

Development and Application of Rice Starch Based Edible Coating to Improve the Postharvest Storage Potential and Quality of Plum Fruit (*Prunus salicina*)

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1 **Development and Application of Rice Starch Based Edible Coating to Improve the**
2 **Postharvest Storage Potential and Quality of Plum Fruit (*Prunus salicina*)**

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23 **Abstract**

24 The study investigated the possibility of enhancing the shelf life of plum fruit coated with
25 rice starch- ι -carrageenan (RS- ι -car) composite coating blended with sucrose fatty acid esters
26 (FAEs). Film solution (starch 3%, carrageenan 1.5% and FAEs 2%) was prepared by mixing
27 the ingredients and properties of stand-alone films (physical, mechanical, barrier and surface
28 morphology) were studied before applying the coating on fruit surface. Fruit were stored at
29 20°C for 3 weeks and analyzed for weight loss, ethylene production, respiration rate, color
30 change, firmness, and titratable acidity (TA) and soluble solid content (SSC). Surface
31 morphology of stand-alone film and fruit surface (after applying on the plum fruit) was
32 studied using scanning electron microscopy (SEM). Phytochemical analysis was performed
33 during the storage period and total phenolic content (TPC), total antioxidant capacity (TAC),
34 flavonoid content (FC) and free radical scavenging activity were determined. The rice starch
35 composite coating was shown to be effective in reducing both weight loss (WL) and
36 respiration rate and inhibiting the endogenous ethylene production when compared to the
37 uncoated control fruit stored at room temperature ($p < 0.05$). TPC, TAC, FC and free radical
38 scavenging activity was unaffected in the coated fruit throughout the storage period ($p < 0.05$).
39 The findings reported in this study indicate that the RS- ι -car-FAEs coating prolongs the shelf
40 life and maintains the overall quality of plum fruit during storage and could potentially be
41 commercialized as a new edible coating for the plum fruit industry.

42 **Keywords:** Starch; Coating; Plum; Fruit; Postharvest; Shelf-life

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47 **1. Introduction**

48

49 Plum is considered a climacteric fruit (Wu *et al.*, 2011) that softens rapidly during the
50 postharvest supply chain due to rapid senescence. Fruit softening is a natural phenomenon
51 that progresses with storage and compromises final fruit quality leading to significant
52 volumes of fruit being rejected at the marketplace due to firmness levels being below
53 acceptable retail standards (Hussain *et al.*, 2015; Paniagua *et al.*, 2013). Therefore, new
54 research aimed at improving the postharvest shelf life and storage quality of plum fruit is
55 necessary and has great potential value for plum industry.

56 Plum is an important commercial stone fruit, grown in different geographical regions
57 globally. Worldwide annual production currently exceeds 10 million tons (Karaca *et al.*,
58 2014). A number of previous studies have shown low temperature storage and
59 transportation to be an effective means of reducing perishability of plum fruit (Hussain *et*
60 *al.*, 2015; Kumar *et al.*, 2017; Pan *et al.*, 2018). However, this method of preservation
61 often results into severe chilling injury, translucency and red pigment accumulation
62 (bleeding) and flavor loss (Minas *et al.*, 2013). Other techniques have been studied to
63 improve the postharvest life of plum fruit including edible surface coatings, modified
64 atmosphere packaging, fumigation with ethylene antagonists such as 1-MCP, salicylic
65 acid treatment and natural signaling agents such as nitric oxide (Liu *et al.*, 2014;
66 Manjunatha *et al.*, 2010; Pan *et al.*, 2016; Singh *et al.*, 2009). The use of edible films and
67 coatings has recently emerged as an innovative and effective solution to extending the
68 shelf life of fresh horticulture produce. These surface coatings extend postharvest life by
69 regulating gaseous exchange and slowing moisture loss through the formation of cohesive
70 molecular semipermeable network covering the fruit surface (Vargas-Torres *et al.*, 2017).
71 Edible coatings can also improve the texture quality of fruit (Choi *et al.*, 2016; Karaca *et*

72 al., 2014) and reduce the incidence of skin bruising during handling. Novel coating
73 materials previously utilised on plums include chitosan (Kumar et al., 2017),
74 hydroxypropylmethyl cellulose (Choi et al., 2016), aloe vera (Guillén *et al.*, 2013),
75 xanthan and gellan gums and sodium alginate (Vargas-Torres et al., 2017). However,
76 these combinations still have permeability and tensile strength limitations in improving
77 the postharvest quality of plum fruit and new, more compatible biopolymer coating
78 materials therefore need to be developed to overcome the current limitations.

79 Rice starch is an underutilized conventional biodegradable material that has not
80 previously been explored alone or in combination with other compatible biopolymers for
81 its fruit coating potential. The approach of composite formulations has been investigated
82 widely, as they often result in synergistic effects (Liu et al., 2014). Compatibility
83 between starch and carrageenan in coating formulations and their ability to form a strong,
84 complex polymer network has previously been reported (Huc *et al.*, 2014; Lascombes *et*
85 *al.*, 2017; Thakur *et al.*, 2016). So there is no doubt in their potential to improve the
86 postharvest stability of horticultural produce where respiration is a critical factor. Thakur
87 *et al.* (2018) demonstrated that edible films manufactured from starch composite
88 possessed significantly improved permeability and mechanical properties and can be a
89 potential solution to improve the quality of plum fruit. Moreover, there is no evidence in
90 the current literature of the use of rice starch-carrageenan-fatty acid esters composite
91 materials for fruit coating applications. Therefore, the objective of this study was to
92 investigate the coating properties of starch-t-carrageenan coating blended with sucrose
93 fatty acid esters and their impact on the physiology and shelf life of plum fruit.

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95

96 **2. Materials and methods**

97 **2.1 Materials**

98 Rice grains (*Oryza sativa* var. Doongara) were obtained from Sunrice (Sun Rice, Leeton
99 Australia). The ι-carrageenan (*Chondrus crispus*) was purchased from Melbourne Food
100 ingredient depot, Victoria, Australia. Glycerol (Ajax fine-chem Pty. Ltd, Australia) was used
101 as plasticizing agent in the film formulation. Starch isolation and characterisation of its
102 chemical composition is described elsewhere (Thakur et al., 2016). Sucrose fatty acid ester
103 was purchased from Xi'an Plant Bio-Engineering Co., Ltd, China.

104 **2.2 Preparation of film/coating solution**

105 Based on the laboratory trials and preliminary study, the optimum volume required to coat
106 the fruit was identified and used for subsequent coating experiment. Rice starch (3%, w/w),
107 ι-car (1.5%, w/w), FAEs (2%%, w/w) and glycerol (1 %, w/w) were mixed in a two-step
108 procedure. In the first step, starch solution (2%) was prepared by heating a starch-water
109 mixture at 85 °C using a hot plate magnetic stirrer for 15 min. In the second step ι-car gelling
110 solution was prepared by heating the ι-car-H₂O mixture at 80°C for 20 min. until a clear
111 transparent gel was formed. The solution from step 1 and step 2 were mixed together with a
112 subsequent addition of FAEs and glycerol and stirred for a further 20 min.

113 **2.2.1 Formation of edible film:** The final film solution (20 mL) was poured into Petri plates
114 and dried in the oven for 24 h under controlled conditions (35°C, RH 50%). For evaluation,
115 dried films were peeled from the plate surface and dried in a desiccator prior to the final
116 thickness being determined. For water vapor permeability measurement, films (three
117 replication with six films each) were conditioned at 27°C, RH 60% for 72 h prior to
118 measurement.

119 **2.3 Properties of rice starch- κ -car film**

120 **2.3.1 Thickness, water vapour permeability (WVP) and tensile properties**

121 Thickness of film was measured using a digital micro-meter (Mitutoyo, Co., Code No. 543-
122 551-1, Model ID-F125, 139 Japan; sensitivity= 0.001 mm). Ten measurements were taken
123 from random positions for each film samples and mean value calculated to analyse WVP and
124 optical properties. WVP was measured according to a previously reported method by Thakur
125 et al. (2016). Tensile strength (TS) and elongation at break (EAB) were determined using a
126 Texture Analyzer (LLOYD Instrument LTD, Fareham, UK). Preconditioned (60% RH) films
127 (15 x 40 mm) were placed in the tensile grip with initial grip distance 40 mm and 1 mm/s
128 crosshead speed. Ten samples from every single film preparation were studied for the
129 mechanical properties of the film.

130 **2.4 Fruit coating and design of experiment**

131 Mature plum fruit (*Prunus salicina*) without visual defects, were collected from a local
132 market (Central AVE. Shepparton East, NSW, Australia) and coated on the same day of
133 purchase. A randomized experimental design, comprising 60 homogeneous lots (based on
134 color and size) of 7 fruit each were assembled randomly. Four lots were used to measure the
135 fruit properties at harvest (0 day) and the remaining 56 lots were divided into two groups for
136 the following treatments in four replicates i.e., four lots (with 7 fruit per replication) from
137 each treatment were assessed on the sampling day for the different properties. Two
138 treatments, coated (rice starch- κ -carrageenan-FAEs) and control (without any coating) were
139 used in this experiment and treated accordingly. For coating: cooled emulsion (0.5mL) was
140 applied over the individual fruit using hand coating method ensuring the whole surface of the
141 fruit including calyx and epicalyx were coated uniformly. The coatings were then dried using
142 hair dryer placed at a distance of 60 cm from the fruit to avoid thermal damage. After drying,

143 the fruit were stored at 20°C/RH 55±5% and their quality monitored every third day to assess
144 the effectiveness of applied coating on physiological parameters.

145 **2.4.1 Measurement of plum ethylene and respiration rate**

146 Plum fruit, (n=4) from each replicate were sealed in a 0.5 L hermetic glass jar (2 fruit per jar,
147 selected randomly) with a septum and a lid for gas sampling after 2h. The jars were stored at
148 ambient temperature of 20°C and gas sampling was carried out using a needle probe through
149 the rubber septum. After 2h incubation, a sample of headspace gas was used to measure the
150 rate of CO₂ production. For ethylene measurement, 1 mL of gas sample was withdrawn from
151 the vessel and inserted into a gas chromatograph (Gow-Mac 580, Bridgewater NJ) fitted with
152 a 6' x 1/8'' activated alumina stainless steel carbowax silico steel 80/100 column and
153 equipped with a flame ionization detector. Nitrogen was used as the carrier gas for all
154 experiments. The injector, column and detector temperatures were set at 65°C, 85°C and 105
155 °C respectively. The ethylene production rate was expressed in μL C₂H₄/kg h and calculated
156 as.

$$157 \text{ Ethylene rate } (\mu \text{ L C}_2\text{H}_4/\text{kg h}) = C_2\text{H}_4 (\mu \text{L L}^{-1}) \times \text{volume (mL)} / \text{weight (kg)} \times \text{time (h)} \quad (1)$$

158 The respiration rate was determine by measuring CO₂ in 5 mL of gas sample withdrawn from
159 the vessel and injected into a using an ICA40 series low-volume gas analysis system
160 (International Controlled Atmosphere Ltd., Kent, UK). Respiration rate was calculated using
161 the following equation:

$$162 \text{ CO}_2 (\text{ml Kg}^{-1}\text{h}^{-1}) = (\text{CO}_2(\%) \times \text{volume (mL)}) / (\text{weight (kg)} \times \text{time (h)} \times 100) \quad (2)$$

163 **2.4.2 Measurement of plum firmness**

164 The flesh firmness of starch uncoated and coated plums was measured using HortPlus™
165 Penetrometer after 0 day, days 3, 6, 9, 12, 15, 18 and 21 days storage at 20 °C. The average

166 of two readings from each side of the fruit was recorded. For measuring the fruit firmness,
167 fruit skin (1 x 1cm) was peeled off using a sharp knife to expose the flesh from two ends one
168 opposite to each other. A 7 mm diameter stainless steel probe was inserted into the fruit and
169 corresponding values were recorded using computer software. The maximum penetration
170 force (N) was defined as the maximum force required pushing the probe into the plum
171 surface to a depth of 2 mm at a cross-head speed of 1 mm/s.

172 **2.4.3 Weight loss**

173 The weight loss (%) was determined by weighing the plum fruit before and after the storage
174 period

175 **2.4.5 Measurement of color change**

176 The color of the plum surface was determined by a Chroma meter CR-400 (Konica Minolta
177 Sensing Inc., Japan). The CIELAB software was employed to measure the L*, a*, and b*
178 values.

179 **2.4.6 TSS and TA**

180 For the assessment of total soluble solids (TSS) and titratable acidity (TA), fruit samples
181 were chopped into small pieces and squeezed until no more juice was released. TSS was
182 determined with a digital hand-held refractometer (Atago PAL-1, Japan). TA was determined
183 by titrating 5 mL of juice with 0.05 M NaOH to pH 8.2 using an automatic titrator (Mettler
184 Toledo T50, Switzerland) and the result was expressed as a percentage of malic acid.

185 **2.5 Surface morphology (SEM)**

186 Stand-alone film and fruit surface morphology were studied by using scanning electron
187 microscope (JEOL, JSM 6300 SEM, JEOL, and Tokyo, Japan). Film samples were stored in

188 desiccator for 1 week to ensure the dryness (theoretical RH in desiccator 0%). For fruit,
189 samples were freeze dried completely and stored in the desiccator prior to analysis. The
190 microscopic analysis of film and fruit was determined by mounting the sample pieces on the
191 copper stubs, gold coated and observed using an accelerating voltage of 10 kV under high
192 vacuum mode.

193 **2.6 Polyphenols determination**

194 **2.6.1 Determination of total polyphenolic content (TPC)**

195 A modified Folin-Ciocalteu method as described by (Bhuyan *et al.*, 2015) was used for the
196 determination of total polyphenolic content (TPC). Briefly, diluted juice sample (1 ml of fruit
197 juice in 50 mL of water) was mixed with 5 mL of Folin-Ciocalteu reagent and 4 mL of 7.5%
198 Na₂CO₃ solution. The mixture was left at room temperature for 1h to complete the reaction.
199 The optical absorbance was measured at 765nm using UV-spectrophotometer (Varian
200 Australia Pty. Ltd., Victoria, Australia). A calibration curve (R² 0.998) was constructed with
201 ten points using Gallic acid as a pure standard. The results expressed as gallic acid
202 equivalents (GAE) mg GAE mL⁻¹ of fresh fruit juice sample.

203 **2.6.2 Total flavonoids content (TFC)**

204 The total flavonoid content was measured by AlCl₃ colorimetric assay as described by Šamec
205 *et al.* (2016) with some modifications. Briefly, to 0.5 mL of diluted sample 2 mL of H₂O and
206 0.15 mL of 5% (w/v) NaNO₂ were added and left at RT for 6 minutes. Then, 0.15 mL of 10%
207 (w/v) AlCl₃ was added and left at RT for another 6 minutes. It was followed by the addition
208 of 2 mL of 4% (w/v) NaOH and 0.7 mL of H₂O with the final solution being mixed well and
209 left at RT for a further 15 minutes before the absorbance was measured at 510 nm using a UV

210 spectrophotometer. Rutin was used as the standard for a calibration curve (R^2 0.994) and the
211 results were expressed rutin equivalents (mg RUE mL⁻¹ of juice sample).

212 **2. 7 Determination of antioxidant capacity**

213 **2. 7.1 DPPH radical scavenging activity determination and cupric acid antioxidant** 214 **capacity (CUPRAC)**

215 The DPPH (1,1-diphenyl-2-2picrylhydrazyl) radical scavenging activity and the antioxidant
216 capacity of the plum fruit samples was determined using the assays described previously
217 (Bhuyan et al., 2015; Jatoi *et al.*, 2017). Briefly, 1 mL of 10mM CuCl₂.2H₂O was mixed with
218 1 mL of 7.5mM neocuproine solution and 1 M NH₄CH₃COO solution. Filtered juice sample
219 (0.5 mL) was added to the above solution and final volume was completed to 4.1 mL with
220 pure distilled water. The solution was let to stand at room temperature for 1.5h to achieve
221 equilibrium. Absorbance measurements of the resulting cuprous-neocuproine complex was
222 measured at 450 nm against a reagent blank. Trolox was used as standard and results
223 expressed as millimole Trolox equivalent (mg TE mL⁻¹ juice sample).

224 **2.8 Statistical analysis**

225 Analysis of variance (ANOVA) was performed on the test data by using the SPSS software
226 package, v. 24.0 for Windows (SPSS, Inc., Chicago, IL). Analyses of films samples were
227 carried out in triplicate. For the fruit quality assessment, fruit samples with four replications
228 including seven fruit per replications were used. Tukey's test was used to examine whether
229 the differences among the treatments were significant at $p < 0.05$.

230 3. Results and discussion

231

232 The evaluation of coating performance under in vitro conditions (on the fruit surface) is
233 necessary to assess their performance characteristics for future industrial applications. It is
234 however, equally important to understand the physical and chemical behavior of coating
235 formulations as standalone entities in order to be able to adapt to the commercial
236 requirements. Film thickness, tensile strength, adhesion and gas and moisture exchange
237 characteristics may affect the coating integrity during the prolonged storage of fresh
238 horticulture produce, therefore, films prepared from rice starch- ι -car-FAEs composite
239 material were analyzed for physical, mechanical and barrier properties.

240 The results showed that the final casted film has an average thickness of 0.07mm, tensile
241 strength 253.5 N/m², EAB 35 mm and WVP 2.8×10^{-11} gs⁻¹m⁻¹Pa⁻¹ respectively. Compared to
242 the properties of a standalone film, actual coating performance is affected by coating
243 distribution over the fruit surface for example whether it forms a continuous uniform layer
244 over the fruit surface (Fagundes *et al.*, 2015). Therefore, film morphology becomes more
245 important aspect of the analyses of film surface features. SEM images of the manufactured
246 films showed no solid granule remnants or aggregates within their structure, indicating high
247 miscibility of the formulation ingredients (Fig 1). A recent study by Huc *et al.* (2014)
248 reported favorable miscibility between polysaccharides and carrageenan molecules to result
249 from the formation of a strong networking complex arising from the incorporation of
250 carrageenan strands into the helical structures of amylose and amylopectin. RS- ι -car-FAEs
251 films showed smooth surfaces, free of defect (pores or cracks) and no sign of phase
252 separation. The smooth surface further reflects the stronger inter- and intra- molecular
253 interactions between the components. In summary, these morphological observations confirm

254 that rice starch- γ -car-FAEs composite combination resulted in a strong semi-permeable
255 membrane with a uniform distribution over the fruit surface.

256 **3.1 Analytical determinations**

257 **3.1.1 Weight loss (WL) (%)**

258 Moisture loss is an important aspect of storage and is driven by a difference in water vapour
259 pressure between the fruit surface and the environment (Brasil and Siddiqui, 2018). Rice- γ -
260 car-FAEs treatment employed in this study showed a significant impact on the weight loss of
261 plum fruit during the three weeks storage period (Fig 2). As expected, weight loss increased
262 during storage for both control and coated fruit. The control fruit showed higher weight loss
263 (1%) compared to coated fruit (<0.8%) during 21 days of storage. The reduction in the weight
264 loss in the coated fruit was attributed to the beneficial effect of the polysaccharides-based
265 edible coating, and has previously been demonstrated to be effective in a wide range of
266 commercial fruit including mango, pomegranate, pineapple and strawberry (Bierhals *et al.*,
267 2011; Chiumarelli *et al.*, 2010; García *et al.*, 2001). The complex network formed between
268 the starch-FAEs and starch with other ingredients retarded the mass loss in the plum fruit.
269 Loss of water vapour from the fruit surface is a natural aspect of fruit metabolic processes
270 that occur through the pores and cracks on skin. From the SEM micrographs (Fig 1) it is clear
271 that there were some cracks at the fruit surface in the uncoated fruit which might have
272 facilitated accelerated moisture and weight loss. In the coated fruit, the coating covers the
273 pores and cracks, thereby limiting transpiration while allowing gaseous exchange to continue
274 ($WVP = 2.8 \times 10^{-11} \text{ gs}^{-1}\text{m}^{-1}\text{Pa}^{-1}$). Loss of moisture from the control fruit surface can also be
275 explained as a poor function of cuticle wax layer, which might have lost its integrity during
276 washing and handling thus unmasking the skin pores at some areas. Respiration has also been
277 considered as an important factor behind the weight loss. The heat generated during the
278 respiration process leads to temperature elevation within the fruit which in turn increases

279 internal water vapor pressures leading to increased transpiration. Moreover, a strong
280 correlation (R^2 0.86%) exists between weight loss and respiration signifying that increased
281 respiration rate has contributed in the weight loss throughout the storage period.

282

283 **3.1.2 Ethylene production rate**

284 Endogenous ethylene production is a primary characteristic of ripening in climacteric fruit.
285 Fig 3 shows the rate of ethylene production for uncoated and coated fruit during the three
286 weeks storage period at ambient temperature (20°C). A significant increase ($p < 0.05$) in the
287 ethylene production was observed from the first week (from 0.03 to 9.76 $\mu\text{L C}_2\text{H}_2/\text{Kg/h}$)
288 which was 8.08 $\mu\text{L C}_2\text{H}_2/\text{Kg/h}$ higher than coated fruit at the end of storage. These effects
289 were similar to those obtained with other edible coatings (Martínez-Romero *et al.*, 2017; Pan
290 *et al.*, 2016). Biosynthesis of ethylene occurs as ripening progresses in the fruit and is
291 regulated by ripening enzyme 1-aminocyclopropane-1-carboxylic acid (ACC) synthase and
292 oxidase. ACC synthase convert the ACC into S-adenosyl-methionine (SAM) to ACC which
293 is subsequently converted to ethylene via the action of a second enzyme - ACC oxidase.
294 (Wills and Golding, 2016). The decreased levels of ethylene expressed by the coated fruit
295 signify that coating has provided an effective gas barrier between the fruit and the
296 surrounding atmosphere. The semi anaerobic conditions formed inside the fruit might have
297 decreased the catalytic activity of ACC oxidase thus the ethylene production was effectively
298 maintained by the coated fruit during the storage (Both *et al.*, 2016; Deng *et al.*, 2017).

299

300 **3.1.3 Firmness**

301 Flesh firmness is one of the most noticeable physical changes used to assess the quality of the
302 fresh produce and is closely aligned to the rate of water loss as well as metabolic changes
303 within the fruit including loss of membrane integrity, hydrolysis of cellulose and

304 hemicellulose as well as depolymerisation of pectin and starch (Mditshwa *et al.*, 2017). Flesh
305 firmness in the control fruit declined continuously during the storage period (Fig 4a),
306 decreasing from 2.25N (Day 3) to 0.05N (Day 21). Across the same storage period, firmness
307 in the coated fruit remained consistently greater than the control ($p<0.05$), indicating that the
308 starch composite coating had significant, beneficial impact on fruit quality. The semi-
309 permeability of membrane in the coated fruit restricted metabolic gas exchange (O_2 and CO_2)
310 through the coating barrier, resulting in a slowdown in metabolic activity including the
311 effectiveness of oxidizing enzymes leading to retention of firmness. The differential in the
312 rate of tissue softening between treated and control fruit was greatest during the third week of
313 storage and is consistent with previously findings by Tesfay and Magwaza (2017) and
314 Mahfoudhi and Hamdi (2015) who concluded that oxidizing enzymes (of polygalacturonase,
315 β -galactosidase and pectin methyl esterase) play a significant role in maintaining the firmness
316 of the coated fruit. The activity of these enzymes could be suppressed by the internal low O_2
317 concentration in case of the coated fruit. Another important parameter that affects the fruit
318 firmness is the loss of water during storage. Firmness results are supported by fruit weight
319 loss % which was higher in the control fruit (R^2 0.91) (Fig 4b). Similar results were observed
320 (Paniagua *et al.*, 2013) who found that fruit firmness and softening is influenced by
321 transpiration induced moisture loss. Water loss as a form of stress has the potential to elicit
322 senescence like response, which may also explain or contribute to the induced firmness
323 changes in these studies.

324

325 **3.1.4 Respiration rate**

326 Atmospheric gases, particularly O₂, serve as a crucial substrate of many biochemical
327 reactions in the fruit (Dongen and Licausi, 2015). The respiration rate for control and coated
328 fruit is presented in Fig 5 and shows that the treatment apparently suppressed the respiration
329 rate during storage. In general fruit metabolic process, higher the energy metabolism rate
330 (respiration), more quickly will be the consumption of nutrients and faster the ripening rate.
331 Differences in the respiration rate of the fruit reveal that coating was a sensitive indicator for
332 the gas exchange abilities of edible coating. Permeability of gases is a function of Fick's law
333 of diffusion and Henry's law of solubility and can be used to express the steady state
334 permeability of a permeate through a non-porous barrier of known thickness, hence the need to
335 design films critically with the thickness as low as possible (Thakur *et al.*, 2017). An
336 impermeable coating will prevent the fruit respiration process and cause anaerobic conditions
337 that leads to the accumulation of off-flavor volatiles (Arnon *et al.*, 2015). On the other hand,
338 a film with high permeability will not sufficiently modify the atmosphere to retard the
339 respiration (Baldwin *et al.*, 1999). Respiration rate was lower than control fruit throughout
340 the storage period however no statistical difference was observed until 18D ($p>0.05$). The
341 possible fluctuations in the respiration graph could be due to the fact that true equilibrium of
342 gases between system (fruit) and surrounding was hard to achieve since the fruit were
343 continuously ripening. It is interesting to note that control and coated fruit undergo an abrupt
344 increase during the 3rd week of storage from 24.99 to 45.09 mLCO₂Kg⁻¹h⁻¹ and 22.06 to
345 30.85 mLCO₂Kg⁻¹h⁻¹ and a significant difference was observed in the control and coated fruit
346 ($p<0.05$). The most possible reason for this trend could be the widening of stomatal pores due
347 to the rapid process of ripening leading the fruit to consume more oxygen. However, no such
348 information related to this event is available in the literature hence further study is
349 recommended to understand the behavior of plum respiration rate under the conditions

350 experimented in this experiment. In summary, the slow rate of fruit respiration combined
351 with relatively low concentration of CO₂ was observed due to the modified atmosphere
352 created by the coating over the fruit surface.

353

354 **3.1.5 SSC & TA**

355 Sugars represent a fundamental component of fruit edible quality, predominantly conferring
356 sweetness and importantly influencing the consumer satisfaction for plum fruit. Organic
357 acids, as a respiratory substrate, begin degrading as ripening progresses, resulting in
358 increased sugar loading (Kowalczyk *et al.*, 2017). As shown in the Fig 6, no significant
359 difference ($p>0.05$) between the SSC content of the control and coated fruit was observed
360 during the storage period, signifying no negative impact of the coating material. From the
361 titratable acidity results shown in Fig 6b, it could be seen that no significant difference
362 between the treated and control fruit was observed ($p>0.05$) however, there was an overall
363 decrease in TA values during 3 weeks of storage period. The decrease in TA during
364 postharvest storage of plums has been attributed to the use of organic acids as substrate for
365 the respiratory metabolism in the fruit (Valero *et al.*, 2013).

366

367 **3.1.6 Color**

368 In the process of ripening and senescence, plum fruit color changes from light red to dark red
369 due to the biosynthesis of anthocyanins. The variations in fruit skin color as represented by
370 the hue angle and shown in Fig 7. Starch coating delayed the synthesis of anthocyanin in
371 control and coated fruit without any significant differences between them ($p>0.05$). The
372 possible reason for the lower value of hue° in the coated fruit could be the suppressed
373 metabolic activities that ultimately led to the inhibition of anthocyanin synthesis. Similar

374 explanation has been provided by earlier by Valero et al. (2013) who reported that edible
375 coating delayed the color development in plum fruit.

376

377 **3.1.7 Total phenolic content**

378 Phenolic compounds are synthesized during maturation as secondary metabolites; however
379 they are also synthesized during the ripening of fruit (Andrade *et al.*, 2017). Table 1 shows
380 the phytochemical profile of coated and control fruit analyzed on different sampling time
381 stored for 21 days. From the data it is clear that concentrations of phenolic compounds
382 generally decreased with the storage time regardless of the treatment. However, starch
383 coating suppressed the decline in the phenolic content during storage. The concentration of
384 phenolic compounds for the uncoated plums was markedly reduced for first 6 d (1.14 mg
385 GAE. ml⁻¹ juice) showing lowest concentration of phenolic compounds among the fruit. The
386 decrease in the phenolic components at the end of storage could be due to the cell structural
387 breakdown as a part of senescence during storage. Similar explanation has been provided in
388 previous reported studies (Ghasemnezhad *et al.*, 2013; Nadim *et al.*, 2015) for decrease in
389 total phenolic content in the fruit. (Kim *et al.*, 2013) explained the activities of phenol
390 oxidase and peroxidase for the decrease in phenolic content for the plum fruit. However, the
391 concentration reaches to its higher content (1.74 mg GAE. ml⁻¹ juice) at the end of 12 d and
392 started declining when stored further. The phenolic content was higher in the coated plums
393 during the first and last week of storage however no statistical significant differences were
394 observed between control and coated fruit ($p>0.05$). The total flavonoids content of control
395 and coated fruit was between 16.98 to 27.09 mg RT. ml⁻¹ and 17.75 to 34.80 mg RT. ml⁻¹
396 juice respectively (Table 1). For coated fruit, flavonoid content was higher at the end of
397 storage period however no significant difference ($p>0.05$) was observed among the treated
398 and untreated fruit. These results signifies that suggests that modified atmosphere created by

399 edible coating has not promoted the biosynthesis of these secondary metabolites during
400 storage.

401 **3.1.8 The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity and reducing** 402 **power**

403 The DPPH scavenging activity of uncoated and coated fruit samples are shown in table 1.
404 Scavenging activity was reported to decline with ripening (Sivakumar *et al.*, 2012) and
405 similar behavior was observed in this study in the case of uncoated fruit. However, the
406 application of rice starch coating improved the retention of scavenging activity of plum fruit
407 stored at 20°C. A correlation between CUPRAC and TFC (R^2 0.86) at the 0.05% level was
408 observed during the storage study of plum fruit signifying that total antioxidant activity was
409 significantly influenced by the flavonoid content of the fruit ($p < 0.05$). On the contrary, no
410 significant influence was found between flavonoids and free radical scavenging activity
411 where a moderate correlation was observed ($p > 0.05$) and a moderate correlation between
412 TFC and DPPH (R^2 0.36, 0.05%). The phytochemical profile is different in other fruit as
413 reported by Kim *et al.* (2007) who found that scavenging activity was influenced by the
414 flavonoids content in the fruit.

415

416 **Conclusion**

417 Results presented in this study demonstrated that RS-t-car-FAEs delayed the increase in
418 respiration rate and inhibiting the ethylene production. Control fruit lost marketability within
419 two weeks of storage due to loss of firmness while coated plums remained firm with good
420 color for up to three weeks at room temperature for coated plums. The delay in ripening was
421 also reflected in accumulation of phytochemicals and the concentration of phenolics,

422 flavonoids was higher at the end of storage period. However, more future study is required to
423 elucidate the enzymatic mechanisms involved in the delay in ripening behavior of plum fruit.

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