

1 **Development of a whey protein concentrate/apple pomace edible coating**
2 **for extending shelf life of fresh-cut apple**

3 **Running title: Novel edible coating for fresh fruits**

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24 **Abstract**

25 The present study aimed to develop a novel edible coating using whey protein
26 concentrate (WPC) and apple pomace extract (APE) to extend shelf life of fresh-
27 cut apple. Apple slices were coated with a mixture of WPC and APE at
28 concentration of 0.5, 1 and 1.5% and were stored at 5°C for 12 days. The total
29 phenolic content and DPPH radical scavenging activity of APE were determined.
30 The weight loss, color, browning index, microbiological analysis and sensory
31 evaluation of coated and uncoated apple slices were estimated. A total phenolic
32 content of ethanolic APE was 6.77 ± 0.339 mg gallic acid equivalent/g dry apple
33 pomace. Apple pomace extract contained the total of 15 phenolic compounds.
34 Also, a significant antioxidant activity was observed for apple pomace extract using
35 the DPPH method and the inhibitory concentration (IC_{50}) was 51.97 ± 1.576 μ g
36 gallic acid equivalent/mL extract compared with BHT (21.80 ± 0.424 μ g/mL).
37 Coating apple slices with WPC/APE decreased the weight loss compared to the
38 uncoated and the apple slices coated with WPC only. The coated apple slices with
39 WPC/1.5% APE had the highest lightness compared to other coated and uncoated
40 apple slices after 12 days of storage. In addition, the coated apple slices with WPC/
41 1 and 1.5% APE exhibited the lowest browning index compared to the uncoated
42 apple slices. Using WPC and APE as coating agents showed antimicrobial activity
43 and they had little effect on the sensory evaluation of apple slices.

44 **Keywords:** Whey protein, apple pomace, edible coating, fresh-cut apple.

45 **Introduction**

46 Fresh-cut fruits and vegetables are the most preferable foods for
47 consumers because they are highly nutritious, convenient and healthful
48 commodities. However, their market is still perfectly limited due to fast damage
49 through storage and distribution. In recent years, there has been an increasing
50 interest in achieving novel strategies to enhance storage ability, shelf life and the
51 microbiological safety of fresh-cut products. The methods of edible coatings and
52 films have been considered prospective strategies for meeting this demand. Edible
53 coatings constitute a thin layer of edible agent composed as a coating over a food
54 product and this coating can be eaten with the product (Kuorwel *et al.*, 2015;
55 Tavassoli-Kafrani *et al.*, 2016). Edible coatings can preserve vegetables and fruits
56 in fresh form by enhancing the retention of flavor, sugar, acid, and color to prolong
57 shelf life and keep nutritional characteristics (Fakhouri *et al.*, 2015; Kerch, 2015).
58 Furthermore, edible coatings used to prevent undesirable mass transports
59 (moisture, oxygen and flavor), improve visual properties and act as carriers to
60 deliver active components such as antimicrobial, antioxidant and nutraceuticals
61 agents (Reinoso *et al.*, 2008).

62 Lipids, proteins, and polysaccharides can be utilized as biopolymers for
63 edible coatings creation (Schmid *et al.*, 2015; Jahed *et al.*, 2017; Martelli *et al.*,
64 2017; Niamlang *et al.*, 2017). Generally, proteins have been given a great attention
65 in edible coating technology for their plenty of food processing residuals. Also, the
66 reactive amino acids enable the proteins to be modified and cross-linked through
67 chemical and physical treatments to form new polymeric structures (Gennadios,
68 2002). Whey protein isolates have excellent barrier function for gas, aroma

69 compounds, and oil as compared to the films made with polysaccharides and lipids
70 (Krochta, 2002 and Feng *et al.*, 2018). Commercially, whey proteins are available
71 as whey protein concentrates (WPC) or whey protein isolates (WPI), with protein
72 contents of 20-85 and >90%, respectively (Khwaldia *et al.*, 2004). Since whey
73 protein coatings are edible, they are perfect carriers for nutraceuticals to improve
74 the nutritional value of the coated food product. The applications of whey protein
75 films are to be utilized mainly as antimicrobial agents and as protective barrier
76 coatings to increase the shelf-life of food products (Seydim and Sarikus, 2006).

77 Apple pomace is an industrial solid waste of apple manufacturing, and it
78 represents around 30% of the original fruit. Wet pomace, generated by cider
79 pressing, represents up to 25% of the fresh fruit weight and its moisture content is
80 about 70-85% after being pressed. Apple pomace is a heterogeneous mixture
81 consisting of the apple peels, leftover flesh, core with seeds and stems (Jung *et al.*,
82 *et al.*, 2015; Kara and Doymaz, 2015). It has been proved that apple pomace affects
83 pharmacological targets. Nutraceutically, apple pomace appeared to have various
84 pharmacological benefits where the preliminary studies reported promising anti-
85 inflammatory, antiviral, antioxidative and antibacterial activities (Waldbauer *et al.*,
86 2017). Many studies, aiming to have value-added products, have used apple
87 pomace to produce protein-enriched feeds, ethanol, enzymes and natural
88 antioxidants (Shrikot *et al.*, 2004; Paganini *et al.*, 2005; Medeiros *et al.*, 2006;
89 Albuquerque *et al.*, 2006; Vendruscolo *et al.*, 2008). The polyphenols content of
90 apple pomace and its ability to scavenge DPPH radicals have been reported
91 (Gharedaghi *et al.*, 2019, Cetkovic *et al.*, 2008 and Rana *et al.*, 2014)

92 Based on the previously mentioned information, our study aimed to develop
93 whey protein-based coating incorporating apple pomace extract (APE) as an anti-
94 browning, anti-microbial and antioxidative agent for apple slices under cold
95 storage.

96 **Materials and methods**

97 *Materials*

98 Whey protein concentrate (WPC, 80%), glycerol (99.5%), 1,1-diphenyl-2-
99 picrylhydrazyl (DPPH) and Butylated hydroxytoluene (BHT) were obtained from
100 CP Kelco, a Huber Company (Georgia, USA) and Sigma-Aldrich (St. Louis, MO,
101 USA). Apple fruits (*Malus domestica* var. *anna*) were purchased from a local
102 supermarket in January 2019.

103 *Preparation of apple pomace extract (APE)*

104 Ten kg of apple fruits were washed and cut into small pieces then squeezed
105 in a domestic food processor (Moulinex, Compact Kitchen Machine, Egypt) and
106 finally filtrated through muslin cloth. Apple pomace was oven-dried at 50°C then
107 milled and sieved at 50 mesh. About 200 g apple pomace powder was extracted
108 by ethanol 80% at a ratio of 1:20 (w/v) using a homogenizer for 30 min. The mixture
109 was passed through filter paper (Whatman No. 1). The filtrate was concentrated
110 by rotary evaporator at 40°C. The concentrate was lyophilized and stored at 5°C
111 before coating application.

112 *Coating preparation*

113 The apple fruits were cut into similar thick slices (2 cm). The pieces were
114 divided into 5 parts. The first part was dipped in distilled water and served as a

115 control. The second part was dipped in an aqueous solution of 10% (w/w) WPC
116 and 3% (w/w) glycerol. The third, fourth and fifth parts were coated with the same
117 solution incorporated with 0.5, 1 and 1.5% of APE, respectively. Dipping process
118 was performed for 2 min. The excess of the immersion solutions on the apple slices
119 was drained off for 5 min. Then these apple slices were placed in polypropylene
120 packages and thermally sealed by stretch film before storage at 2-5°C and 80%
121 RH for 12 days for analyses. At least three batches for each treatment were
122 performed.

123 *Total phenolic content and antioxidant activity of apple pomace extract (APE)*

124 Total phenolic compounds of ethanolic APE were determined using the
125 Folin-Ciocalteu reagent, and gallic acid was used as a standard. The results were
126 expressed as mg gallic acid equivalent/g dry matter according to Khalifa *et al.*
127 (2017). The ability of APE to scavenge DPPH radicals was measured according to
128 Marquez *et al.* (2017).

129 *Determination of phenolic compounds of apple pomace extract (APE)*

130 Determination of phenolic compounds was performed by high-performance
131 liquid chromatography (HPLC). Samples were analyzed using an Agilent 1260
132 series HPLC system. The separation was carried out using Agilent Zorbax C₁₈
133 column (4.6 mm i.d. x 250 mm., 5 µm, Agilent Technologies Co. Ltd., CA, USA).
134 The mobile phase consisted of water (A) and acetonitrile (B) at a flow rate of 1
135 mL/min. The mobile phase was programmed consecutively in a linear gradient as
136 follows: 0–5 min (80% A); 5-8 min (40% A); 8-12 min (50% A); and 12-16 min (80%

137 A). The multi-wavelength detector was monitored at 280 nm. Each sample was
138 injected at 10 µL. The column temperature was maintained at 35°C.

139 *Determination of weight loss*

140 Weight loss of different apple slices was estimated in triplicate after 1, 4, 8
141 and 12 days of storage as follows:

$$142 \quad \text{Weight loss (\%)} = \frac{(\text{initial weight} - \text{final weight})}{(\text{initial weight})} \times 100$$

143 *Measurement of color*

144 Color of apple pieces was measured with a chromameter Minolta CR-400
145 (Minolta. Inc., Tokyo, Japan) using the CIE color parameters L^* , a^* , b^* . The samples
146 were measured after 1, 4, 8 and 12 days of storage. The browning index (BI) was
147 calculated according to Olivas *et al.* (2007) as follows:

$$148 \quad BI = \frac{100(x - 0.31)}{0.172}$$
$$149 \quad x = \frac{(a^* + 1.75L^*)}{(5.645L^* + a^* - 3.01b^*)}$$

150 *Antimicrobial activity of apple pomace extract*

151 The antimicrobial activity of apple pomace extract was determined using
152 agar well diffusion method against gram positive bacteria (*Staphylococcus aureus*
153 and *Bacillus subtilis* NRRL B-543), gram negative bacteria (*Escherichia coli* ATCC
154 25955 and *Proteus vulgaris* ATCC13315) and fungi (*Aspergillus fumigates* and
155 *Candida albicans* ATCC 10231) as described by Boyanova *et al.* (2005).

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157 *Microbiological examination*

158 Each sample of apple slice (10 g) was homogenized aseptically with 90 mL
159 of Ringer's solution as described by ICMSF, 1978. Serial dilutions were made
160 using Ringer's solution and they were poured onto sterile standard plate count
161 agar plates. The plates were incubated at 32°C for 48 h for the enumeration of total
162 bacterial count or at 7°C for 5 days for the enumeration of psychrotrophic bacteria.
163 Colonies were counted and results are expressed as log CFU/g of the sample.

164 *Sensory evaluation*

165 Ten panelists from the staff members of the Food Science Department,
166 Faculty of Agriculture, Cairo University, Egypt, used a quality rating scorecard for
167 the evaluation of treated apple slices for taste, odor, texture, and overall
168 acceptability. Based on their preference and liking, panelists were demanded to
169 classify the samples on a ten-point hedonic scale; 1 is unacceptable and 10 is very
170 much like.

171 *Statistical analysis*

172 The results were expressed as the mean \pm standard deviation. All data
173 were analyzed in three replications for each parameter. Statistical analysis was
174 performed using XLSTAT 2014 (5.03) software (USA). Significant differences
175 ($p < 0.05$) between means were determined by Tukey's test.

176 **Results and discussion**

177 *Total phenolic content and phenolic composition*

178 The phenolic compounds are responsible for most of the antioxidant activity
179 as well as the health benefits of apple consumption (Feng *et al.*, 2018). Total
180 phenolic content of ethanolic APE was 6.77 ± 0.339 mg gallic acid equivalent/g dry

181 apple pomace, while it was 14.969 ± 0.359 mg gallic acid equivalent/g dry
182 lyophilized extract. Suárez *et al.* (2010) found that the total phenolic content of
183 apple pomace methanolic and acetonetic extracts was 3.63 and 6.48 mg gallic acid
184 equivalent/g of dry wt. pomace, respectively.

185 Apple pomace extract was subjected to HPLC analysis and the total
186 identified phenolic compounds were 15 (Figure 1). The major polyphenols of APE
187 were ellagic acid (2494.93 mg/L), salicylic acid (174.83 mg/L), quinol (138.65
188 mg/L), gallic acid (73.39 mg/L), benzoic acid (70.95 mg/L), rosmarinic acid (50.51
189 mg/L), syringic acid (16.05 mg/L), chlorogenic acid (12.13 mg/L), o-coumaric acid
190 (9.39 mg/L), and vanillin (9.18 mg/L) while the main identified flavonoids were
191 myricetin (3184.31 mg/L), naringin (94.93 mg/L), kampherol (57.62 mg/L),
192 quercetin (12.79 mg/L) and rutin (7.90 mg/L). Suárez *et al.*, (2010)-indicated the
193 presence of chlorogenic acid, (-)-epicatechin, quercetrin, protocatechuic acid, and
194 caffeic acid in apple pomace methanol extract.

195 *Free radical scavenging capacity of apple pomace extract (APE)*

196 The obtained results showed that the antioxidant activity of APE increased
197 as polyphenols concentration increased. The IC_{50} (concentration of APE that is
198 required to inhibit 50% of DPPH free radicals) value of APE was 51.97 ± 1.576 μ g
199 gallic acid equivalent/ mL whereas the IC_{50} value of BHT was 21.80 ± 0.424 μ g/mL.
200 The radical scavenging capacity of APE could be attributed to the presence of
201 ellagic acid which is the main phenolic compound in apple pomace (Hayes *et al.*,
202 2011). Cetkovic *et al.*, (2008) found that IC_{50}^{DPPH} radical scavenging activity of apple
203 pomace methanol extract ranged from 6.33 to 15.72 mg/mL. They used 5 apple

204 varieties. Rana *et al.*, (2014) found that the IC_{50}^{DPPH} of ethyl acetate fraction of APE
205 was 7.37 mg/mL.

206 *Weight loss of apple slices*

207 The effect of WPC/ APE coating on the weight loss of apple slices during
208 cold storage is shown in Figure 2. The weight loss of WPC/ APE coated samples
209 was < 0.25 % after 4 days of storage instead of >2% in the case of uncoated and
210 WPC coated apple slices. After 12 days of cold storage, uncoated apple slices
211 showed the highest weight loss (3.07 ± 0.042 %). However, the weight loss of
212 coated apple slices with WPC incorporated with apple pomace extract at
213 concentrations of 0.5, 1 and 1.5% decreased significantly ($p < 0.05$) to 1.06 ± 0.021 ,
214 0.98 ± 0.014 and 0.58 ± 0.028 %, respectively compared to the uncoated and coated
215 apple slices with WPC only. Increasing apple pomace concentration in the coating
216 mixture to 1.5% was significantly ($p < 0.05$) effective in reducing the weight loss of
217 apples. This could be due to the high sugar content of the dried apple pomace as
218 reported by O'Shea *et al.* (2015).

219 Weight loss of uncoated apple slices varied from 0.53% to 1.29% after 12
220 days of cold storage at 2°C according to the investigated cultivars (Kim *et al.*,
221 1993). Khalifa *et al.* (2017) found that the weight loss of uncoated apple samples
222 reached 3.03% and 8.50% after 21 and 35 days of storage. Marquez *et al.* (2017)
223 reported that coating apple pieces with whey protein concentrate decreased the
224 weight loss from 10% to 8% after 10 days of storage at 4-6°C. McHugh and Krochta
225 (2014) concluded that protein-based films have high sensitivity to moisture and
226 poor water vapor barrier properties due to their hydrophilic nature. Umaraw and

227 Verma (2017) reported that whey protein isolates (WPI) have high water vapor
228 permeability owing to the high degree of hydrophilic amino acids in their structure.
229 In addition, Alves *et al.* (2017) found that coating formula containing sodium
230 ascorbate (10 g/L) was more effective in controlling weight loss of apple slices than
231 that without antioxidants. This result may be due to the additional protective effect
232 provided by the interactions of the antioxidants with compounds at the surface of
233 the apples.

234 *Color changes and browning assessment of apple slices*

235 Extending storage period of apple slices was accompanied by an increase
236 in the enzymatic browning as indicated by an increase in a^* and b^* values and
237 decrease in lightness (L^*) and hue values (Perez-Gago *et al.*, 2006). In Figure 3a,
238 results indicated that the uncoated apple slices and those coated with WPC
239 significantly ($p < 0.05$) recorded the lowest lightness after 12 days of storage. L^*
240 values of apple slices coated with WPC/APE were not significantly ($p > 0.05$)
241 different at the end of storage, regardless level of apple pomace used in the coating
242 formula. Coating apple slices with WPC/ APE kept its L^* values for 12 days not
243 significantly ($p > 0.05$) different from those of WPC coated apple slices that stored
244 for 4 days.

245 A significant ($p < 0.05$) increase in (a^*) value of all treatments was observed
246 during the cold storage period (Figure 3b). The lowest increase of a^* value after
247 12 days of storage was recorded for apple slices coated with WPC/ APE (1.5%).
248 The highest ($+b^*$) value (33.81 ± 0.168) was recorded for the uncoated apple slices
249 at the end of storage (Figure 3c). There was no significant ($p > 0.05$) change

250 between (+b*) value of apple slices coated with WPC/APE at 1% or 1.5% after 12
251 days of storage and that of the freshly cut apple slices (uncoated).

252 Extending storage time of all treatments to 12 days was accompanied by a
253 significant ($p<0.05$) increase in the chroma (C*) values (Figure 3d). The chroma
254 (C*) values of WPC / APE (1% or 1.5%) coated apple samples, at the end of
255 storage period, was not significantly ($p>0.05$) different from those of the 4 days
256 stored uncoated or WPC coated apple slices.

257 Hue values decreased slightly during storage of the uncoated and coated
258 apple slices (Figure 3e). After 12 days of storage, the hue value of the WPC / APE
259 (1.5%) coated apple samples was not significantly ($p>0.05$) different from that of
260 the uncoated slices at zero-time storage.

261 The browning index is an indicator for tissue decay. The results in Figure
262 3f showed that browning index increased during the cold storage of all treatments.
263 The uncoated apple slices significantly ($p<0.05$) recorded the highest browning
264 index (60.57 ± 0.338) at the end of the cold storage period. Meanwhile, browning
265 index of apple slices coated with WPC only was 58.54 ± 0.453 . On the other hand,
266 enriching coating formula with APE at levels of 0.5, 1 and 1.5% significantly
267 ($p<0.05$) reduced the increase of browning index to 37.66 ± 0.174 , 37.10 ± 0.425 and
268 35.01 ± 0.200 , respectively. Coatings which incorporated with antioxidants reduced
269 oxygen permeability and affect polyphenol oxidase activity (Alves *et al.*, 2017). In
270 this regard, Perez-Gago *et al.* (2006) found that apple pieces coated with whey
271 protein-based coatings had higher L* and lower b* and a* values. They reported

272 that browning index values of the coated apple pieces were lower than those of
273 the uncoated ones.

274 *Microbiological examination of apple slices*

275 The antimicrobial activity of apple pomace extract (200 mg/ mL) was
276 determined against six species of spoilage and pathogenic microorganisms and
277 the results showed that the apple pomace extract showed large zone of inhibition
278 (8.00 mm) for *Staphylococcus aureus* NRRL B-543 and *Escherichia coli* ATCC
279 25955. However, no inhibition zones were detected for other investigated
280 microorganisms. These results are in consistent with those of Younis and Ahmad
281 (2015). The growth inhibition property of apple pomace is attributed to the
282 presence of polyphenols (Agourram *et al.*, 2013).

283 As shown in Table 1, coating apple slices with WPC or WPC/APE at 0.5%
284 or 1.0% decreased significantly ($p<0.05$) the TBC during storage period.
285 Increasing APE concentration in the coating mixture from 0.5% to 1% decreased
286 significantly ($p<0.05$) the TBC during storage of apple slices. The phenolic
287 compounds of apple pomace have antimicrobial activity (Zhang *et al.* 2016 and
288 Riaz *et al.*, 2018).

289 The lowest bacterial count was recorded for the WPC/APE (1%) coated
290 samples that was significantly ($p<0.05$) different from all other investigated
291 treatments. Extending storage time to 12 days did not significantly ($p<0.05$) affect
292 the bacterial count of WPC/APE (1%) coated samples. On the other hand, coating
293 apple slices with WPC/APE at 1.5% did not significantly ($p<0.05$) decrease TBC
294 during the first 4 days of storage, after which the TBC decreased significantly

295 ($p < 0.05$). These results indicated that whey protein concentrate and apple pomace
296 may have antimicrobial activity. In this respect, Marquez *et al.* (2017) found that
297 the whey protein/pectin/transglutaminase edible coating is efficacious to obviate
298 fresh-cut apple spoilage during the ten days of storage, as demonstrated by
299 microbial growth prevention.

300 *Sensory evaluation of apple slices*

301 The utilization of functional ingredients as coating agents to the fruits may alter the
302 sensory attributes of the fruits that might cause a decrease in consumer
303 acceptability. Consequently, it is essential to study the changes in sensory
304 attributes of apple slices as a result of using whey proteins and apple pomace as
305 coating agents. The sensory attributes of taste, odor and texture of coated and
306 uncoated apple slices are listed in Table 2. On day 1 of cold storage, no significant
307 ($p < 0.05$) differences were noted in all treatments for the taste and odor sensory
308 attributes. The highest scores for texture and overall acceptability were recorded
309 for WPC/APE (1% and 1.5 %) coated samples at the first day of storage. During
310 the cold storage period, it was observed that there was a gradual decrease in the
311 sensory properties of all samples, except WPC/APE1.5% coated samples, till the
312 end of the storage period. Coating with WPC/APE1.5% kept sensory attributes of
313 apple slices during the first 4 days of storage without significant ($p < 0.05$) difference
314 from those of the freshly cut slices. Extending storage time to 12 days did not
315 significantly affect the overall acceptability of the WPC/APE1.5% coated samples.
316 Our findings pointed out that using whey protein concentrate and apple pomace
317 as coating agents attained the sensory properties of apple slices during storage.

318 In this regard, Javanmard (2011) found that WPC-gellan coating maintained the
319 color, firmness, glossiness and overall acceptability of apple during storage.
320 Hassani *et al.* (2012) reported that using a composite of WPC and rice bran oil as
321 a coating agent was effective in the preservation of color, firmness, taste and
322 overall acceptability of the kiwifruit during storage. Marquez *et al.* (2017) observed
323 no significant differences in acceptability scores for the texture and flavor of the
324 coated samples with whey protein/pectin/transglutaminase edible coating after
325 storage compared to all samples tested before storage.

326 **Conclusion**

327 The present study revealed that the use of a mixture of whey protein
328 concentrate and apple pomace extract as an edible coating was effective to
329 obviate fresh apple slices damage or spoilage during the 12 days of cold storage.
330 This edible coating led to the reduction of weight loss, color changes, browning
331 index and microbial growth of fresh apple slices. Also, coating apple slices with
332 WPC and apple pomace did not have a negative effect on the sensory attributes
333 of apple slices. Finally, a blend of WPC and apple pomace can be used as coating
334 agents for fresh-cut fruit without affecting their properties during the cold storage
335 period.

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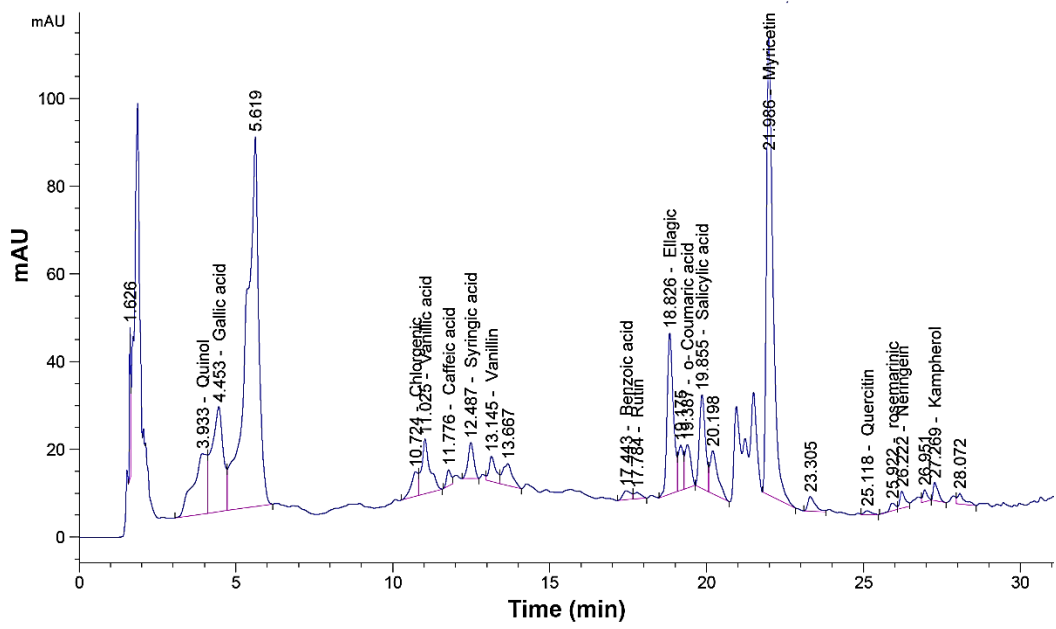
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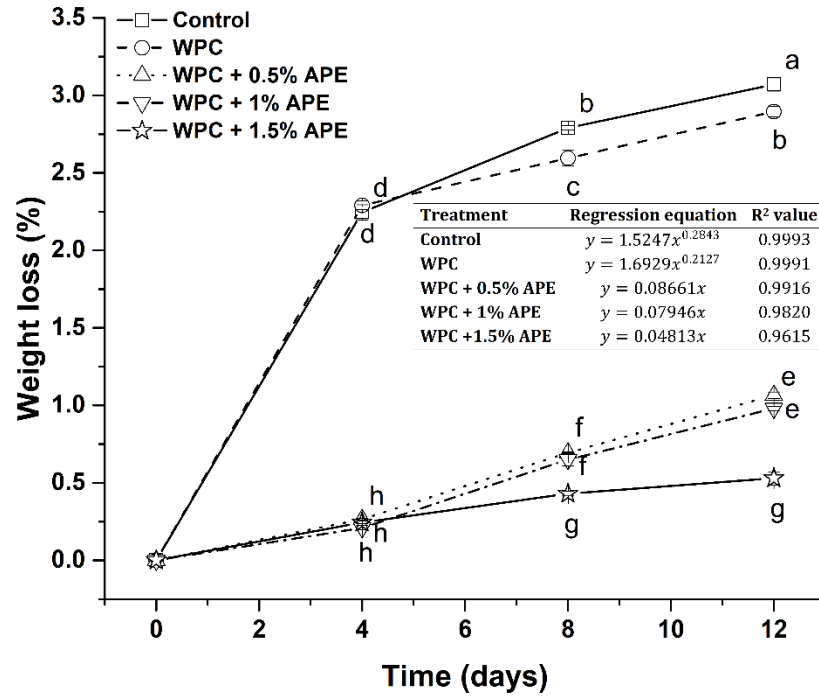
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486 **Figure 1.** HPLC profile of phenolic compounds in apple pomace extract



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Figure 2. Weight loss of apple treated with different coating formulations during cold storage

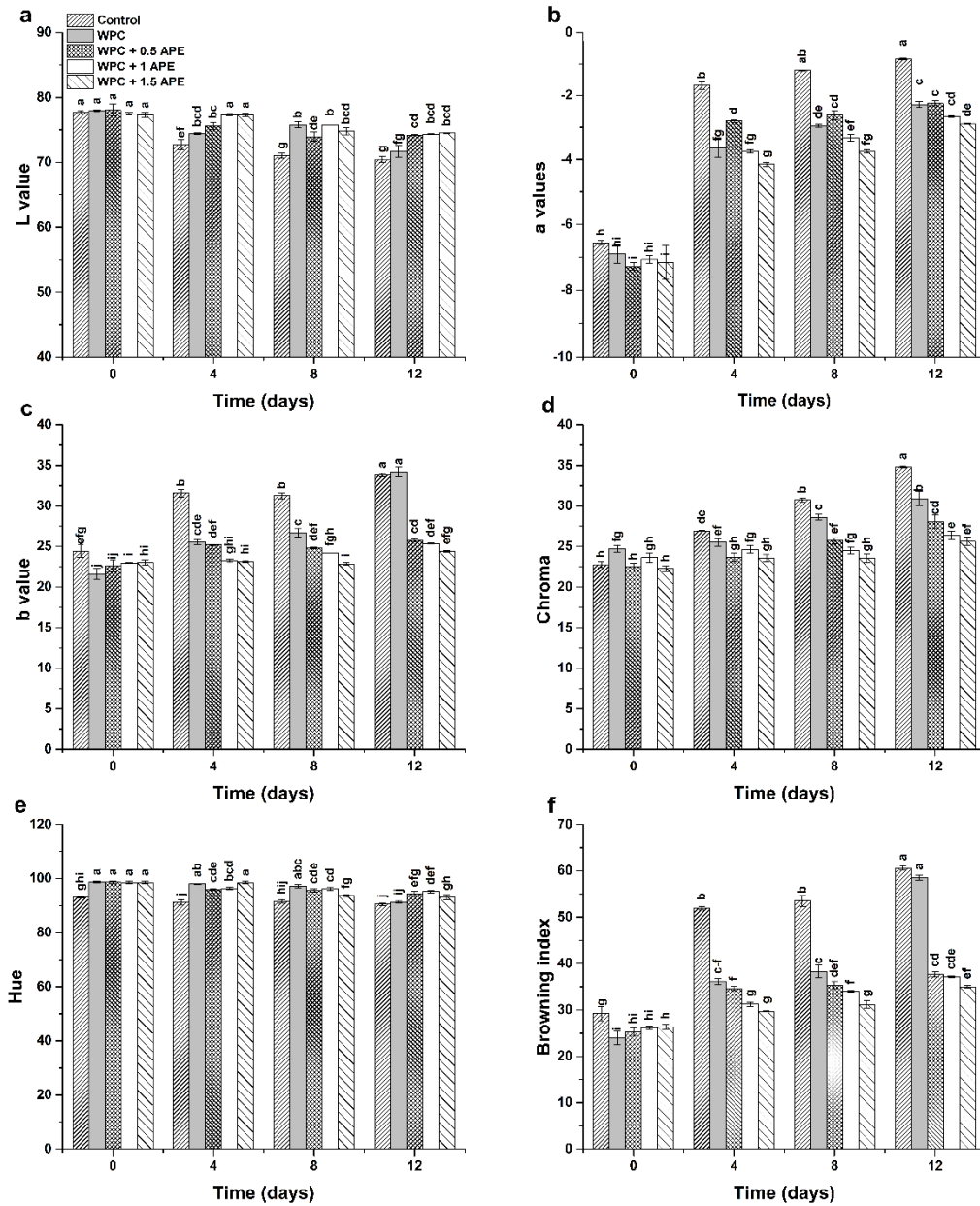


Figure 3. Color changes and browning index of apple treated with different coating formulations during cold storage

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490 **Table 1. Total bacterial count (log CFU/g) of apple treated with different coating**
 491 **formulations during cold storage**

Treatments	Storage days			
	1	4	8	12
Control	2.17±0.01 ^c	2.16±0.01 ^c	2.26±0.02 ^b	2.38±0.01 ^a
WPC	1.55±0.05 ^{fg}	1.97±0.01 ^d	1.82±0.05 ^e	1.52±0.02 ^g
WPC+0.5%APE	1.28±0.02 ^h	2.10±0.04 ^c	1.89±0.01 ^e	1.49±0.04 ^g
WPC+1 % APE	1.16±0.02 ⁱ	1.21±0.04 ^{hi}	1.18±0.01 ⁱ	1.14±0.04 ⁱ
WPC+1.5% APE	2.17±0.02 ^c	2.16±0.03 ^c	2.13±0.02 ^c	1.62±0.03 ^f

492 Values are means ± standard deviation. Means with the same letter indicated no significant
 493 difference at 5% level of probability by Tukey's test.

494 Control: uncoated apple; WPC: whey protein concentrate; APE: apple pomace extract.
 495 Pyschrotrophic bacteria were not detected in all treatments at day 1 and during storage.

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Table 2. Sensory evaluation of apple treated with different coating formulations during cold storage

Treatments	Storage days	Taste	Odor	Texture	Over all acceptability
Control	1	9.62±0.45 ^a	9.87±0.33 ^a	8.50±0.50 ^{cd}	9.62±0.48 ^{abc}
	4	8.03±0.27 ^c	6.93±0.24 ^g	7.81±0.52 ^g	8.18±0.52 ^{cd}
	8	5.62±0.24 ^d	4.25±0.82 ^j	5.62±0.55 ⁱ	5.75±0.24 ^g
	12	3.81±0.94 ^f	3.37±0.72 ⁱ	2.31±0.59 ^l	3.87±0.48 ^k
WPC	1	9.62±0.45 ^a	9.80±0.40 ^a	9.00±0.36 ^b	9.86±0.44 ^{ab}
	4	8.20±0.24 ^c	8.46±0.80 ^c	8.00±0.36 ^e	8.66±0.59 ^e
	8	6.20±0.67 ^h	6.33±0.69 ^e	6.80±0.54 ^h	7.86±0.24 ^c
	12	4.86±0.88 ^e	3.46±0.74 ^k	5.00±0.44 ^j	5.33±0.59 ^h
WPC+0.5%APE	1	9.70±0.40 ^a	9.73±0.44 ^a	9.40±0.48 ^{cd}	9.93±0.24 ^a
	4	8.30±0.52 ^{bc}	8.66±0.47 ^{bc}	9.06±0.85 ^{de}	9.13±0.33 ^b
	8	8.00±0.24 ^c	7.33±0.73 ^e	7.93±0.59 ^e	8.33±0.57 ^f
	12	5.33±0.59 ^e	6.20±0.40 ^f	5.73±0.44 ⁱ	7.00±0.36 ^h
WPC+1 % APE	1	9.64±0.23 ^a	10.00±0.54 ^a	10.00±0.33 ^a	10.00±0.33 ^a
	4	8.16±0.23 ^c	8.93±0.24 ^b	9.26±0.57 ^{cd}	9.60±0.48 ^a
	8	8.80±0.24 ^b	8.80±0.40 ^{bc}	8.66±0.33 ^{ef}	9.60±0.40 ^a
	12	5.53±0.49 ^e	7.00±0.24 ^f	7.13±0.71 ^h	7.40±0.71 ^f
WPC+1.5%APE	1	9.87±0.21 ^a	9.75±0.43 ^a	10.00±0.33 ^a	10.00±0.33 ^a
	4	9.62±0.34 ^a	9.75±0.42 ^a	9.62±0.48 ^a	10.00±0.49 ^a
	8	8.34±0.24 ^{bc}	8.93±0.74 ^b	9.43±0.49 ^{bcd}	9.62±0.39 ^a
	12	7.93±0.82 ^c	8.81±0.48 ^b	8.18±0.88 ^{de}	10.00±0.33 ^a

511 Values are means ± standard deviation. Means for each parameter with different superscript letters
512 differ significantly ($p < 0.05$).

513 Control: uncoated apple; WPC: whey protein concentrate; APE: apple pomace extract.

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