Microstructure of films was affected by addition of EO's which produced smoother surfaces when compared to WPIF. A sponge-like structure was observed for all of the films with WPI being the predominant phase and for WPIGF, WPIGOF, and WPIOF an additional layer of EO's appeared on the top of the structure. Improved physic-chemical and mechanical properties were obtained for WPIF and WPIOF, which presented homogeneous distribution of OEO. This study demonstrated that an edible wrap made of WPI and containing OEO can be developed with potential cost-effective applications for food packaging.

3.5 References

Alyanak, D. (2004). *Water vapour permeable edible membranes.* Izmir Institute of Technology.

Anker, M., Berntsen, J., Hermansson, A.-M., & Stading, M. (2002). Improved water vapor barrier of whey protein films by addition of an acetylated monoglyceride. *Innovative Food Science & Emerging Technologies, 3*(1), 81-92.

Banker, G. S., Gore, A. Y., & Swarbrick, J. (1966). Water vapour transmission properties of free polymer films*. *Journal of Pharmacy and Pharmacology, 18*(7), 457-466.

Barrer, R. M. (1941). *Diffusion in and through Solids*: CUP Archive.

Bech-Larsen, T. (1996). Danish consumers' attitudes to the functional and environmental characteristics of food packaging. *Journal of Consumer Policy, 19*(3), 339-363.

Bravin, B., Peressini, D., & Sensidoni, A. (2006). Development and application of polysaccharide–lipid edible coating to extend shelf-life of dry bakery products. *Journal of Food Engineering, 76*(3), 280-290.

Cabrera, C., Artacho, R., & Giménez, R. (2006). Beneficial effects of green tea—a review. *Journal of the American College of Nutrition, 25*(2), 79-99.

Campos, C., Gerschenson, L., & Flores, S. (2011). Development of Edible Films and Coatings with Antimicrobial Activity. *Food and Bioprocess Technology, 4*(6), 849-875. doi: 10.1007/s11947-010-0434-1

Chambi, H. N. M., & Grosso, C. R. F. (2011). Mechanical and water vapor permeability properties of biodegradables films based on methylcellulose, glucomannan, pectin and gelatin. *Food Science and Technology (Campinas), 31*(3), 739-746.

Chantre, P., & Lairon, D. (2002). Recent findings of green tea extract AR25 (Exolise) and its activity for the treatment of obesity. *Phytomedicine, 9*(1), 3-8.

Chien, P.-J., Sheu, F., & Yang, F.-H. (2007). Effects of edible chitosan coating on quality and shelf life of sliced mango fruit. *Journal of Food Engineering, 78*(1), 225-229.

Damodaran, S., Parkin, K. L., & Fennema, O. R. (2007). *Fennema's Food Chemistry, Fourth Edition*: CRC Press.

Debeaufort, F., & Voilley, A. (2009). Lipid-based edible films and coatings *Edible Films and Coatings for Food Applications* (pp. 135-168): Springer.

Del-Valle, V., Hernández-Muñoz, P., Guarda, A., & Galotto, M. (2005). Development of a cactus-mucilage edible coating (Opuntia ficus indica) and its application to extend strawberry (Fragaria ananassa) shelf-life. *Food Chemistry, 91*(4), 751-756.

Derraik, J. G. (2002). The pollution of the marine environment by plastic debris: a review. *Marine pollution bulletin, 44*(9), 842-852.

Dulloo, A. G., Duret, C., Rohrer, D., Girardier, L., Mensi, N., Fathi, M., . . . Vandermander, J. (1999). Efficacy of a green tea extract rich in catechin polyphenols and caffeine in increasing 24-h energy expenditure and fat oxidation in humans. *The American journal of clinical nutrition, 70*(6), 1040-1045.

Ekthamasut, K., & Akesowan, A. (2001). Effect of vegetable oils on physical characteristics of edible Konjac films. *AU Journal of Technology, 5*(2), 22-28.

Fang, Y., Tung, M. A., Britt, I. J., Yada, S., & Dalgleish, D. G. (2002). Tensile and barrier properties of edible films made from whey proteins. *Journal of Food Science, 67*(1), 188-193.

Fernández-Pan, I., Royo, M., & Ignacio Maté, J. (2012). Antimicrobial activity of whey protein isolate edible films with essential oils against food spoilers and foodborne pathogens. *Journal of food science, 77*(7), M383-M390.

Gómez-Estaca, J., Bravo, L., Gómez-Guillén, M., Alemán, A., & Montero, P. (2009a). Antioxidant properties of tuna-skin and bovine-hide gelatin films induced by the addition of oregano and rosemary extracts. *Food Chemistry, 112*(1), 18-25.

Gómez-Estaca, J., Montero, P., Fernández-Martín, F., Alemán, A., & Gómez-Guillén, M. C. (2009b). Physical and chemical properties of tuna-skin and bovine-hide gelatin films with added aqueous oregano and rosemary extracts. *Food Hydrocolloids, 23*(5), 1334-1341.

Gómez-Guillén, M., Ihl, M., Bifani, V., Silva, A., & Montero, P. (2007). Edible films made from tuna-fish gelatin with antioxidant extracts of two different murta ecotypes leaves (*Ugni molinae Turcz*). *Food Hydrocolloids, 21*(7), 1133-1143.

Gounga, M. E., Xu, S.-Y., & Wang, Z. (2007). Whey protein isolate-based edible films as affected by protein concentration, glycerol ratio and pullulan addition in film formation. *Journal of Food Engineering, 83*(4), 521-530.

Guilbert, S., Gontard, N., & Gorris, L. G. (1996). Prolongation of the shelf-life of perishable food products using biodegradable films and coatings. *LWT-Food Science and Technology, 29*(1), 10-17.

Henning, S. M., Niu, Y., Lee, N. H., Thames, G. D., Minutti, R. R., Wang, H., . . . Heber, D. (2004). Bioavailability and antioxidant activity of tea flavanols after consumption of green tea, black tea, or a green tea extract supplement. *The American journal of clinical nutrition, 80*(6), 1558-1564.

Hong, Y. H., Lim, G. O., & Song, K. (2009). Physical properties of Gelidium corneum–gelatin blend films containing grapefruit seed extract or green tea extract and its application in the packaging of pork loins. *Journal of food science, 74*(1), C6-C10.

Huang, M.-T., Ho, C.-T., Wang, Z. Y., Ferraro, T., Finnegan-Olive, T., Lou, Y.-R., . . . Yang, C. S. (1992). Inhibitory effect of topical application of a green tea polyphenol fraction on tumor initiation and promotion in mouse skin. *Carcinogenesis, 13*(6), 947-954.

Joerger, R. D. (2007). Antimicrobial films for food applications: a quantitative analysis of their effectiveness. *Packaging Technology and Science, 20*(4), 231-273.

Juhel, C., Armand, M., Pafumi, Y., Rosier, C., Vandermander, J., & Lairon, D. (2000). Green tea extract (AR25®) inhibits lipolysis of triglycerides in gastric and duodenal medium in vitro. *The Journal of nutritional biochemistry, 11*(1), 45-51.

Karel, M. (1980). Lipid oxidation, secondary reactions, and water activity of foods *Autoxidation in food and biological systems* (pp. 191-206): Springer.

Maltini, E., Torreggiani, D., Venir, E., & Bertolo, G. (2003). Water activity and the preservation of plant foods. *Food Chemistry, 82*(1), 79-86.

Maté, J. I., & Krochta, J. M. (1996). Comparison of oxygen and water vapor permeabilities of whey protein isolate and β-lactoglobulin edible films. *Journal of agricultural and food chemistry, 44*(10), 3001-3004.

McHugh, T. H., Avena-Bustillos, R., & Krochta, J. M. (1993). Hydrophilic edible films: modified procedure for water vapor permeability and explanation of thickness effects. *Journal of Food Science, 58*(4), 899-903.

McHugh, T. H., & Krochta, J. M. (1994). Milk-protein-based edible films and coatings. *Food technology, 48*(1), 97-103.

McHugh, T. H., & Krochta, J. M. (1994a). Sorbitol-vs glycerol-plasticized whey protein edible films: integrated oxygen permeability and tensile property evaluation. *Journal of Agricultural and Food Chemistry, 42*(4), 841-845.

McHugh, T. H., & Krochta, J. M. (1994b). Water vapor permeability properties of edible whey protein-lipid emulsion films. *Journal of the American Oil Chemists' Society, 71*(3), 307-312.

Mukhtar, H., Katiyar, S. K., & Agarwal, R. (1994). Green tea and skin--anticarcinogenic effects. *Journal of investigative dermatology, 102*(1), 3-7.

Nagao, T., Hase, T., & Tokimitsu, I. (2007). A green tea extract high in catechins reduces body fat and cardiovascular risks in humans. *Obesity, 15*(6), 1473-1483.

Parris, N., & Coffin, D. R. (1997). Composition factors affecting the water vapor permeability and tensile properties of hydrophilic zein films. *Journal of Agricultural and Food Chemistry, 45*(5), 1596-1599.

Peressini, D., Bravin, B., Lapasin, R., Rizzotti, C., & Sensidoni, A. (2003). Starch–methylcellulose based edible films: rheological properties of film-forming dispersions. *Journal of Food Engineering, 59*(1), 25-32.

Pérez-Gago, M. B., & Krochta, J. M. (2001). Lipid particle size effect on water vapor permeability and mechanical properties of whey protein/beeswax emulsion films. *Journal of agricultural and food chemistry, 49*(2), 996-1002.

Pérez-Gago, M. B., Nadaud, P., & Krochta, J. M. (1999). Water Vapor Permeability, Solubility, and Tensile Properties of Heat-denatured versus Native Whey Protein Films. *Journal of Food Science, 64*(6), 1034-1037.

Rojas-Graü, M. A., Raybaudi-Massilia, R. M., Soliva-Fortuny, R. C., Avena-Bustillos, R. J., McHugh, T. H., & Martín-Belloso, O. (2007). Apple puree-alginate edible coating as carrier of antimicrobial agents to prolong shelf-life of fresh-cut apples. *Postharvest Biology and Technology, 45*(2), 254-264.

Royo, M., Fernández-Pan, I., & Maté, J. I. (2010). Antimicrobial effectiveness of oregano and sage essential oils incorporated into whey protein films or cellulose-based filter paper. *Journal of the Science of Food and Agriculture, 90*(9), 1513-1519.

Sakanaka, S., Kim, M., Taniguchi, M., & Yamamoto, T. (1989). Antibacterial substances in Japanese green tea extract against Streptococcus mutans, a cariogenic bacterium. *Agricultural and Biological Chemistry, 53*(9), 2307-2311.

Seydim, A., & Sarikus, G. (2006). Antimicrobial activity of whey protein based edible films incorporated with oregano, rosemary and garlic essential oils. *Food research international, 39*(5), 639-644.

Shaw, N., Monahan, F., O'riordan, E., & O'sullivan, M. (2002). Physical properties of WPI films plasticized with glycerol, xylitol, or sorbitol. *Journal of Food Science, 67*(1), 164-167.

Shellhammer, T., & Krochta, J. (1997). Whey protein emulsion film performance as affected by lipid type and amount. *Journal of Food Science, 62*(2), 390-394.

Siripatrawan, U., & Harte, B. R. (2010). Physical properties and antioxidant activity of an active film from chitosan incorporated with green tea extract. *Food Hydrocolloids, 24*(8), 770-775.

Sobral, P. d. A., Menegalli, F., Hubinger, M., & Roques, M. (2001). Mechanical, water vapor barrier and thermal properties of gelatin based edible films. *Food Hydrocolloids, 15*(4), 423-432.

Song, X., Zhou, C., Fu, F., Chen, Z., & Wu, Q. (2013). Effect of high-pressure homogenization on particle size and film properties of soy protein isolate. *Industrial Crops and Products, 43*, 538-544.

Standard, A. (1995). Standard test methods for water vapor transmission of materials. *Annual book of ASTM standards. Designation E96-E80*, 730-739.

Standard, A. (2002). D882 (2002) Standard test method for tensile properties of thin plastic sheeting. ASTM International, West Conshohocken, PA, 2003, doi: 10.1520/D0882-02.

Wang, L., Auty, M. A., & Kerry, J. P. (2010). Physical assessment of composite biodegradable films manufactured using whey protein isolate, gelatin and sodium alginate. *Journal of food engineering, 96*(2), 199-207.

Yaman, Ö., & Bayoındırlı, L. (2002). Effects of an edible coating and cold storage on shelf-life and quality of cherries. *LWT-Food science and Technology, 35*(2), 146-150.

Yoshida, C., & Antunes, A. (2004). Characterization of whey protein emulsion films. *Brazilian Journal of Chemical Engineering, 21*(2), 247-252.

Zinoviadou, K. G., Koutsoumanis, K. P., & Biliaderis, C. G. (2009). Physico-chemical properties of whey protein isolate films containing oregano oil and their antimicrobial action against spoilage flora of fresh beef. *Meat Science, 82*(3), 338-345.

Zivanovic, S., Chi, S., & Draughon, A. F. (2005). Antimicrobial activity of chitosan films enriched with essential oils. *Journal of food science, 70*(1), M45-M51.

CHAPTER 4. DEVELOPMENT OF QUESO BLANCO CONTAINING FLAXSEED OIL

4.1 Introduction

The lipid fraction composition of cheeses is an important nutritional factor that contributes to cheeses specific flavor and other sensorial characteristics mainly by free fatty acid type and concentration. Milk fat composition mainly consists of saturated fatty acids myristic (C14:0) and palmitic (C16:0) and lower amounts of monounsaturated (MUFA) like cis C18:1 and polyunsaturated fatty acids (PUFA). According to the Wisconsin Milk Marketing Board, ideal milk fat composition should be less than 10% PUFA; up to 8% saturated fatty acids and 82% MUF. However, regular milk does not meet such desirable standards as it has around 70% saturated fatty acids. Thus, the marked predominance of palmitic and myristic fatty acids in milk represents a nutritional concern for consumers and researchers (Kennelly, 1996). On the other hand, rumenic acid (RA, cis-9 trans-11 C18:2), which is an isomer of conjugated linoleic acid (CLA, 18:2 n-6) is present in ruminant derived foods. Several studies aimed to increase the RA concentration in foods because of its nutritional benefits when consumed by animals and during in vitro assays have been reported (Connor, 2000; Simopoulos, 1991). The rumen can perform incomplete hydrogenation of linoleic acid leading to RA production but the most common pathway for RA production is through desaturation of vaccenic acid (VA, trans-11 C18:1) in the mammary gland (Luna, Bach, Juárez, & de la Fuente, 2008). Ruminants' diet modifications through addition of lipids that increase energy have been performed for several years (Benchaar et al., 2006; Chilliard, Ferlay, & Doreau, 2001; Chilliard et al., 2000; Givens, Cottrill, Davies, Lee, Mansbridge, & Moss, 2000; Gómez-Cortés, Frutos, Mantecón, Juárez, De La Fuente, & Hervás, 2008; Loor,

Ueda, Ferlay, Chilliard, & Doreau, 2004). Long chain omega-3 polyunsaturated fatty acids (LC Ω-3PUFA) are important in the composition of foods as they have been proven to benefit health in decreasing cardiovascular and immune-inflammatory diseases. Moreover, they are essential for the well-functioning of brain, retina, and testis having a structural role in cell functioning (Kidd, 2007). Many oils from vegetables sources like canola, fish, flaxseed, soybean, and walnut are rich in α-linolenic acid (ALA C18:3 n3) and are usually used to provide foods with omega-3 fatty acids (Covington, 2004; Surette, 2008; Whelan & Rust, 2006). On the other hand, the recommended daily intake of LC Ω-3PUFA (<100-200 mg/d), polyunsaturated fatty acids (650 mg/d), and ALA (2.2 g/d) is not met by the United States population (Simopoulos, 2008). Consequently, there is an increasing tendency in the production of omega-3 foods (e.g. bread, milk, yogurt, cheese, pasta, and infant meals). Yet, incorporation of omega-3 fatty acids faces stability challenges regarding oxidation and more research related to its sensorial characteristics and consumer´s acceptability is needed. Dairy products are attractive candidates for omega-3 incorporation due to their refrigerated storage which may decrease lipid oxidation (Hilliam, 1998).

Today, studies are also focused on addition of oils and/or fats in order to improve the fatty acid (FA) profile of different types of milk and its derivatives to supply humans with better nutritional quality foods (AbuGhazaleh, Schingoethe, Hippen, & Kalscheur, 2003; Chilliard & Ferlay, 2004; Dunstan, Mitoulas, Dixon, Doherty, Hartmann, Simmer, & Prescott, 2007; Kelly, Berry, Dwyer, Griinari, Chouinard, Van Amburgh, & Bauman, 1998; Kolanowski & Laufenberg, 2006; Lock & Bauman, 2004; Shingfield, Ahvenjarvi, Toivonen, Arola, Nurmela, Huhtanen, & Griinari, 2003). In fact, previous studies have

demonstrated that feeding ruminants with linseed oil and other vegetable oils (e.g. peanut and sunflower) rich in linolenic, linoleic, and/or oleic acids can increase CLA content in milk fat (Bu, Wang, Dhiman, & Liu, 2007; Chilliard et al., 2000; Dhiman, Satter, Pariza, Galli, Albright, & Tolosa, 2000; Kelly et al., 1998).

The objectives of this project were: (1) determine the step in queso blanco elaboration at which better retention of flaxseed oil (FO) can be achieved, and (2) evaluate physicochemical and microbiological characteristics of cheeses containing flaxseed oil during one month storage period.

4.2 Materials and methods

4.2.1 Elaboration of queso blanco and queso blanco containing flaxseed oil

The method described by Carroll (2010) was used for queso blanco elaboration and the method of Bermúdez-Aguirre and Barbosa-Cánovas (2011) was used for flaxseed oil (FO) addition using 3.785 L of milk. Grade A- pasteurized whole milk was purchased from a local producer (Rocking R Dairy, Inc. Tylertown, MS, USA) and citric acid (Anhydrous coarse granular 14-30 mesh PT. BUDI ACID JAYA SUNGAI) was used to separate the curds from whey. Queso-blanco control (QU) was prepared by heating whole-non-homogenized-milk at 88°C, subsequently adding citric acid (2.17g/L), pouring the curds in cheese-cloth-lined-colander, followed by manual salting (3.96g/L), and pressing (2.5 h, 30 PSI) in cheese presses. Addition of FO (1.27g/L) separately at three stages of cheese elaboration (1) during heating at 38°C, (2) salting, and (3) during homogenization at 34,010 x *g* for 10 min at 38°C with an homogenizer (UltraShear M, Omni International) produced batches FQ, SFQ, and HFQ, respectively. Batches were analyzed by triplicates. FQ, SFQ, HFQ, and QU were removed from the press and

refrigerated at 8°C with sunlight and fluorescent light exposure (750 lumens) until analyses were performed.

4.2.2 Moisture content and fat content determination of cheeses containing flaxseed oil and control cheese

Moisture content of FQ, SFQ, HFQ, and QU was determined according to the Standard Methods for the Examination of Dairy Products, SM 15.10 C (Bradley, Arnold, Barbano, Semerad, Smith, & Vines, 1992) with a variant of time as suggested by Barbano and Sherbon (1984) using a forced- air- convection oven (VWR Scientific 1330FM Sheldon Manufacturing Inc, Cornelius, OR, USA). Cheese samples of 3 g were finely shredded, weighed in an analytical balance (Sartorius ED 224s Data Weighting Systems, Inc., Elk Grove IL, USA), and placed into disposable aluminum dishes inside the oven for 24 h at 100 °C. Moisture content wet basis determination for cheeses was calculated according to equation 10 by triplicates on each cheese batch.

$$MC_{wb} = \left[\frac{(Ms+d)-(Mds+d)}{Ms}\right][100] \qquad (10)$$

Where MCwb= moisture content wet basis (%); Ms= mass of moist sample (g); d= mass of aluminum dish (g); and Mds= mass of dry solids (g).

Fat content of unwrapped freshly prepared cheeses (FQ, SFQ, HFQ, and QU) was determined according to the AOCS (American Oil Chemists' Society) method, Ai 3.75 (Society, 1988) using a continuous Soxhlet unit and petroleum ether (Fisher Scientific E139-4, Fair Lawn, NJ, USA) as a solvent. Shredded cheeses samples (4-5 g) were first dried at 100 °C under pressure= 660 mmHg for about 5 h according to AOAC method, AOAC 934.01 (Chemists, 1991). Samples of pre-dried cheeses were placed into pre-dried porous extraction thimbles. The weight of pre-died boiling flasks was registered and boiling flasks, Soxhlet flasks, and condensers were assembled for extraction.

Extraction was performed by heating the solvent in flasks. After fat was extracted, the boiling flasks were dried in an air oven at 100 °C for 30 min, cooled in a desiccator, and weighed. Fat content of cheeses was determined by weight of the fat removed in wet basis and by triplicates of each cheese batch.

4.2.3 Color of cheeses containing flaxseed oil and control cheese

Color of FQ, SFQ, HFQ, and QU was determined using a Lab Scan XE colorimeter (Hunter Associates Laboratory Inc., Reston, VA) at days 1 and 30 of refrigerated storage with sunlight and fluorescent light (750 lumens) exposure. The spectrophotometer was first standardized using white and black tiles. Cheese samples (10 g) were placed in polystyrene hexagonal weigh boats (Fisherbrand®, Fisher Scientific, Pittsburg, PA) for color determination. Results were reported in L* (0-darkness, 100-lightness), a* (-greenness + redness), and b* (-blueness + yellowness) values by triplicates of each cheese batch.

4.2.4 Thiobarbituric acid reactive substances of cheeses containing flaxseed oil and control cheese

FQ, SFQ, HFQ, and QU were analyzed for Thiobarbituric Acid Reactive Substances (TBARS) according to the method described by Lemon (1975) at days 1,15, and 30 of refrigerated storage with sunlight and fluorescent (750 lumens) light exposure. Moisture content of samples was first determined by the method mentioned above in order to calculate TBARS values. TBARS results were expressed in µmol of malondialdehyde (MDA) per kg of sample. All chemicals were purchased from Sigma Chemical Co, St. Louis, MO, USA. Reagents were prepared according to Lemon (1975) and the analysis was performed using 15 g of blended cheese. Extraction solution (30 mL) was mixed with blended cheese for 2 min in a stomacher (Tekmar Co., Cincinnati, OH, USA) using

stomacher bags (Whirl-pack, Nasco Co, Fort Atkinson, WI, USA). The resulting homogenate was filtered with Whatman No. 1filter paper; 5 mL of the clear filtrate were then mixed with 5 mL of TBA solution in test tubes using a vortex mixer (Mini vortexer MV 1, VWR Scientific, Ika-Works, Inc., Wilmington, NC, USA) and placed in a boiling-water-flask for 40 min. After heating, test tubes were cooled down to 21°C with tap water for 10 min and absorbance at 530 nm against a blank of 5 mL of water plus 5 mL of TBA solution was read using a UV spectrophotometer (Thermo Scientific GENESYS 20, Thermo Fisher Scientific, Inc., Madison, WI, USA). Triplicates per cheese batch were analyzed and TBA value was calculated from a standard curve constructed by using TEP (1, 1, 3, 3 tetraethoxypropane).

4.2.5 Total yeast and mold count and total coliform count of cheeses containing flaxseed oil and control cheese

Total yeast and mold count (TYMC) and total coliform count (TCC) of FQ, SFQ, HFQ, and QU were performed at days 1 and 30 of refrigerated storage with sunlight and fluorescent light (750 lumens) exposure. For each cheese batch, triplicate samples (5g) were diluted with peptone water (45 mL) and blended with a stomacher (Tekmar Co., Cincinnati, OH, USA) using stomacher bags (Whirl-pack, Nasco Co, Fort Atkinson, WI, USA) for 2 min. All microbiological analyses were performed using petrifilms (3M Microbiology, St. Paul, MN, USA). Duplicate plantings for each triplicate was performed according to AOAC methods for foods AOAC 997.02 (Chemists & Horwitz, 1995) for TYMC and dairy products, AOAC 989.10 (Chemists & Horwitz) for TCC. Incubation period and temperature for TYMC were 5 days and 23 °C, respectively and for TCC 24h and 32°C, respectively.

4.2.6 pH of cheeses containing flaxseed oil and control cheese

pHs of FQ, SFQ, HFQ, and QU were measured with a previously calibrated portable pH meter (ExStik pH Meter PH100, Extech Instruments Corporation, USA) by immersing the probe into each cheese batch at least three times in different locations. Measurements were performed at day 1 and 30 of refrigerated storage with sunlight and fluorescent light exposure (750 lumens).

4.2.7 Texture profile analysis of cheeses containing flaxseed oil and control cheese

Texture profile analysis (TPA) of FQ, SFQ, HFQ, and QU to characterize the cheeses for hardness (maximum force to compress the sample: Newton, N), cohesiveness (ratio between the positive areas of a TPA plot: dimensionless), springiness (height of cheese recovered after first bite and before the second one: mm), and chewiness (relation between gumminess and springiness: N mm^2) was performed on each cheese triplicate per cheese batch as described by Tunick and Van Hekken (2002) using an Instron Universal testing instrument Model 5544 (Instron Co. Canton, MA, USA). Cylinders of each cheese batch of 15 mm diameter and 15 mm height were obtained and compressed in two cycles by 75% at a crosshead speed of 100 mm/min by triplicate. Hardness, cohesiveness, springiness, and chewiness were calculated by the system software from the force-distance curve.

4.2.8 Statistical Analysis

The collected data were analyzed using Statistical Analysis System (SAS, Version 9.2, SAS Institute Inc., Cary, NC, USA). Triplicate experiments were conducted and Tukey's studentized range mean separation test was used to detect statistical differences (α=0.05).

4.3 Results and discussion

4.3.1 Moisture content and fat content determination of cheeses containing flaxseed oil and control cheese

Percentage moisture content wet basis (MC) and percentage fat content wet basis (FC) of FQ, SFQ, HFQ, and QU are showed in Table 7. According to these results, MC was greatest ($p < 0.05$) for QU and lower ($p < 0.05$) for the rest of the cheeses. On the contrary, FC was greater ($p < 0.05$) for HFQ when compared to QU, but not significantly different ($p > 0.05$) from FQ and SFQ. Similar results in queso blanco composition were obtained by Guo, Van Hekken, Tomasula, Shieh, and Tunick (2011) and Hill, Bullock, and Irvine (1982) in which the cheese remained stable for an 8 week storage period. The results from our study suggest that FO can be incorporated into queso blanco, which are in agreement with the study of Bermúdez-Aguirre and Barbosa-Cánovas (2011), who reported queso blanco best omega-3 retention when compared to cheddar and mozzarella cheeses and decreased moisture content when compared to control (no omega-3 added). In this study addition of FO for queso blanco was by direct addition during heating whereas for our study the FO addition was done directly during heating, during salting, and during homogenization, which has not been previously documented.

Table 7. Moisture content and fat content of cheeses containing flaxseed oil and control cheese

Treatment	MC*[1]	FC*[2]
FQ	48.06 ± 0.26^{BC}	20.11 ± 0.25^{AB}
SFQ	48.95 ± 0.39^{B}	20.2 ± 0.52^{AB}
HFQ	46.82 ± 0.30^{C}	20.82 ± 0.45^{A}
QU	51.74 ± 1.10^{A}	19.44 ± 0.49^{B}

* Means with different letters (AB) within columns are significantly different (P<0.05).
MC[1] = Percentage of moisture content wet basis, FC[2]= Percentage of fat content wet basis.
FQ= cheese with flaxseed oil (FO) added directly during heating, SFQ= cheese with FO added during salting, HFQ= cheese with FO added during homogenization, QU: Control (no FO).

4.3.2 Color of cheeses containing flaxseed oil and control cheese

Darkness-Lightness (L*), greenness-redness (a*), and blueness-yellowness (b*) values of FQ, SFQ, HFQ, and QU are shown in Table 8. According to these results, lightness of FQ, SFQ, and HFQ was not significantly different ($p>0.05$) from QU at day 1 and day 30. Yet, SFQ and QU significantly ($p<0.05$) increased their L* value in time. On the other hand, a* results show that no significant differences ($p>0.05$) between the treatments in the red-greenness scale was observed at day 1. However, at day 30 of storage, FQ was significantly ($p<0.05$) more red than SFQ and HFQ and was the only one treatment that significantly ($p<0.05$) increased its redness in time. This can be attributed to the development of certain different types of yeasts and molds on the surface of FQ; similar results were reported by Bermúdez - Aguirre and Barbosa - Cánovas (2010) when queso fresco was thermo-sonicated resulting in color variability specially in tendency towards redness.

Table 8. Color of cheeses containing flaxseed oil and control cheese

Color	Treatment	Day 1	Day 30
L*	FQ	$78.63 \pm 4.14^{A(a)}$	$82.00 \pm 1.68^{A(a)}$
	SFQ	$78.38 \pm 1.15^{A(b)}$	$83.61 \pm 0.34^{A(a)}$
	HFQ	$79.03 \pm 2.57^{A(a)}$	$81.80 \pm 2.74^{A(a)}$
	QU	$80.41 \pm 1.61^{A(b)}$	$83.87 \pm 0.43^{A(a)}$
a*	FQ	$-0.48 \pm 0.23^{A(b)}$	$0.10 \pm 0.19^{A(a)}$
	SFQ	$-0.74 \pm 0.16^{A(a)}$	$-0.78 \pm 0.23^{B(a)}$
	HFQ	$-0.46 \pm 0.27^{A(a)}$	$-0.60 \pm 0.25^{B(a)}$
	QU	$-0.29 \pm 0.35^{A(a)}$	$-0.37 \pm 0.09^{AB(a)}$
b*	FQ	$19.39 \pm 1.18^{A(a)}$	$19.59 \pm 2.48^{A(a)}$
	SFQ	$21.97 \pm 0.57^{A(a)}$	$18.72 \pm 1.35^{A(b)}$
	HFQ	$22.34 \pm 1.84^{A(a)}$	$18.15 \pm 2.15^{A(a)}$
	QU	$21.55 \pm 0.92^{A(a)}$	$17.94 \pm 3.03^{A(a)}$

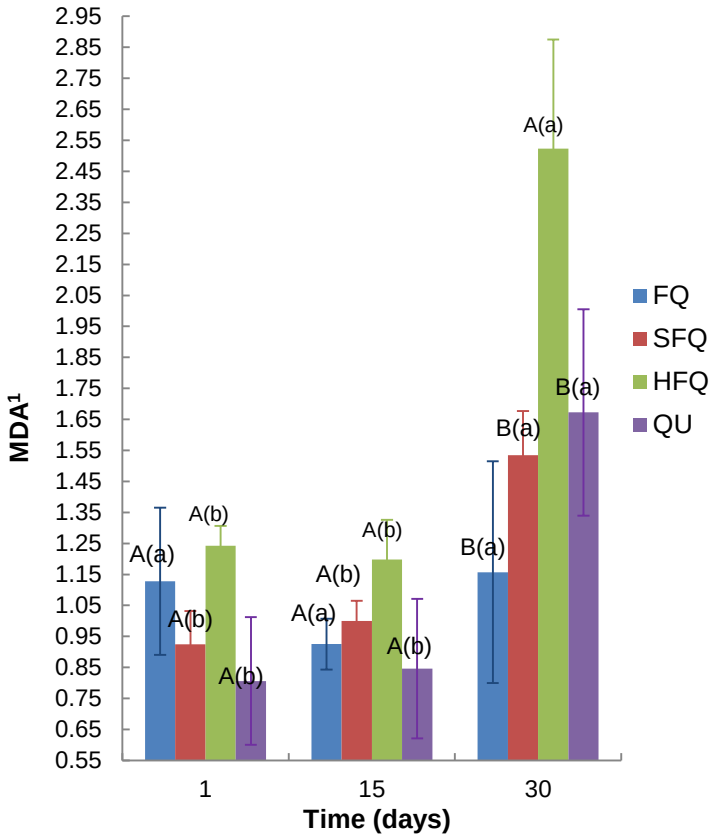
Means with different letters (AB) within columns are significantly different ($P<0.05$).
Means with different letters (ab) within rows are significantly different ($P<0.05$).
FQ= cheese with flaxseed oil (FO) added directly during heating, SFQ= cheese with FO added during salting, HFQ= cheese with FO added during homogenization, QU: Control (no FO).
L*= (0-darkness, 100-lightness), a*= (- greenness +redness), b*= (- blueness + yellowness).

Finally, b* values showed no significant differences ($p>0.05$) between the treatments at day 1 and day 30 but showed significantly ($p<0.05$) decreased yellowness values in time for SFQ. This can be explained by the mechanism of oil incorporation in SFQ, which was performed manually during the salting process after curd formation.

4.3.3 Thiobarbituric Acid Reactive Substances of cheeses containing flaxseed oil and control cheese

Malondialdehyde (MDA), a three carbon dialdehyde having carbonyl groups at position 1 and 3 is a decomposition product of hydroperoxided-polyunsaturated omega-3 and omega-6 fatty acids. It has been found that MDA is a carcinogenic agent that can damage and alter functioning of cells. Hence, quantification of MDA in foods is used as an indicator of freshness and healthiness and high values are attributed to rancid foods (Papastergiadis, Fatouh, Jacxsens, Lachat, Shrestha, Daelman, Kolsteren, Van Langenhove, & De Meulenaer, 2014). Oxidation of FQ, SFQ, HFQ, and QU expressed as µmol of malondialdehyde per kg of cheese (TBARS) is presented in Figure 8. According to these results at day 1 and day 15, TBARS value was not significantly different ($p>0.05$) between cheeses and neither was it in time, meaning that 15 days was not enough to significantly oxidize flaxseed oil (FO) present in FQ, SFQ, and HFQ. However, at day 30 of storage, HFQ presented highest ($p<0.05$) TBARS compared to the rest of the cheeses. These results suggest higher FO retention in the cheese matrix when it is incorporated during homogenization allowing a more homogeneous distribution of the oil within the cheese, which is in accordance with the results of fat determination. An unequal distribution of the polyunsaturated fatty acids (mainly present in FO), made FQ and SFQ less susceptible to oxidation (Kamal-Eldin, 2006; Rudnik,

Szczucinska, Gwardiak, Szulc, & Winiarska, 2001) and caused similar levels of oxidation when compared to QU.



Means with different letters (AB) are treatments significantly different (P<0.05).
Means with different letters (ab) are treatments significantly different within time (P<0.05).
MDA[1]= µmol of malondialdehyde per kg of cheese.
FQ= cheese with flaxseed oil (FO) added directly during heating, SFQ= cheese with FO added during salting, HFQ= cheese with FO added during homogenization, QU: Control (no FO).

Figure 8. Thiobarbituric Acid Reactive Substances (TBARS) of cheeses containing flaxseed oil and control cheese.

Also, homogenization performed in HFQ resulted in disruption of FO droplets and fat globules into lower-size particles and increased surface area that made them more susceptible to the attack of free radicals and production of hydroperoxides and its decomposition products in presence of oxygen (Damodaran et al., 2007). These results are supported by the observation of cheese microstructure at days 1 and 30 in section 5.3.5; a change in the microstructure of HFQ affected its stability to oxidation exhibiting

a less dense protein matrix with numerous openings that were occupied by fat globules and FO droplets. In general, in spite of addition of FO, cheeses presented relatively low values of TBARS during the storage period, which is consistent with previous studies on cheese oxidation (Shamberger, Shamberger, & Willis, 1977).

4.3.4 Total yeast and mold count and total coliform count of cheeses containing flaxseed oil and control cheese

Total yeast and mold count (TYMC) of FQ, SFQ, HFQ, and QU is presented in Figure 9.

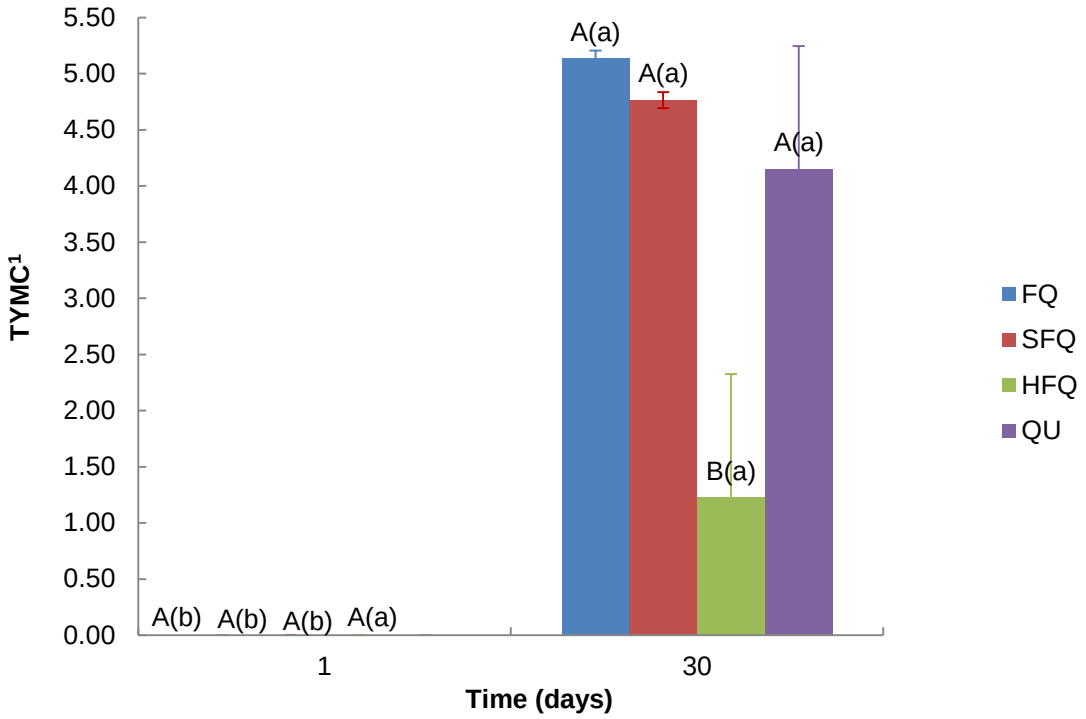


Figure 9. Total Yeast and Mold Count of cheeses containing flaxseed oil and control cheese.

Means with different letters (AB) are treatments significantly different (P<0.05).
Means with different letters (ab) are treatments significantly different within time (P<0.05)
TYMC[1]= Log colony forming units of total yeast and mold count per g of cheese.
FQ= cheese with flaxseed oil (FO) added directly during heating, SFQ= cheese with FO added during salting, HFQ= cheese with FO added during homogenization, QU: Control (no FO).

At day 1 of storage all the cheeses exhibited no counts and thus, were not significantly different ($p>0.05$). However, at day 30 of storage HFQ presented least ($p<0.05$) TYMC when compared to the other treatments. In addition, all of the treatments presented

significant ($p<0.05$) increase in time for TYMC except HFQ. These results suggest that FO being homogeneously distributed and better retained in HFQ exerts an antifungal effect on queso blanco. Previous studies have reported antimicrobial properties of the protein fraction of flaxseed against *Alternaria solani*, *Alternaria alternate*, *Candida albicans* (Borgmeyer, Smith, & Huynh, 1992; Vigers, Roberts, & Selitrennikoff, 1991), and food spoilage fungi like *Penicillium chrysogenum*, *Fusarium graminearum*, and *Aspergillus flavus* (Xu, Hall, & Wolf-Hall, 2008). Moreover, flaxseed oil has also been studied for antimicrobial properties (Kaithwas, Mukerjee, Kumar, & Majumdar, 2011) resulting in an efficient alternative for treating bovine mastitis and microbial counts in milk samples which are in accordance with these results. Total coliform count was 0 CFU/g product (data not shown) for all the cheeses during 1 month of refrigerated storage, which means that sanitary conditions and good manufacture practices (GMP) were performed during cheese making process.

4.3.5 pH of cheeses containing flaxseed oil and control cheese

pHs of FQ, SFQ, HFQ, and QU are presented in Table 9.

Table 9. pH of cheeses containing flaxseed oil and control cheese

	pH	
Treatment	Day 1	Day 30
FQ	$6.07 \pm 0.12^{A(a)}$	$5.60 \pm 0.00^{B(b)}$
SFQ	$6.01 \pm 0.04^{A(a)}$	$5.50 \pm 0.17^{B(b)}$
HFQ	$5.98 \pm 0.12^{A(a)}$	$5.40 \pm 0.20^{B(b)}$
QU	$6.03 \pm 0.07^{A(a)}$	$6.37 \pm 0.23^{A(a)}$

Means with different letters (AB) within columns are significantly different ($P<0.05$).
Means with different letters (ab) within rows are significantly different ($P<0.05$).
FQ= cheese with flaxseed oil (FO) added directly during heating, SFQ= cheese with FO added during salting, HFQ= cheese with FO added during homogenization, QU: Control (no FO).

According to these results, at day 1 of storage no significant ($p>0.05$) differences between treatments were observed. However, at day 30, QU resulted in higher ($p<0.05$)

pH when compared to the rest of the cheeses. Moreover, QU was the only one treatment that showed no significant ($p>0.05$) change in pH in 1 month of storage. Similar values of pH have been reported for queso blanco produced by acid precipitation (Hill et al., 1982). Literature reports that pH decreases as starter-bacteria grow in dairy foods converting lactose to lactic acid, but queso blanco was not inoculated with any starter culture, and therefore, a decrease in pH is attributed to natural microflora that survived the heating process or was a post-pasteurization contaminant (Guo et al., 2011).

4.3.6 Texture profile analysis of cheeses containing flaxseed oil and control cheese

Texture profile analysis (TPA) of FQ, SFQ, HFQ, and QU is presented in Table 10.

Table 10. Texture profile analysis of cheeses containing flaxseed oil and control cheese

Texture Parameters	Treatment	Day 1	Day 30
Hardness (N)	FQ	$69.96 \pm 18.24^{A(a)}$	$82.70 \pm 3.39^{A(a)}$
	SFQ	$50.82 \pm 8.05^{A(a)}$	$51.69 \pm 6.68^{B(a)}$
	HFQ	$78.48 \pm 7.90^{A(a)}$	$74.71 \pm 2.23^{A(a)}$
	QU	$70.56 \pm 5.80^{A(a)}$	$77.69 \pm 11.97^{A(a)}$
Springiness (mm)	FQ	$4.55 \pm 0.10^{AB(a)}$	$5.32 \pm 0.77^{AB(a)}$
	SFQ	$4.39 \pm 0.12^{B(a)}$	$4.86 \pm 0.57^{AB(a)}$
	HFQ	$4.50 \pm 0.04^{AB(a)}$	$4.99 \pm 0.75^{B(a)}$
	QU	$5.40 \pm 0.72^{A(a)}$	$5.98 \pm 0.11^{A(a)}$
Cohesiveness	FQ	$0.28 \pm 0.05^{A(a)}$	$0.27 \pm 0.01^{A(a)}$
	SFQ	$0.26 \pm 0.02^{A(a)}$	$0.30 \pm 0.02^{A(a)}$
	HFQ	$0.26 \pm 0.04^{A(a)}$	$0.30 \pm 0.04^{A(a)}$
	QU	$0.25 \pm 0.02^{A(b)}$	$0.30 \pm 0.03^{A(a)}$
Chewiness (mJ)	FQ	$87.12 \pm 17.78^{A(a)}$	$117.55 \pm 19.52^{AB(a)}$
	SFQ	$59.18 \pm 12.44^{A(a)}$	$74.06 \pm 7.86^{C(a)}$
	HFQ	$91.05 \pm 13.83^{A(a)}$	$100.45 \pm 11.21^{BC(a)}$
	QU	$93.23 \pm 6.22^{A(b)}$	$137.90 \pm 9.06^{A(a)}$

Means with different letters (AB) within columns are significantly different ($P<0.05$).
Means with different letters (ab) within rows are significantly different ($P<0.05$).
FQ= Queso blanco with flaxseed oil (FO) added directly, SFQ= Queso blanco with FO added during salting, HFQ=Queso blanco with FO added during homogenization, QU= Queso blanco without FO (control).

According to these results, no difference was found on hardness, cohesiveness, and chewiness between treatments at day 1. Yet, QU presented higher ($p< 0.05$) springiness (mm) when compared to SFQ. This can be attributed to the higher moisture content of QU. In contrast, at day 30 of storage significant ($p<0.05$) differences between treatments TPA were found except for cohesiveness. Hardness was higher ($p<0.05$) for FQ, QU, and HFQ when compared to SFQ. Springiness was lower for HFQ when compared to QU but not significantly ($p> 0.05$) different from FQ and SFQ, which can be explained by lower water content of HFQ. Finally, chewiness was lower ($p< 0.05$) for SFQ when compared to FQ and QU but not significantly ($p>0.05$) different from HFQ. In congruence with the study of Guo et al. (2011), all the treatments showed no significant ($p> 0.05$) difference between days for any of the TPA parameters except by QU, which showed increased cohesiveness and chewiness at day 30. Literature reports that for most fresh, not aged cheeses, hardness increases due to syneresis whereas cohesiveness does not display significant changes as proteolysis of α_{s1} into smaller peptides, which softens cheese structure is not significant in unripened cheeses, but for aged cheeses the contrary occurs because of minimal moisture loss and significant proteolysis. Another variant has been observed for elastic cheeses like mozzarella and brick which increase their cohesiveness during time (Tunick & Van Hekken, 2002).

Changes in TPA of cheeses has been attributed to the interactions between milk fat globules, casein micelles, and the source of omega 3, but mainly to the method of coagulation used for curd formation (acidic or enzymatic) as it will determine the structure and body of the cheese (Pinho, Mendes, Alves, & Ferreira, 2004). Other authors categorize the TPA as an empirical test because there is no correction factor for

changes in shapes and can be used for comparisons but not to determine real rheological properties (Tunick & Van Hekken, 2002).

4.4 Conclusions

A queso blanco containing flaxseed oil (FO) was produced by incorporation of FO to the cheese during three stages of cheese elaboration: (1) heating, (2) salting, and (3) homogenization. QU had highest moisture content (MC) whereas HFQ had highest fat content (FC) suggesting better FO retention during homogenization. No major changes in the color of omega-3 cheeses were noticeable during 30 days of storage except for a* (-red +greenness) values in FQ after 1month which suggested different yeast and molds growing on its surface. Also, decreasing b* (-blueness +yellowness) values for SFQ in time indicates a non-homogeneous distribution of FO during salting. Thiobarbituric Acid Reactive Substances (TBARS) analysis showed significant differences between treatments until 1 month of storage, at which HFQ had the greatest value indicating highest oxidation due to omega-3 fatty acids successfully incorporated during homogenization. Non-homogeneous distribution of FO caused similar oxidation levels for QU, FQ, and SFQ. Still TBARS values were low for all cheeses at the end of the 1 month storage period. Total Yeast and Mold Count (TYMC) revealed an antifungal effect of FO when incorporated homogeneously as HFQ presented the least counts in time and when compared to the other cheeses. Acidity of cheeses was different only at day 30 and was attributed to natural microflora that survived the heating process or was a post-pasteurization contaminant. Texture Profile Analysis (TPA) showed significant changes until day 30 of storage showing the least hardness for SFQ. Other TPA parameters variations were attributed to differences in MC of cheeses. Incorporation of

FO was better achieved during homogenization process but higher oxidation also occurred. Protection of the PUFA present in FO is necessary in order to take advantage of its antifungal properties when incorporated in queso blanco.

4.5 References

AbuGhazaleh, A., Schingoethe, D., Hippen, A., & Kalscheur, K. (2003). Milk conjugated linoleic acid response to fish oil supplementation of diets differing in fatty acid profiles. *Journal of dairy science, 86*(3), 944-953.

Barbano, D., & Sherbon, J. (1984). Cheddar cheese yields in New York. *Journal of Dairy Science, 67*(8), 1873-1883.

Benchaar, C., Petit, H., Berthiaume, R., Whyte, T., & Chouinard, P. (2006). Effects of addition of essential oils and monensin premix on digestion, ruminal fermentation, milk production, and milk composition in dairy cows. *Journal of dairy science, 89*(11), 4352-4364.

Bermúdez-Aguirre, D., & Barbosa-Cánovas, G. V. (2011). Quality of selected cheeses fortified with vegetable and animal sources of omega-3. *LWT-Food Science and Technology, 44*(7), 1577-1584.

Bermúdez-Aguirre, D., & Barbosa-Cánovas, G. V. (2010). Processing of Soft Hispanic Cheese ("Queso Fresco") Using Thermo-Sonicated Milk: A Study of Physicochemical Characteristics and Storage Life. *Journal of food science, 75*(9), S548-S558.

Borgmeyer, J. R., Smith, C. E., & Huynh, Q. K. (1992). Isolation and characterization of a 25 kDa antifungal protein from flax seeds. *Biochemical and biophysical research communications, 187*(1), 480-487.

Bradley, R., Arnold, E., Barbano, D., Semerad, R., Smith, D., & Vines, B. (1992). Chemical and physical methods. *Standard methods for the examination of dairy products, 16*, 433-531.

Bu, D., Wang, J., Dhiman, T., & Liu, S. (2007). Effectiveness of oils rich in linoleic and linolenic acids to enhance conjugated linoleic acid in milk from dairy cows. *Journal of Dairy Science, 90*(2), 998-1007.

Carroll, R. (2010). *Home Cheese Making: Recipes for 75 Homemade Cheeses*: Storey Publishing, LLC.

Chemists, A. o. O. A. (1991). *Journal of the Association of Official Analytical Chemists*: The Association.

Chemists, A. o. O. A., & Horwitz, W. *Official Methods of Analysis of the AOAC International*: The Association.

Chemists, A. o. O. A., & Horwitz, W. (1995). *Official Methods of Analysis of the Association of Official Analytical Chemists*: The Association.

Chilliard, Y., & Ferlay, A. (2004). Dietary lipids and forages interactions on cow and goat milk fatty acid composition and sensory properties. *Reproduction Nutrition Development, 44*(5), 467-492.

Chilliard, Y., Ferlay, A., & Doreau, M. (2001). Effect of different types of forages, animal fat or marine oils in cow's diet on milk fat secretion and composition, especially conjugated linoleic acid (CLA) and polyunsaturated fatty acids. *Livestock Production Science, 70*(1), 31-48.

Chilliard, Y., Ferlay, A., Mansbridge, R. M., & Doreau, M. (2000). *Ruminant milk fat plasticity: nutritional control of saturated, polyunsaturated, trans and conjugated fatty acids.* Paper presented at the Annales de Zootechnie.

Connor, W. E. (2000). Importance of n− 3 fatty acids in health and disease. *The American journal of clinical nutrition, 71*(1), 171S-175S.

Covington, M. B. (2004). Omega-3 fatty acids. *Atlantic, 1*, 2.0.

Damodaran, S., Parkin, K. L., & Fennema, O. R. (2007). *Fennema's Food Chemistry, Fourth Edition*: CRC Press.

Dhiman, T., Satter, L., Pariza, M., Galli, M., Albright, K., & Tolosa, M. (2000). Conjugated linoleic acid (CLA) content of milk from cows offered diets rich in linoleic and linolenic acid. *Journal of Dairy Science, 83*(5), 1016-1027.

Dunstan, J. A., Mitoulas, L. R., Dixon, G., Doherty, D. A., Hartmann, P. E., Simmer, K., & Prescott, S. L. (2007). The effects of fish oil supplementation in pregnancy on breast milk fatty acid composition over the course of lactation: a randomized controlled trial. *Pediatric research, 62*(6), 689-694.

Givens, D., Cottrill, B., Davies, M., Lee, P. A., Mansbridge, R., & Moss, A. R. (2000). *Sources of n-3 polyunsaturated fatty acids additional to fish oil for livestock diets-a review.* Paper presented at the Nutrition Abstracts and Reviews. Series B, Livestock Feeds and Feeding.

Gómez-Cortés, P., Frutos, P., Mantecón, A., Juárez, M., De La Fuente, M., & Hervás, G. (2008). Addition of olive oil to dairy ewe diets: Effect on milk fatty acid profile and animal performance. *Journal of dairy science, 91*(8), 3119-3127.

Guo, L., Van Hekken, D. L., Tomasula, P. M., Shieh, J., & Tunick, M. H. (2011). Effect of salt on the chemical, functional, and rheological properties of Queso Fresco during storage. *International Dairy Journal, 21*(5), 352-357.

Hill, A., Bullock, D., & Irvine, D. (1982). Manufacturing parameters of Queso Blanco made from milk and recombined milk. *Canadian Institute of Food Science and Technology Journal, 15*(1), 47-53.

Kaithwas, G., Mukerjee, A., Kumar, P., & Majumdar, D. K. (2011). *Linum usitatissimum* (linseed/flaxseed) fixed oil: antimicrobial activity and efficacy in bovine mastitis. *Inflammopharmacology, 19*(1), 45-52.

Kamal-Eldin, A. (2006). Effect of fatty acids and tocopherols on the oxidative stability of vegetable oils. *European Journal of Lipid Science and Technology, 108*(12), 1051-1061.

Kelly, M. L., Berry, J. R., Dwyer, D. A., Griinari, J., Chouinard, P. Y., Van Amburgh, M. E., & Bauman, D. E. (1998). Dietary fatty acid sources affect conjugated linoleic acid concentrations in milk from lactating dairy cows. *The Journal of nutrition, 128*(5), 881-885.

Kennelly, J. J. (1996). The fatty acid composition of milk fat as influenced by feeding oilseeds. *Animal Feed Science and Technology, 60*(3), 137-152.

Kolanowski, W., & Laufenberg, G. (2006). Enrichment of food products with polyunsaturated fatty acids by fish oil addition. *European Food Research and Technology, 222*(3-4), 472-477.

Lemon, D. (1975). An improved TBA test for rancidity. New Series Circular No. 51. Environment Canada. *Fisheries and Marine Service, Halifax, NS*.

Lock, A. L., & Bauman, D. E. (2004). Modifying milk fat composition of dairy cows to enhance fatty acids beneficial to human health. *Lipids, 39*(12), 1197-1206.

Loor, J. J., Ueda, K., Ferlay, A., Chilliard, Y., & Doreau, M. (2004). Biohydrogenation, Duodenal Flow, and Intestinal Digestibility of *Trans* Fatty Acids and Conjugated Linoleic Acids in Response to Dietary Forage: Concentrate Ratio and Linseed Oil in Dairy Cows. *Journal of Dairy Science, 87*(8), 2472-2485.

Luna, P., Bach, A., Juárez, M., & de la Fuente, M. A. (2008). Influence of diets rich in flax seed and sunflower oil on the fatty acid composition of ewes' milk fat especially on the level of conjugated linoleic acid, n-3 and n-6 fatty acids. *International dairy journal, 18*(1), 99-107.

Papastergiadis, A., Fatouh, A., Jacxsens, L., Lachat, C., Shrestha, K., Daelman, J., . . . De Meulenaer, B. (2014). Exposure assessment of Malondialdehyde, 4-Hydroxy-2-(E)-Nonenal and 4-Hydroxy-2-(E)-Hexenal through specific foods available in Belgium. *Food and Chemical Toxicology, 73*, 51-58.

Pinho, O., Mendes, E., Alves, M., & Ferreira, I. (2004). Chemical, physical, and sensorial characteristics of "Terrincho" ewe cheese: changes during ripening and intravarietal comparison. *Journal of dairy science, 87*(2), 249-257.

Rudnik, E., Szczucinska, A., Gwardiak, H., Szulc, A., & Winiarska, A. (2001). Comparative studies of oxidative stability of linseed oil. *Thermochimica Acta, 370*(1), 135-140.

Shamberger, R. J., Shamberger, B. A., & Willis, C. E. (1977). Malonaldehyde content of food. *The Journal of nutrition, 107*(8), 1404-1409.

Shingfield, K., Ahvenjarvi, S., Toivonen, V., Arola, A., Nurmela, K., Huhtanen, P., & Griinari, J. M. (2003). Effect of dietary fish oil on biohydrogenation of fatty acids and milk fatty acid content in cows. *Animal Science-Glasgow Then Penicuik-, 77*(1), 165-180.

Simopoulos, A. P. (1991). Omega-3 fatty acids in health and disease and in growth and development. *The American journal of clinical nutrition, 54*(3), 438-463.

Society, A. O. C. (1988). *Official and Tentative Methods*.

Surette, M. E. (2008). The science behind dietary omega-3 fatty acids. *Canadian Medical Association Journal, 178*(2), 177-180.

Tunick, M., & Van Hekken, D. (2002). Torsion gelometry of cheese. *Journal of dairy science, 85*(11), 2743-2749.

Vigers, A. J., Roberts, W., & Selitrennikoff, C. P. (1991). A new family of plant antifungal proteins. *Mol Plant Microbe Interact, 4*(4), 315-323.

Whelan, J., & Rust, C. (2006). Innovative dietary sources of n-3 fatty acids. *Annu. Rev. Nutr., 26*, 75-103.

Xu, Y., Hall, C., & Wolf-Hall, C. (2008). Antifungal activity stability of flaxseed protein extract using response surface methodology. *Journal of food science, 73*(1), M9-M14.

CHAPTER 5. APPLICATION OF EDIBLE FILMS CONTAINING OREGANO (*ORIGANUM VULGARE*) ESSENTIAL OIL TO EXTEND THE SHELF LIFE OF QUESO BLANCO CONTAINING FLAXSEED OIL

5.1 Introduction

Queso blanco is a type of fresh cheese with soft texture and elaborated traditionally from raw-cows' milk. It is originally from Latin-American countries and presents mild, salty flavor and crumbly texture. The increase in Latin-American population in the US has also led to an increase in the consumption of nostalgic products such as queso blanco. However, queso blanco has a short shelf life limitation due to the early development of yeasts and molds, which represents product loss and decreased profits to manufacturers (Sabikhi, 2007; Sandra, Stanford, & Goddik, 2004). Another limitation for queso blanco is the low content of polyunsaturated fatty acids (PUFA) and high content of saturated fatty acids.

The shelf life of cheeses can be extended by adding chemical preservatives, but as the demand for natural products increases, the use of chemicals in foods to extend their shelf life decreases. The use of natural antioxidants and antimicrobials in foods has been widely documented showing successful potential applications to extend shelf life of foods (Oussalah, Caillet, Saucier, & Lacroix, 2007). Oregano essential oil (OEO) has been recognized for its antimicrobial and antioxidant properties (Lagouri et al., 1993; Royo et al., 2010; Seydim & Sarikus, 2006). However, due to the high intensity of its flavor compounds it cannot be incorporated directly to all types of food. OEO may be incorporated within a whey-protein-isolate edible film that can be used to carry antimicrobial and antioxidant benefits of OEO to minimize surface microbial

development of cheese while keeping important attributes of the wrapped product (Zinoviadou et al., 2009).

Cheeses' composition differs depending on the origin of the milk, type of cheese, coagulation method, storage conditions, and additives used in their elaboration (Arispe & Westhoff, 1984). Queso blanco has a high content of the saturated fatty acids myristic (C14:0) and palmitic (C16:0) (Parnell-Clunies et al., 1985a). These represents a concern for consumers because high intake of saturated fatty acids has been linked to the development of cardiovascular diseases (Christiansen, Schnider, Palmvig, Tauber-Lassen, & Pedersen, 1997; Sabikhi, 2007). Hence, addition of polyunsaturated fatty acids (PUFA) has been done in foods in order to improve their nutritional profile (Whelan & Rust, 2006). Addition of flaxseed oil (FO) to queso blanco could improve its fatty acid profile but also affect its shelf life due to oxidative susceptibility. Free radicals such as superoxide ions (O2-), hydroxyl radicals (OH), and non-free radical compounds, have been identified as precursors for lipid oxidation in food and important diseases (Alma et al., 2003). Oxidation products include hydroperoxides, which are tasteless and odorless but are further decomposed to secondary products like aldehydes. Aldehydes impart off flavors to food and have been documented to be precursors of carcinogenesis in cells (Papastergiadis et al., 2014). On the other hand, most popular antioxidants namely butylated hydroxyanisole (BHA) and butylated hydroxy- toluene (BHT), which are generally recognized as safe (GRASS) have detrimental side effects at doses of 500 mg/kg body wt/day (5000 times the estimated average consumption per capita) including enzymatic, lipid, and pathological negative side effects in rodents and monkeys. High fat content, especially polyunsaturated fats make foods more

susceptible to oxidation. Protection of the polyunsaturated fatty acids (PUFA) is necessary to obtain longer shelf life in high-fat products. The use of antioxidants in order to extend the shelf-life of food is widely documented achieving shelf life values of 15-200% higher (Branen, 1975). Hence, natural antioxidants like plant derived essential oils are being incorporated in foods and packaging to diminish lipid oxidation. Oregano essential oil (OEO) due to its high phenolic content (carvacrol, carvacrol methyl ether, and thymol methyl ether) has gained attention as a potential natural antioxidant (Lagouri et al., 1993; Milos et al., 2000; Tsimidou et al., 1995) and may reduce the lipid oxidation of queso blanco.

The objective of this project was to evaluate the effect of wrapping with a whey-protein-isolate (WPI) edible film containing oregano essential oil (OEO) on the physicochemical and microbiological characteristics of cheeses containing flaxseed oil (FO) during one month of refrigerated storage.

5.2 Materials and methods

5.2.1 Elaboration of edible films

Edible films, WPIF (WPI edible film) and WPIOF (WPI edible film with OEO) were prepared as previously described in Chapter 3, section 3.2.1.

5.2.2 Elaboration of queso blanco and queso blanco containing flaxseed oil

Elaboration of control cheese (QU) and omega-3 cheese with flaxseed oil (HFQ) batches was performed as described in Chapter 4, section 4.2.1. After pressing, HFQ was wrapped by triplicate with whey-protein-isolate edible film (WPIF) and whey-protein-isolate edible film with oregano essential oil (WPIOF), producing WHFQ and WOHFQ batches, respectively. All cheeses (QU, HFQ, WHFQ, and WOHFQ) were

refrigerated at 8 °C with exposure to sunlight and fluorescent light (750 lumens) until analyses were performed. Batches of cheeses where analyzed by triplicates.

5.2.3 Color of control, cheese containing flaxseed oil, and wrapped cheeses containing flaxseed oil

Color of QU, HFQ, WHFQ, and WOHFQ was performed as described in Chapter 4, section 4.2.3 at days 1 and 30 of refrigerated storage with sunlight and fluorescent light (750 lumens) exposure.

5.2.4 Peroxide value and thiobarbituric acid reactive substances of flaxseed oil

Peroxide value (PV) and thiobarbituric acid reactive substances (TBARS) of flaxseed oil (FO) were determined at days 1 and 30 by triplicate during its refrigerated storage with sunlight and fluorescent light (750 lumens) exposure. PV was determined according to the official method of the American Oil Chemists Society (AOCS Ja 8-87). Oil samples of 5 (± 0.05) g were weighed into 250 ml Erlenmeyer flasks. Acetic acid –chloroform 1.5:1 (v/v) solution, saturated potassium iodide solution (stored in dark), and 0.1N sodium thiosulfate solution were prepared for the analysis and 1% starch solution was used as indicator. To each oil sample, 30 mL of acetic acid chloroform solution were added followed by agitation with a magnetic stirrer for 3 min. Using a micropipette, 0.5 mL of saturated potassium iodide solution were added to Erlenmeyer flask followed by stirring during 1 min. After that, 30 mL of deionized water were added to the Erlenmeyer flask and was shaken vigorously to liberate the iodine from the chloroform layer. Using a micropipette 1 mL of starch solution was added to the Erlenmeyer flask and titration with 0.1N sodium thiosulfate was performed until the blue-gray color disappeared in the upper layer. The amount of 0.1N sodium thiosulfate used to titrate each sample was recorded and PV was calculated according to eq. 11:

$$PV = \frac{(S-B)\ X\ N\ x\ 1000}{w} \qquad (11)$$

Where PV= Peroxide Value (meq. of active oxygen per kg of oil); S= volume of titrant used for sample; B= volume of titrant used for blank; N= normality of titrant; and w= weight of sample.

TBARS of FO were determined according to the method used by Mei, McClements, Wu, and Decker (1998) with some modifications. A solution of thiobarbituric acid (TBA) was prepared by mixing 15 g of trichloroacetic acid, 0.375 g of TBA, 1.76 mL of 12 N HCL, and 82.9 mL of H_2O. Of the resulting TBA solution, 2 mL were mixed with 1 mL of a sample prepared by diluting 0.008g of FO in 0.092 mL of deionized water. The mixture agitated in a vortex unit for 10 s and heated in a boiling water bath for 15 min followed by cooling with tap water for 10 min. After cooling the sample was centrifuged at 15,244 x *g* for 10 min (Eppendorf centrifuge 5417C Brinkmann Instruments Inc. Westbury, NY, USA). The absorbance of the supernatant was measured at 530 nm. A standard curve with 1, 1, 3, 3-tetraethoxypropane was constructed to determine TBARS concentrations of 0-0.02 µmoles/mL. The results were expressed in mmol of malondialdehyde per kg oil.

5.2.5 Thiobarbituric acid reactive substances of control, cheese containing flaxseed oil, and wrapped cheeses containing flaxseed oil

Thiobarbituric acid reactive substances (TBARS) of QU, HFQ, WHFQ, and WOHFQ were determined according to the method described in Chapter 4, section 4.2.4 at days 1 and 30 of refrigerated storage with sunlight and fluorescent light (750 lumens) exposure.

5.2.6 Total yeast and mold count and total coliform count of control, cheese containing flaxseed oil, and wrapped cheeses containing flaxseed oil

Total yeast and mold count (TYMC) and total coliform count (TCC) of QU, HFQ, WHFQ, and WOHFQ was performed as described in Chapter 4, section 4.2.5 at days 1 and 30 of refrigerated storage with sunlight and fluorescent light (750 lumens) exposure.

5.2.7 pH of control, cheese containing flaxseed oil, and wrapped cheeses containing flaxseed oil

pHs of QU, HFQ, WHFQ, and WOHFQ were measured as described in Chapter 4, section 4.2.6. Measurements were performed at day 1 and 30 of refrigerated storage with sunlight and fluorescent light (750 lumens) exposure.

5.2.8 Microstructure of control cheese and cheese containing flaxseed oil

Scanning electron microscopy (SEM) was used to examine the microstructure and surface area of QU and HFQ at days 1 and 30 using the method described by Lobato-Calleros, Robles-Martinez, Caballero-Perez, Vernon-Carter, and Aguirre-Mandujano (2001). Squared samples of 0.5 cm x 0.5 cm that were fixed in a buffer (pH 7.2) and consequently dehydrated in increasing concentrations of ethanol solutions (50%, 70%, 80%, 90% and 100%, 30 min in each and 3x with 100%) were critical-point dried in a DCP-1 critical point drying apparatus (Denton Vacuum, Inc.). The cheese samples were mounted on aluminum stubs with double-stick tape and sputter-coated with a gold-palladium layer of 10nm (Leica EM ACE600). SEM was performed using a high vacuum Scanning Electron Microscope (JSM-6610LV, Jeol Ltd., Akishima, Japan), at 10 kV.

5.2.9 Statistical Analysis

The collected data were analyzed using Statistical Analysis System (SAS, Version 9.2, SAS Institute Inc., Cary, NC., USA). Triplicate experiments were conducted and Tukey's studentized range mean separation test was used to detect statistical differences (α=0.05).

5.3 Results and discussion

5.3.1 Color of control, cheese containing flaxseed oil, and wrapped cheeses containing flaxseed oil

Darkness-Lightness (L*), greenness-redness (a*), and blueness-yellowness (b*) values of QU, HFQ, WHFQ, and WOHFQ are presented in Table 11. According to these results, WHFQ and WOHFQ presented lowest ($p<0.05$) lightness at day 1 and 30 of storage when compared to HFQ but were not significantly different from QU.

Table 11. Color of control, cheese containing flaxseed oil, and wrapped cheeses containing flaxseed oil

Color	Treatment	Day 1	Day 30
L*	QU	$84.72 \pm 0.32^{AB(a)}$	$85.51 \pm 0.61^{AB(a)}$
	HFQ	$85.03 \pm 1.24^{A(a)}$	$86.71 \pm 0.67^{A(a)}$
	WHFQ	$81.91 \pm 1.06^{B(b)}$	$84.48 \pm 0.20^{B(a)}$
	WOHFQ	$81.73 \pm 1.65^{B(b)}$	$84.79 \pm 0.51^{B(a)}$
a*	QU	$-0.51 \pm 0.09^{A(a)}$	$-0.97 \pm 0.13^{B(b)}$
	HFQ	$-0.59 \pm 0.15^{A(a)}$	$-0.63 \pm 0.08^{A(a)}$
	WHFQ	$-0.56 \pm 0.07^{A(a)}$	$-0.90 \pm 0.04^{B(b)}$
	WOHFQ	$-0.56 \pm 0.14^{A(a)}$	$-0.52 \pm 0.08^{A(a)}$
b*	QU	$17.41 \pm 0.91^{A(a)}$	$16.31 \pm 0.53^{C(a)}$
	HFQ	$18.41 \pm 0.97^{A(a)}$	$17.84 \pm 1.31^{BC(a)}$
	WHFQ	$18.78 \pm 0.92^{A(b)}$	$20.64 \pm 0.35^{A(a)}$
	WOHFQ	$17.87 \pm 1.04^{A(a)}$	$19.30 \pm 0.35^{AB(a)}$

Means with different letters (AB) within columns are significantly different ($P<0.05$).
Means with different letters (ab) within rows are significantly different ($P<0.05$).
QU: Control (no flaxseed oil, FO), HFQ: cheese with FO added during homogenization, WHFQ= HFQ wrapped with whey protein isolate film (WPIF), WOHFQ= HFQ wrapped with WPIF containing oregano essential oil (WPIOF).
L*= (0-darkness, 100-lightness), a*= (- greenness +redness), b*= (- blueness + yellowness).

On the other hand, a* values indicated no significant ($p>0.05$) differences between cheeses at day 1 but HFQ and WOHFQ were significantly more red ($p<0.05$) than QU and WHFQ at day 30. This can be attributed to the presence of different types of yeast and molds on cheeses (Bermúdez-Aguirre & Barbosa-Cánovas, 2010). In addition, only QU and WHFQ presented significant ($p<0.05$) tendency towards greenness in time. Finally, b* values of cheeses were not significantly ($p>0.05$) different at day 1 but at day 30 QU and HFQ had lower ($p<0.05$) values than WHFQ which was not significantly ($p>0.05$) from WOHFQ. Only WHFQ significantly ($p<0.05$) increased b* value in time. Different colonies of yeast may have contributed to blueness tendency in QU and HFQ (Bermúdez-Aguirre & Barbosa-Cánovas, 2010). The results from this study indicated that there was major effect in color of cheeses by wrapping with edible films.

5.3.2 Peroxide value and thiobarbituric acid reactive substances of flaxseed oil

Peroxide value (PV) and thiobarbituric acid reactive substances (TBARS) of flaxseed oil (FO) during refrigerated storage with fluorescent light and sunlight exposure expressed as miliequivalents of active oxygen per kilogram of oil and mmol of malondialdehyde per kilogram of cheese respectively are shown in Table 12.

Table 12. Peroxide value and thiobarbituric acid reactive substances of flaxseed oil

Oxidation parameter	Day 1	Day 30
PV[1]	45.87 ± 0.12B	95.49 ± 2.01A
TBARS[2]	1.45 ± 0.29B	144.37 ± 9.12A

Means with different letters (AB) within rows are significantly different ($P<0.05$).
PV[1]: Peroxide value (miliequivalents of active oxygen per kg of oil)
TBARS[2]: Thiobarbituric Acid Reactive Substances (mmol of malondialdehyde per kg of oil)

These results show increased oxidation of FO in 30 days of storage according to both parameters, PV and TBARS. In fact, according to the FAO (Food and Agriculture

Organization) codex standard, the maximum tolerance for unrefined, cold –pressed, or virgin oils is 15 miliequivalents of active oxygen per kg of oil which is not met by FO even at day 1. There is no standard for a maximum tolerance of TBARS in oils but an increase of almost 100 fold clearly demonstrates susceptibility of FO to oxidation under refrigerated conditions and with exposure of fluorescent light and sunlight (same conditions applied to cheeses). These results are in accordance with several studies and literature about oxidation of polyunsaturated oils (Gardner, 1989; Leyton, Drury, & Crawford, 1987).

5.3.3 Thiobarbituric acid reactive substances of control, cheese containing flaxseed oil, and wrapped cheeses containing flaxseed oil

Our previous study showed that queso blanco incorporated with flaxseed oil (FO) during homogenization (HFQ) resulted in higher oxidation when compared to cheeses with FO added at different stages of the manufacture. Hence, it was recognized that protection of omega-3 fatty acids in cheese was needed. Development of edible films made of whey-protein-isolate (WPIF) and containing oregano essential oil (WPIOF) was performed to wrap the cheeses in order to decrease their oxidation. Results from Thiobarbituric Acid Reactive Substances (TBARS) expressed as μmol of malondialdehyde per kilogram of cheese of QU, HFQ, WHFQ, and WOHFQ are showed in Table 13. According to these results, at day 1 of storage HFQ had highest ($p<0.05$) TBARS than the rest of cheeses. These results showed that FO was highly susceptible to oxidation even at the low level of addition in cheese (1% w/w). This is in accordance with previous studies of oxidation-susceptibility of polyunsaturated fatty acids present in FO (Jenski, Sturdevant, Ehringer, & Stillwell, 1993). Moreover, addition of edible films resulted in lower TBARS than HFQ but only WPIOF (present in WOHFQ) showed no

significant ($p>0.05$) differences with respect to QU at day 1. In addition, at day 30 of storage, only HFQ was significantly ($p<0.05$) more oxidized than the rest of the cheeses. Furthermore, only HFQ presented a significant ($p<0.05$) increase of TBARS in time.

Table 13. Thiobarbituric Acid Reactive Substances (TBARS) of control, cheese containing flaxseed oil, and wrapped cheeses containing flaxseed oil

	TBARS[1]	
Treatment	Day 1	Day 30
QU	$1.70 \pm 0.05^{C(a)}$	$1.72 \pm 0.04^{B(a)}$
HFQ	$2.39 \pm 0.05^{A(b)}$	$2.73 \pm 0.13^{A(a)}$
WHFQ	$1.91 \pm 0.14^{B(a)}$	$1.88 \pm 0.18^{B(a)}$
WOHFQ	$1.65 \pm 0.04^{C(a)}$	$1.60 \pm 0.26^{B(a)}$

TBARS[1]= Thiobarbituric Acid Reactive Substances (µmol of malondialdehyde per kg of cheese).
Means with different letters (AB) within columns are significantly different ($P<0.05$).
Means with different letters (ab) within rows are significantly different ($P<0.05$).
QU: Control (no flaxseed oil, FO), HFQ: cheese with FO added during homogenization, WHFQ= HFQ wrapped with whey protein isolate film (WPIF), WOHFQ= HFQ wrapped with WPIF containing oregano essential oil (WPIOF).

Previous studies performed on fresh and aged cheeses found no relationship between the ripening period and oxidation levels (Fedele & Bergamo, 2001) which supports the results found for QU. Our study demonstrated that both, WPIOF and WPIF were effective in protecting omega-3 cheese (HFQ) from oxidation. Antioxidative effect of WPIOF can be attributed to OEO high carvacrol content (Aeschbach et al., 1994; Lambert et al., 2001; Mastelic, Jerkovic, Blažević, Poljak-Blaži, Borović, Ivančić-Baće, Smrečki, Žarković, Brčić-Kostic, & Vikić-Topić, 2008; Michiels, Missotten, Fremaut, De Smet, & Dierick, 2007). WHFQ also had the same level of oxidation as QU at 30 days of storage although FO was not present in QU, demonstrating therefore a protective effect attributed to efficient but not total oxygen barrier properties of the whey proteins present in WPIF (Elias, Kellerby, & Decker, 2008; Hu, McClements, & Decker, 2003; Peña-Ramos & Xiong, 2003; Peng, Xiong, & Kong, 2009; Tien, Vachon, Mateescu, & Lacroix, 2001; L. Tong, S. Sasaki, D. McClements, & E. Decker, 2000; L. M. Tong, S. Sasaki, D. J. McClements, & E. A. Decker, 2000). Milk proteins antioxidative properties are

attributed to cysteine SH groups which can link quinones producing stable, colorless compounds (Dudley & Hotchkiss, 1989). Also histidine residues and its derivatives, which have an imidazole group, can donate hydrogen molecules exerting an antioxidant effect (Kohen, Yamamoto, Cundy, & Ames, 1988). Besides cysteine and histidine, other aromatic amino acids like tyrosine and tryptophan can stabilize free radicals (Berlett & Stadtman, 1997). Previous studies have reported antioxidant activity of acid whey by small peptides (Kohen et al., 1988) and lactose influence in contributing to radical scavenging activity (Wehmeier & Mooradian, 1994).

5.3.3 Total yeast and mold count and total coliform count of control, cheese containing flaxseed oil, and wrapped cheeses containing flaxseed oil

Total yeast and mold count (TYMC) of QU, HFQ, WHFQ, and WOHFQ is presented in Table 14. According to these results, no significant ($p>0.05$) differences were observed at day 1 of storage as development of spoilage yeast and molds in fresh cheeses usually requires more time (1-8 weeks) (Petersen, Nielsen, Bertelsen, Lawther, Olsen, Nilsson, & Mortensen, 1999a). However, at day 30 of storage QU presented highest ($p<0.05$) counts when compared to the rest of the treatments showing an expected short shelf life (Altieri, Scrocco, Sinigaglia, & Del Nobile, 2005; Chandan, 1996; Del Nobile, Gammariello, Conte, & Attanasio, 2009). Nevertheless, when flaxseed oil (FO) was incorporated to the cheese matrix in HFQ, WHFQ, and WOHFQ, an antimicrobial effect was observed. WOHFQ had lower ($p<0.05$) counts than WHFQ but WOHFQ was not significantly ($p>0.05$) different from HFQ. These results suggest that a greater storage period (at least 60 days) is required in order to observe significant differences between HFQ and WOHFQ. Similarly, QU and WHFQ presented increased TYMC in time whereas HFQ and WOHFQ did not. Wrapping with WPIF could have retained

water from syneresis which further favored the growth of yeasts and molds. Literature has previously reported antimicrobial activity of oregano essential oil (OEO) applied to foods and packaging (Royo et al., 2010; Seydim & Sarikus, 2006; Zinoviadou et al., 2009), which supports these findings. Likewise, some studies have reported strong antifungal activity of a protein fraction in flaxseeds (Borgmeyer et al., 1992) and moderate antifungal and antibacterial properties in lignans (phenolic compounds) present in flaxseed and its derivatives (Barbary, El-Sohaimy, El-Saadani, & Zeitoun, 2010). Total coliform count was 0 CFU/g cheese (data not shown) for unwrapped and wrapped cheeses during one month of refrigerated storage as sanitary conditions were applied during the cheese making process.

Table 14. Total Yeast and Mold Count (TYMC) of control, cheese containing flaxseed oil, and wrapped cheeses containing flaxseed oil

	TYMC[1]	
Treatment	Day 1	Day 30
QU	$< 1.00 \pm 0.00^{A(b)}$	$2.43 \pm 0.08^{A(a)}$
HFQ	$< 1.00 \pm 0.00^{A(a)}$	$< 1.00 \pm 0.40^{BC(a)}$
WHFQ	$< 1.00 \pm 0.00^{A(b)}$	$1.10 \pm 0.46^{B(a)}$
WOHFQ	$< 1.00 \pm 0.00^{A(a)}$	$< 1.00 \pm 0.00^{C(a)}$

TYMC[1]= Log Colony Forming Units of Total Yeast and Mold Count per g of cheese.
Means with different letters (AB) within columns are significantly different (P<0.05).
Means with different letters (ab) within rows are significantly different (P<0.05).
QU: Control (no flaxseed oil, FO), HFQ: cheese with FO added during homogenization, WHFQ= HFQ wrapped with whey protein isolate film (WPIF), WOHFQ= HFQ wrapped with WPIF containing oregano essential oil (WPIOF).

5.3.4 pH of control, cheese containing flaxseed oil, and wrapped cheeses containing flaxseed oil

The pHs of QU, HFQ, WHFQ, and WOHFQ are presented in Table 15. According to these results, at day 1 of storage QU had significant ($p<0.05$) higher pH than WHFQ and WOHFQ but not significantly ($p>0.05$) different from HFQ. However, at day 30 WOHFQ and HFQ presented significantly ($p<0.05$) lower pH when compared to QU and WHFQ. Regarding time effect, only WHFQ and WOHFQ showed significant ($p<0.05$)

increase in pH whereas HFQ had significant ($p<0.05$) decrease in pH. Values of pH for queso blanco vary between methods of elaboration (being acid or enzymatic precipitation the main cause of variation), type and amount of acid or enzyme used for solids recovery, and type of milk used (Hill et al., 1982).

Table 15. pH of control, cheese containing flaxseed oil, and wrapped cheeses containing flaxseed oil

	pH	
Treatment	Day 1	Day 30
QU	$6.03 \pm 0.07^{A(a)}$	$6.37 \pm 0.23^{A(a)}$
HFQ	$5.98 \pm 0.12^{A(a)}$	$5.40 \pm 0.20^{B(b)}$
WHFQ	$5.40 \pm 0.00^{B(b)}$	$6.39 \pm 0.16^{A(a)}$
WOHFQ	$5.47 \pm 0.06^{B(b)}$	$5.65 \pm 0.03^{Ba)}$

Means with different letters (AB) within columns are significantly different ($P<0.05$).
Means with different letters (ab) within rows are significantly different ($P<0.05$).
QU: Control (no flaxseed oil, FO), HFQ: cheese with FO added during homogenization, WHFQ= HFQ wrapped with whey protein isolate film (WPIF), WOHFQ= HFQ wrapped with WPIF containing oregano essential oil (WPIOF).

Literature reports pH values ranging 5.15 -5.5 (Parnell-Clunies et al., 1985a; Parnell-Clunies, Irvine, & Bullock, 1985b) with 5.2-5.3 being the optimum range to develop desired flavor and body (Glass, Prasad, Schlyter, Uljas, Farkye, & Luchansky, 1995; Hill et al., 1982). However, higher pH values ranging from 6 to ≥6.5 have also been found in Latin-American style cheeses (Genigeorgis, Toledo, & Garayzabal, 1991; Uhlich, Luchansky, Tamplin, Molina-Corral, Anandan, & Porto-Fett, 2006) and have correlated positively with the presence of *Listeria monocytogenes.* Lower values of pH at day 1 in wrapped cheeses could be attributed to the natural microflora present during its wrapping (post-pasteurization contaminants) rather than to presence of flaxseed oil or film effect. Significant increase of pH of WHFQ and WOHFQ after 1 month of storage could be attributed to a lower water content of cheese caused by syneresis, which concentrates salt level and to the presence of yeasts that can deacidify the cheese (Beresford, Fitzsimons, Brennan, & Cogan, 2001). Several studies have reported

decreased moisture content when storing cheeses at refrigeration temperatures and higher temperatures (Arispe & Westhoff, 1984; Farkye, 2004). Decreasing pH values of HFQ might be attributed to lactose consumption by fermenting, post-contaminant bacteria that may have produced lactic acid (Turner & Thomas, 1980).

5.3.5 Microstructure of control cheese and cheese containing flaxseed oil

Scanning electron micrographs of QU and HFQ at day 1 are showed in Figures 10 and 11.

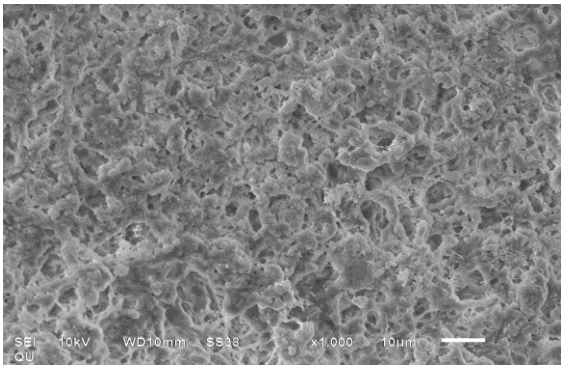


Figure 10. Scanning electron micrograph of QU (control cheese, no flaxseed oil).

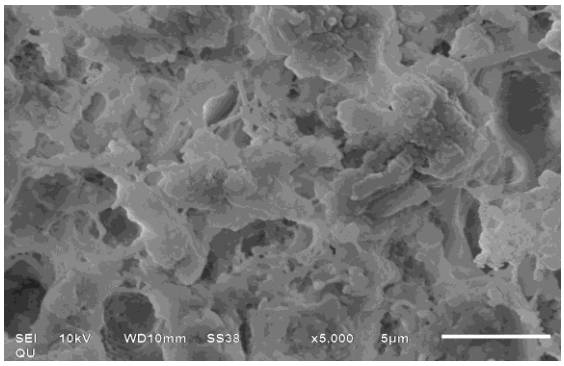


Figure 11. Scanning electron micrograph of QU (control cheese, no flaxseed oil).

High milk heating temperatures (88 ºC) used during cheese elaboration produced a compacted structure with random distribution of casein particles. Moreover, a “core-lining” structure was also observed in figures 10, 11, 12, and 13 being heat treatment and pH range between 5.2-5.5 being particularly responsible for its development (Sainani, Vyas, & Tong, 2004).

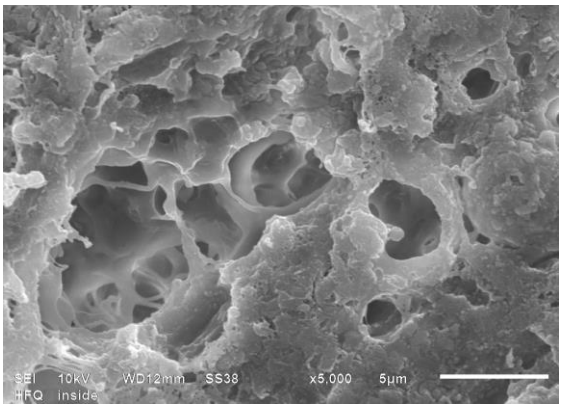


Figure 12. Scanning electron micrograph of HFQ (queso blanco with flaxseed oil added during homogenization).

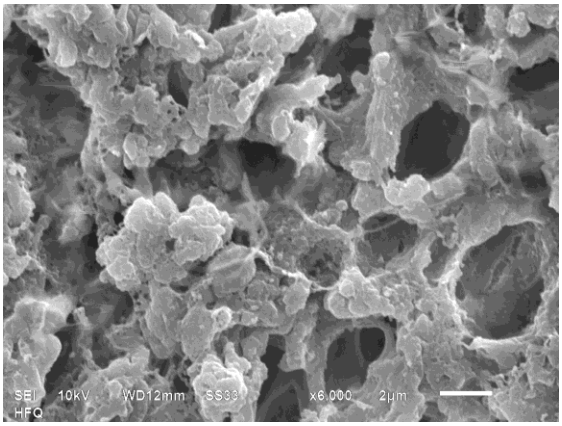


Figure 13. Scanning electron micrograph of HFQ (queso blanco with flaxseed oil added during homogenization).

This structure consists of casein micelles with a nucleus (>300 nm diameter) surrounded by an outer layer (30-50 nm thick) and separated by an empty space (50-80nm wide).The proposed mechanism of core-lining structure formation lies on the heat-induced interaction between β- lactoglobulin and κ-casein that leads to formation of protuberances. Then,β-casein after dissociation from casein micelles caused by heat treatment precipitates on filamentous protuberances forming a layer and leaving a ringed space between casein nucleus and the outer layer formed (D. Lee & Merson, 1975). On the other hand, pH allows casein micelles to acquire adequate volume avoiding sedimentation and presence of colloidal calcium phosphate (Farkye, 2004) . Particulate appearance of micrographs in both, QU and HFQ was due to destabilized

casein micelles that constitute the matrix when aggregated (Guo et al., 2011). On the other hand, differences in the microstructure of QU and HFQ were also noticed by bigger void spaces, less dense protein matrix, and less crosslinking in HFQ and can be attributed to enhanced proteolysis of matrix during the homogenization step (34,010 x *g* for 10min) in HFQ to incorporate flaxseed oil (FO). In addition proteolytic enzymes could have been present in greater amount in HFQ during its elaboration, or could come from milk and survived pasteurization, or produced by post-pasteurization bacteria resulting in a breaking down of the cheese matrix. It has also been reported that fat globules can merge and form larger droplets that appear like holes in the protein network (Guo et al., 2011; Hernando, Pérez-Munuera, & Lluch, 2000) which demonstrates the FO incorporation by HFQ matrix as numerous and larger cavities were observed in HFQ than in QU. Scanning Electron micrographs of QU at day 30 are shown in Figure 14.

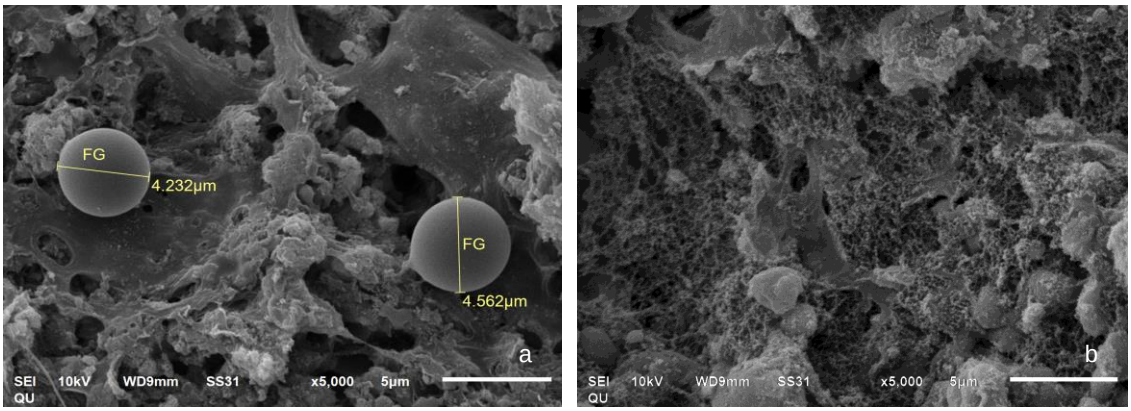


Figure 14. Scanning electron micrographs of QU (control cheese, no flaxseed oil): (a) milk fat globules (FG) integrated in the protein matrix; (b) protein network.

Control cheese (QU) presented an open protein matrix (Figures 14a and 14b) interrupted by fat globules (Figure 14a). Different sizes of FG were observed and confirmed by variation of the void spaces that they originally occupied. The diameter size observed for FG (4.2-4.5 µm) was similar to the one reported by Lobato-Calleros, Reyes-Hernández, Beristain, Hornelas-Uribe, Sánchez-García, and Vernon-Carter

(2007). Figure 14a shows FG enclosed in the protein matrix, the filamentous links can be attributed to casein and/or whey proteins. Similarly, Figure 14b shows protein matrix interrupted by immersed FG with a granular appearance due to adsorbed casein micelles possibly during the first heat treatment (pasteurization) with filamentous fibers from casein and whey chains. The presence of such adsorbed proteins in the surface of FG probably lead to further assimilation to the protein network of QU causing a more compact structure (Lobato-Calleros, Sosa-Pérez, Rodríguez-Tafoya, Sandoval-Castilla, Pérez-Alonso, & Vernon-Carter, 2008).

Scanning Electron micrographs of HFQ at day 30 are shown in Figure 15. Queso blanco with FO added during homogenization (HFQ) presented a more open structure with less degree of linking between protein chains (Marchesseau, Gastaldi, Lagaude, & Cuq, 1997), which promoted shorter filaments (Lobato-Calleros et al., 2007). Small void spaces represent the originally spaces occupied by FO droplets, and larger void spaces represent the originally spaces occupied by milk fat globules (FG).

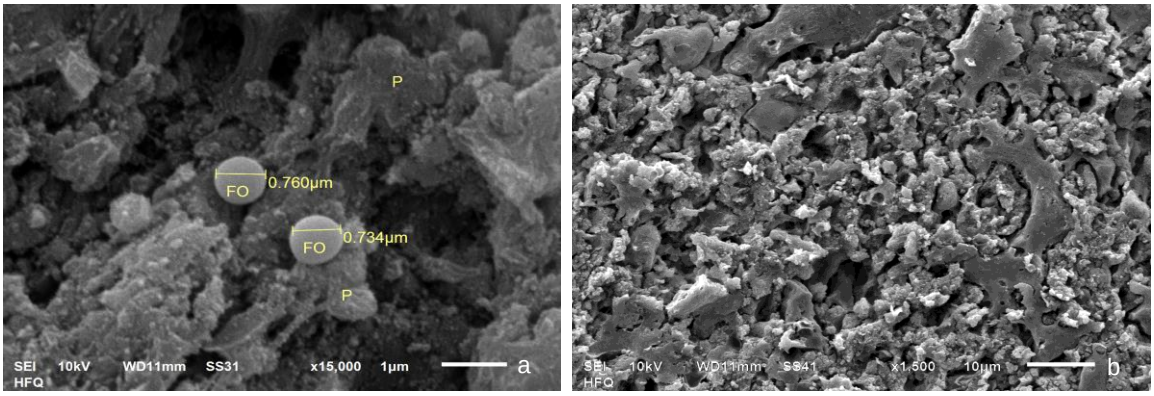


Figure 15. Scanning electron micrographs of HFQ (queso blanco with flaxseed oil added during homogenization): (a) flaxseed oil droplets; (b) protein network.

Due to the numerous void spaces of different sizes, the interactions between casein and whey chains was affected and lead to a more porous open structure when compared to QU (Hassan & Awad, 2005). Homogenization of milk in HFQ during FO addition also

contributed to the openness of the network as smaller sizes of both FG and FO droplets was achieved. These resulted in variable droplets size with the smaller size droplets attributed to FO and the larger ones to FG; the increased surface area of FO droplets present in HFQ could have favored its oxidation, which supports the results found in the oxidation section of this study. In Figure 15a two FO droplets can be observed with a diameter of 0.7 μm, some of these droplets are covered by a porous protein network (Figure 15b) (Ong, Dagastine, Kentish, & Gras, 2011).

5.4 Conclusions

Antioxidant and antimicrobial edible film with oregano essential oil (OEO) extended the shelf life of queso blanco containing flaxseed oil (FO) during 30 days of refrigerated storage. Both, whey-protein- isolate edible film (WPIF) and WPIF with oregano essential oil (WPIOF) exhibited improved barrier properties against pro-oxidant agents (UV light, oxygen, free radicals, etc.) demonstrated by lower oxidation rate when compared to omega 3 cheeses with no film (HFQ). In fact, wrapping cheeses with edible films (WPIF and WPIOF) resulted in no significant increase of TBARS in time and the same level of oxidation as control (QU) after 1 month of storage. WPIOF was more efficient in controlling the development of yeasts and molds in queso blanco than WPIF but did not differ significantly from HFQ, which also showed antifungal properties due to FO's lignan content. Incorporation of FO into queso blanco was successfully conducted by homogenization of FO in the milk producing HFQ. This study demonstrated the advantage of applying a biodegradable packaging to protect cheese from oxidation and early microbial spoilage. Scanning electron micrographs revealed retention of FO in HFQ, which presented an opened structure and less dense protein matrix than QU due

to homogenization of FO, which increased its surface area and susceptibility to oxidation. A core-lining structure was observed for both, QU and HFQ. Minor changes in the color of cheeses were detected from addition of oil and effect of type of edible film. Changes in pH occurred due to natural microflora of cheese, moisture loss, and development of yeasts and molds.

5.5 References

Aeschbach, R., Löliger, J., Scott, B., Murcia, A., Butler, J., Halliwell, B., & Aruoma, O. (1994). Antioxidant actions of thymol, carvacrol, 6-gingerol, zingerone and hydroxytyrosol. *Food and Chemical Toxicology, 32*(1), 31-36.

Alma, M. H., Mavi, A., Yildirim, A., Digrak, M., & Hirata, T. (2003). Screening chemical composition and in vitro antioxidant and antimicrobial activities of the essential oils from Origanum syriacum L. growing in Turkey. *Biological and Pharmaceutical Bulletin, 26*(12), 1725-1729.

Altieri, C., Scrocco, C., Sinigaglia, M., & Del Nobile, M. (2005). Use of chitosan to prolong mozzarella cheese shelf life. *Journal of dairy science, 88*(8), 2683-2688.

Arispe, I., & Westhoff, D. (1984). Manufacture and quality of Venezuelan white cheese. *Journal of Food Science, 49*(4), 1005-1010.

Barbary, O., El-Sohaimy, S., El-Saadani, M., & Zeitoun, A. (2010). Antioxidant, antimicrobial and anti-HCV activities of lignan extracted from flaxseed. *Res J Agric & Biol Sci, 6*(3), 247-256.

Beresford, T. P., Fitzsimons, N. A., Brennan, N. L., & Cogan, T. M. (2001). Recent advances in cheese microbiology. *International Dairy Journal, 11*(4), 259-274.

Berlett, B. S., & Stadtman, E. R. (1997). Protein oxidation in aging, disease, and oxidative stress. *Journal of Biological Chemistry, 272*(33), 20313-20316.

Bermúdez-Aguirre, D., & Barbosa-Cánovas, G. V. (2010). Processing of Soft Hispanic Cheese (“Queso Fresco”) Using Thermo-Sonicated Milk: A Study of Physicochemical Characteristics and Storage Life. *Journal of food science, 75*(9), S548-S558.

Borgmeyer, J. R., Smith, C. E., & Huynh, Q. K. (1992). Isolation and characterization of a 25 kDa antifungal protein from flax seeds. *Biochemical and biophysical research communications, 187*(1), 480-487.

Branen, A. (1975). Toxicology and biochemistry of butylated hydroxyanisole and butylated hydroxytoluene. *Journal of the American Oil Chemists' Society, 52*(2), 59-63.

Chandan, R. (1996). *Cheeses made by direct acidification*: Woodhead Publishing Limited, England.

Christiansen, E., Schnider, S., Palmvig, B., Tauber-Lassen, E., & Pedersen, O. (1997). Intake of a diet high in trans monounsaturated fatty acids or saturated fatty acids: effects on postprandial insulinemia and glycemia in obese patients with NIDDM. *Diabetes care, 20*(5), 881-887.

Del Nobile, M. A., Gammariello, D., Conte, A., & Attanasio, M. (2009). A combination of chitosan, coating and modified atmosphere packaging for prolonging Fior di latte cheese shelf life. *Carbohydrate polymers, 78*(1), 151-156.

Dudley, E. D., & Hotchkiss, J. H. (1989). Cysteine as an inhibitor of polyphenol oxidase. *Journal of Food Biochemistry, 13*(1), 65-75.

Elias, R. J., Kellerby, S. S., & Decker, E. A. (2008). Antioxidant activity of proteins and peptides. *Critical reviews in food science and nutrition, 48*(5), 430-441.

Farkye, N. (2004). Acid-and acid/rennet-curd cheeses. Part C: Acid-heat coagulated cheeses. *Cheese Chemistry, Physics and Microbiology, 2*, 343-348.

Fedele, E., & Bergamo, P. (2001). Protein and lipid oxidative stresses during cheese manufacture. *Journal of Food science, 66*(7), 932-935.

Gardner, H. W. (1989). Oxygen radical chemistry of polyunsaturated fatty acids. *Free Radical Biology and Medicine, 7*(1), 65-86.

Genigeorgis, C., Toledo, J. H., & Garayzabal, F. J. (1991). Selected microbiological and chemical characteristics of illegally produced and marketed soft Hispanic-style cheeses in California. *Journal of Food Protection®, 54*(8), 598-601.

Glass, K. A., Prasad, B. B., Schlyter, J. H., Uljas, H. E., Farkye, N. Y., & Luchansky, J. B. (1995). Effects of acid type and Alta™ 2341 on Listeria monocytogenes in a Queso Blanco type of cheese. *Journal of Food Protection®, 58*(7), 737-741.

Guo, L., Van Hekken, D. L., Tomasula, P. M., Shieh, J., & Tunick, M. H. (2011). Effect of salt on the chemical, functional, and rheological properties of Queso Fresco during storage. *International Dairy Journal, 21*(5), 352-357.

Hassan, A., & Awad, S. (2005). Application of exopolysaccharide-producing cultures in reduced-fat Cheddar cheese: Cryo-scanning electron microscopy observations. *Journal of dairy science, 88*(12), 4214-4220.

Hernando, I., Pérez-Munuera, I., & Lluch, M. (2000). Caracterización microestructural del queso de Burgos mediante diferentes técnicas microscópicas/Microstructural characterization of Burgos cheese using different microscopy techniques. *Food science and technology international, 6*(2), 151-157.

Hill, A., Bullock, D., & Irvine, D. (1982). Manufacturing parameters of Queso Blanco made from milk and recombined milk. *Canadian Institute of Food Science and Technology Journal, 15*(1), 47-53.

Hu, M., McClements, D. J., & Decker, E. A. (2003). Impact of whey protein emulsifiers on the oxidative stability of salmon oil-in-water emulsions. *Journal of Agricultural and Food Chemistry, 51*(5), 1435-1439.

Jenski, L. J., Sturdevant, L. K., Ehringer, W. D., & Stillwell, W. (1993). Omega-3 Fatty Acid Modification of Membrane Structure and Function. I. Dietary Manipulation of Tumor Cell Susceptibility to Cell-and Complement-Mediated Lysis.

Kohen, R., Yamamoto, Y., Cundy, K. C., & Ames, B. N. (1988). Antioxidant activity of carnosine, homocarnosine, and anserine present in muscle and brain. *Proceedings of the National Academy of Sciences, 85*(9), 3175-3179.

Lagouri, V., Blekas, G., Tsimidou, M., Kokkini, S., & Boskou, D. (1993). Composition and antioxidant activity of essential oils from oregano plants grown wild in Greece. *Zeitschrift für Lebensmittel-Untersuchung und Forschung, 197*(1), 20-23.

Lambert, R., Skandamis, P. N., Coote, P. J., & Nychas, G. J. (2001). A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. *Journal of applied microbiology, 91*(3), 453-462.

Lee, D., & Merson, R. (1975). Examination of cottage cheese whey proteins by scanning electron microscopy: relationship to membrane fouling during ultrafiltration. *Journal of dairy science, 58*(10), 1423-1432.

Leyton, J., Drury, P., & Crawford, M. (1987). Differential oxidation of saturated and unsaturated fatty acids in vivo in the rat. *British Journal of Nutrition, 57*(03), 383-393.

Lobato-Calleros, C., Reyes-Hernández, J., Beristain, C., Hornelas-Uribe, Y., Sánchez-García, J., & Vernon-Carter, E. (2007). Microstructure and texture of white fresh cheese made with canola oil and whey protein concentrate in partial or total replacement of milk fat. *Food research international, 40*(4), 529-537.

Lobato-Calleros, C., Sosa-Pérez, A., Rodríguez-Tafoya, J., Sandoval-Castilla, O., Pérez-Alonso, C., & Vernon-Carter, E. (2008). Structural and textural characteristics of reduced-fat cheese-like products made from W 1/O/W 2 emulsions and skim milk. *LWT-Food Science and Technology, 41*(10), 1847-1856.

Lobato-Calleros, C., Robles-Martinez, J., Caballero-Perez, J., Vernon-Carter, E., & Aguirre-Mandujano, E. (2001). Fat Replacers in Low-Fat Mexican Manchego Cheese. *Journal of Texture Studies, 32*(1), 1-14.

Marchesseau, S., Gastaldi, E., Lagaude, A., & Cuq, J.-L. (1997). Influence of pH on protein interactions and microstructure of process cheese. *Journal of Dairy Science, 80*(8), 1483-1489.

Mastelic, J., Jerkovic, I., Blažević, I., Poljak-Blaži, M., Borović, S., Ivančić-Baće, I., . . . Vikić-Topić, D. (2008). Comparative study on the antioxidant and biological activities of carvacrol, thymol, and eugenol derivatives. *Journal of agricultural and food chemistry, 56*(11), 3989-3996.

Mei, L., McClements, D. J., Wu, J., & Decker, E. A. (1998). Iron-catalyzed lipid oxidation in emulsion as affected by surfactant, pH and NaCl. *Food chemistry, 61*(3), 307-312.

Michiels, J., Missotten, J., Fremaut, D., De Smet, S., & Dierick, N. (2007). *In vitro* dose–response of carvacrol, thymol, eugenol and *trans*-cinnamaldehyde and interaction of combinations for the antimicrobial activity against the pig gut flora. *Livestock Science, 109*(1), 157-160.

Milos, M., Mastelic, J., & Jerkovic, I. (2000). Chemical composition and antioxidant effect of glycosidically bound volatile compounds from oregano (*Origanum vulgare L. ssp. hirtum*). *Food Chemistry, 71*(1), 79-83.

Ong, L., Dagastine, R. R., Kentish, S. E., & Gras, S. L. (2011). Microstructure of milk gel and cheese curd observed using cryo scanning electron microscopy and confocal microscopy. *LWT-Food Science and Technology, 44*(5), 1291-1302.

Oussalah, M., Caillet, S., Saucier, L., & Lacroix, M. (2007). Inhibitory effects of selected plant essential oils on the growth of four pathogenic bacteria: *E. coli* O157: H7, *Salmonella typhimurium*, *Staphylococcus aureus*, and *Listeria monocytogenes*. *Food control, 18*(5), 414-420.

Papastergiadis, A., Fatouh, A., Jacxsens, L., Lachat, C., Shrestha, K., Daelman, J., . . . De Meulenaer, B. (2014). Exposure assessment of Malondialdehyde, 4-Hydroxy-2-(E)-Nonenal and 4-Hydroxy-2-(E)-Hexenal through specific foods available in Belgium. *Food and Chemical Toxicology, 73*, 51-58.

Parnell-Clunies, E. M., Irvine, D., & Bullock, D. (1985a). Composition and yield studies for Queso Blanco made in pilot plant and commercial trials with dilute acidulant solutions. *Journal of Dairy Science, 68*(11), 3095-3103.

Parnell-Clunies, E. M., Irvine, D., & Bullock, D. (1985b). Heat treatment and homogenization of milk for Queso Blanco (Latin American white cheese) manufacture. *Canadian Institute of Food Science and Technology Journal, 18*(2), 133-136.

Peña-Ramos, E. A., & Xiong, Y. L. (2003). Whey and soy protein hydrolysates inhibit lipid oxidation in cooked pork patties. *Meat Science, 64*(3), 259-263.

Peng, X., Xiong, Y. L., & Kong, B. (2009). Antioxidant activity of peptide fractions from whey protein hydrolysates as measured by electron spin resonance. *Food Chemistry, 113*(1), 196-201.

Petersen, K., Nielsen, P. V., Bertelsen, G., Lawther, M., Olsen, M. B., Nilsson, N. H., & Mortensen, G. (1999). Potential of biobased materials for food packaging. *Trends in Food Science & Technology, 10*(2), 52-68.

Royo, M., Fernández-Pan, I., & Maté, J. I. (2010). Antimicrobial effectiveness of oregano and sage essential oils incorporated into whey protein films or cellulose-based filter paper. *Journal of the Science of Food and Agriculture, 90*(9), 1513-1519.

Sabikhi, L. (2007). Designer milk. *Advances in Food and Nutrition Research, 53*, 161-198.

Sainani, M., Vyas, H., & Tong, P. (2004). Characterization of particles in cream cheese. *Journal of dairy science, 87*(9), 2854-2863.

Sandra, S., Stanford, M., & Goddik, L. M. (2004). The Use of High-pressure Processing in the Production of Queso Fresco Cheese. *Journal of food science, 69*(4), FEP153-FEP158.

Seydim, A., & Sarikus, G. (2006). Antimicrobial activity of whey protein based edible films incorporated with oregano, rosemary and garlic essential oils. *Food research international, 39*(5), 639-644.

Tien, C., Vachon, C., Mateescu, M. A., & Lacroix, M. (2001). Milk protein coatings prevent oxidative browning of apples and potatoes. *Journal of Food Science, 66*(4), 512-516.

Tong, L., Sasaki, S., McClements, D., & Decker, E. (2000). Antioxidant activity of whey in a salmon oil emulsion. *Journal of Food Science, 65*(8), 1325-1329.

Tong, L. M., Sasaki, S., McClements, D. J., & Decker, E. A. (2000). Mechanisms of the antioxidant activity of a high molecular weight fraction of whey. *Journal of Agricultural and Food Chemistry, 48*(5), 1473-1478.

Tsimidou, M., Papavergou, E., & Boskou, D. (1995). Evaluation of oregano antioxidant activity in mackerel oil. *Food research international, 28*(4), 431-433.

Turner, K., & Thomas, T. (1980). Lactose fermentation in Cheddar cheese and the effect of salt. *New Zealand Journal of Dairy Science and Technology, 15*(3), 265-276.

Uhlich, G. A., Luchansky, J. B., Tamplin, M. L., Molina-Corral, F. J., Anandan, S., & Porto-Fett, A. (2006). Effect of storage temperature on the growth of *Listeria monocytogenes* on queso blanco slices. *Journal of food safety, 26*(3), 202-214.

Wehmeier, K. R., & Mooradian, A. D. (1994). Autoxidative and antioxidative potential of simple carbohydrates. *Free Radical Biology and Medicine, 17*(1), 83-86.

Whelan, J., & Rust, C. (2006). Innovative dietary sources of n-3 fatty acids. *Annu. Rev. Nutr., 26*, 75-103.

Zinoviadou, K. G., Koutsoumanis, K. P., & Biliaderis, C. G. (2009). Physico-chemical properties of whey protein isolate films containing oregano oil and their antimicrobial action against spoilage flora of fresh beef. *Meat Science, 82*(3), 338-345.

CHAPTER 6. SUMMARY AND CONCLUSIONS

This study was designed to evaluate the effectiveness of a whey-protein-isolate (WPI) edible film incorporated with oregano essential oil (OEO) in extending the shelf life of queso blanco with respect to oxidation and spoilage microbial development. As the first objective, edible films were characterized for physical, chemical, and, mechanical properties, respectively. From this project, a whey-protein-isolate edible film containing OEO with potential antimicrobial and/or antioxidant properties was obtained.

The saturated fat content in cheeses represents a concern for consumers as a high intake of saturated fats may increase the risk of coronary diseases. In order to improve the fatty acid profile of queso blanco, flaxseed oil (FO) was incorporated during different stages of its manufacture. Addition of FO during homogenization was the best way of its incorporation into the cheese matrix. Although development of yeasts and molds was reduced when FO was incorporated into cheese, a greater rate of oxidation was also observed. Deterioration of omega 3 fatty acids, oxidative rancidity, and harmful health effects can occur by repeated ingestion of oxidized foods.

In order to evaluate potential antioxidant and antimicrobial effect of whey-protein-isolate edible film (WPIF) and WPIF with OEO (WPIOF) and protect queso blanco containing FO added during homogenization (HFQ) from oxidative stress, HFQ batches were elaborated and wrapped with edible films. Real store conditions were applied during refrigerated storage (8ºC in presence of fluorescent light and exposure to sunlight) and cheeses were evaluated for physical, chemical, and microbiological characteristics. Wrapping with WPIOF (WOHFQ) and WPIF (WHFQ) resulted in lower lipid oxidation than HFQ after 30 days of storage. Moreover, WPIOF was also more effective than

WPIF in controlling growth of yeasts and molds and had lower spoilage than QU. Addition of FO itself to cheese (HFQ) also contributed to retard the development of yeasts and molds having a similar effect to WOHFQ.

This study demonstrated that WPIOF can be used as a wrapping material to extend the shelf life of queso blanco containing FO regarding oxidation and yeast and mold development. Slower oxidation rate of omega-3 fatty acids present in FO was achieved while keeping important organoleptic characteristics of queso blanco.

APPENDIX. RESULTS OF THE PRODUCTION OF QUESO BLANCO WRAPPED WITH EDIBLE FILMS CONTAINING OREGANO (*ORIGANUM VULGARE*) ESSENTIAL OIL AND/OR GREEN TEA (*CAMELLIA SINENSIS*) EXTRACT

Table 1. Rheological properties of film forming solutions

Treatment	K^1(Pa. s^n)	η^2	μ^3 (Pa s)
WPIS	0.03 ± 0.00^{AB}	0.89 ± 0.01^{B}	0.02 ± 0.00^{A}
WPIGS	0.04 ± 0.02^{A}	0.89 ± 0.12^{B}	0.02 ± 0.00^{A}
WPIGOS	0.01 ± 0.00^{B}	1.05 ± 0.01^{AB}	0.01 ± 0.00^{A}
WPIOS	0.01 ± 0.00^{B}	1.11 ± 0.02^{A}	0.02 ± 0.00^{A}

Means with different letters (AB) within columns are significantly different (P<0.05).
WPIS= whey-protein-isolate-film-solution, WPIGS= WPIS with green tea extract (GTE), WPIGOS= WPIS with GTE plus oregano essential oil (OEO), WPIOS= WPIS with OEO
K^1= consistency index, n^2= flow behavior index, μ^3= apparent viscosity

Table 2. Queso blanco moisture and fat content determination.

Sample	MC^1	FC^2
QU	51.74 ± 1.10	19.44 ± 0.49

MC^1 = Percentage of moisture content wet basis, FC^2= Percentage of fat content wet basis.

Table 3. Water activity (a_w) of QWPIF, QWPIGF, QWPIGOF, QWPIOF, and QU.

Treatments	a_w[1] Day 1	a_w[1] Day 15	a_w[1] Day 30	a_w[1] Day 45	a_w[1] Day 60
QWPIF	0.84 ± 0.04 A(a)	0.84 ± 0.03 A(a)	0.84 ± 0.04 A(a)	0.82 ± 0.04 A(a)	0.86 ± 0.04 A(a)
QWPIGF	0.84 ± 0.07 A(a)	0.86 ± 0.02 A(a)	0.85 ± 0.02 A(a)	0.85 ± 0.03 A(a)	0.86 ± 0.01 A(a)
QWPIGOF	0.85 ± 0.04 A(a)	0.86 ± 0.01 A(a)	0.84 ± 0.03 A(a)	0.88 ± 0.02 A(a)	0.86 ± 0.02 A(a)
QWPIOF	0.86 ± 0.01 A(a)	0.82 ± 0.03 A(a)	0.80 ± 0.11 A(a)	0.88 ± 0.03 A(a)	0.87 ± 0.02 A(a)
QU	0.84 ± 0.02A(a)	0.87 ± 0.05 A(a)	0.86 ± 0.05 A(a)	0.88 ± 0.01 A(a)	0.87 ± 0.02 A(a)

Means with same letters (A) within columns are not significantly different (P>0.05).
Means with same letters (a) within rows are not significantly different (P>0.05).
QWPIF= cheese wrapped with whey-protein-isolate-film, QWPIGF= cheese wrapped with WPIF containing green tea extract (GTE), QWPIGOF= cheese wrapped with WPIF containing GTE plus oregano essential oil (OEO), QWPIOF= cheese wrapped with WPIF containing OEO
a_w[1] = Water activity.

Table 4. Color of QWPIF, QWPIGF, QWPIGOF, QWPIOF, and QU.

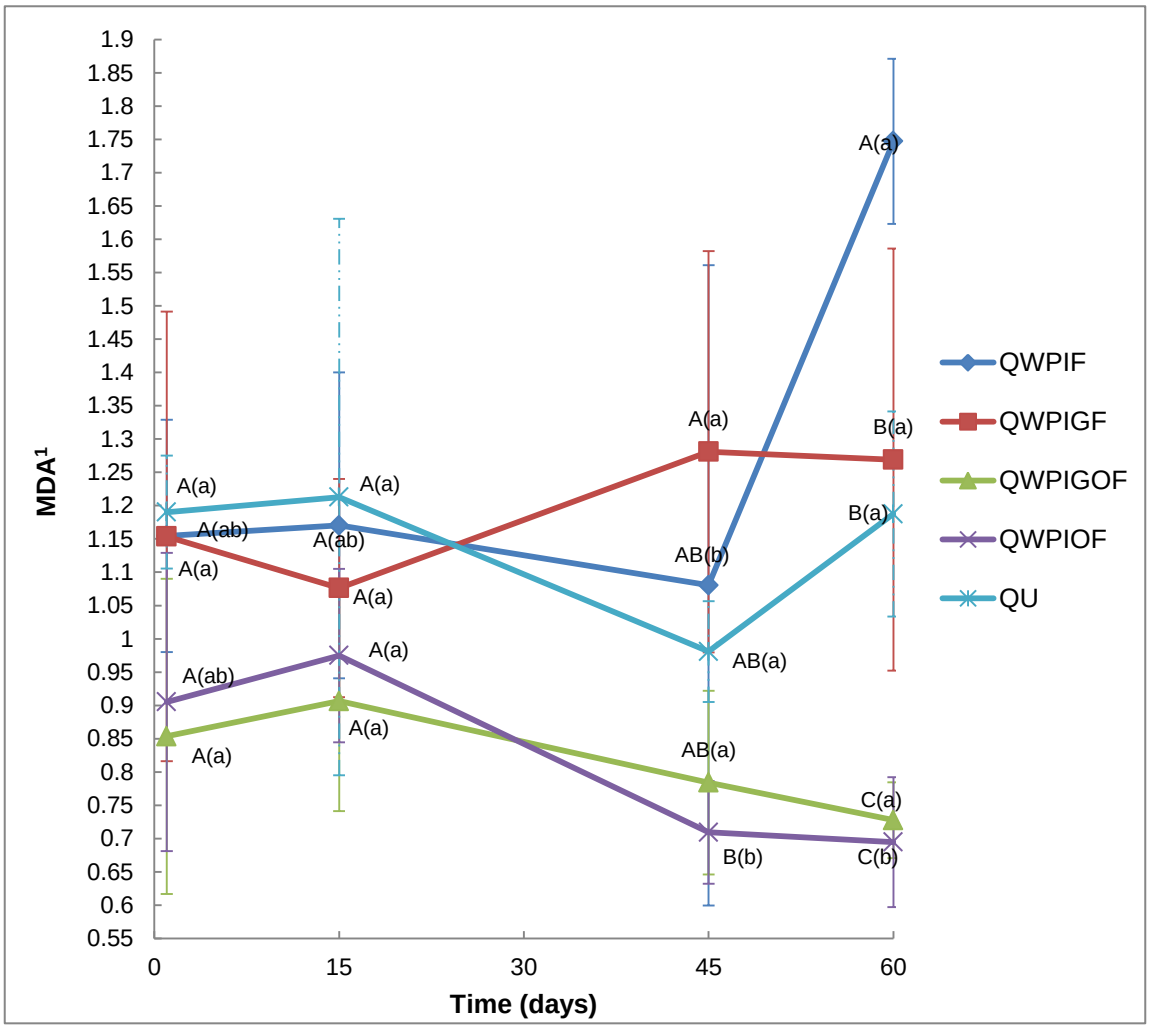
Color	Treatment	Day 1	Day 7	Day 30	Day 45	Day 60
	QWPIF	$79.20 \pm 3.40^{A(a)}$	$79.46 \pm 6.04^{A(a)}$	$81.21 \pm 1.28^{AB(a)}$	$82.86 \pm 2.63^{A(a)}$	$80.79 \pm 1.20^{A(a)}$
	QWPIGF	$75.94 \pm 5.86^{A(a)}$	$78.46 \pm 8.15^{A(a)}$	$80.78 \pm 0.93^{AB(a)}$	$81.96 \pm 0.99^{A(a)}$	$76.75 \pm 3.65^{A(a)}$
L^*	QWPIGOF	$78.99 \pm 3.12^{A(a)}$	$79.44 \pm 3.74^{A(a)}$	$77.26 \pm 2.07^{AB(a)}$	$79.03 \pm 0.96^{A(a)}$	$78.02 \pm 1.37^{A(a)}$
	QWPIOF	$82.90 \pm 2.49^{A(a)}$	$79.74 \pm 5.75^{A(a)}$	$81.65 \pm 0.38^{A(a)}$	$81.12 \pm 1.45^{A(a)}$	$80.32 \pm 1.32^{A(a)}$
	QU	$75.70 \pm 2.21^{A(b)}$	$90.18 \pm 0.35^{A(a)}$	$75.87 \pm 3.68^{B(b)}$	$81.22 \pm 1.48^{A(b)}$	$78.17 \pm 2.79^{A(b)}$
	QWPIF	$-0.80 \pm 0.20^{A(ab)}$	$-1.00 \pm 0.09^{A(b)}$	$-0.76 \pm 0.13^{B(ab)}$	$-0.72 \pm 0.07^{B(ab)}$	$-0.55 \pm 0.27^{AB(a)}$
	QWPIGF	$-0.79 \pm 0.35^{A(a)}$	$-1.53 \pm 1.34^{AB(a)}$	$-0.25 \pm 0.04^{A(a)}$	$-0.30 \pm 0.29^{B(a)}$	$0.06 \pm 0.28^{A(a)}$
a^*	QWPIGOF	$-0.70 \pm 0.20^{A(b)}$	$-0.66 \pm 0.35^{A(b)}$	$-0.77 \pm 0.19^{B(b)}$	$0.21 \pm 0.26^{A(a)}$	$-0.22 \pm 0.37^{A(ab)}$
	QWPIOF	$-0.74 \pm 0.20^{A(a)}$	$-0.77 \pm 0.12^{A(a)}$	$-0.61 \pm 0.06^{AB(a)}$	$-0.59 \pm 0.07^{B(a)}$	$-0.65 \pm 0.12^{AB(a)}$
	QU	$-0.37 \pm 0.34^{A(a)}$	$-2.85 \pm 0.18^{B(d)}$	$-1.35 \pm 0.22^{C(c)}$	$-0.68 \pm 0.11^{B(ab)}$	$-1.31 \pm 0.33^{B(bc)}$
	QWPIF	$16.03 \pm 0.87^{B(a)}$	$15.63 \pm 2.14^{C(a)}$	$19.70 \pm 0.94^{A(a)}$	$18.25 \pm 1.44^{B(a)}$	$18.52 \pm 2.86^{A(a)}$
	QWPIGF	$16.47 \pm 1.76^{B(c)}$	$17.07 \pm 0.38^{BC(bc)}$	$21.19 \pm 0.77^{A(a)}$	$19.88 \pm 0.79^{AB(ab)}$	$21.12 \pm 1.88^{A(a)}$
b^*	QWPIGOF	$21.25 \pm 0.92^{A(a)}$	$21.28 \pm 0.89^{A(a)}$	$20.55 \pm 1.63^{A(a)}$	$22.06 \pm 0.23^{A(a)}$	$22.04 \pm 0.53^{A(a)}$
	QWPIOF	$19.18 \pm 1.77^{AB(a)}$	$20.73 \pm 1.98^{AB(a)}$	$19.15 \pm 0.71^{A(a)}$	$20.42 \pm 0.19^{AB(a)}$	$19.40 \pm 1.55^{A(a)}$
	QU	$17.55 \pm 0.38^{B(abc)}$	$16.25 \pm 1.09^{C(bc)}$	$16.14 \pm 1.14^{B(c)}$	$19.59 \pm 1.06^{B(a)}$	$19.29 \pm 1.65^{A(ab)}$

Means with different letters (AB) within columns are significantly different ($P<0.05$).
Means with different letters (ab) within rows are significantly different ($P<0.05$).
QWPIF= cheese wrapped with whey-protein-isolate-film, QWPIGF= cheese wrapped with WPIF containing green tea extract (GTE), QWPIGOF= cheese wrapped with WPIF containing GTE plus oregano essential oil (OEO), QWPIOF= cheese wrapped with WPIF containing OEO.
L^*= 0-darkness, 100-lightness; a^*=- greenness, +redness; b^*=-blueness, +yellowness.

Table 5. Peroxide value of QWPIF, QWPIGF, QWPIGOF, QWPIOF, and QU.

Treatment	PV[1] Day 1	PV[1] Day 7	PV[1] Day 30	PV[1] Day 45	PV[1] Day 60
QWPIF	$0.25 \pm 0.03^{A(a)}$	$0.24 \pm 0.06^{A(a)}$	$0.24 \pm 0.05^{A(a)}$	$0.24 \pm 0.02^{A(a)}$	$0.17 \pm 0.04^{B(a)}$
QWPIGF	$0.28 \pm 0.02^{A(a)}$	$0.20 \pm 0.03^{A(a)}$	$0.22 \pm 0.07^{A(a)}$	$0.30 \pm 0.09^{A(a)}$	$0.19 \pm 0.03^{B(a)}$
QWPIGOF	$0.23 \pm 0.05^{A(a)}$	$0.26 \pm 0.10^{A(a)}$	$0.26 \pm 0.03^{A(a)}$	$0.23 \pm 0.02^{A(a)}$	$0.27 \pm 0.03^{AB(a)}$
QWPIOF	$0.26 \pm 0.10^{A(a)}$	$0.39 \pm 0.12^{A(a)}$	$0.23 \pm 0.02^{A(a)}$	$0.17 \pm 0.11^{A(a)}$	$0.32 \pm 0.07^{A(a)}$
QU	$0.26 \pm 0.09^{A(a)}$	$0.26 \pm 0.03^{A(a)}$	$0.25 \pm 0.06^{A(a)}$	$0.32 \pm 0.03^{A(a)}$	$0.20 \pm 0.03^{B(a)}$

Means with different letters (AB) within columns are significantly different ($P<0.05$).
Means with same letters (a) within rows are not significantly different ($P>0.05$)
PV[1]= Peroxide value (meq. of peroxide per kg of oil)
QWPIF= cheese wrapped with whey-protein-isolate-film, QWPIGF= cheese wrapped with WPIF containing green tea extract (GTE), QWPIGOF= cheese wrapped with WPIF containing GTE plus oregano essential oil (OEO), QWPIOF= cheese wrapped with WPIF containing OEO, QU= control (bare cheese).



Means with different letters (AB) are treatments significantly different (P<0.05).

Means with different letters (ab) are treatments significantly different within time (P<0.05).

MDA[1]= µmol of malondialdehyde per kg of sample.

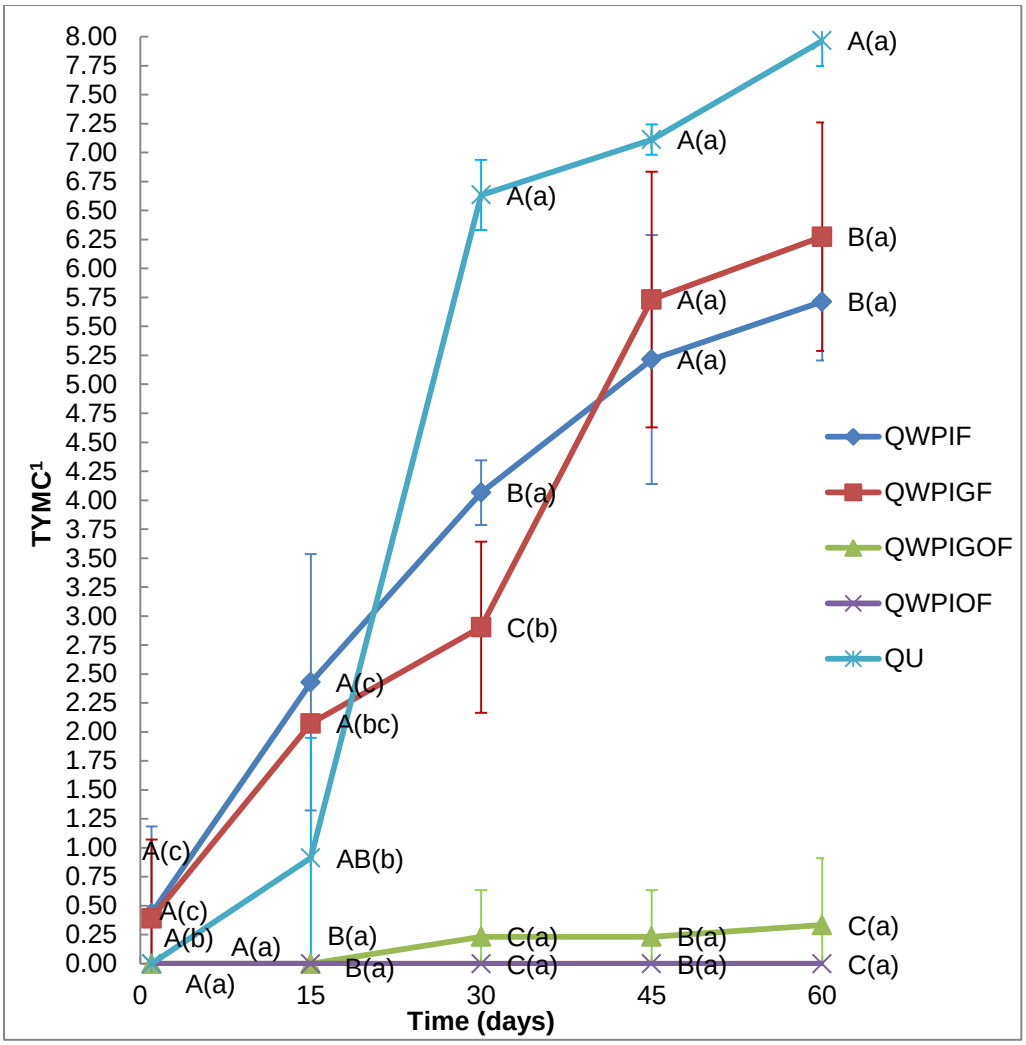
QWPIF= cheese wrapped with whey-protein-isolate-film, QWPIGF= cheese wrapped with WPIF containing green tea extract (GTE), QWPIGOF= cheese wrapped with WPIF containing GTE plus oregano essential oil (OEO), QWPIOF= cheese wrapped with WPIF containing OEO, QU= control (bare cheese).

Figure 1. Thiobarbituric acid reactive substances of QWPIF, QWPIGF, QWPIGOF, QWPIOF, and QU.

Table 6. Total aerobic count (TAC) of cheeses during 60 days of refrigerated storage

Treatments	TAC[1] day 1	TAC[1] day 15	TAC[1] day 30	TAC[1] day 45	TAC[1] day 60
QWPIF	$1.60 \pm 0.00^{A(a)}$	$1.24 \pm 0.54^{A(a)}$	$< 1.00 \pm 0.00^{AB(a)}$	$1.79 \pm 0.80^{AB(a)}$	$1.33 \pm 0.15^{B(a)}$
QWPIGF	$1.00 \pm 0.00^{A(d)}$	$1.54 \pm 0.06^{A(bc)}$	$1.18 \pm 0.00^{A(cd)}$	$1.68 \pm 0.38^{B(b)}$	$2.30 \pm 0.00^{A(a)}$
QWPIGOF	$1.39 \pm 0.09^{A(a)}$	$1.40 \pm 0.00^{A(a)}$	$< 1.00 \pm 0.15^{B(b)}$	$1.06 \pm 0.10^{BC(a)}$	$< 1.00 \pm 0.00^{C(b)}$
QWPIOF	$1.47 \pm 0.87^{A(a)}$	$1.10 \pm 0.17^{A(ab)}$	$< 1.00 \pm 0.58^{AB(ab)}$	$< 1.00 \pm 0.00^{C(b)}$	$< 1.00 \pm 0.51^{BC(ab)}$
QU	$1.37 \pm 0.37^{A(b)}$	$1.18 \pm 0.00^{A(b)}$	$1.18 \pm 0.00^{A(b)}$	$2.79 \pm 0.21^{A(a)}$	$2.63 \pm 0.00^{A(a)}$

Means with different letters (AB) within columns are significantly different (P<0.05).
Means with different letters (ab) within rows are significantly different (P<0.05).
TAC[1]= Log colony forming units of total aerobic count per g of cheese
QWPIF= cheese wrapped with whey-protein-isolate-film, QWPIGF= cheese wrapped with WPIF containing green tea extract (GTE), QWPIGOF= cheese wrapped with WPIF containing GTE plus oregano essential oil (OEO), QWPIOF= cheese wrapped with WPIF containing OEO, QU= control (bare cheese).



Means with different letters (AB) are significantly different between treatments (P<0.05).
Means with different letters (ab) are significantly different between days (P<0.05).
TYMC[1]=Log colony forming units of total yeast and mold count per g of cheese.
QWPIF= cheese wrapped with whey-protein-isolate-film, QWPIGF= cheese wrapped with WPIF containing green tea extract (GTE), QWPIGOF=cheese wrapped with WPIF containing GTE plus oregano essential oil (OEO), QWPIOF= cheese wrapped with WPIF containing OEO, QU= control (bare cheese).

Figure 2. Total Yeast and Mold count (TYMC) of cheeses during 60 days of refrigerated storage

VITA

Cristhiam Eugenia Gurdian Curran was born in January 1991 in Leon, Nicaragua. She earned her Bachelor of Sciences in Food Science and Technology in Zamorano University, Valle del Yeguare, Honduras, in December 2011. After studying English for 2 months, she joined the Food Science Department at Louisiana State University as an intern in May 2012. She became a graduate student in Food Science department in January 2013 at LSU and expects to graduate in May 2015.