1	Alginate Based Aloe Vera Gel And/Or Pomegranate Seed
2	Essential Oil Added Edible Coating Film Production Using
3	Casting Method To Extend Strawberries and Cherries Shelf Life
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#### ABSTRACT

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27 Many types of material used for coating food to extend shelf life and that may be eaten together with 28 the coated food. Today, environmentally friendly technologies, like edible films produced from 29 biodegradable materials, are preferred in food coating. In this study alginate-based (3g) aloe vera 30 gel (1.5g) and/or pomegranate seed essential oil (1%) added plasticized with glycerol (2g) edible 31 films were produced by casting method for use in food coating at 5 different composition. Aloe 32 vera gel and pomegranate seed essential oil (3%) added edible composite films have the lowest 33 (72.56%) water solubility value. Pomegranate seed essential oil added edible films have lower water 34 vapor transmission rate (0,0897\*10-3), and lower moisture content (19.95%) values. 1% 35 pomegranate seed essential oil added film has the lowest film thickness (0.125 mm). FTIR spectra 36 of the films confirmed the interactions between alginate, aloe vera gel and pomegranate seed 37 essential oil thus proving that the films were prepared successfully. Strawberries and cherries were 38 coated with produced edible coating films and weight loss and color analysis were carried out to 39 observe coating performance. It was seen that alginate based and pomegranate seed essential oil 40 added edible coatings reducing moisture loss, color change and surface deformation of strawberries 41 and cherries. The biggest difference of this study from previous studies is that pomegranate seed 42 essential oil was used for the first time in this study, for edible coating film production and the results 43 were quite successful especially for the cherry's coating.

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<sup>45</sup> **KEYWORDS:** Edible film, sodium alginate, aloe vera gel, pomegranate seed oil

#### 1. INTRODUCTION

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53 Utilisation of environmentally friendly polymer-based edible films (like 54 polysaccharides, lipids, and proteins) that extend shelf life of foods has been increased day by day. Edible films protect firmness, limit microbial growth, resist 55 56 the weight loss of the foods and move as a carrier for many active components like antimicrobials and antioxidants to extend the efficiency in the preservation of food. 57 58 Polysaccharide and lipid based edible films are chemically stable and have good 59 physical, mechanical, and gas barrier properties. Consequently, biopolymer based edible films have appeared as a food coating alternative material <sup>[1-3]</sup>. Alginates, 60 61 widely used in medicine and food industries, due to its characteristics such as 62 thickening, stabilizing, suspending, film forming, gel production, emulsion stabilization, non-flammability, biodegradability, and biocompatibility are suitable 63 for use in edible film production. They are produced through the alkali treatment of 64 alginic acid which is extracted from Phaeophyceae plant <sup>[4-6]</sup>. Numerous edible 65 films originated from alginate can't shield the UV light which has many negative 66 effects on food. Aloe vera gel, extracted from the aloe vera plant, is a promising 67 additive for the improvement of the UV-protecting and antimicrobial properties of 68 edible coatings <sup>[7,8]</sup>. Essential oils obtained by aromatic plants contain functional 69 70 bioactive compounds providing strong antioxidant and antimicrobial properties. 71 They are effective natural agents against microorganisms, eco friendly and 72 biodegradable. The addition of essential oils to edible films help in the inhibition of 73 bacterial and fungal growth. Therefore, essential oils can move as natural

preservatives for perishable foods as well as fillers to produce functional coating 74 materials <sup>[9,10]</sup>. Pomegranate (Punica granatum L.) is one of the most widely 75 76 cultivated and processed fruits. Consumption of pomegranate products has 77 increased recently due to their high antioxidant and health-promoting properties. Pomegranate peel and its extract are a rich source of phenolics and flavonoids, 78 79 whith antimicrobial and antioxidant properties. Pomegranate seed oil has many applications from food production to preservation. The nutritional value of 80 81 pomegranate, which is the best natural source of punicic acid and punicalagin, is 82 excellent. Many advantages of food coating materials enriched with pomegranate 83 seed oil, in addition to their antimicrobial activities, reveal their role in food preservation <sup>[11-13]</sup>. Plasticizers are also incorporated within alginates films to 84 improve their mechanical properties. Among plasticizers, glycerol and sorbitol are 85 the most extensively employed plasticizer in alginate films <sup>[14,15]</sup>. 86

87 The aim of this study is to investigate the possibility of producing a new alginate based, aloe vera gel and/or pomegranate seed essential oil added, biodegradable 88 89 edible coating film with casting method. To determine and compare the aloe vera gel and pomegranate seed essential oil effects, they were added to the coating 90 91 solution at 5 different ratios. The most important difference of this study from 92 previous studies is the development of aloe vera gel and pomegranate seed oil incorporated alginate-based edible films for the first time, and its application as a 93 coating for the preservation of strawberry and cherrie fruits. 94

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97	2. MATERIAL AND METHODS
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99	2.1. Materials
100	Sodium alginate used in this study was purchased from AFG Bioscience, Glycerol
101	was from Merck (Germany) and D-sorbitol from Carlo Erba (France). Aloe vera
102	leaves, strawberries and cherries were purchased from local markets and PSEO
103	from Arifoğlu in Istanbul, Turkey.
104	
105	2.2. Aloe vera gel extraction
106	Aloe vera gel (AVG) was obtained according to the previos studies with some
107	modification. Fresh aloe vera leaves were washed with tap water and then by pure
108	water to eliminate surface impurities. Then, they were peeled with a knife to remove
109	the outer green layer. The obtained AVG was blended and mixed for 20 min with
110	ultrasonic probe, to form a uniform solution. Then, it was filtered to remove
111	impurities arising from cell walls. Finally, AVG was pasteurized at 70 °C for 45
112	min and was cooled to a room temperature (22+0.5 °C). The prepared gel extract
113	was stored at a refrigerator <sup>[16,17]</sup> .
114	
115	2.3. Alginate/ aloe vera gel/ pomegranate seed essential oil film production.
116	3 a sodium alginate and 2g glycerol were dissolved in 100 mL distilled water and

116 3 g sodium alginate and 2g glycerol were dissolved in 100 mL distilled water and 117 the obtained solution (C<sub>1</sub>) was stirred in a magnetic stirrer at 500 rpm for 60 min., 118 at 60 °C. After that, AVG (0-1.5g) and/or PSEO (1%-3%) were added to the 119 obtained solution at different ratios according to the compositions listed in Table 1

120	(C <sub>2</sub> , C <sub>3</sub> , C <sub>4</sub> and C <sub>5</sub> ), to determine AVG and PSEO effects. The alginate, glycerol,
121	AVG and PSEO ratios were determined by preliminary tests. In order to obtain
122	more homogeneous film solutions, they were first mixed with ultrasonic probe for
123	10 min. Next, they were placed in an ultrasonic water bath for 10 min., at 50 °C,
124	until the mixtures were completely solubilized. Biodegradable edible films were
125	prepared by using solution-casting method. The film solutions were cast in petri
126	dishes with a diameter of 10 cm followed by drying at 40 $^{0}$ C in an oven for 24 h.
127	Finally, the films were peeled off and conditioned in a desiccator, at 50% RH and
128	room temperature (22+0.5 °C) until analyzed, after cooling $^{[7, 18]}$ .

**Table 1** Formulation of alginate/ aloe vera gel/ pomegranate seed essential oil
 edible films.

Formulation	Alginate (g)	Glycerol (g)	AVG (g)	PSEO (v/W%)
C1	3	2	-	-
C <sub>2</sub>	3	2	1.5	-
Сз	3	2	-	1
<b>C</b> 4	3	2	-	3
C5	3	2	1.5	1
C <sub>6</sub>	3	2	1.5	3



Fig. 1. Alginate/ aloe vera gel/ pomegranate seed essential oil edible films

#### 139 **2.4. Characterization of the films**

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### 141 2.4.1 Fourier transform infrared spectroscopy

The chemical structure of the films was analyzed by FTIR using a PerkinElmer Spectrum One FTIR spectrometer (Waltham, MA, USA) equipped with a universal attenuation total reflectance sampling accessory with a spectral range between 4000 and 400 cm<sup>-1</sup> with a resolution of 4 cm<sup>-1.</sup> The films were cut to  $10 \times 10$  mm and placed onto the ATR platform.

#### 147 2.4.2 Water solubility

148 The water solubility of the films was determined as described by Girgin and Tugrul <sup>[16]</sup> and Oliveira Filho et al. <sup>[19]</sup> with some modifications. The film samples were 149 dried in a hot air oven at  $105 \pm 2$  <sup>0</sup>C for 24 h. Dried films were cut to 2×2 cm and 150 151 then they were weighed. The samples were then immersed in 10 mL of distilled water and mixed at 100 rpm at room temperature (22+0.5 °C) for 6 h. The 152 unsolvable part of the film samples was filtered, dried at  $105 \pm 2$  <sup>0</sup>C for 24 h. and 153 weighed. All the experiments were carried out in triplicate. Water solubility 154 calculated using equation (1): 155

156 Water solubility (%) = 
$$\frac{W_1 - W_2}{W_1} \times 100$$
 (1)

157  $W_1 = dry$  weight and  $W_2 = dry$  weight of the insoluble fraction of the film.

#### 158 2.4.3 Water vapor transmission rate (WVTR)

The WVTR (g/sm<sup>2</sup>) of the films was determined by the drying method, according to the procedure described by Li et al. <sup>[20]</sup> and Shafie et al. <sup>[21]</sup> with some modifications. The film samples were dried in a hot air oven at  $105 \pm 2$  <sup>0</sup>C for 24 h

until they reach a constant weight. Then the glass test tubes were filled with 5 g of
silica gel, the mouth of the tubes (diameter of 13 mm) was closed with the film
sample dried in the oven and surrounded with paraffin (for the tube to be completely
closed). The prepared samples were weighed every 24 h (at the same time every
day) for 5 days. All the experiments were carried out in triplicate. The WVTR was
calculated according to the formula below:

168 WVTR = 
$$\Delta W / (\Delta t x A)$$
 (2)

169  $\Delta W/\Delta t$  = the amount of water transferred (g) per unit time (s), and A = the exposed 170 area (m<sup>2</sup>).

#### 171 2.4.4 Moisture content

The films moisture content was determined as described by Costa et al. <sup>[22]</sup> and Mutlu <sup>[23]</sup> with some modifications. Edible films (2 cm × 2 cm) were cut and weighed, then dried at  $105 \pm 1$  <sup>0</sup>C during 24 h until the equilibrium weight was reached. The sample weight loss was determined, and moisture content was calculated as the percentage of water removed from the system using equation (3).

177 Moisture (%) = 
$$\frac{(M_0 - M_1)}{M_0} \times 100$$
 (3)

178  $M_0 = initial weight, M_1 = dry weight of the films$ 

180 The thicknesses values of the films (with 0,01 mm. sensitivity) were measured from

- 181 five different points using Mitutoyo micrometer (Tokyo, Japan). The measurements
- 182 were taken from five different point and the average value was recorded <sup>[24]</sup>.

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#### 185 2.4.6 Color

186 The color values of the samples were assessed using a handheld colorimeter. Hunter 187 scale values for the positive a\* values represent redness, the negative ones represent 188 greenness, the positive b\* values represent yellowness, and the negative ones 189 represents blueness. All the experiments were carried out in triplicate. Total color 190 changes ( $\Delta E$ ) were calculated from the following equation:

191 
$$\Delta \mathbf{E} = \sqrt{(\mathbf{L}_0 - \mathbf{L})^2 + (\mathbf{a}_0 - \mathbf{a})^2 + (\mathbf{b}_0 - \mathbf{b})^2}$$

192 (4)

where  $L_0$ ,  $a_0$  and b0 are the color values of the control films and L, a and b are the color values of the produced films.

#### 195 2.4.7 Application of edible coating films on strawberries and cherries

196 Strawberries and cherries of homogeneous size and colour were selected, they were washed using a 1% sodium hypochlorite solution for 1 minute and dried at room 197 198 temperature (22+0.5 °C). All the fruits were dipped to 3 different coating solutions 199 for 1 min: alginate based film solution, alginate and 1% PSEO based film solution, 200 and alginate and 3% PSEO based film solution. Their weight was measured first 201 and eighth days drying. The weight loss rate was calculated based on the ratio of 202 the reduction in the weight of fruit the to the initial weight. Photographs of the coated strawberries and cherries were taken on the first and eighth days to observe 203 the spoilage <sup>[25-28]</sup>. 204

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### 210 **3.1 FTIR spectroscopy**







220 **Table 2** Physical properties of starch-based control films.

Formulatio n	Solubility (%)	WVTR (g / (s.m <sup>2</sup> )	Moisture (%)	Thickness (mm)
C1	41.31	0,8793*10 <sup>-3</sup>	27.93	0.090
C <sub>2</sub>	87.57	0,4914*10-3	22.56	0.130
С3	86.22	0,1091*10-3	28.05	0.080
<b>C</b> 4	83.36	0,0897*10 <sup>-3</sup>	19.95	0.140
C5	84.95	0,1267*10-3	29.40	0.125
C6	72.56	0,1207*10 <sup>-3</sup>	22.90	0.178

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As exhibited in Fig. 2, Fig. 3 and Fig. 4, strong peak observed at around  $3300 \text{ cm}^{-1}$ 222 showed the typical bands of alginate; -OH stretching vibrations which confirms the 223 224 presence polysaccharides, phenols and carboxylic acids present in AV and polyphenols in the PSEO. The band observed at around  $1700 \text{ cm}^{-1}$  represented the 225 stretching of C=O and N-H groups of PSEO. The presence of carbonyl C = C group 226 was confirmed through the presence of a strong band at about  $1600 \text{ cm}^{-1}$ . The peak 227 at about 1400 cm<sup>-1</sup> was related to CH<sub>3</sub> bending vibration, indicating the presence 228 of COOH groups. The relevant vibration peaks around 1000 cm<sup>-1</sup> were due to the 229 presence of O-C-O group in polysaccharides, and the presence of C-C, C-O and C-230

- 231 N bounds in complex polysaccharides. FTIR results explain the changes in physical
- properties of the PSEO added films <sup>[2, 7, 29, 30]</sup>
- 233

#### 234 **3.2 Water solubility**

When the water solubility values of the alginate based edible films given at Table 235 236 2 were examined, it was seen that the solubility increases with the addition of AVG 237 from 41.31% to 87.57%. The solubility value was increased from 41.31% to 238 86.22% when 1% PSEO was added and it was increased from 41.31% to 83.36% 239 when 3% PSEO was added. Water solubility decreased when the PSEO 240 concentration increased. This may be caused by the hydrophobic nature of the 241 PSEO and high water solubility of the AVG components (sugars, organic acids, 242 amino acids) which could be solubilized from the edible films increasing water solubility. Similar results have been obtained by Nazir and Wani<sup>[31]</sup> and Pinzon et 243 al. <sup>[32]</sup>. Edible films having low solubility does not degrade quickly and can be used 244 as a food coating film. Alginate based, AVG and PSEO added composite edible 245 246 films water solubility is lower than only AVG and only PSEO added alginate based films (84.95% when1% PSEO was added and 72.56% when 3% PSEO was added). 247 248 It was seen that alginate based edible composite films water solubility are suitable 249 than the others films produced.

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### 251 **3.3 Water vapor transmission rate (WVTR)**

Water vapor transmission rate of the alginate based edible films was given at Table2. Edible films used in food coating must have low WVTR values to reduce the

transfer rate of water from food to its environment. The addition of AVG to alginate 254 based edible film reduced the WVTR value from  $0.8793 \times 10^{-3}$  (g/(s.m<sup>2</sup>) to 255  $0,4914*10^{-3}$ . (g/(s.m<sup>2</sup>). This reduction, is due to the interactions between alginate 256 and AVG, which decrease the availability of the hydrophilic groups of alginate and 257 prevent the interaction with water. Similar results have been obtained by Pinzon et 258 al. <sup>[32]</sup> and Salama and Aziz <sup>[33]</sup>. WVTR of the alginate based edible films was 259 decreased also after PSEO addition from 0,8793\*10<sup>-3</sup> (g/(s.m<sup>2</sup>) to 0,1091\*10<sup>-3</sup> 260  $(g/(s.m^2))$  (1% PSEO) and to 0,0897\*10<sup>-3</sup> (3% PSEO). This is because essential oils 261 moved as the hydrophobic dispersed phase. The increase of the PSEO concentration 262 263 increased the distance travelled by water molecules diffusing through the film 264 matrix. So, the transfer rate of water molecules and the WVTR of edible film were reduced. Similar results have been obtained by Zhang et al. <sup>[34]</sup>. Edible composite 265 films WVTR values were 0,1267\*10<sup>-3</sup> with 1% PSEO addition and to 0,1207\*10<sup>-3</sup> 266 267 with 3% PSEO addition respectively. PSEO added alginate based edible films have lower WVTR values. So they are suitable for using at food coating. 268

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#### 270 **3.4 Moisture content**

The moisture content of an edible film is determined by the total amount of water molecules present in the microstructure networks of the film. The moisture content of the films indicated the film hydrophilicity and affect water permeability of the films. The results given at Table 2 indicated that AVG addition decreased edible films moisture content from 27.93% to 22.56%. This can be caused by the formation of interaction between the free hydroxyl groups of alginate chains and

AVG, which leads to the decrease of free hydroxyl groups and the reduction of the 277 moisture content of the film. The increase of PSEO concentration from 1% to 3% 278 279 decreased the moisture content of the edible films from 28.05% to 19.95%, because, 280 the essential oil components may interact with the hydroxyl groups of the film matrix to reduce the film's affinity for water. Edible composite films moisture 281 282 content values were 29.40% for 1% PSEO added films and 22.90% for 3% PSEO added composite film <sup>[34-37]</sup>. Lower moisture content value was obtained from the 283 284 PSEO added alginate based edible films.

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#### 286 3.5 Film Thickness

287 The thickness of the alginate based edible films is related to the total dry mass in 288 the film-forming solution. It is preferred that the film thickness is less than 0.3 mm. When the thickness values of the films given at Table 2 were examined, it was seen 289 290 that the film thickness increased with the addition of AVG, from 0.090 mm to 0.130 mm. Similar results have been obtained by Gutiérrez and Álvarez <sup>[38]</sup>. When the 291 PSEO concentration increased from 1% to 3%, the film thickness increased from 292 0.080 mm to 0.140 mm. Similar results have been obtained by Roshandel-hesari et 293 al <sup>[9]</sup>. It is preferred that the film thickness is less than 0.3 mm so that the packaged 294 295 food can be eaten together with the edible film. Produced edible composite films thickness values was 0.125 mm for 1% PSEO added films and 0.178 mm for 3% 296 PSEO added composite film. 297

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#### 300 **3.6 Application of edible coating films on strawberries and cherries**

Weight loss is an important characteristics of fruits during storage. Strawberrie and 301 302 cherrie fruits display weight losses at long storage times. Water loss caused by respiration and water evaporation leads to fruit shrinking <sup>[39-40]</sup>. The weight loss (%) 303 of coated and uncoated strawberries and cherries during 8 days storage at 4<sup>0</sup>C, were 304 305 given in Table 3. The results showed that maximum weight loss values were seen 306 at uncoated strawberries and cherries, 15.83% and 15.02% respectively. Alginate 307 coated strawberries weight loss was 12.25% lower than the uncoated strawberries. 308 Alginate and 1% PSEO coated strawberries weight loss was 23.06% lower than the 309 uncoated strawberries. And alginate and 3% PSEO coated strawberries weight loss 310 was 28.99% lower than the uncoated strawberries. Alginate coated cherries weight 311 loss was 52.20% lower than the uncoated cherries. Alginate and 1% PSEO coated cherries weight loss was 70.37% lower than the uncoated cherries. And alginate and 312 313 3% PSEO coated cherries weight loss was 77.90% lower than the uncoated cherries. As a result, it was seen that edible coating film reduced the weight loss of the fruits, 314 315 moving as a water barrier between the fruits and the environment. Increasing PSEO % in coating solution decreases weight loss %, and the lowest weight loss was 316 317 observed for fruits coated with alginate and 3% PSEO. The color change is one of 318 the fruit quality parameter taken care of by consumers. The color change of coated fruits were confirmed using L<sup>\*</sup>, a<sup>\*</sup>, b<sup>\*</sup>, and  $\Delta E$  values (Table 4). The L<sup>\*</sup> (lightness) 319 values of strawberries and cherries reduced after 8 days storage at 4<sup>o</sup>C. This 320 decrease may be caused by water loss of the fruits. At day 8 maximum L\* value 321 was obtained by alginate and 3% PSEO coated fruits. a\* (redness), b\* (yellowness) 322

323	and $\Delta E$ values were decreased also similar to lightness <sup>[41-43]</sup> . Maximum a* value
324	was obtained by alginate and 1% PSEO coated fruits, maximum b* value was
325	obtained by alginate coated fruits and maximum $\Delta E$ value was obtained by alginate
326	and 1% PSEO coated fruits. The results indicated that alginate based and PSEO
327	added edible coatings reducing moisture loss, color change and surface deformation
328	of fruits. Images of uncoated and coated strawberries and cherries were given at
329	Fig.5.

Fruit Type	<b>Coating Solution</b>	Weight Loss (%
	Uncoated	15.83
Strawberries	Alginate	13.89
	Alginate and 1% PSEO	12.18
	Alginate and 3% PSEO	11.24
	Uncoated	15.02
	Alginate	7.18
	Alginate and 1% PSEO	4.45
	Alginate and 3% PSEO	3.32

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Table 4 Color parameters of strawberries and cherries after 8 days storage at 4<sup>o</sup>C 332

Time	Fruit Type	<b>Coating Solution</b>	$\mathbf{L}^{*}$	a <sup>*</sup>	$b^*$	ΔΕ
	Strawberries	Uncoated	31.42	21.27	17.89	40.63
		Alginate	35.49	25.56	20.90	46.50
		Alginate and 1% PSEO	43.99	19.28	16.66	48.65
D 1		Alginate and 3% PSEO	39.91	17.74	8.51	44.37
Day1	Cherries	Uncoated	34.36	25.17	15.26	43.82
		Alginate	35.71	28.57	14.27	47.91
		Alginate and 1% PSEO	34.78	23.02	12.52	43.54
		Alginate and 3% PSEO	35.93	21.31	7.89	42.51
	Strawberries	Uncoated	30.09	14.48	14.53	32.72
		Alginate	27.83	15.91	15.80	38.27
		Alginate and 1% PSEO	27.77	17.64	7.73	44.88
D 0		Alginate and 3% PSEO	31.27	15.81	7.77	42.51
Day8	Cherries	Uncoated	29.39	20.65	10.36	37.57
		Alginate	28.71	18.05	10.99	35.46
		Alginate and 1% PSEO	30.01	20.05	9.80	42.15
		Alginate and 3% PSEO	30.54	14.74	4.86	41.25

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strawberries weight loss was 28.99% lower than the uncoated strawberries, and 351 alginate and 3% PSEO coated cherries weight loss was 77.90% lower than the 352 353 uncoated cherries. It was concluded that alginate based and PSEO added edible coatings reducing moisture loss, color change and surface deformation of 354 strawberries and cherries. The biggest difference of this study from previous studies 355 356 is that, PSEO was used for the first time in this study, for edible coating film 357 production and the results were quite successful especially for the cherrys coating. 358 359 REFERENCES 360 361 [1]. Lim, L.I, Tan, H.L., & Pui, L.P. (2021). Development and characterization 362 of alginate-based edible flm incorporated with hawthorn berry (Crataegus pinnatifda) extract. Journal of Food Measurement and Characterization, 15, 2540-363 364 2548. https://doi.org/10.1007/s11694-021-00847-4 [2] Bishnoi, S., Trifol, J., Moriana, R., & Mendes, A.C. (2022). Adjustable 365 polysaccharides-proteins films made of aqueous wheat proteins and alginate 366 367 solutions. Food Chemistry, 391, Article 133196. https://doi.org/10.1016/j.foodchem.2022.133196 368 369 [3] Fabra, M.J., Falco, I., Randazzo, W., Sanchez, G., & Lopez-Rubio, A. (2018). Antiviral and antioxidant properties of active alginate edible films containing 370 371 phenolic Hydrocolloids, 81, 96-103. extracts. Food 372 https://doi.org/10.1016/j.foodhyd.2018.02.026

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