THE USE OF NANOEMULSION-BASED EDIBLE COATINGS TO PROLONG THE SHELF-LIFE OF CHEESE

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Abstract

In this work, the pectin/oregano oil nanoemulsion coatings were investigated, in order to prolong the shelf-life of cheese. The water vapor barrier, mechanical and antimicrobial activity of prepared nanoemulsion coatings were examined. In addition, the textural properties of uncoated and pectin/oregano oil coated cheeses were followed for 2 weeks. The results showed that obtained coatings possessed high antimicrobial activity toward E. coli, S. aureus and C. albicans and satisfied mechanical and water vapor barrier resistance. The coatings were also effective on reducing losses of weight and firmness of cheeses. Overall, antimicrobial nanoemulsion/edible films from renewable sources were successfully produced and showed high potential to be applicable in food industry to prolong the shelf-life of cheeses.

Key words: *oregano oil, edible coating, pectin*

1. INTRODUCTION

The rapid grow of pollution levels derived from commercial plastics, has lead the food industry to develop new innovative materials which allow consumers to better sustain their health and well-being. Therefore, there is an emphasized need for the production of novel biobased, biodegradable and edible food packaging materials with the final goal to maintain the food quality, to enhance the shelf-life of food products and to reduce the amount of plastic packaging that causes environmental concerns. Structuring biopolymers, such as polysaccharides, proteins, and lipids have been used for the formulation of edible films (Bourtoom, 2009; Bertolino et al., 2016; Cavallaro et al., 2011; Cavallaro et al., 2011; Cavallaro et al., 2013). Among the polysaccharides, starch (Famá et al., 2012; Gutiérrez et al., 2015; Souza et al. 2012), alginate (Azaraksh et al. 2014; Juck et al. 2010; Song et al. 2011) and chitosan (Rivero et al., 2009; Srinivasa et al., 2007) are widely investigated as a matrix for synthesis of edible food packaging films because of their wide abundant, nontoxicity and versatile properties. In this work, special attention will be given to development of pectin-based edible coatings. The choice is related to the fact that pectins are renewable, nontoxic and biodegradable resource present in fruits, mostly apples and citrus. Pectins are family of natural polysaccharides comprising chains of α-(1-4)-linked D-galacturonic acid (Mohnen 2008). In nature, around 80% of carboxylic groups of galacturonic acid are methlyesterified. The ratio of esterified to non-esterified galacturonic acid determines the behavior of pectin in solutions, influencing the properties of pectin-based materials, as well (Thakur et al. 2009). Its unique property to gel at acidic conditions or in presence of divalent cations, allows the widen use in food and beverage technology as colloidal stabilizer, thickening and gelling agent (May 1990). Although pectin is widely applicable as food ingredient due to good gelling properties, water retention ability and safety concerns, there are only few pectin-based films for food packaging application reported in literature (Giosafatto et al. 2014; Maftoonazad et al. 2007; Guerra-Rosa et al. 2016).

In order to control undesirable microorganisms on food surfaces, various antimicrobial agents were incorporated into pectin matrix. Through this approach, the antimicrobial agents are released slowly to the surface of food inhibiting spoilage microorganisms on food surfaces and suppressing the food-borne illness microbial that potentially contaminates food products. The essential oils (EO) from many aromatic spices (oregano, thyme, salvia, parsley, clove, coriander, garlic and onion) has gained increasing attention, because they contain a high percentage of phenolic compounds such as carvacrol, eugenol and thymol, which are the main responsible for antimicrobial activity of EO (Lambert et al. 2001). In this study, the synthesis and characterization (mechanical, barrier and antimicrobial activity) of low methylated pectin-based films added with different concentrations of oregano essential oil was investigated. Oregano EO show to be very efficient antimicrobial additive due to relatively high levels of carvacrol, thymol, γ -terpinene and ρ-cymene (Dafarerea et al. 2007). The aim of this work is to investigate physical properties of pectin/oregano oil nanoemulsion coatings and test their efficiency to prolong the shelf-life of cheese.

2. EXPERIMENTAL WORK

The material used for film formation included food-grade amidated pectin (AP) LA-110, M_w= 15 kDa, with a degree of methylation of 35.9 and a degree of amidation 15.9 and 48.2% free galacturonic acid residues (Danisco, Denmark). Glycerol used as a plasticization agent was obtained from Merck Co., (Darmstadt, Germany). Oregano essential oil (OEO), obtained from plant *Origanum minutiflorum* by steam-distillation was provided by commercial company in Serbia. Chemical composition of oregano oil is presented in Table 1.

Table 1. Chemical composition of oregano oil

Constituents	Content, %
Carvacrol	84.6
p-cymene	4.2
γ-terpinene	3.3
Thymol	1.7
p-myrcene	1.5
Borneol	0.5
Bornylacetate	0.8
α-terpinene	0.8

2.1. Film preparation

The aqueous phase was prepared by solving amidated pectin in distilled water at room temperature for 1 h. After dissolution under continuous stirring, 20 wt% of glycerol was added as plasticizer. An accurate amount of the lipid phase consisted of the mixture of OEO and Tween 80 at room temperature was added to the aqueous phase, and blended with the high-shear homogenizer at 25 000 rpm for 30 min, leading to coarse emulsions. The solutions were cast onto petri dishes 9 cm in diameter and allowed to dry in an oven at 25 ^{o}C for approximately 24 h. The dried films were then removed from the petri dishes and stored in fridge at 8 ^{o}C until being used.

2.2. Methods

2.2.1. Mechanical analysis

Mechanical tests in terms of Young's modulus, tensile strength and strain at break were performed by Instron M 1185. The width and the length of characterized films were respectively 4.92 mm and 20 mm, while the thickness of each film was measured at five random points using a micrometer and the result was expressed as the average value. Prior to testing, the films were conditioned in an environmental chamber at 25 ^{o}C and 63% relative humidity (RH) for 48 h. All the measurements were carried out at room temperature and at crosshead rate of 2 mm/ min. The reported data are the average values of five measurements.

2.2.2. Water vapor analysis

Water vapor permeability (WVP) was performed gravimetrically at 25 ºC, using the ASTM E96-95 method. Prior to testing, all films were equilibrated at 63% RH at 25±2 ºC for 48 h. Afterwards, test films were fixed onto opening cells containing distilled water (100 %RH) and the cells were placed in an controlled humidity chamber at 63±2% RH and 25±2 ºC. The film area exposed for water vapor transmission was 27 cm^2. The cells were weighed hourly over a period of 8 hours. WVP was calculated from the following equation:

$$WVP = \frac{WVTR \times L}{\Delta p}$$

where WVTR (g/ m^2 s) indicates the water vapor permeability rate calculated by dividing the slope of weight vs. time plot by the surface area of the cup, L (m) is thickness of pectin films and Δp is the water pressure difference between both sides of the film (Pa). All measurements were made in triplicate.

2.2.3. Antimicrobial tests

The antimicrobial effect of the films was estimated against Gram-positive bacterium *Staphylococcus aureus*, Gram-negative bacterium *Escherichia coli* and fungus *Candida albicans* using disc diffusion method. A 100 µl of appropriate dilution (~10^8 CFU/ml) of overnight culture of tested pathogen was seeded by spread plate technique on sterile Tryptic Soy Agar plates. Plates were stored at 4 ºC for 30 minutes before placing the film samples. The films were cut into discs 6 mm in diameter and sterilized by exposing to UV light for 15 min on each side before the test. Inoculated plates with film samples were incubated at 37 ºC for 24h. Antimicrobial potential of the films was evaluated by measuring the diameters of clear inhibition zones, including the diameter of the disc.

2.2.4. Cheese coating and quality assessment of coated cheese

The cheeses (3.0 cm × 2.0 cm × 2.0 cm) were coated with the pectin/oregano oil solution by brushing the surface until all of it was covered. Then the cheeses were left to dry for 30 minutes at room temperature. Packaged and uncontrolled samples were stored at 8 ºC for 21 days for evaluation of moisture content and weight loss. The moisture content in cheese was determined by the gravimetric method, after being dried at 105 ºC for 24 h in triplicate. The weight loss was evaluated by weighing the cheeses at the beginning of the experiment and during the storage period.

3. RESULTS

3.1. Mechanical analysis

Mechanical parameters of neat plasticized pectin and pectin/oregano oil films, in terms of tensile strength and elongation at break, are shown in Figure 1. Generally, the addition of essential oil into polymer matrix has led to decrease in tensile strength and an increase in elasticity of films due to difference in polarities between film components and formation of weak and heterogeneous polymer network structure (Taqi et al., 2014; Martucci et al., 2015). In this work, pectin/glycerol control film had a tensile strength value of 10 MPa, while addition of oregano oil caused an increase in tensile strength and reached a value of 18 MPa. The crosslinking effect of oregano oil at some level could be possible explanation for unusual trend, which lowered the effect of plasticizer. On the other hand, an increase in elongation at break with introduction of oregano oil was observed. Hence, pectin/oregano oil films showed synergic effect to mechanical properties, due to improved tensile strength and elasticity of pectin films with addition of oregano oil.

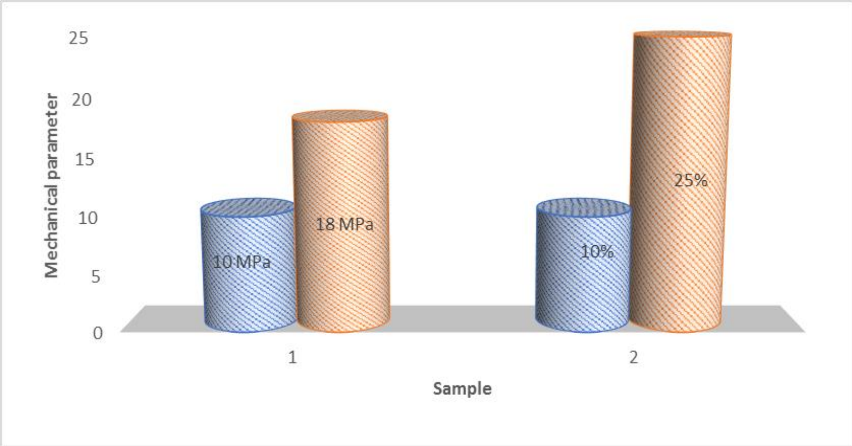

Figure 1. Mechanical properties of control pectin film (blue) and pectin/oregano oil film (red).

3.2. Water vapor permeability

The important role of any antimicrobial coating is barrier to water vapor, because the presence of moisture significantly affects the quality and ripening rate of food products. The water vapor permeability of neat plasticized pectin film and films encapsulated with oregano essential oil was examined at 25 ^{o}C at RH=63% and these results are shown in Figure 2. The addition of oregano oil enhanced the water vapor barrier properties in comparison to the control pectin film, due to the increased hydrophobicity of films influenced by the presence of oregano oil which limited the water vapor transfer through films. The WVP values of pectin/oregano oil films obtained in this work are in the range of 0.44 to 0.9 g mm/m^2 kPa h and these values are significantly lower than alginate/apple puree/oregano oil films (5.25 g mm/m^2 kPa h)(Rojas-Graü et al., 2007); starch/gelatin/oregano oil films(4.6 g mm/m^2 kPa h) (Acosta et al., 2016); whey protein isolate/oregano oil films (range between 8.5 and 11 g mm/m^2 kPa h) (Zinoviadou et al., 2009) and alginate/oregano oil (range between 2.7E-9 and 3.1E-9 g/m Pa s)(Benavides et al., 2012); thus confirmed matching of pectin as choice of biopolymer matrix and oregano oil, which led to significant improvement in water vapor barrier property.

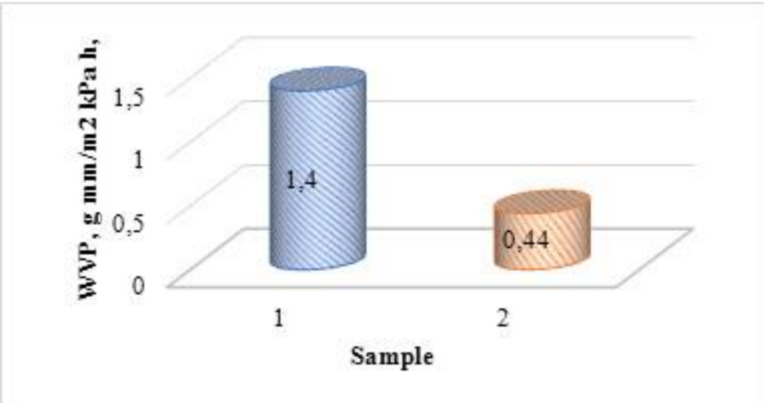

Figure 2. Water vapor permeability of control pectin film (blue) and pectin/oregano oil film (red).

3.3. Antimicrobial activity

The pictures of the inhibitory effect of pectin films incorporated with oregano essential oil against all tested microorganisms are shown in Figure 3. The control film (without oregano oil) did not show any antimicrobial effect against all studied microorganisms and resulted in no inhibition zones. The addition of oregano oil into amidated pectin films caused the largest inhibition zones against gram-positive bacteria *Staphylococcus aureus*. The lower antibacterial activity against *E. coli*, the gram negative bacteria was ascribed to the presence of an outer membrane surrounding the cell wall in gram-negative bacteria, which limits diffusion of hydrophobic substances via its lipopolysaccharide covering. These results proved that oregano oil could be immobilized in pectin films and consequently released, thereby efficiently prevented the growth of selected pathogens.

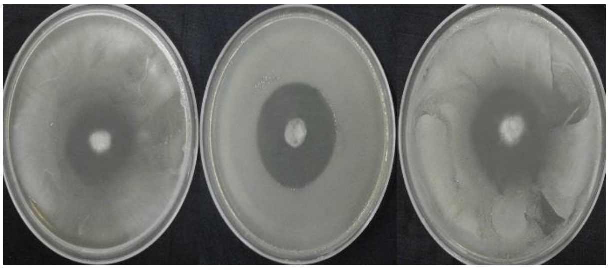

Figure 3. Antimicrobial activity of pectin/oregano oil coating toward *E. coli*, *S. aureus* and *C. albicans*, respectively.

3.4 Quality assessment of cheese coating

Weight loss analyses provided information on how the moisture loss during the storage period was influenced by the temperature and the presence of coating and if those factors can change the moisture loss profile of the cheese. Fig. 4 shows the weight loss in the coated and uncoated cheeses stored at 8 ºC. In all cases the weight loss increased during the storage time, where the increase was higher for the cheeses without coating. These results demonstrated that only coating could retard the water loss.

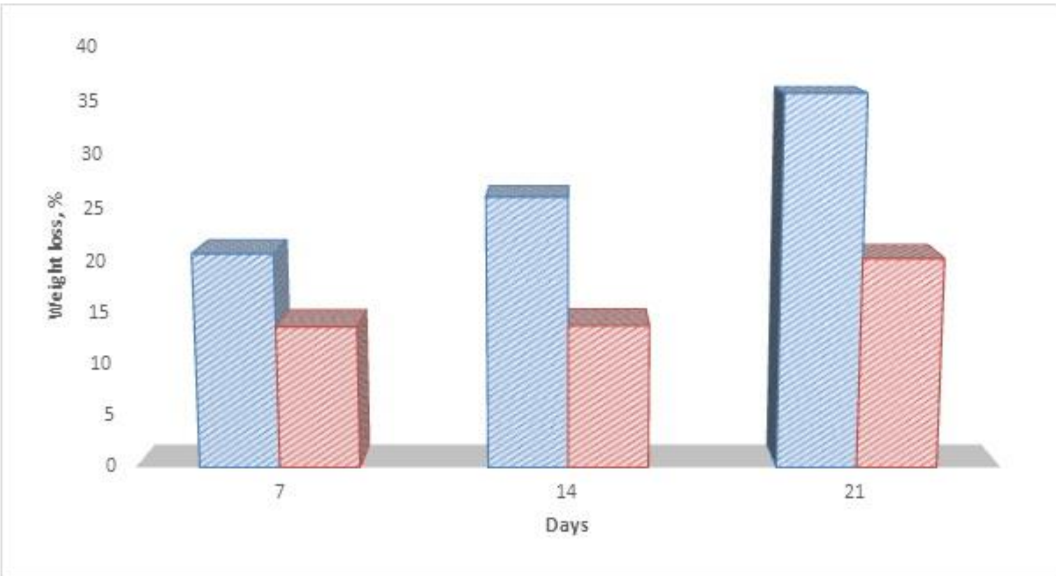

Figure 4. The weight loss of uncoated (blue) and coated cheeses (red) within the time.

The moisture content decreased in all cases within the time. The lowest moisture content was observed for uncoated cheeses and after 21 days reached a value of 12.8%, whereas the moisture content for coated cheeses was 20.2%. These results are in agreement with previously obtained results of weight loss and water vapor permeability tests. Both weight loss and moisture loss are related to the water loss of the cheese that depends on the kinetics of water permeation through the used coating.

CONCLUSIONS

The objectives of this work were to prepare the pectin/oregano oil nanoemulsion coatings and evaluate their potential to prolong the shelf-life of cheeses. The pectin/oregano oil coatings have shown great antimicrobial activity toward different pathogens, moderate mechanical stability and satisfied water vapor barrier property. The presence of the coating onto cheeses decreased the weight loss and increased the moisture content of cheeses. Nevertheless, to be potentially used in food packaging sector to prolong the shelf-life of cheeses. Further investigations of microbiological quality of coated cheeses will be assessed and will be object of an incoming paper.

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