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Optimization of edible coating with essential oils in blueberries

Otimização de cobertura comestível com óleos essenciais em mirtilo

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ABSTRACT

The application of edible coatings containing natural antimicrobials is a postharvest conservation technology in fruits that have generated interest. This research aimed the determination of the edible coating composition and the concentration of essential oil that allows optimizing the physical-mechanical characteristics for its application in the conservation of blueberries. The antimicrobial activity of the essential oils of cinnamon and lemon was determined, resulting in a minimum inhibitory concentration of 0.3% in both cases. After applying the Box Behnken design of the Response Surface Methodology (RSM), the optimal treatment for edible coating with cinnamon essential oil 0.3% was determined: aloe vera gel 18.40%, gelatin 2%, and glycerol 0.055% obtaining values of 27.95% solubility, 0.90 mm of deformation and 3.34 N of breaking strength. Likewise, the same procedure was followed for the coating with lemon essential oil 0.3%, determining as optimal 23.94% aloe vera gel, 2% gelatin, and 0.05% glycerol, getting values of 28.06% solubility, 0.45 mm deformation, and 4.53 N of breaking strength. Finally, their applications in Biloxi blueberries were validated, preserving the main physicochemical and microbiological quality attributes during 28 days of storage at 2 °C, compared, to a control sample.

Index terms: Minimal processed; postharvest; berries.

RESUMO

A aplicação de revestimentos comestíveis com antimicrobianos naturais é uma tecnologia de conservação pós-colheita em frutas de interesse. O objetivo da pesquisa foi determinar os componentes da cobertura comestível e concentração de óleo essencial que permitem otimizar as características físico-mecânicas para aplicação na conservação de mirtilos. A atividade antimicrobiana dos óleos essenciais de canela e limão foi determinada, encontrando uma concentração inibitória mínima de 0,3% em ambos os casos. Após a aplicação do desenho Box Behnken da Metodologia de Superfície de Resposta (MSR), determinou-se o tratamento ótimo para cobertura comestível com óleo essencial de 0,3% de canela, 18,40% de gel de aloe vera, 2% de gelatina e 0,055% de glicerol obtendo valores de 27,95% de solubilidade, 0,90 mm de deformação e 3,34 N de resistência à ruptura. Da mesma forma, foi feito um trabalho de cobertura com 0,3% de óleo essencial de limão, determinando como valores, 23,94% de gel de aloe vera, 2% de gelatina e 0,05% de glicerol obtendo valores de 28,06% de solubilidade, 0,45 mm de deformação e 4,53 N de quebra força. Por fim, a aplicação foi validada em mirtilos da variedade Biloxi, conservando seus principais atributos de qualidade físico-química e microbiológica em alto nível durante 28 dias de armazenamento a 2 °C, em comparação, com uma amostra controle.

Termos para indexação: Mínimo processado; pós-colheita; bagas.

INTRODUCTION

During the last decade, the inclusion of fresh products in the human diet has constantly risen due to the higher consumer awareness of the associated health benefits (Falcó et al., 2019). Biopolymer-based packaging materials show great potential for improving food quality and safety and include among its main advantages the individual packaging of small foods, carriers of functionally active substances, and nutritional supplements. Therefore, there is a growing interest in using renewable, degradable, and compostable films and coatings based on polysaccharides, proteins, and lipids (Nandane; Dave; Rao, 2017).

Because of the rejection of sulfites, synthetic additive benzoic acid or its derived salt generally used to control the growth of microorganisms in food, same as the natural antimicrobial agents used with the same objective, have focused more and more attention (Ju et al., 2018). Essential oils are natural volatile substances or secondary metabolites extracted from plants with antioxidant and antimicrobial properties.

Some of their components have been isolated and identified as factors implicated in the observed antimicrobial activity. The main constituent of lemon essential oil is limonene, which is present in a considerable quantity, between 30-35% (Rezaeifar et al., 2020). The cinnamon essential oil is abundant in cinnamaldehyde (81, 97%), b-caryophyllene, linalool, and others terpenes (Murmu; Mishra, 2017). According to the FDA (U.S. Food and Drug Administration), essential oils are generally recognized as safe (GRAS), which means that they can be used as a potential substitute for synthetic additives (Ju et al., 2018; Sun et al., 2014).

An edible coating containing the essential oil is a thin layer resulting from mixing the oil with biologic polymers. It avoids the exchange of oxygen, water, carbon dioxide, and other constituents of external substances with food and delays food spoilage having a crucial role in conservation (Ju et al., 2018).

The plasticizer is one of the principal components of edible coatings. Generally, they are tiny molecules, such as glycerol, which intersperse between biopolymer chains. Its application is necessary because otherwise, the coating would be fragile due to an excess of cohesive force. During coating formation, the incompatibility of fruits with the principal biopolymer in the former dispersion of the coat increases the cohesion and consequently decreases the film strength (Lin et al., 2018). Hydrophilic coating's permeability can be affected by factors such as the hydrophilic-hydrophobic nature of the polymeric matrix and the added components and by the morphology, thickness, and homogeneity of the matrix (Nandame; Dave; Rao, 2017).

RSM is applied for optimizing coatings because it has helped find the relations between different independent and response variables while minimizing the number of experiments and usage of resources in the development of edible coatings for fruits and vegetables. (Thakur et al., 2017). This research aimed to determine the components of the edible coating and the essential oil concentration that optimize the physic-mechanic characteristics for its application in the conservation of blueberries.

MATERIAL AND METHODS

Material

The experimental tests and physic-chemical, microbiological, and sensory analysis took place in the Food Engineering laboratories of Antenor Orrego Private University, Peru. Fresh blueberries (*Vaccinium corymbosum L.*, cv. Biloxi) at commercial ripeness (90 – 100% blue) were brought from Blueberries farm located in Chao, Viru, La Libertad). For the preparation of the edible coating, the following were used: aloe vera leaves brought from the local market La Hermelinda, Trujillo, neutral jelly 280 Bloom (Gel Base), glycerol (Now Foods), cinnamon and lemon essential oil (Núa essences), drinking water (Agua Fiel), and citric acid (NorBright). Plastic clamshells (Pamolsa S.A.) of 170 g capacity were used for storing fresh berries.

Edible coating preparation

Iodine from aloe vera leaves was eliminated by submerging them in water for 24 h before coating preparation. After peeling, the mucilaginous gel was pureed in a blender (Oster, model BRLY07) for 30 s, then filtered to eliminate the fibers. A 10-30% (p/v) aqueous base solution of aloe vera gel was prepared and regulated to a value of pH equivalent to 4 by adding citric acid. Between 1 and 2% (p/v) of gelatin was incorporated then the solution was pasteurized at 90 °C for 10 min. Later 0.05 to 0.5 % of glycerol was added. Finally cooling at 30 °C, the mixture was divided into four groups for applying 0.1 or 0.3% of cinnamon or lemon essential oil to determine the optimization (Adetunji; Fadaji; Aboyiji, 2014).

Statistic design

The SRM Box Behnken design (Table 1) was generated with the Design Expert 7.0 software (Stat-Ease, Inc., Minn., USA) to optimize the edible coating content of aloe vera gel, gelatin, and glycerol that, joined to the minimum concentration of essential oil inhibitory *Botrytis cinerea*, provide desirable mechanical and barrier properties for extending shelf life of fresh blueberries.

Application of the optimized edible coatings with the addition of essential oils

The damaged blueberries were eliminated, and the good ones were selected by uniform size (10-12 mm) and color. They were then placed on a stainless mesh to apply the edible coating with a spray-type atomizer. After, letting them dry at room temperature (25 °C) for 30 min, the berries were placed in clamshells at a weight of 125 g approximately and finally stored in a cooling chamber with temperature control for 28 days at 2 °C ± 1 °C y 85 - 90 % ± 0.1 % relative humidity, parameter carried out with a thermo-hygrometer (Fluke 971, Netherlands).

Physicochemical and microbiological results were evaluated through analysis of variance (ANOVA) and Duncan's multiple comparisons. All statistical analyses were performed with Minitab 19 software (Minitab, LLC, USA) at a 95% confidence level.

Table 1: Box Behnken experimental design employed with codified and decodified values for formulation of the edible coating.

Run of test	Aloe vera codified	Gelatin codified	Glycerol codified	Aloe vera (%)	Gelatin (%)	Glycerol (%)
1	-1	-1	0	10.0	1.0	0.275
2	+1	-1	0	30.0	1.0	0.275
3	-1	+1	0	10.0	2.0	0.275
4	+1	+1	0	30.0	2.0	0.275
5	-1	0	-1	10.0	1.5	0.050
6	+1	0	-1	30.0	1.5	0.050
7	-1	0	+1	10.0	1.5	0.500
8	+1	0	+1	30.0	1.5	0.500
9	0	-1	-1	20.0	1.0	0.050
10	0	+1	-1	20.0	2.0	0.050
11	0	-1	+1	20.0	1.0	0.500
12	0	+1	+1	20.0	2.0	0.500
13*	0	0	0	20.0	1.5	0.275
14*	0	0	0	20.0	1.5	0.275
15*	0	0	0	20.0	1.5	0.275
16*	0	0	0	20.0	1.5	0.275
17*	0	0	0	20.0	1.5	0.275

*Center Point Repeats

Analysis method

Inhibition of radial growth

The strain of *Botrytis cinerea* was provided by the Phitopatology laboratory at Antenor Orrego Private University, Trujillo, to establish the minimum inhibitory concentration for growth (Behdani et al., 2012; Behshti et al., 2020), a portion of the fungi was placed by puncture in a petri plate with Rose Bengal Chloramphenicol agar (RBC) mixed with Tween (0.1%) and cinnamon or lemon essential oil at a concentration equivalent to 5-100 mL/L agar. The plates were incubated at 20 °C, and the area in cm^2 of growth was measured every three days for nine days (Behshti et al., 2020). Images of growth were analyzed by ImageJ free software. The inhibition percentage was determined by comparing them with a negative plate without essential oil and applying the Equations 1 and 2:

$$\%\ Growth = \frac{Area\ of\ fungi\ in\ place\ with\ oil}{Area\ of\ fungi\ in\ negative\ plate} \times 100 \quad (1)$$

$$\%\ Inhibition = 100 - \%\ Growth \quad (2)$$

Characterization of the edible films

Prior to the addition of the edible coating in blueberries, the various formulations in the form of film were characterized.

Breaking strength and deformation

Were determinated using a TA.HD plus texturometer (Stable Micro System, Surrey, UK) with a 30 N load cell. Film samples were cut to 2 mm wide and 8 mm length and subjected to a tensile test at a crosshead speed of 1 mm/s, the results were obtained directly from the software of the equipment (Anchundia; Santacruz; Coloma, 2016).

Water-vapor permeability (WVP)

It was determined by gravimetry using the method described in the ASTM E96-00 (2000) standard and by Anchundia, Santacruz and Coloma (2016). Acrylic cells with an exposed area of approximately 15 cm^2 were filled with distilled water, maintaining a headspace of 10 mm. The test films were sealed with vacuum grease to assure hermeticity and then placed in a desiccator inside an oven with a controlled temperature of 25 °C for 24 h. Three

weights were recorded during the 24 h of storage, and the WVP was calculated with the Equation 3:

$$WVP = \frac{\Delta m \times I}{\Delta t \times A \times \Delta p} \quad (3)$$

Where Δm/Δt is moisture loss per unit time (g s^{-1}), A is the area of the exposed film (m^2), I is the film thickness (m), Δ*p* is the difference between water vapor pressure of both sides of film (Pa), determined with the Equation 4:

$$\Delta p = \frac{\Delta RH}{100} * PV\ AP\ SAT \quad (4)$$

Where is the relative humidity gradient between the cell and the surroundings and is the saturated vapor pressure of pure water (3160 Pa a 25 °C).

Thickness

The center and five points equally distributed around the circle were measured using a digital micrometer caliper (Mitutoyo, Japan). Their average was considered the final value (Cai; Wang; Cao, 2020).

Opacity

The procedure described by Cai, Wang and Cao (2020) was performed. Coating films were cut into 40 x10 mm rectangular strips and placed in the inner wall of a quartz cuvette while an empty one was used as control. Opacity was measured using a UV-Vis spectrophotometer (UV-VIS, Genesys 6, USA) at 600 nm wavelength and calculated by the Equation 5:

$$Opacity = \frac{absorbance_{600nm}}{film\ thickness\ (mm)} \quad (5)$$

Solubility

The method described by Anchundia, Santacruz and Coloma (2016) was followed. Coatings were dried in the oven (Venticel 111, Germany) at 100 °C until constant weight (w_i). Each sample was placed in a beaker containing 50 mL distilled water with stirring (Boeco, Germany) at room temperature for 24 h. The remaining coating was dried in the oven at 100 °C for 24h and weighed (w_f). Solubility percentage was calculated with the Equation 6:

$$Solubility\ (\%) = \frac{w_i - w_f}{w_i} \times 100 \quad (6)$$

In vitro evaluation of the antifungal effect of the coating

It was carried out using the agar diffusion method (Scartazzini et al., 2019). Coating disks of 20 mm diameter were placed in the center of a Petri plate with RBC agar previously inoculated with a suspension of *Botrytis cinerea* spores at $5x10^6$ cfu/mL. Coating without adding essential oil was also analyzed and considered as control. Finally, it was incubated at 20 °C for 72 h. The inhibition halo diameter was measured at 48 and 72 h of incubation and analyzed using the free ImageJ software. The results were expressed in mm (diameter of the zone of inhibition).

Evaluation of quality characteristics in blueberry

Loss of weight

It was determined weighing before and after each storage period. The results were expressed as a percentage of weight loss compared to the initial weight (Guerreiro et al., 2015b).

Color

The color was measured using the CIELAB system with the CR-400 Kónica-Minolta colorimeter. The equipment was heated for 10 min and calibrated with a standard blank. Then the luminosity value (L*) was determined (L*=0 for black and L*=100 for white), reporting the average of 5 measurements (Guerreiro et al., 2015b).

Firmness

It was determined with an Instron model 3342 texturemeter performing a puncture test at 1.0 mm/s. The post-test speed was 10.0 mm/s (Guerreiro et al., 2015a). An average of 5 measurements was reported.

Total phenol content

It was determined according to the Folin Ciocalteu colorimetric method. 80 µL blueberry juice and 20 µL sodium carbonate (75 g/L) were added to 100 µL Folin Ciocalteu (10% w/v). After 30 min of reaction at room temperature, the absorbance at 765 nm was measured. A standard Gallic acid calibration curve was developed (Guerreiro et al., 2015a).

Total anthocyanin content

The total anthocyanin content was determined by the pH differential method. Two sample dilutions were prepared by placing 1 mL berry juice in two test tubes, one with 9 mL potassium chloride buffer at pH 1 and the other with 9 mL sodium acetate buffer at pH 4.5.

After homogenizing, they were analyzed with the spectrophotometer using distilled water was used as a blank. The total anthocyanin content was calculated with the Equation 7.

$$A = (A_{vis\,\max} - A_{700})_{pH=1} - A = (A_{vis\,\max} - A_{700})_{pH=4.5} \quad (7)$$

Where $A_{vis.\,máx}$ is the value of the highest peak at pH 1 y pH 4.5, and A_{700}, is the value of the absorbance at a wavelength of 700 nm at pH 1 y pH 4.5. The value of total anthocyanin concentration was calculated with the Equation 8:

$$Anthocyanin = \frac{A * PM * FD * (V / W)}{\varepsilon * L} * 100 \quad (8)$$

Where A is the absorbance, PM is the Molecular weight (449.2 g/mol), FD is the dilution factor, V is the extraction volume, W is the weight of sample, ε is the molar extintion coeficient (26900 L/mol*cm), and L is the cell length (1 cm). The results were expressed as mg cyaniding-3-O-glucoside/100 g (Hassanpour, 2015).

Microbial count

It was determined by the pour plate method on Plate Count Agar (PCA) for viable mesophiles and by the spread plate method on RBC agar for molds and yeasts. Ten grams of each sample was aseptically transferred to 90 mL of 0.1% peptone water and homogenized. Serial dilutions up to 10^{-3} were made, transferring 1 mL of the suspension. The plates were incubated at 30 °C for 48 h. Results were expressed as colony-forming units per gram of fresh sample (cfu/g) (Guerreiro et al., 2015a).

RESULTS AND DISCUSSION

Antifungal activity of essential oils

Table 2 shows the evaluation results of radial growth inhibition of *Botrytis cinerea* using cinnamon and lemon essential oil as natural antifungals.

Many authors have studied the antifungal activity of cinnamon and lemon essential oils over *Botrytis cinerea* and reported total inhibition with less than 30 µL (Munhuweyi et al., 2017; Arrebola et al., 2009). In fact, Siripornvisal, Rungprom and Sawatdikarn (2009) and Pazmiño et al. (2017) reached total inhibition of this gray mold with 25 and 30 µL of cinnamon and lemon essential oil, respectively.

After verifying the antifungal action of cinnamon and lemon essential oils on *Botrytis cinerea* strains, the *in vitro* evaluation of these oils on the edible coating based on aloe vera gel and gelatin was carried out (Table 3), showing an adequate efficacy (greater than 20 mm) equivalent to 15% on average, with respect to the control from 0.1%; for both cases.

Table 2: Percentage inhibition of *Botrytis cinerea* growth using different cinnamon and lemon essential oil concentrations.

Oil concentration (ml/L)	% Inhibition cinnamon	% Inhibition lemon
5	8.88	3.01
10	14.76	12.78
30	100.00	34.79
50	100.00	100.00
100	100.00	100.00

Table 3: *In vitro* evaluation of the antifungal effect on *Botrytis cinerea* of the cinnamon and lemon essential oils in the edible cover based on aloe vera gel and gelatin.

Coating base	Oil	Concentration (%)	Diameter (mm)
10% aloe vera 2% gelatin	Control	0.0	22.5
	Lemon	0.1	24.5
		0.3	25.2
	Cinnamon	0.1	25.2
		0.3	26.3
30% aloe vera 2% gelatin	Control	0.0	22.7
	Lemon	0.1	25.8
		0.3	26.7
	Cinnamon	0.1	26.2
		0.3	27.3

Scartazzini et al. (2019) determined the *in vitro* activity of peppermint essential oil in a gelatin-based edible film over *Botrytis cinerea*, using a concentration of 0.38%. Mohammadi, Hashemi and Hosseini (2015) found the *in vitro* activity of cinnamon essential oil in a chitosan-based edible coating applied on strawberries on *Botrytis cinerea* from a concentration of 0.15%.

Regression models and optimization of edible coatings

Table 4 and Table 5 present the characterization results of the edible coatings with cinnamon and lemon essential oil, respectively. Aloe vera gel and gelatin increased thickness; the increase of aloe vera gel

increased opacity; the decrease of gelatin and glycerol increased solubility; as gelatin increased, the resistance to rupture increased; as gelatin and glycerol increased, the deformation increased; and as glycerol increased, the permeability to water vapor increased. In addition, a concentration of 0.3% was used to guarantee the inhibition of pathogenic molds during postharvest application.

Table 6 and Table 7 show the results of the analysis of variance (ANOVA) of the edible coatings with cinnamon and lemon essential oils. The parameters considered were the different sources of variation such as lack of fit, coefficient of determination (R^2), and regression. These results indicated that the SRM model adequately fixed the properties that characterize the coatings, from obtaining R^2 between 0.919 to 0.999, and lack of fit ($p > 0.05$) in all cases. Likewise, the quadratic model is the one that best modeled the data in the independent variables as in many interactions, indicating a significant effect in the design of the model, high efficiency in the prediction of the properties of the coating, and its reliability to predict these characteristics studied.

Peretto et al. (2014) evaluated the effectiveness of using carvacrol, methyl cinnamate, and their combinations (0.25 to 1.25%) to inhibit *Botrytis cinerea* on strawberries; and reported a similar model goodness-of-fit in the optimization of the edible coating based on alginate and calcium chloride using a central composite rotatable design. Takur et al. (2017) studied the optimization of an edible coating with pea starch (0.5-1.5%), chitosan (1-2%), and glycerol (0.5-1%) by applying a Box Behnken design; their results denoted high goodness-of-fit of the proposed model. Singh, Kumar and Sahoo (2014) studied the optimization of an edible coating with chitosan (1.5-2.5%) and glycerol (0.5-1%) by applying a Box Behnken design, denoting R^2 values from 0.839 to 0.997, lack of fit ($p > 0.05$) and the regression model that best modeled the data was quadratic.

The application of the multiple regression analysis allowed the development of the empirical models fitted to the experimental data obtained from the Box Behnken design into a second-order polynomial equation for each characterization property of the edible coating studied. The model fitted using the regression coefficients to investigate the relationship between the independent variables and the responses obtained from the following equations expressed with real values:

Table 4: Box Behenken design used for evaluating the edible coatings with cinnamon essential oil 0.3%.

	Cinnamon essential oil 0.3%								
Run of test	X_1 Aloe vera (%)	X_2 Gelatin (%)	X_3 Glycerol (%)	Thickness (mm)	Opacity (Abs)	Solubility (%)	Breaking strength (N)	Deformation (mm)	Water vapor permeability (x 10^{-8} $gmPa^{-1}s^{-1}m^{-2}$)
1	10	1.0	0.275	0.0617	1.179	36.49	0.365	6.990	1.195
2	30	1.0	0.275	0.0704	1.709	32.51	0.428	0.791	1.437
3	10	2.0	0.275	0.1052	1.018	15.75	2.550	1.175	2.149
4	30	2.0	0.275	0.1039	1.232	35.08	1.290	6.930	1.094
5	10	1.5	0.050	0.1071	1.449	26.00	1.227	0.239	2.019
6	30	1.5	0.050	0.0731	1.532	32.40	1.650	8.410	2.161
7	10	1.5	0.500	0.0996	0.460	19.05	0.480	37.075	3.251
8	30	1.5	0.500	0.1267	1.386	37.35	0.430	21.930	2.316
9	20	1.0	0.050	0.0571	2.582	36.70	0.610	1.815	1.692
10	20	2.0	0.050	0.0905	1.598	28.37	3.450	2.035	1.210
11	20	1.0	0.500	0.0769	1.138	40.17	0.415	28.270	2.019
12	20	2.0	0.500	0.1045	1.359	31.30	0.780	18.310	2.200
13	20	1.5	0.275	0.0940	1.072	32.19	1.150	3.090	1.219
14	20	1.5	0.275	0.0915	1.106	38.60	1.560	3.740	1.206
15	20	1.5	0.275	0.1109	1.044	33.91	1.700	5.920	1.598
16	20	1.5	0.275	0.1020	0.958	35.88	1.100	3.080	1.215
17	20	1.5	0.275	0.0867	0.849	33.84	1.296	2.210	1.410

Table 5: Box Behenken design used for evaluating the edible coatings with lemon essential oil 0.3%.

Lemon essential oil 0.3%									
Run of test	X_1 Aloe vera (%)	X_2 Gelatin (%)	X_3 Glycerol (%)	Thickness (mm)	Opacity (Abs)	Solubility (%)	Breaking strength (N)	Deformation (mm)	Water vapor permeability (x 10^{-8} $gmPa^{-1}s^{-1}m^{-2}$)
1	10	1.0	0.275	0.111	1.139	41.67	0.151	0.386	13.81
2	30	1.0	0.275	0.071	2.165	36.34	1.418	0.811	10.18
3	10	2.0	0.275	0.101	1.019	16.51	3.794	2.732	6.85
4	30	2.0	0.275	0.113	0.902	35.18	1.815	3.440	6.41
5	10	1.5	0.050	0.099	1.205	19.05	1.721	0.975	13.71
6	30	1.5	0.050	0.082	2.409	34.88	2.450	0.323	11.40
7	10	1.5	0.500	0.097	1.275	30.26	0.630	19.990	21.77
8	30	1.5	0.500	0.099	1.131	39.94	0.430	21.930	14.63
9	20	1.0	0.050	0.077	2.085	34.42	0.550	7.825	16.52
10	20	2.0	0.050	0.091	1.523	22.62	5.100	0.410	18.02
11	20	1.0	0.500	0.098	1.867	46.89	0.260	16.680	31.57
12	20	2.0	0.500	0.105	1.282	33.72	0.780	31.710	18.91
13	20	1.5	0.275	0.087	1.083	31.35	1.480	3.030	9.83
14	20	1.5	0.275	0.085	1.238	26.38	1.770	3.170	7.07
15	20	1.5	0.275	0.092	1.573	33.33	1.340	3.090	8.42
16	20	1.5	0.275	0.095	1.255	31.25	1.520	3.680	12.71
17	20	1.5	0.275	0.085	1.165	34.97	1.900	2.600	11.03

Table 6: ANOVA (p-value) for the regression equation fitted to the experimental response values obtained from optimizing the concentration of aloe vera gel, gelatin, and glycerol in the edible coating with cinnamon essential oil 0.3%.

Source	Thickness (mm)	Opacity (Abs)	Solubility (%)	Breaking strength (N)	Deformation (mm)	Water vapor permeability (x 10^{-7} $gmPa^{-1}s^{-1}m^{-2}$)
X_1	1.000	0.004	0.005	0.326	0.135	0.030
X_2	0.007	0.009	0.007	0.001	0.077	0.560
X_3	0.040	0.001	0.562	0.003	0.000	0.005
X_1^2	0.457	0.142	0.012	0.088	0.027	0.009
$X_1.X_2$	0.658	0.201	0.009	0.064	0.013	0.020
$X_1.X_3$	0.032	0.015	0.072	0.415	0.001	0.036
X_2^2	0.027	0.002	0.841	0.553	0.045	0.037
$X_2.X_3$	0.729	0.004	0.917	0.009	0.022	0.127
X_3^2	0.874	0.004	0.450	0.365	0.000	0.001
Regression	0.004	0.000	0.002	0.002	0.000	0.000
Lack of fit	0.822	0.343	0.446	0.253	0.097	0.424
R^2	0.923	0.972	0.937	0.938	0.982	0.958
R^2-ajusted	0.824	0.937	0.855	0.859	0.960	0.905

X_1, X_2, X_3: aloe vera gel, gelatin y glycerol.

Table 7: ANOVA (p value) for the regression equation fitted to the experimental response values obtained from the optimization of the concentration of aloe vera gel, gelatin and glycerol in the edible coating with lemon essential oil 0.3%.

Source	Thickness (mm)	Opacity (Abs)	Solubility (%)	Breaking strength (N)	Deformation (mm)	Water vapor permeability (x 10^{-8} $gmPa^{-1}s^{-1}m^{-2}$)
X_1	0.030	0.020	0.013	0.790	0.091	0.095
X_2	0.014	0.009	0.005	0.000	0.000	0.024
X_3	0.018	0.034	0.012	0.000	0.000	0.012
X_1^2	0.050	0.483	0.487	0.481	0.000	0.041
$X_1.X_2$	0.005	0.037	0.021	0.002	0.732	0.507
$X_1.X_3$	0.080	0.023	0.395	0.111	0.028	0.333
X_2^2	0.154	0.278	0.239	0.066	0.005	0.066
$X_2.X_3$	0.424	0.954	0.843	0.001	0.000	0.032
X_3^2	0.949	0.026	0.646	0.134	0.000	0.001
Regression	0.005	0.003	0.003	0.000	0.000	0.000
Lack of fit	0.368	0.570	0.550	0.111	0.254	0.738
R^2	0.919	0.930	0.930	0.969	0.999	0.961
R^2-ajusted	0.815	0.840	0.841	0.929	0.998	0.910

X_1, X_2, X_3: aloe vera gel, gelatin y glycerol.

0.3% Cinnamon essential oil

$$\text{Thickness} = -0.0746784 - 0.00269889X_1 + 0.236478X_2 - 0.0761975X_3 + 0.00003775X_1^2 - 0.00045X_1X_2 + 0.00677778X_1X_3 - 0.0629X_2^2 - 0.0155556X_2X_3 + 0.0153086X_3^2$$

$$\text{Opacity} = 6.0747 + 0.0566822\,X_1 - 5.21934\,X_2 - 10.6341\,X_3 - 0.00092025\,X_1^2 - 0.0158\,X_1X_2 + 0.0935556\,X_1X_3 + 1.4829\,X_2^2 + 2.67778\,X_2X_3 + 5.78222\,X_3^2$$

$$\text{Solubility} = 59.2792 + 0.461204\,X_1 - 34.8824\,X_2 - 11.3478\,X_3 - 0.0518058\,X_1^2 + 1.1653\,X_1X_2 + 1.32278\,X_1X_3 + 1.0227\,X_2^2 - 1.20444\,X_2X_3 - 19.7546\,X_3^2$$

$$\text{Breaking} = -5.17647 + 0.217368\,X_1 + 3.4142\,X_2 + 8.02331\,X_3 - 0.00284975\,X_1^2 - 0.06615\,X_1X_2 - 0.0525556\,X_1X_3 + 0.3281\,X_2^2 - 5.5\,X_2X_3 - 2.55753\,X_3^2$$

$$\text{Deformation} = -2.89115 - 1.21074X_1 + 15.5681X_2 + 18.2727\,X_3 + 0.0233475\,X_1^2 + 0.5977\,X_1X_2 - 2.59067\,X_1X_3 - 7.885\,X_2^2 - 22.6222\,X_2X_3 + 216.706\,X_3^2$$

$$\text{Permeability} = -4.71344\times10^{-8} - 4.90217\times10^{-9}\,X_1 + 4.07343\times10^{-7}\,X_2 - 6.02069\times10^{-7}\,X_3 + 3.97825\times10^{-10}\,X_1^2 - 6.485\times10^{-9}\,X_1X_2 - 1.19667\times10^{-8}\,X_1X_3 - 1.0347\times10^{-7}\,X_2^2 + 1.47333\times10^{-7}\,X_2X_3 + 1.40114\times10^{-6}\,X_3^2$$

0.3% Lemon essential oil

$$\text{Thickness} = 0.212765 - 0.00743167X_1 - 0.0790611X_2 + 0.00885185X_3 + 0.000061X_1^2 + 0.00255X_1X_2 + 0.00233333X_1X_3 + 0.0154\,X_2^2 - 0.0177778\,X_2X_3 - 0.00296296X_3^2$$

$$\text{Opacity} = 0.625702 + 0.179659X_1 - 0.843094X_2 - 1.24977X_3 - 0.00070175X_1^2 - 0.0572X_1X_2 - 0.149778X_1X_3 + 0.4563X_2^2 - 0.0511111X_2X_3 + 6.17432X_3^2$$

$$\text{Solubility} = 81.7891 - 0.644105X_1 - 62.0824X_2 + 31.8835X_3 - 0.0120355X_1^2 + 1.1996X_1X_2 - 0.684X_1X_3 + 8.7018X_2^2 - 3.04\,X_2X_3 + 15.436\,X_3^2$$

$$\text{Breaking} = -7.86757 + 0.303999X_1 + 4.64278X_2 + 13.4694X_3 - 0.00086125X_1^2 - 0.1623X_1X_2 - 0.103222X_1X_3 + 1.1145X_2^2 - 8.95556X_2X_3 - 4.11111X_3^2$$

$$\text{Deformation} = 23.0843 + 0.854538X_1 - 23.3319X_2 - 144.367X_3 - 0.0231175X_1^2 + 0.01415X_1X_2 + 0.288X_1X_3 + 4.16X_2^2 + 49.8778X_2X_3 + 197.575X_3^2$$

$$\text{Permeability} = 3.06056\times10^{-7} + 1.01517\times10^{-8}X_1 - 3.22653\times10^{-7}X_2 - 2.20367\times10^{-7}X_3 - 3.18942\times10^{-10}X_1^2 + 1.5945\times10^{-9}X_1X_2 - 5.36667\times10^{-9}X_1X_3 + 1.07523\times10^{-7}X_2^2 - 3.14667\times10^{-7}\,X_2X_3 + 1.7290\times10^{-6}X_3^2$$

The optimization of the independent variables about solubility, deformation, and breaking strength, considered the most critical parameters for the adherence and stability of the coating applied on fruits during refrigerated storage, is shown (Figure 1a edible coating with cinnamon essential oil 0.3% and Figure 1b edible coating with lemon essential oil 0.3%). The overlay of regions of interest of the contour surfaces determined that

the optimum area for coatings with cinnamon essential oil 0.3% fits 18.40% aloe vera gel, 2% gelatin, and 0.055% glycerol, obtaining 27.95% solubility, 0.90 mm deformation, and 3.34 N breaking strength.

Likewise, the optimal zone for coating with lemon essential oil 0.3% was 23.94% of aloe vera gel, 2% gelatin, and 0.05% glycerol, obtaining 28.06% solubility, 0.45 mm of deformation, and 4.53 N breaking strength.

Application of edible coatings on blueberries

Optimized edible coatings with cinnamon and lemon essential oil were applied on blueberries and stored at 2 °C for 28 days to evaluate their effectiveness as a postharvest preservation technology. The ANOVA determined that the addition of essential oils in the edible coating and storage time had a significant effect ($p<0.05$) on the physicochemical and microbiological characteristics of blueberries (Table 8).

Color

Table 8 shows that samples became lighter (higher L*) on day 28 of storage, mainly the blueberries with coatings and essential oils. Since luminosity (L*) indicates fruit darkening, a brightness modification in blueberry peel with alginate-based edible coating points out the delay of the ripening process (Chiabrando; Giacalone, 2017). De Souza et al. (2017) reported similar results in applying alginate-based edible coating with 0.2% of lemon essential oil and 0.1% of orange essential oil on raspberries stored at 4 °C for 15 days. Furthermore, Guerreiro et al. (2016) evaluated edible coating based on alginate 2% and pectin 1% with citral 0.15% and eugenol 0.1% on raspberries stored at 0.5 °C for 15 days.

Weight loss

Weight loss is an indicator of freshness in fruits. This study denotes that weight loss increased in berries as storage time progressed, reaching percentages between 3.06 to 4.14% on day 28 (Table 8). However, this decrease was lower in the coatings with essential oils. Choi, Singh and Lee (2016) indicated a more significant loss of weight in plums coated only with hydroxypropyl methylcellulose (HPMC) 2%, compared, to those with bergamot and oregano essential oil (0.5 – 2%, in both cases) during 30 days of storage at 5 °C. They suggest that this behavior is due to the hydrophobicity that essential oils generate in HPMC coatings, which serves as an excellent water barrier to reduce moisture evaporation and, consequently, fruit weight loss, surface depression, and deformation of the fruit.

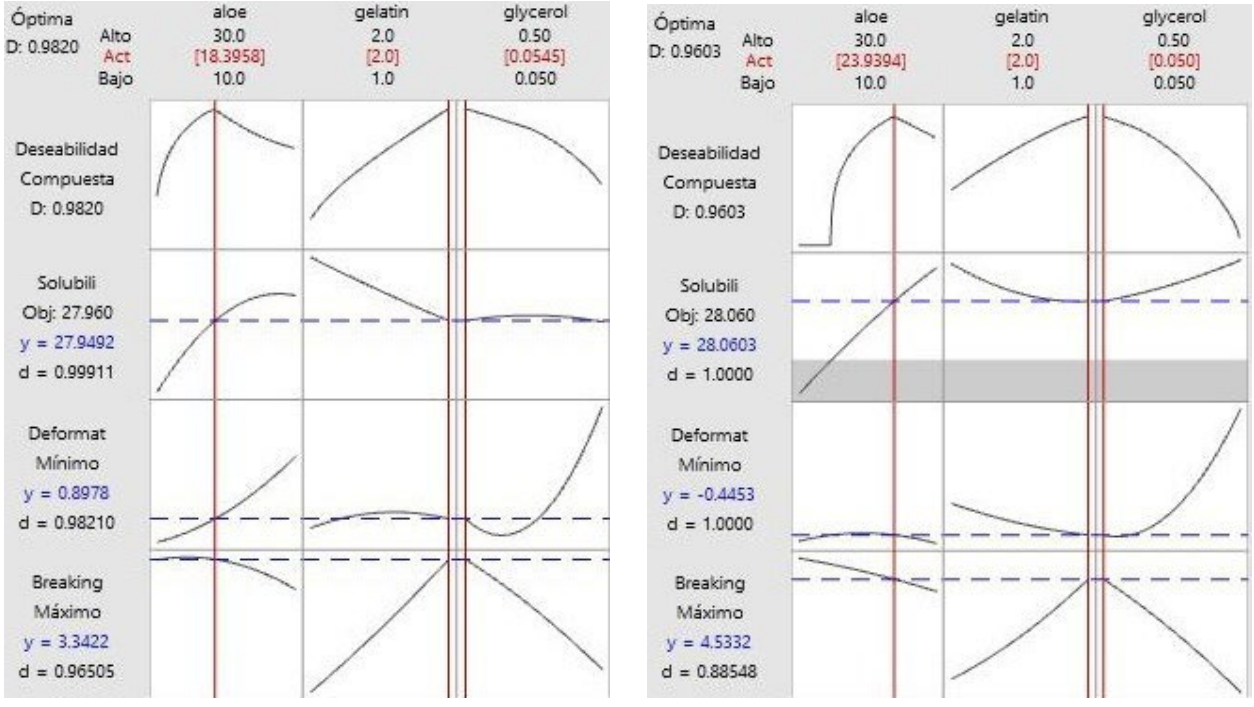

Figure 1: Optimization of edible coatings with 0.3% of cinnamon essential oil (a) and 0.3% of lemon essential oil (b).

Table 8: Effect of the application of edible coatings with essential oils on physicochemical and microbiological characteristics of blueberries during storage at 2 °C.

Treatment	Color (L)	Weight loss (%)	Firmness (N)	Phenol content (mg AG/100 g)	Anthocyanin (mg cyaniding 3-glucosido/100 g)	Viable mesophilic aerobes (cfu/g)	Molds and yeasts (cfu/g)
Control-0 days	29.540^{EFG}	0.000^{G}	2.497^{AB}	298.970^{F}	21.9133^{I}	10.00^{H}	10.00^{I}
Control-7 days	28.220^{FG}	1.140^{E}	1.760^{D}	304.220^{EF}	23.273^{HI}	46.67^{FG}	307.70^{GH}
Control-14 days	28.097^{G}	2.103^{D}	1.540^{DE}	309.090^{DE}	24.047^{GH}	87.67^{CD}	846.00^{D}
Control-21 days	30.753^{DE}	3.320^{BC}	1.357^{EF}	313.467^{CD}	26.857^{E}	144.33^{B}	1714.30^{B}
Control-28 days	32.073^{BC}	4.140^{A}	0.963^{G}	316.883^{BC}	30.113^{CD}	176.33^{A}	2245.30^{A}
Cinnamon-0 days	31.393^{CD}	0.000^{G}	2.737^{A}	304.440^{EF}	23.173^{HI}	10.00^{H}	0.00^{I}
Cinnamon-7 days	29.383^{EFG}	0.330^{G}	2.323^{B}	308.677^{DE}	25.797^{EF}	31.00^{GH}	185.70^{H}
Cinnamon-14 days	29.693^{EF}	0.947^{EF}	2.027^{C}	313.150^{CD}	28.903^{D}	46.00^{FG}	459.30^{FG}
Cinnamon-21 days	31.631^{CD}	1.863^{D}	1.717^{D}	318.440^{ABC}	32.217^{B}	78.33^{DE}	691.33^{E}
Cinnamon-28 days	33.413^{AB}	3.057^{C}	1.42^{EF}	324.103^{A}	35.233^{A}	103.33^{C}	905.00^{CD}
Lemon-0 days	32.354^{ABC}	0.000^{G}	2.700^{A}	304.300^{EF}	22.420^{I}	10.00^{H}	0.00^{I}
Lemon-7 days	28.737^{FG}	0.710^{F}	2.290^{BC}	306.143^{E}	24.720^{FG}	39.33^{FG}	255.33^{H}
Lemon-14 days	29.737^{EF}	1.233^{E}	1.753^{D}	309.733^{DE}	27.130^{E}	58.33^{EF}	571.30^{EF}
Lemon-21 days	31.823^{CD}	2.220^{D}	1.573^{DE}	314.230^{CD}	30.947^{BC}	90.67^{CD}	852.00^{D}
Lemon-28 days	33.892^{A}	3.573^{B}	1.267^{F}	320.780^{AB}	33.950^{A}	130.67^{B}	1012.67^{C}

Firmness

Firmness is one of the main physical attributes considered in the postharvest quality of berries (Table 8). As storage time progressed, firmness decreased in all treatments until they reached values between 0.96 to 1.42 N on day 28 of storage. The decrease of firmness was slighter in berries with coatings with essential oils. Choi, Singh and Lee (2016) studied the application of coatings with hydroxypropyl methylcellulose (HPMC) 2%, with bergamot and oregano essential oil (0.5 - 2%, in both cases). They reported a more significant decrease in firmness in control plums than those containing essential oils during 30 days of storage at 5 °C, possibly attributed to the fact that fruits with coatings and essential oils were more effective in delaying ripening progress.

Firmness is one of the most critical quality attributes influencing consumer appeal and marketability of fresh fruit. Blueberries typically soften during the postharvest chain, which decreases their shelf life and reduces market value. Coatings with added essential oils inhibit the development of microorganisms, which contributes to the maintenance of firmness in blueberries (Guerreiro et al., 2015b).

Total phenols

Table 8 shows a gradual increase of phenol content in blueberries, mainly at the end of storage in berries with coatings and essential oils, where values from 316.72 to 325.16 (mg AG/100 g) were found at day 28. Similar behavior was observed by Guerreiro et al. (2016) in edible coating based on alginate 2% and pectin 1% with citral 0.15% and eugenol 0.1% in raspberries stored at 0.5 °C for 15 days. Chiabrando and Giacalone (2017) mention that the increase in total phenol content during storage is affected by several causes of physiological stress, promoting the enzymatic oxidation of these compounds. Genskowsky et al. (2015) mention that the content of phenolic compounds, including anthocyanins and flavonoids, can be used as powerful indicators of antioxidant capacity, which can be used as a preliminary assay for any product when intended as a natural source of antioxidants in functional foods.

Total anthocyanins

Anthocyanins are considered essential antioxidants in berries. Table 8 exhibits a gradual increase of anthocyanin content in blueberries, mainly at the end of storage of berries with coatings containing essential oils, where values between 29.91 to 34.67 (mg cyanidin-3-glucoside/100 g) were obtained on day 28. Similar behavior was observed by Hassanpour (2015) in edible coating with aloe vera gel (25 - 75%) on raspberry stored at 4 °C for 8 days. The increase during storage may be due to the continuous biosynthesis of phenolic compounds after harvest, related to ripening processes. In addition, it is likely that aloe vera gel positively regulates anthocyanin production by stimulating gene expression of enzymes in the anthocyanin biosynthetic pathway, such as phenylalanine-ammonium-lyase (PAL).

Aerobios mesófilos viables

Food decomposition microorganisms are one of the leading causes of fresh fruit spoilage. The main objective of using essential oils and their constituents in edible coatings is their effect as antimicrobial agents. Sun et al. (2014) suggest 1% chitosan-based edible coating with carvacrol, cinnamaldehyde, and trans-cinnamaldehyde (between 0.1 - 0.5%) in all cases substantially reduces microbial populations of mold and yeast bacteria in blueberries; regardless of storage temperature and duration. Table 8 shows a minimal increase in viable mesophilic aerobic bacteria count in blueberries, more evident in the control sample than in the coating with essential oil, with values at day 28 ranging from 103 to 176 (cfu/g). Similar behavior was observed by Guerreiro et al. (2015b) in alginate-based edible coating (1 and 2%) with citral (0.15 and 0.3%) and eugenol (0.1 and 0.2%) on raspberries stored at 0.5 °C for 14 days.

Mold and yeasts

Table 8 displays an increase in the microbiological molds and yeasts count of blueberries, more noticeable in the control sample than in the coatings with essential oils, finding the values between 905 to 2245 (cfu/g) on day 28. Similar behavior was observed by De Souza et al. (2017) in applying an alginate-based edible coating with 0.2% lemon essential oil and 0.1 % orange essential on raspberries stored at 4 °C for 15 days. The action mechanism of essential oils against microbial cells is not yet fully understood; however, most research suggests that the cell wall and membrane are targets of antimicrobial agents, disrupting ATP production and pH homeostasis. In addition, the combined action of the edible coating with incorporated essential oils results in low gas diffusion, which limits the availability of oxygen to the tissues.

CONCLUSIONS

The antimicrobial activity of the cinnamon and lemon essential oils had a minimum inhibitory concentration of 0.3%, the cinnamon essential oil was the one that showed greater efficacy, at the same concentration. Edible coatings were made based on aloe vera gel, gelatin, and glycerol, characterizing its physical-mechanical properties. The SRM contour overlay technique optimized each coating with essential oil for solubility, deformation, and breaking strength. The application in blueberries was validated, conserving its physicochemical and microbiological quality attributes during 28 days of storage at 2 °C.

AUTHOR CONTRIBUTION

Conceptual Idea: Márquez-Villacorta, L.; Pretell-Vásquez, C.; Hayayumi-Valdivia, M.; Methodology design: Márquez-Villacorta, L.; Pretell-Vásquez, C.; Hayayumi-Valdivia, M.; Data collection: Hayayumi-Valdivia, M.; Data analysis and interpretation: Márquez-Villacorta, L.; Pretell-Vásquez, C.; Hayayumi-Valdivia, M., and Writing and editing: Márquez-Villacorta, L.; Pretell-Vásquez, C.; Hayayumi-Valdivia, M.

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REFERENCES

ADETUNJI, C. O.; FADAJI, A. E.; ABOYIJI, O. O. Effect of chitosan coating combined aloe vera gel on cucumber (*Cucumis sativa* L.) post-harvest quality during ambient storage. Journal of Emerging Trends in Engineering and Applied Sciences, 5(6):391-397, 2014.

ANCHUNDIA, K.; SANTACRUZ, S.; COLOMA, J. Caracterización física de películas comestibles a base de cáscara de plátano (*Musa paradisiaca*). Revista Chilena de Nutrición, 43(4):394-399, 2016.

ARREBOLA, E et al. Combined application of antagonist *Bacillus amyloliquefaciens* and essential oils for the control of peach postharvest diseases. Crop Protection, 29(4):369-377, 2009.

ASTM INTERNATIONAL. ASTM E96-00: Standard test method for water vapor transmission of materials. 2000. Available in: <https://www.astm.org/e0096-00.html>. Access in: August 09, 2022.

BEHDANI, M.; POOYAN, M.; ABBASI, S. Evaluation of antifungal activity of some medicinal plants essential oils against botrytis cinerea, causal agent of postharvest apple rot, *in vitro*. International Journal of Agriculture and Crop Sciences, 4(14):1012-1016, 2012.

BEHSHTI, M et al. Essential oils to control *Botrytis cinerea in vitro* and *in vivo* on grapefruits. Journal of Horticulture and Postharvest Research, 3(2):161-172, 2020.

CAI, L.; WANG, Y.; CAO, A. The physiochemical and preservation properties of fish sarcoplasmic protein/chitosan composite films containing ginger essential oil emulsions. Journal of Food Process Engineering, 43(10): e13495, 2020.

CHIABRANDO, V.; GIACALONE, G. Quality evaluation of blueberries coated with chitosan and sodium alginate during postharvest storage. International Food Research Journal, 24(4):1553-1561, 2017.

CHOI, W. S.; SINGH, S.; LEE, Y. S. Characterization of edible film containing essential oils in hydroxypropyl methylcellulose and its effect on quality attributes of 'Formosa' plum (*Prunus salicina* L.). LWT, 70:213-222, 2016.

DE SOUZA, M. et al. Effect of edible coatings with essential oils on the quality of red raspberries over shelf-life. Journal of the Science of Food and Agriculture, 97:92-938, 2017.

FALCÓ, I. et al. On the use of carrageenan matrices for the development of antiviral edible coatings of interest in berries. Food Hydrocolloids, 92:74-85, 2019.

GENSKOWSKY, E. et al. Assessment of antibacterial and antioxidant properties of chitosan edible films incorporated with maqui berry (*Aristotelia chilensis*). LWT - Food Science and Technology, 64(2):1057-1062, 2015.

GUERREIRO, A. C. et al. The effect of alginate-based edible coatings enriched with essential oils constituents on *Arbutus unedo* L. fresh fruit storage. Postharvest Biology and Technology, 100:226-233, 2015a.

GUERREIRO, A. C. et al. Raspberry fresh fruit quality as affected by pectin- and alginate-based edible coatings enriched with essential oils. Scientia Horticulturae, 194:138-146, 2015b.

GUERREIRO, A. C. et al. The influence of edible coatings enriched with citral and eugenol on the raspberry storage ability, nutritional and sensory quality. Food Packaging and Shelf Life, 9:20-28, 2016.

HASSANPOUR, H. Effect of *Aloe* vera gel coating on antioxidant capacity, antioxidant enzyme activities and decay in raspberry fruit. LWT - Food Science and Technology, 60(1):495-501, 2015.

JU, J. et al. Inhibitory effects of cinnamon and clove essential oils on mold growth on baked foods. Food Chemistry, 240:850-855, 2018.

LIN, M. G. et al. Effect of chitosan and carrageenan-based edible coatings on post-harvested longan (*Dimocarpus longan*) fruits. CYTA - Journal of Food, 16(1):490-497, 2018.

MOHAMMADI, A.; HASHEMI, M.; HOSSEINI, S. The control of Botrytis fruit rot in strawberry using combined treatments of chitosan with zataria multiflora or *Cinnamomum zeylanicum* essential oil. Journal of Food Science and Technology, 52:7441-7448, 2015.

MUNHUWEYI, K. et al. *In vitro* and in vivo antifungal activity of chitosan-essential oils against pomegranate fruit pathogens. Postharvest Biology and Technology, 129:9-22, 2017.

MURMU, S. B.; MISHRA, H. The effect of edible coating based on Arabic gum, sodium caseinate and essential oil of cinnamon and lemon grass on guava. Food Chemistry, 245:820-828, 2017.

NANDANE, A. S.; DAVE, R. K.; RAO, T. V. Optimization of edible coating formulations for improving postharvest quality and shelf life of pear fruit using response surface methodology. Journal of Food Science and Technology, 54:1-8, 2017.

PAZMIÑO, M. et al. Efecto de los extractos hidro-etanólicos de canela (*Cinnamomum zelanicum*) y cola de caballo (*Equisetum arvense L.*) sobre la incidencia y severidad de *Botrytis cinerea* en fresa. Journal of the Selva Andina Biosphere, 5(1):29-38, 2017.

PERETTO, G. et al. Optimization of antimicrobial and physical properties of alginate coatings containing carvacrol and methyl cinnamate for strawberry application. Journal of Agriculture and Food Chemistry, 62(4):984-990, 2014.

REZAEIFAR, M. et al. Effect of chitosan edible coating enriched with lemon verbena extract and essential oil on the shelf life of vacuum rainbow trout (*Oncorhynchus mykiss*). Journal of Food Safety, 40(3):e12781, 2020.

SCARTAZZINI, L. et al. Gelatin edible coatings with mint essential oil (*Mentha arvensis*): Film characterization and antifungal properties. Journal of Food Science and Technology, 56:4045-4056, 2019.

SINGH, T. P.; KUMAR, M.; SAHOO, J. Development of chitosan based edible films: Process optimization using response surface methodology. Journal of Food Scientists & Technologists, 52:2530-2543, 2014.

SIRIPORNVISAL, S.; RUNGPROM, W.; SAWATDIKARN, S. Antifungal activity of essential oils derived from some medicinal plants against grey mould (*Botrytis cinerea*). Asian Journal of Food and Agro-Industry, S229-S233, 2009.

SUN, X. et al. Effects of chitosan-essential oil coatings on safety and quality of fresh blueberries. Journal of Food Science, 79(5):955-960, 2014.

THAKUR, R. et al. Use of response surface methodology (RSM) to optimize pea starch–chitosan novel edible film formulation. Journal of Food Science and Technology, 54:2270-2278, 2017.