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Edible Film Production from Effluents of Potato Industry Incorporated with *Origanum onites* Volatile Oils and Changes Its Textural Behaviors under High Hydrostatic Pressure

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Abstract

Development and characterization of edible film incorporated with *Origanum onites* volatile oil from the effluents of potato industry, determination of changes on its textural properties of force and elongation at break (EAB) under high hydrostatic pressure (HHP) in addition to its antimicrobial effect against *Escherichia coli* O157:H7 and *Salmonella* Enteritis were prompted. The optimum operational conditions under HHP for maximum force and EAB were achieved with 350 MPa pressure, 8 min operational time, and addition of 45 µL *O. onites* volatile oil concentration (VOC). Inhibition zones for *S.* Enteritis and *E. coli* O157:H7 at the optimum conditions were 1.7 ± 0.109 and 2.386 ± 0.07 cm, respectively. Textural properties of force and EAB of the HHP-processed films ranged from 2.27 ± 0.52 to 5.23 ± 0.79 N, and from 7.47 ± 1.68 to 15.71 ± 0.65 mm, respectively. Thermal transition of the edible film was observed at 86.77°C for 7.19 min. The microscopic observation of the film surfaces shoowed homogenous and translucent structure. The improved textural properties with HHP and VOC revealed that it carries a potential to be used as a food packaging material.

Keywords: Edible film, High hydrostatic pressure, Potato industry effluent, Antimicrobial activity, Textural properties

Introduction

Recent interests have intensified the search for biodegradable food packaging and edible polymers (films) to replace plastics or synthetic polymers due to the growing environmental concerns (Borah et al., 2017; Lopez-Rubio et al., 2017). The high cost of biopolymers, on the other hand, makes agricultural by-products a viable feedstock to produce edible films or coatings. Different edible films developed from polysaccharide, protein, and lipid biopolymers have potential to be considered as food packaging or coating to enhance food quality and safety (Nandane and Jain, 2018; Park et al., 2017). Because it carries advantages of having lower cost, being available, higher thermoplasticity, and inherent biodegradability; starch, as the carbohydrate-based polymers, is one of the most suitable and promising material for biodegradable, and edible packaging (Ehiyet et al., 2011).

According to data released by FAOSTAT in 2019, Turkey ranked in the 14th place, with its 4.8 million tons of potato production (Anonymous, 2019). Potato industry produced about 0.16 tons of solid waste per ton of processed potato such as pulp, potato wastewater, and peel (Pathak et al., 2018). In general, waste from potato industry has been utilized as components of microbial media (Kot et al., 2020) as well as production of edible film from potato peel waste (Othman et al., 2017).

Active food packaging is a rapidly developing food packaging technology actively allowing to eliminate contamination of foods (De Kruijf et al., 2002). Active packages are mostly incorporated with antioxidants, vitamins, antimicrobial agents, or flavoring agents as biologically active compounds to increase its functionality (Quintavalla and Vicini, 2002), and thus antimicrobial agents containing packaging can be as an excellent packaging alternative to improve the safety of food products.

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Essential plant oils of origanum species as antimicrobial agents are getting more interests because of their potential antimicrobial properties and consumer demand for less synthetic and artificial additives in foods (Ehivet et al., 2011; De Kruijf et al., 2002; Quintavalla and Vicini, 2002; Burt, 2004; Min and Oh, 2009).

High hydrostatic pressure (HHP) processing involves application of high pressures from 100 to 800 MPa to liquid and solid foods at ambient, lower, and higher than the ambient temperature (Farkas and Hoover, 2000; Torres and Velazquez, 2005). HHP is mainly used for food pasteurization, but studies related to effect of HHP on the buckwheat, tapioca starch film, and modified amaranth proteins preparation and its effect on starch gelatinization as well as film properties were reported (Condés et al., 2015; Kim et al., 2018). Even though development of edible films and measurement of their textural and mechanical properties are reported in the literature, information regarding the use of food industry by-products in the development of potential food-packaging materials, measurement of its properties, and determination of changes in its properties under food processing technologies such as HHP when used as food packaging material is not reported in the literature. Thus, the objective of this study was to produce an edible film from effluents of potato production industry having potential to be food packaging incorporated with *Origanum onites* volatile oil to carry antimicrobial properties and determine its textural behaviors under HHP.

Materials and Method

Effluents of potato production treatment plant

Effluents were sampled from a potato processing plant based in Bolu, Turkey (Köksal Patates Sanayi ve Ticaret A.Ş.). The samples were taken at the same day when discharge was started.

Extraction and determination of major constituents of *Origanum onites* volatile oil

Dried leaves of *Origanum onites* were purchased from local stores (Bolu, Turkey). Leaves (30 g) were placed in a 2 L-volume Clevenger type apparatus and mixed with 500 mL water before extraction. Temperature of the heater was gradually increased up to 250 ºC for 2 h for extraction. Temperature of the extracted oil was lowered to room temperature with cooling coils connected to tap water. Obtained oil was kept in an amber-colored bottle at 4 ºC until its further use (Dadalioglu and Evrendilek, 2004).

In order to determine the major constituents, 30 µL of the *O. onites* volatile oil were mixed with 1.5 mL hexane before injecting to a gas chromatography mass spectroscopy (GC-MS) (Shimadzu 2010 QP 2010 Plus GC-MS Kyoto, Japan) coupled with HP-5 MS capillary column (30 m x 0.25 µm x 0.25 µm) with 250 ºC injection block temperature. A maximum column temperature of 325 ºC was used starting at 50 ºC for 5 min, with a 2 ºC/min increase to 90 ºC, then a 5 ºC/min increase to 210 ºC for 5 min. Helium as a carrier gas at a flow-rate of 1.5 mL/min. Mass spectrum of each compound was taken over the 35–350 *m/z* range with the electron ionization mode of 70 eV. Volatile oil components (VOC) were identified with comparison of their retention indices (RIs) and mass spectra to Wiley (275) library (Dadalioglu and Evrendilek, 2004).

Preparation of the edible film

Effluent samples contained pieces of solid potato particles and peels, thus liquid phase was removed at room temperature after the samples were settled for three hours. After removal of macro particles, the fraction contained starch was centrifuged at 0.45 x *g* for 5 min. Pellets from the effluent were dried at 37 ºC for 24 h, and grinded with an 8-mm sieve. About 300 g dry residue were obtained from 5 L effluent.

Twenty-five g of dried residue mixed with 250 mL water (1:10 w/v ratio) and 5 % (v/v) plasticizer (glycerol). The magnetic hot plate (Wisestir, Germany) at 240 rpm was set to 150 ºC, and the mixture at 85-90 ºC was heated for at least 75 min. pH was adjusted to 2.6 with 0.1M HCl (Sigma Aldrich, Steinheim, Germany). Food grade green colorant (0.2 mL) and *O. onites* volatile oil at three different concentrations of 30, 45, and 60 µL/mL were added to edible film mixtures (Table 1). The mixture solution was stirred until colorant and *O. onites* oil are homogenously mixed. The final solution was cooled at room temperature and poured into an aluminum casting tray (26 x 17 cm) placed into a natural convection oven at 40 ºC for 10 hours to dry the film samples.

Bacterial cultures

Activation of *Escherichia coli* O157:H7 (ATCC 35218) and *Salmonella* Enteritis (OSU 799) cultures (The Ohio State University, Columbus, OH) was realized by transferring both cultures to Tryptic Soya Broth (TSB, Fluka, Seelze, Germany). Both cultures were incubated at 35 ± 2 ºC overnight. After transferring grown cells into sterile tubes; the samples were centrifuged at 0.49 x *g* for 15 min, separately, and the supernatants of the liquids were removed. Obtained pellets were resuspended with 10 mL pre-sterilized phosphate buffered saline (Sigma Chemical Co., Stockholm, Sweden). Cell resuspensions were washed with PBS in several times to remove TSB. Number of viable cells as log cfu/mL were determined by preparing a 10-fold serial dilutions of 0.1 mL aliquot plated on TSA plates. Prepared plated were incubated at 35 ± 2 ºC for 24 h.

High hydrostatic pressure processing

A 2-L capacity pilot-scale HHP equipment (Avure, Middletown, OH, USA) with water used as pressured fluid was used to process the film samples. The pouches were put into the flexible pouches composed of a multilayer polymer/aluminum/polymer film (polyethylene–aluminum–polypropylene) (APACK Packaging Technologies, Istanbul, Turkey). After heat sealing of the pouches, the samples were processed by the pressures changing from 200 to 500 MPa, treatment time from 1 to 15 min, *O. onites* volatile oil volumes (VOC) from 30 to 60 µL (Table 1), and the maximum processing temperature of 29 ºC.

Table 1. (Un)coded variables of Box-Behnken design for textural properties of biopolymer with *Origanum onites* volatile oil.

Run Order	HHP pressure (*P*, MPa) X_1	Volatile oil concentration (*VOC*, µL) X_2	Time (*T*, min) X_3	Force (*N*)	Elongation at break (mm)
Control	-	-	-	3.60 ± 0.23^{bc}	8.83 ± 0.14^{d}
1	500	45	1	4.57 ± 0.07^{a}	11.78 ± 1.35^{b}
2	500	60	8	4.90 ± 0.60^{a}	15.71 ± 0.65^{a}
3	350	30	1	5.23 ± 0.79^{a}	12.53 ± 0.62^{b}
4	200	60	8	4.64 ± 0.51^{a}	10.25 ± 1.09^{c}
5	500	45	15	3.79 ± 0.26^{bc}	12.79 ± 1.49^{b}
6	200	30	8	4.70 ± 0.39^{a}	12.71 ± 2.72^{bc}
7	350	45	8	4.43 ± 0.28^{ab}	9.82 ± 1.20^{c}
8	200	45	15	2.27 ± 0.52^{d}	7.47 ± 1.68^{e}
9	500	30	8	4.68 ± 0.16^{a}	10.78 ± 1.13^{c}
10	350	30	15	3.95 ± 0.08^{c}	12.67 ± 1.33^{bc}
11	350	60	1	3.92 ± 0.54^{b}	12.49 ± 1.65^{bc}
12	200	45	1	2.68 ± 0.01	9.04 ± 0.00^{d}
13	350	60	15	3.85 ± 0.39	12.30 ± 2.46^{b}

*Data with different superscript letters in the same response column show a significant difference at $p \leq 0.05$.
**Data is presented as average ± standard deviation

Properties of the effluent

pH of the effluents was measured at room temperature with 10 mL of the samples (Orion perpHectlogR meter, Inolab WTW, Germany).

Conductivity (mS/cm) measurement was performed by conductivity meter (Sension 5 model, HACH, CO, USA) with 50 mL of the samples at room temperature.

Twenty-five mL of the effluent samples at room temperature was used for turbidity measurement. Turbidity (NTU) was conducted by the turbidimeter (Micro TPI, HF Scientific, FL, USA).

Fifty mL of the samples after filtration through Whatman 42 filter paper dried at 105 °C for 30-40 min. Suspended solid matter (SSM) was calculated by taking into account the initial weight (g) of the paper after drying (A) and the weight (g) of the paper after filtration (B) (Rice et al., 2005).

$$SSM\left(\frac{mg}{L}\right) = \frac{(B-A)}{V} x100 \quad (1)$$

Spectroquant cell tests (DIN ISO 15705, Merck, Germany) were used to determine chemical oxygen demand (COD). The effluent samples were homogenized, and then diluted with distilled water at 1:50 ratio. Three mL of the diluted samples were taken and placed into the COD tubes heated at 148 °C in thermoreactor for 120 min. When the tubes were cooled to room temperature, the measurement was performed using the photometer.

Initial microbial load of effluent

Appropriate dilutions of effluent samples prepared with 0.1 % peptone were plated onto potato dextrose agar (PDA, Fluka, Seelze, Germany) to count total mold and yeast count (TMY); and plate count agar (PCA, Fluka, Seelze, Germany) to count total aerobic mesophilic bacteria (TAMB), respectively. PCA plates were incubated at 35 ± 2 °C for 24 to 48 h, while PDA plates were incubated at 22 ± 2 °C for 3 to 5 days. Results were expressed in log cfu/mL.

Properties of the edible film

Weight and thickness of edible films cut in an 8.64-mm diameter using a mold were measured using a balance with 0.001 g sensitivity and micrometer (Mitutoyo, Japan) with 0.0001 mm sensitivity, respectively (Seydim and Sarikus, 2006).

Edible films were cut at 40 x 40 mm^2 dimensions, and each piece was transferred into beaker containing 50 mL of water for the determination of water solubility. Each beaker was sealed with parafilm and they were transferred into shaking water bath at 25 °C for 24 h. Insoluble fraction of the film samples was dried at 70 °C for 10 min until a constant weight was reached. Results were calculated as follows (Razavi et al., 2015):

$$\%\ water\ solubility = \frac{W_0 - W_{24}}{W_0} * 100 \quad (2)$$

where W_0 is the initial dry weight of the film before drying (g), while W_{24} is the final dry weight (g) of the insoluble film after drying.

L^*, a^*, and b^* color parameters of the edible film were measured using a colorimeter (Konica Minolta CR-400, Osaka, Japan) Total color difference (ΔE) was calculated as follows;

$$\Delta E = \sqrt{(L_0 - L*)^2 + (a_0 - a*)^2 + (b_0 - b*)^2} \quad (3)$$

Both the control and the HHP treated films samples having *O. onites* volatile oil were cut in 80 x 25 mm dimensions using a standard mold at 25 °C and 53 ± 2% relative humidity for 48 h before textural measurements. Elongation at break (EAB) and force of the film samples were measured using a TA-XT2 model texture analyzer (Stable Micro Systems, Surrey, England) with a tensile grip at 1 mm/s speed, and calculated by the software (Texture

Expert Exceed 2.3, Stable Micro System, Survey, England) (Chaichi et al., 2017).

Thermal properties of edible film

Differential scanning calorimeter (DSC, TA Q20 Instruments, Shimadzu, Japan) was used in order to determine the thermal properties of biopolymers. Calibration was performed with the indium standard (156.6 ºC melting temperature and 28.5 J/g melting enthalpy) under the nitrogen atmosphere with 30 mL/min flow rate. Thermograms were obtained with 2-3 mg of the samples weighed into sampling cups, and scanning was made between 20 and 100 ºC at an interval of 10 ºC/min.

Surface properties of biopolymer was observed using a binocular microscopy (BK 5000 L modal, Soif Optical Instruments, China) at 4x magnification. X-ray diffraction (XRD) patterns of the films were measured using a MRD X-ray diffractometer (Rigaku, Neu-Isenburg, Germany) with Coka radiation. The samples were prepared by placing the square shape of each film (2 cm x 2 cm) on a glass side. A nickel monochromator filtering wave was used at 36 kV and 26 mA with scanning at $2\Theta = 5^0$- 80^0 at a rate of 2.20^0/min and with a step size of 0.02^0.

Antimicrobial activity of edible film

Antimicrobial activity of the edible film was tested against *S.* Enteritis and *E. coli* O157:H7, separately. Both cultures at the 10^6 cfu/mL cell count were plated onto PCA agar plates, and the plates were incubated for 48 h at 35 ± 2 ºC for surface growth. An edible film disc of 8.64 mm with 45 µL *O. onites* volatile oil (concentration at optimum point) added during film preparation was placed onto surface of PCA agar. The plates were incubated for additional 24 h at 35 ± 2 ºC, and zone of inhibition was measured using a micrometer. Control samples were prepared the same but without addition of *O. onites* volatile oil.

Data analyses

The force and EAB responses of the edible film production were optimized as a function of pressure (200 to 500 MPa), volatile oil concentration (30 to 60 µL), and processing time (1 to 15 min) using the Box-Behnken design (BBD) with a quadratic model. The levels of these settings were determined by initial experiments. The overall BBD configuration with its (un)coded predictors is presented in Table 1. Data analyses were conducted by MINITAB 17.0 (Minitab Inc. State College, PA, USA). A quadratic regression model was used to best-fit the experimental data:

$$Y_n = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 \quad (4)$$

where Y_n is the response variable of force and EAB; b_0 to b_{33} are slope coefficients; and X_1, X_2 and X_3 are pressure (MPa), VOC (µL), and processing time (sec), respectively. Additional experiments, to validate the models, were carried out under the optimal conditions. Analysis of variance (ANOVA) and regression models were applied to determine the significant terms of the predictive model ($p < 0.05$). Tukey's test was further utilized to determine the multiple comparisons. Verification of the predicted model was conducted by the coefficient of variation (CV, %) as follows:

$$CV = \frac{\sigma}{\overline{X}} 100 \quad (5)$$

where σ is sample standard deviation, and $\overline{C}$ is sample mean.

Results and Discussion

Properties of the effluent and the edible film

The effluent samples had pH of 6.16 ± 0.14, conductivity of 1774 ± 38 mS/cm, and turbidity of 1170.00 ± 57.01 NTU at the temperature of 12.49 ± 0.34 ºC. The mean COD was determined as 50093.75 ± 3361.41 mg/L. The amount of SSM was 0.65 ± 0.13 mg/mL. The initial TMAB and TMY loads were > 9 log cfu/mL.

Average weight and the thickness of the film samples were 0.0177 ± 0.0004 g and 0.370 ± 0.005 mm, consequently. Water solubility of the edible film was estimated at 32.69 ± 4.34%. The color of the edible film changed from cream to green with the addition of green coloring agent. The natural color of the edible film before addition of green color was measured as 79.55 ± 0.64, -0.0011 ± 0.01, and 4.19 ± 0.18 for *L**, *a**, and *b**; whereas they were measured as 76.8 ± 1.84, -21.70 ± 4.92, and 28.94 ± 4.77 after addition of green color, respectively. ΔE of the edible film samples were recorded as 84.89 ± 11.36.

The initial force and EAB values of the film were 3.60 ± 0.23 N and 8.83 ± 0.14 mm, respectively. With HHP treatment, the force ranged from 2.27 ± 0.52 to 5.23 ± 0.79 N. Except for run 8 (200 MPa pressure, 45 µL volatile oil, and 15 min treatment time) and run 12 (200 MPa pressure, 45 µL volatile oil, and 11 min treatment time), HHP treatments regardless of the processing time and VOC, led to higher force values than that of the control samples ($p \leq 0.05$). The highest VOC (60 µL) and pressure (500 MPa) under the moderate processing time (8 min) provided the highest force to film (5.23 ± 0.79 N) on run 3 ($p \leq 0.05$) (Table 1).

EAB of the HHP-treated samples ranged from 7.47 ± 1.68 to 15.71 ± 0.65 mm. Except for run 8 with (200 MPa pressure, 45 µL volatile oil, and 15 min treatment time), all the other HHP processes revealed higher EAB value than that of the control samples. Again, the highest VOC (60 µL) and the pressure (500 MPa) under the moderate processing time (8 min) provided the highest EAB (15.71 ± 0.65 mm) on run 2 ($p \leq 0.05$). The increased force did not linearly increase with the VOC and processing time, but with the pressure and the combination of pressure and VOC (Table 1).

Inhibition zone diameters of *S.* Enteritis and *E. coli* O157:H7 with the addition of 45 mL volatile *O. onites* oil were 1.70 ± 0.109 and 2.39 ± 0.07 cm with the calculated inhibition zone area of 2.15 ± 0.383 and 4.95 ± 0.341 cm^2, respectively. No zone of

inhibition was observed for the edible film samples with no *O. onites* volatile oil.

GC-MS analyses of *O. onites* volatile oil revealed 76 different compounds among which 5-methyl-2-propan-2-ylphenol (thymol, 34.67%), 2-methyl-5-(propan-2-yl) phenol (carvacrol, 28.32%), 3-methylpentane (5.65%), 3,7-dimethylocta-1,6-dien-3-ol (linalool 5.06%), 1-methyl-4-(propan-2-yl) benzene (*p*-cymene, 3.48%), and 2-methylpentane (2.22%), were the highest. High antibacterial activity of *O. onites* volatile oil correlated with the important percentages of thymol (34.67%) and carvacrol (28.32%). Both constituents were also found higher in *O. onites* samples by 5.97 and 71.22% (*23*) and 1.51 and 37.08% (Sevindik et al., 2019). The ability of origanum species to inactivate bacterial strains in synthetic media as well as in food system were related to higher amount of both thymol and carvacrol (Lambert et al., 2001; Knowles et al., 2005; Valero and Francés, 2006). It is also reported that not only the components present in high proportions are responsible for the antimicrobial activity, but also the less abundant ones, like p-cymene should also be considered. Aqueous extract of *O. onites* displayed MIC value of 25 mg/mL against methicillin susceptible *Staphylococcus aureus* (MSSA), methicillin resistant *S. aureus* (MRSA), *Klebsiella pneumoniae*, *Pseuodomonas aeruginosa*, *Bacillus cereus*, and *Enterococcus faecalis*, and 6.25 mg/mL against *E. coli* (*28*). Indeed, it was also presented that carvacrol, thymol and p-cymene of *O. compactum* essential oil have strong antibacterial affect against different bacteria (Andrade-Ochoa et al., 2015; Bouyahya et al., 2017; Chauhan and Kang, 2014; Li and Liu, 2009; Xu et al., 2008).

Starch based active films and food packaging with the addition of plasticizers is a promising alternative due to their low cost, availability and functionality (Castillo et al., 2017). Antimicrobial properties of these films make them suitable for food packaging providing significant inactivation of food borne pathogens and food spoilage microorganisms. For instance, tapioca starch-glycerol edible films incorporated with potassium sorbate controlled growth of *Zygosaccharomycess bailii* contamination in semisolid product (Flores et al., 2007). Starch-clay nanocomposite films contained potassium sorbate presented antimicrobial property against *Aspergillus niger* (Barzegar et al., 2014). Starch film having different concentrations of chitosan and lauric acid exert an inhibitory effect on *Bacillus subtilis* and *E. coli* growth (Salleh et al., 2014).

Due to the antimicrobial properties of Maillard compounds, 1-2 log inactivation was observed on *Listeria innocua* and *E. coli* inoculated in starch based edible film from corn formulated with different oxidation degree and addition of bovine gelatin, glycerol, as a plasticizer, and ethyl lauroyl arginate (LAE) as antimicrobial compound (Moreno et al., 2017). Inhibition efficiency of the starch films incorporated with tea polyphenol (TP) ranged approximately from 60 to 90% for *E. coli* and from 90 to 100% for *S. aureus* revealing good antimicrobial properties (Feng et al., 2018).

It is desired for edible films to have high water resistance because they can be used as protective layers on high as well as intermediate moisture foods (Gontard et al., 1992). In order to prevent the complete dissolution of the film, corporation of antimicrobial agents into edible films are also desired. Antimicrobial edible films having poor water resistance is dissolved very quickly, thus its antimicrobial properties protected (Castillo et al., 2017; Ozdemir and Floros, 2008). Water solubility of the buckwheat starch film prepared using thermal processing (90 ^{o}C for 20 min) and HHP (600 MPa 20 ^{o}C for 20 min) were 19.85 and 11.67%, respectively. It was observed that the water solubility of both films decreased with the application of HHP in spite of the higher moisture content (Kim et al., 2018). The water solubility of the edible film developed in the present study was comparable to those cited in the literature (Kim et al., 2018).

Thermal properties of the edible film

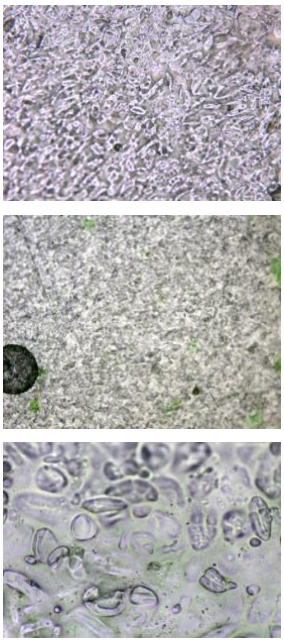
Thermogram results showed that the thermal transition of the edible film was at 86.77 °C for 7.19 min. No activity recovered from the samples after thermal transition temperature indicated that the phase transition was irreversible.

Thermal degradation behavior and stability of starch based materials is important property because it determines the behavior of the film during processing (Liu et al., 2013). Thermo-gravimetric analysis has been widely used to determine the thermal stability and decomposition of starch based film formulated using different sources (Aggarwal et al., 1996; Guinesi et al., 2006; Soares et al., 2005). Both dehydration and decomposition are taken into consideration with the degradation mechanisms of starch (Liu et al., 2009; Liu et al., 2013). These values give information to predict how these materials behave under more realistic atmospheric conditions (Acar et al., 2008; Peterson et al., 2001).

Thermal properties of the film change depending on the base materials and type of the plasticizers used. For example, sage seed gum (SSG) edible films with glycerol and sorbitol presented two exothermic peaks with T_g at about 59.7 and 277.7 °C, which the latter may be related to the thermal decomposition of SSG. T_g of SSG film shifted to 52.2 and 179.1 °C with the presence of glycerol, while sorbitol reduced it to 47.2 and 245.3 °C, respectively. In the glycerol plasticized sample, two additional peaks with T_g of about 286.4 and 340.8 °C were appeared (Razavi et al., 2015).

The microscopic observation of the film surfaces showed that the film was homogeneous and translucent. Even though film formation occurred due to the solubility of starch molecules, some starch molecules were still intact and not completely soluble (Figure 1a). Water-based green color was dissolved in the film and distributed in the continuous starch matrix. HHP processing caused swelling of starch molecules and led to the even distribution of green color (Figure 1b). Starch

molecules grew in size and were dissolved better after the HHP processing (Figure 1c).



a) Control film samples (4X magnification)

b) *O. onites* volatile oil added film samples (4X magnification)

c) *Origanum onites* volatile oil added and HHP processed (200MPa 1 min) film samples (4X magnification)

Figure 1. Microscopic observations of the film samples

Pressure application provides gelatinization of starch in two steps: At first stage, hydration of the amorphous parts of the starch granules occur, and then crystalline region starts to swell (Grossi et al. 2012; Simonin et al., 2011). Applied pressure level, model of pressure application (continuous or cycle), pressure time, processing temperature, as well as the constituent and phase state of the food affect the resistance of starch microstructure to pressure (Simonin et al., 2011). These structural changes after the HHP treatment helped to understand the changes in the textural properties of the film. Depending on the applied HHP processing and VOC concentrations, the texture of the film changed, thus affecting the force and elongation properties

It is reported that HHP caused a decrease in molecular order revealing higher amylopectin amorphous layer (ρ_a) (unlikely), or induced changes in both crystalline and amorphous regions leading to ρ_a decrease (Lopez-Rubio et al., 2017). Applied magnitude of pressure plays an important role in starch structure depending on the starch type. For example, pressure at the magnitude of 200 MPa does not cause a noticeable shifts or changes in the main peaks, but pressure application at 600 MPa for 30 min significantly reduced the peak intensity at 18 °. Dual peaks observed at 17° and 18° were merged into a single peak at 17°, characteristic of B-type starches after the application of 600 MPa at cycle mode. Due to their scattered and flexible amylopectin branching structure, A-type starches are generally believed to be more sensitive to pressure than B- and C-type ones (Deng et al., 2014).

Table 2. ANOVA and regression model results for two functional responses of biopolymer film with *Origanum onites* volatile oil.

	Force (g) (Y_1)		Elongation (mm) (Y_2)	
Term	Coeff	*p*	Coeff	*p*
Linear				
P (X_1)	55.20	0.000	1.45	0.000
VOC (X_2)				
T (X_3)				
Square				
P^2				
VOC^2	43.70	0.003	2.35	0.000
T^2	-79.50	0.000		
Interaction				
*P*VOC*	41.70	0.003	1.85	0.000
*P*T*	-26.50	0.049		
*VOC*T*				
Lack-of-fit		**0.249**		**0.616**
Intercept	442.7	0.000	10.076	0.000
R^2_{adj}	0.77		0.68	

Table 3. Summary of experimental versus predicted values for textural properties of biopolymer film according to BBD.

	Force (Y_1)	Elongation (Y_2)
Predicted values	366.87	12.83
Experimental values	368.06 ± 7.31	12.85 ± 0.08
Coefficient variation (*CV*, %)	0.16	0.07

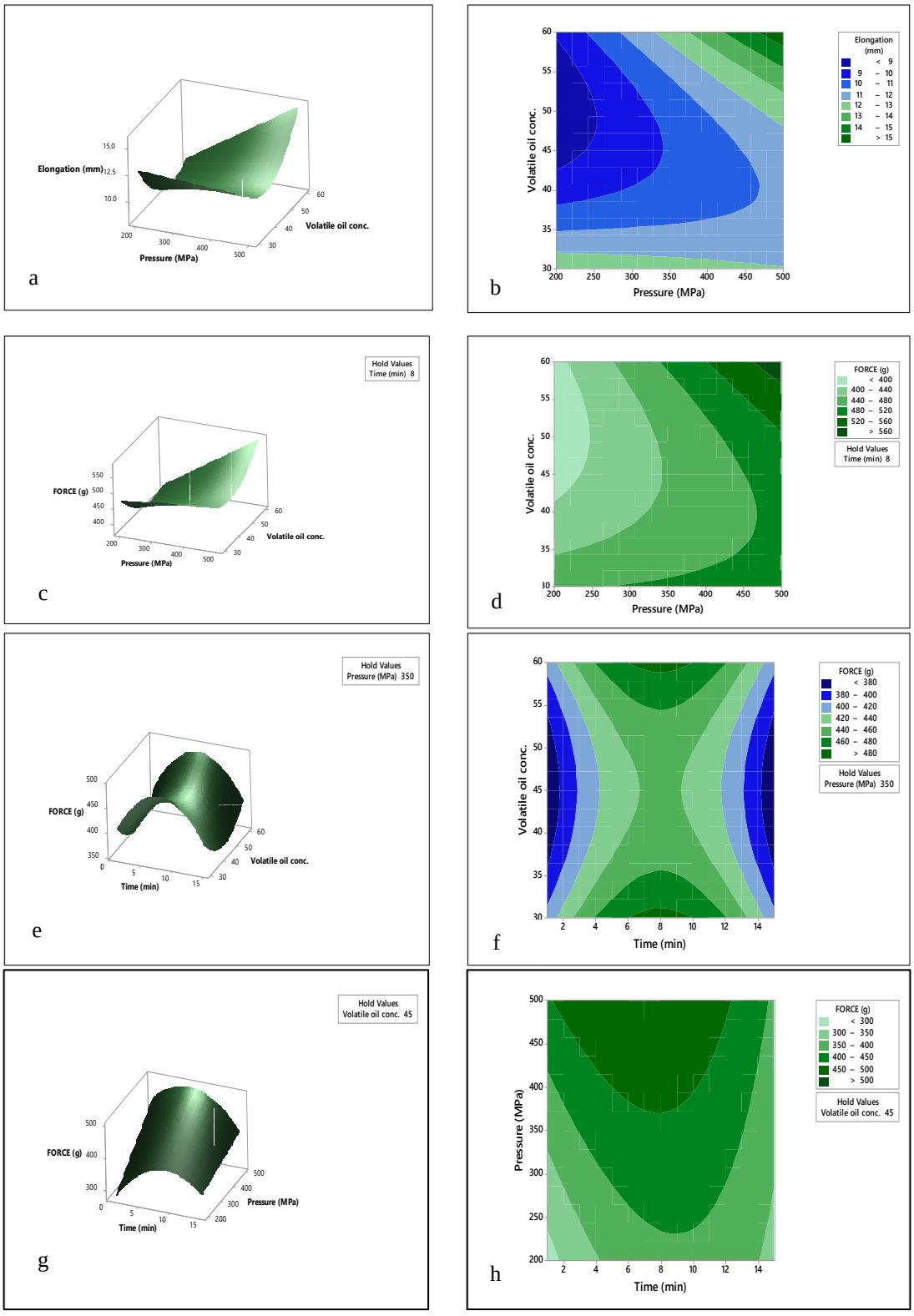


Figure 2. Interaction effects of pressure-volatile oil concentration with treatment time as constant value on elongation for textural properties of biodegradable film showing a) response surface and b) contour plots; interaction effects of pressure-volatile oil concentration on force for textural properties of biodegradable film showing c) response surface and d) contour plots; interaction effects of time-volatile oil concentration on force for textural properties of biodegradable film showing e) response surface and f) contour plots; interaction effects of pressure-time on force for textural properties of biodegradable film showing g) response surface and h) contour plots

Modelling of the textural properties of the edible film

Among the three explanatory variables, only HHP significantly affected ($p \leq 0.05$) both force and EAB of the edible film. According to the best-fit regression models, there existed a positive correlation between pressure and VOC ($p \leq 0.001$) for both textural properties (Table 2).

The significant quadratic terms were found for time ($p = 0.000$) and VOC ($p = 0.003$) with a positive effect on force and VOC ($p = 0.000$) for EAB of the film with a negative effect (Table 2). There was a significant interaction between VOC and pressure ($p \leq 0.05$) with a positive effect, and between time and pressure ($p \leq 0.05$) with a negative effect on force. A significant interaction between pressure and VOC ($p \leq 0.05$) with a positive effect on EAB of the film was also observed (Table 2). The degree of influence of the operational conditions on the force and elongation responses can be inferred from comparing the magnitudes of the coefficients of the quadratic regression models. HHP was the most important factor for both force (55.20) and EAB (1.45) (Table 2). The goodness-of-fit (R^2_{adj}) of the models showed 0.77 and 0.68 % of variations in force and EAB, respectively (Table 2). The insignificant lack-of-fit values for the two models also indicated that the model fitted the experimental data well (Table 2).

According to ANOVA results, the insignificant terms were excluded from the models of the textural properties of the film. The best-fit regression models explained 81 and 71% of variations in force and EAB, respectively. The EAB estimates of the film as a function of HHP, time and VOC are illustrated in Figure 2a. EAB increased with the increased VOC under the highest pressure at an increasing rate (Figure 2a). The highest VOC maximized EAB at the highest HHP of 500 MPa (Figure 2a). Force decreased with the increased VOC under the lowest pressure at a decreasing rate (Figure 2c). The lowest VOC values minimized force at the constant pressure of 350 MPa (Figure 2e). The lowest time minimized force at the lowest pressure (200 MPa) (Figure 2g). Three-dimensional (3D) response contour plots were used to observe the interaction effect of variables in pairs on both EAB and force (Figures 2a, 2c, 2e, 2g).

While circular contour plots indicate a negligible interaction, elliptical or saddle contour plots indicate a significant interaction between the corresponding variables (Murthy et al., 2000). In both figure 2b and 2d, the contour plot showed a significant interaction between VOC and HHP. In figures 2f and 2h, the circular shape of the contour plots indicated a negative time-by-HHP and a negligible time-by-VOC interaction for the force of the film.

Joint optimization and model validations

The overlaid contour plots were used to visualize how the operational settings simultaneously influenced the force and EAB of the film (Figure 3). The solid contour line is the lower bound and the dotted contour is the upper bound (Figure 3). The contours of each response are displayed in a different color. The white area of the plots (feasible region) shows the range of pressure, VOC, and time where the criteria for three response variables are satisfied whereas the shaded area shows regions do not fit the optimization conditions (Figure 3). Figure 3a shows that VOC at 45µL, the HHP range of 200 to 400 MPa with 350 MPa of optimum pressure and 1.7 min for processing time were the optimum setting. With 8 min, both HHP and VOC were maintained in a very narrow range as shown in figure 3b. The two feasible zones (Figure 3c) were obtained with the optimum settings of both time and VOC.

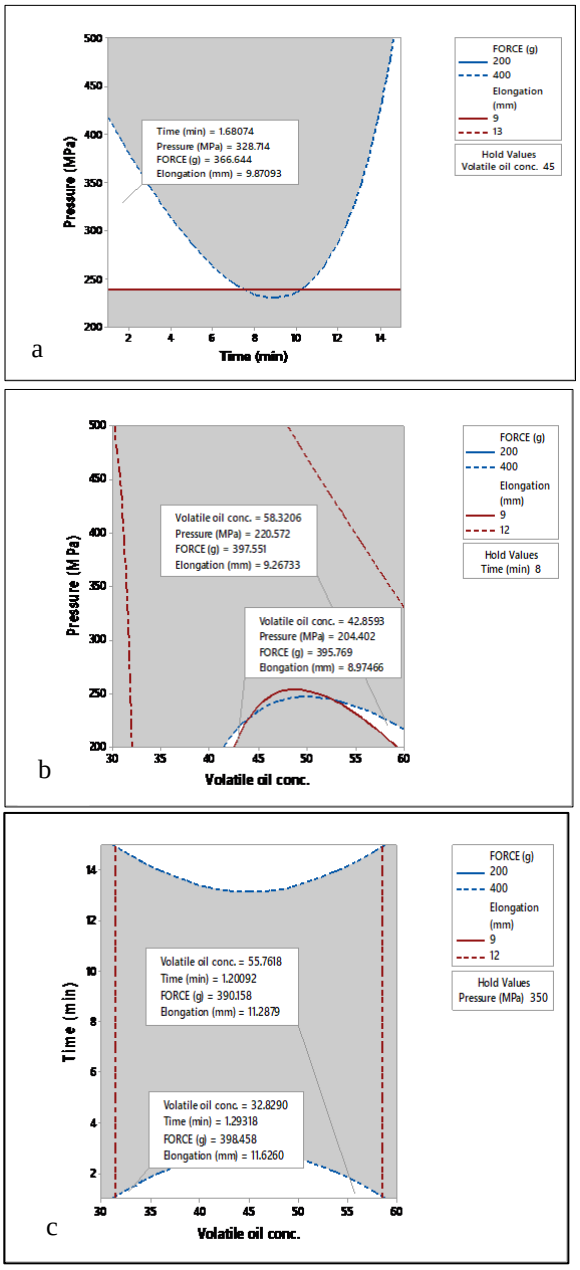


Figure 3. Overlaid contour plots showing feasible operational regions to maintain textural properties of biodegradable film under the constant values of (a) VOC of 45µL, (b) time of 8 min and (c) HHP pressure of 350 MPa

The operational settings for force and EAB of the film production revealed the best solution for the multiple response optimization with 350 MPa pressure, 45 μL VOC, and 8 min operational time. The minimum force (366.87 g) and maximum EAB (12.83 mm) values were obtained with the optimum operational conditions. These conditions were experimentally tested to validate the predictive power of the models. The resultant force and EAB values of 368.06 ± 7.3 g and 12.85 ± 0.08 mm, respectively, indicated no significant difference between the measured and predicted values (Table 3). The smaller CV values showed the better reproducibility of the model.

Conclusions

Although different food industry wastes were used to produce edible films, the production of an edible film from the effluents of potato industry carrying antimicrobial properties with addition of *O. onites*, measurement of its physical properties, and determination of the changes in mechanical properties under HHP are limited. The edible film produced from the effluents of a potato industry was HHP-processed (up to 500 MPa) with the addition of volatile *O. onites* oil. The combination of *O. onites* and HHP-processing provided antimicrobial activity against both *E. coli* O157:H7 and *S.* Enteritis with 2.386 ± 0.07 and 1.7 ± 0.109 cm of inhibition zones and 4.95 ± 0.341 and 2.153 ± 0.383 cm^2 of the calculated inhibition zone areas, respectively. HHP significantly affected both force and EAB of the edible film with a positive correlation between pressure and VOC for both textural properties. The optimum operational conditions obtained for the multiple response optimization were achieved with 350 MPa pressure, 45 μL VOC, and 8 min operational time.

This study focused on the production of the edible film from the effluents of potato industry, and its textural properties and antimicrobial activity. Its flexible and durable structure showed that this film may have potential to be applied as a food packaging, but food-safety, barrier properties, and migration test must be performed in order to evaluate its potential as food packaging. Thus, future studies remain to be conducted to determine the effectiveness of the film on the shelf life extension of different foods.

Compliance with ethical standards

Conflict of interest

The authors have no conflict of interest to declare

Author contribution

Gulsun Akdemir Evrendilek: Supervision, conceptualization, methodology, writing manuscript & editing, data analyses, project administration, funding acquisition; Nurullah Bulut: Data curation, experimental; Sibel Uzuner: Writing manuscript & editing, data analyses, editing.

All the authors read and approved the final manuscript. Text, figures, and tables are approved by all thr authors and they have not been published before.

Ethical approval

Not applicable.

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Data availability

Data will be available upon request.

Consent for publication

Not applicable

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References

Acar, I., Pozan, G.S., Ozgumus, S. (2008). Thermal oxidative degradation kinetics and thermal properties of poly (ethylene terephthalate) modified with poly (lactic acid). Journal of Applied Polymer Science, 109: 2747–2755. Doi: https://doi.org/10.1002/app.28142

Aggarwal, P., Dollimore, D. (1996). A comparative study of the degradation of different starches using thermal analysis. Talanta, 43: 1527–1530. Doi: https://doi.org/10.1016/00399140(96)01930-3

Andrade-Ochoa, S., Nevárez-Moorillón, G.V., Sánchez-Torres, L.E., Villanueva-García, M., Sánchez-Ramírez, B.E., Rodríguez-Valdez, L.M., Rivera-Chavira, B.E. (2015). Quantitative structure-activity relationship of molecules constituent of different essential oils with antimycobacterial activity against *Mycobacterium tuberculosis* and *Mycobacterium bovis*. BMC Complementary Medicine and Therapies, 15(1): 332-343. Doi: 10.1186/s12906-015-0858-2.

Anonymous. (2019). https://www.potatopro.com/world/potato-statistics. (Accessed on 13.03.2019)

Azcan, N., Kara, M., Asilbekova, D.T., Ozek, T., Baser, K.H.C. (2000). Lipids and essential oil of *Origanum onites*. Chemistry of Natural Compounds, 36(2):132-136.

Barzegar, H., Azizi, M.H., Barzegar, M., Hamidi-Esfahani, Z. (2014). Effect of potassium sorbate on antimicrobial and physical properties of starch-clay nanocomposite films. Carbohydrate Polymer, 110: 26-31. Doi: https://doi.org/10.1016/j.carbpol.2014.03.092

Borah, P.R., Das, P., Badwaik, L.S. (2017). Ultrasound treated potato peel and sweet lime pomace based biopolymer film development. Ultrasonic Sonochemistry, 36: 11-19. Doi: https://doi.org/10.1016/j.ultsonch.2016.11.010

Bouyahya, A., Dakka, N., Talbaoui, A., Et-Touys, A., El-Boury, H., Abrini, J., Bakri, Y. (2017). Correlation between phenological changes, chemical composition and biological activities of the essential oil from Moroccan endemic Oregano (*Origanum compactum* Benth). Indian Crop Production, 108 : 729–737. Doi: https://doi.org/10.1016/j.indcrop.2017.07.03

Burt, S. (2004). Essential oils: Their antibacterial properties and potential applications in foods-a review. International Journal of Food Microbiology, 94(3): 223-253. Doi: https://doi.org/10.1016/j.ijfoodmicro.2004.03.022

Castillo, L.A., Farenzena, S., Pintos, E., Rodríguez, M.S., Villar, M.A., García, M.A., López, O.V. (2017). Active films based on thermoplastic corn starch and chitosan oligomer for food packaging applications. Food Packaging and Shelf Life, 14: 128-136. Doi: https://doi.org/10.1016/j.fpsl.2017.10.004

Chaichi, M., Hashemi, M., Badii, F., Mohammadi, A. (2017). Preparation and characterization of a novel bionano composite edible film based on pectin and crystalline nanocellulose. Carbohydrate Polymer, 157, 167-175. Doi: https://doi.org/10.1016/j.carbpol.2016.09.062

Chauhan, A.K., Kang, S.C. (2014). Thymol disrupts the membrane integrity of *Salmonella* ser. Typhimurium in vitro and recovers infected macrophages from oxidative stress in an ex vivo model. Research in Microbiology, 165(7): 559–565. Doi: https://doi.org/10.1016/j.resmic.2014.07.001

Condés, M.C., Añón, M.C., Mauri, A.N. (2015). Amaranth protein films prepared with high-pressure treated proteins. Journal of Food Engineering, 166: 38-44. Doi: https://doi.org/10.1016/j.foodhyd.2015.01.026

Dadalioğlu, 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 Agriculture and Food Chemistry, 52: 8255-8260. Doi: https://dio.org/10.1021/jf049033e

De Kruijf, N., van Beest, M., Rijk, R., Sipilainen-Malm, T., Paseiro Losada, P., De Meulenaer, B. (2002). Active and intelligent packaging: applications and regulatory aspects. Food Additives and Contaminants, 19: 144–62. Doi: https://doi.org/10.1080/02652030110072722

Deng, Y., Jin, Y., Luo, Y., Zhong, Y., Yue, J., Song, X., Zhao, Y. (2014). Impact of continuous or cycle high hydrostatic pressure on the ultrastructure and digestibility of rice starch granules. Journal of Cereal Science, 60: 302-310. Doi: https://doi.org/10.1016/j.jcs.2014.06.005

Ehivet, F.E., Min, B., Park, M.K., Oh, J.H. (2011). Characterization and antimicrobial activity of sweet potato starch-based edible film containing origanum (*Thymus capitatus*) oil. Journal of Food Science, 76(1): 178-184. Doi: https://doi.org/10.1111/j.1750-3841.2010.01961.x

Farkas, D.F., Hoover, D.G. (2000). High pressure processing. Journal of Food Science, 65: 47-64. Doi: https://doi.org/10.1111/j.1750-3841.2000.tb00618.x

Feng, M., Yu, L., Zhu, P., Zhou, X., Liu, H., Yang, Y., Zhou, C., Gao, C., Bao, X., Chen, P. (2018). Development and preparation of active starch films carrying tea polyphenol. Carbohydrate Polymers, 196: 162-167. Doi: https://doi.org/10.1016/j.carbpol.2018.05.043

Flores, S., Haedo, A.S., Campos, C., Gerschenson, L. (2007). Antimicrobial performance of potassium sorbate supported in tapioca starch edible films. European Food Research Technology, 225(3-4): 375–384. Doi: https://doi.org/10.1007/s00217-006-0427-5

Gontard, N., Guilbert, S., Cuq, J.-L. (1992). Edible wheat gluten films: Influence of the main process variables on film properties using response surface methodology. Journal of Food Science, 57: 190-195,199. Doi: https://doi.org/10.1111/j.1365-2621.1992.tb05453.x

Grossi, A., Søltoft-Jensen, J., Knudsen, J.C., Christensen, M., Orlien, V. (2012). Reduction of salt in pork sausages by the addition of carrot fibre or potato starch and high pressure treatment. Meat Science, 92: 481-489. Doi: https://doi.org/10.1016/j.meatsci.2012.05.015

Guinesi, L.S., da Roz. A.L., Corradini, E., Mattoso, L.H.C., Teixeira, E.D.M., Curvelo, A.A.D.S. (2006). Kinetics of thermal degradation applied to starches from different botanical origins by non-isothermal procedures. Thermochimica Acta 447: 190–196. Doi: https://doi.org/10.1016/j.tca.2006.06.002

Kim, S., Yang, S.Y., Chun, H.H., Song, K.B. (2018). High hydrostatic pressure processing for the preparation of buckwheat and tapioca starch films. Food Hydrocolloids, 81: 71-76. Doi: https://doi.org/10.1016/j.foodhyd.2018.02.039

Knowles, J.R., Roller, S., Murray, D.B., Naidu, A.S. (2005). Antimicrobial action of carvacrol at different stages of dual-species biofilm development by *Staphylococcus aureus* and *Salmonella* Enterica serovar Typhimurium. Applied and Environmental Microbiology, 71(2) : 797–803. Doi: https://doi.org /10.1128/AEM.71.2.797-803.2005

Kot, A.M., Pobiega, K., Piwowarek, K., Kieliszek, M., Błażejak, S., Gniewosz, M., Lipińska, E. (2020). Biotechnological methods of management and utilization of potato industry waste-a Review. Potato Research, 1-17. Doi: https://doi.org/10.1007/s11540-019-09449-6

Lambert, R.J.W., Skandamis, P.N., Coote, P.J., Nychas, G.-J.E. (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. Doi: https://doi.org/10.1046/j.1365-2672.2001.01428.x

Li, G.-X., Liu, Z.-Q. (2009). Unusual antioxidant behavior of α- and γ-terpinene in protecting methyl linoleate, DNA, and erythrocyte. Journal of Agriculture and Food Chemistry, 57(9): 3943–3948. Doi: https://doi.org/10.1021/jf803358g

Liu, H., Xie, F., Yu, L., Chen, L., Li, L. (2009). Thermal processing of starch-based polymers. Progress in Polymer Science, 34: 1348-1368. Doi: https://doi.org/10.1016/j.progpolymsci.2009.07.001

Liu, X., Wang, Y., Yu, L., Tong, Z., Chen, L., Liu, H., Li, X. (2013). Thermal degradation and stability of starch under different processing conditions. Starch-Starke, 65: 48-60. Doi: https://doi.org/10.1002/star.201200198

Lopez-Rubio, A., Fabra, M.J., Martinez-Sanz, M., Mendoza, S., Vuong, Q.V. (2017). Biopolymer-based coatings and packaging structures for improved food quality. Journal of Food Quality, 7: 1-3. Doi: https://doi.org/10.1155/2017/2351832

Mahomoodally, M.F., Zengin, G., Aladag, M.O., Ozparlak, H., Diuzheva, A., Jekő, J., Cziáky, Z., Aumeeruddy, M.Z. (2019). HPLC-MS/MS chemical characterization and biological properties of *Origanum onites* extracts: a recent insight. International Journal of Environmental Health Resources, 29(6): 607-621. Doi: https://doi.org/10.1080/09603123.2018.1558184

Min, B.J., Oh, J.-H. (2009). Antimicrobial activity of catfish gelatin coating containing origanum (*Thymus capitatus*) oil against gram-negative pathogenic bacteria. Journal of Food Science, 74: 143–148. Doi: https://doi.org/10.1111/j.1750-3841.2009.01115.x

Moreno, O., Cárdenas, J., Atarés, L., Chiralt, A. (2017). Influence of starch oxidation on the functionality of starch-gelatin based active films. Carbohydrate Polymers, 178: 147-158. Doi: https://doi.org/10.1016/j.carbpol.2017.08.128

Murthy, M.S.R.C., Swaminathan, T., Rakshit, S.K., Kosugi, Y. (2000). Statistical optimization of lipase catalyzed hydrolysis of methyl oleate by response surface methodology. Bioprocessing Engineering, 22: 35-39. https://doi.org/10.1007/PL00009097

Nandane, A.S., Jain, R.K. (2018). Optimization of formulation and process parameters for soy protein-based edible film using response surface methodology. Journal of Packaging Technology Research, 2(3): 203-210. Doi: https://doi.org/10.1007/s41783-018-0045-2

Othman, S.H., Edwal, S.A.M., Risyon, N.P., Basha, R.K., Talib, R.A. (2017). Water sorption and water permeability properties of edible film made from potato peel waste. Food Science and Technology, 37: 63-70. Doi: http://dx.doi.org/10.1590/1678-457x.30216

Ozdemir, M., Floros, J.D. (2008). Optimization of edible whey protein films containing preservatives for water vapor permeability, water solubility and sensory characteristics. Journal of Food Engineering, 86(2): 215-224. Doi: https://doi.org/10.1016/j.jfoodeng.2007.09.028

Park, S.B., Lih, E., Park, K.S., Joung, Y.K., Han, D.K. (2017). Biopolymer-based functional composites for medical applications. Progress in Polymer Science, 68: 77-105. Doi: https://doi.org/10.1016/j.progpolymsci.2016.12.003

Pathak, P.D., Mandavgane, S.A., Puranik, N.M., Jambhulkar, S.J., Kulkarni, B.D. (2018). Valorization of potato peel: a biorefinery approach. Critical Reviews in Biotechnology, 38(2): 218-230. Doi: https://doi.org/10.1080/07388551.2017.1331337

Peterson, J.D., Vyazovkin, S., Wight, C.A. (2001). Kinetics of the thermal and thermo-oxidative degradation of polystyrene: polyethylene and poly(propylene). Macromolecular Chemistry and Physics, 202: 775-784. Doi: https://doi.org/10.1002/1521-3935(20010301)202:6<775::AID-MACP775>3.0.CO;2-G

Quintavalla, S., Vicini, L. (2002). Antimicrobial food packaging in meat industry. Meat Science, 62: 373–80. Doi: https://doi.org/10.1016/S0309-1740(02)00121-3

Razavi, S.M.A., Amini, A.M., Zahedi, A.Y. (2015). Characterisation of a new biodegradable edible film based on sage seed gum: Influence of plasticiser type and concentration. Food Hydrocolloids, 43: 290-298. Doi: https://doi.org/10.1016/j.foodhyd.2014.05.028

Rice, E.W., Baird, R.B., Eaton, A.D. (2005). Standard methods for the examination of water and wastewater, 23rd ed. American Public Health Association, American Water Works Association, Water Environment Federation.

Salleh, E., Muhammad, II., Pahlawi, Q.A. (2014). Spectrum activity and lauric acid release behaviour of antimicrobial starch-based film. Procedia Chemistry, 9: 11-22. Doi: https://doi.org/10.1016/j.proche.2014.05.003

Sevindik, E., Aydin, S., Kurtoglu, C., Tin, B. (2019). Evaluation of essential oil composition of *Origanum onites* L. (lamiaceae) plant and antifungal activity on some strong pathogen fungi. Afs-Advances in Food Science, 2019, 32-35

Seydim, A.C., 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. Doi: https://doi.org/10.1016/j.foodres.2006.01.013

Simonin, H., Guyon, C., Orlowska, M., de Lamballerie, M., Le-Bail, A. (2011). Gelatinization of waxy starches under high pressure as influenced by pH and osmolarity: Gelatinization kinetics, final structure and pasting properties. LWT- Food Science and Technology, 44: 779–786. Doi: https://doi.org/10.1016/j.lwt.2010.07.002

Soares, R.M.D., Lima, A.M.F., Oliveira, R.V.B., Pires, A.T.N., Soldi, V. (2005). Thermal degradation of biodegradable edible films based on xanthan and starches from different sources. Polymer Degradation and Stability, 90: 449–454. Doi: https://doi.org/10.1016/j.polymdegradstab.2005.04.007

Torres, J.A., Velazquez, G. (2005). Commercial opportunities and research challenges in the high pressure processing of foods. Journal of Food Engineering, 67(1-2): 95-112. Doi: https://doi.org/10.1016/j.jfoodeng.2004.05.066

Valero, M., Francés, E. (2006). Synergistic bactericidal effect of carvacrol, cinnamaldehyde or thymol and refrigeration to inhibit *Bacillus cereus* in carrot broth. Food Microbiology, 23(1): 68-73. Doi: https://doi.org/10.1016/j.fm.2005.01.016

Xu, J., Zhou, F., Ji, B.-P., Pei, R.-S., Xu, N. (2008). The antibacterial mechanism of carvacrol and thymol against *Escherichia coli*. Letters in Applied Microbiology, 47(3): 174-179. Doi: https://doi.org/10.1111/j.1472-765X.2008.02407.x