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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R. Thakur [a*], P. Pristijono [a], J. B. Golding [a, c], C. E. Stathopoulos [b], C. J. Scarlett [a], M. Bowyer [a], S.P. Singh [a, c], Q. V. Vuong [a*]

[a] School of Environmental and Life Sciences, University of Newcastle, Ourimbah, NSW 2258, Australia

[b] Division of Food and Drink, School of Science, Engineering and Technology, University of Abertay, Dundee DD1 1HG, UK

[c] NSW Department of Primary Industries, Ourimbah, NSW 2258, Australia

***Correspondence to:**

R. Thakur

E mail: Rahul.thakur@uon.edu.au

School of Environmental and Life Sciences, Faculty of Science and Information Technology, University of Newcastle, Brush Road, Ourimbah, NSW 2258, Australia.

Q. V. Vuong

School of Environmental and Life Sciences, Faculty of Science and Information Technology, University of Newcastle, Brush Road, Ourimbah, NSW 2258, Australia.

Email: vanquan.vuong@newcastle.edu.au

Abstract

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

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

1. Introduction

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

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

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

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

2. Materials and methods

2.1 Materials

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

2.2 Preparation of film/coating solution

Based on the laboratory trials and preliminary study, the optimum volume required to coat the fruit was identified and used for subsequent coating experiment. Rice starch (3%, w/w), ι-car (1.5%, w/w), FAEs (2%%, w/w) and glycerol (1 %, w/w) were mixed in a two-step procedure. In the first step, starch solution (2%) was prepared by heating a starch-water mixture at 85 °C using a hot plate magnetic stirrer for 15 min. In the second step ι-car gelling solution was prepared by heating the ι-car-H_2O mixture at 80ºC for 20 min. until a clear transparent gel was formed. The solution from step 1 and step 2 were mixed together with a subsequent addition of FAEs and glycerol and stirred for a further 20 min.

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

2.3 Properties of rice starch-ι-car film

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

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

2.4 Fruit coating and design of experiment

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

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

2.4.1 Measurement of plum ethylene and respiration rate

Plum fruit, (n=4) from each replicate were sealed in a 0.5 L hermetic glass jar (2 fruit per jar, selected randomly) with a septum and a lid for gas sampling after 2h. The jars were stored at ambient temperature of 20°C and gas sampling was carried out using a needle probe through the rubber septum. After 2h incubation, a sample of headspace gas was used to measure the rate of CO_2 production. For ethylene measurement, 1 mL of gas sample was withdrawn from the vessel and inserted into a gas chromatograph (Gow-Mac 580, Bridgewater NJ) fitted with a 6' x 1/8''activated alumina stainless steel carbowax silico steel 80/100 column and equipped with a flame ionization detector. Nitrogen was used as the carrier gas for all experiments. The injector, column and detector temperatures were set at 65°C, 85°C and 105 °C respectively. The ethylene production rate was expressed in μL C_2H_4/kg h and calculated as.

Ethylene rate (μ L C_2H_4/kg h) = $C_2H_4\ (\mu L\ L^{-1}) x\ volume\ (mL)/weight\ (kg) x\ time\ (h)$ (1)

The respiration rate was determine by measuring CO_2 in 5 mL of gas sample withdrawn from the vessel and injected into a using an ICA40 series low-volume gas analysis system (International Controlled Atmosphere Ltd., Kent, UK). Respiration rate was calculated using the following equation:

CO_2 (ml $Kg^{-1}h^{-1}$) = $(CO_2(\%) x\ volume\ (mL)/(weight\ (kg) x\ time\ (h) x\ 100)$ (2)

2.4.2 Measurement of plum firmness

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

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

2.4.3 Weight loss

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

2.4.5 Measurement of color change

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

2.4.6 TSS and TA

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

2.5 Surface morphology (SEM)

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

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

2.6 Polyphenols determination

2.6.1 Determination of total polyphenolic content (TPC)

A modified Folin-Ciocalteu method as described by (Bhuyan *et al.*, 2015) was used for the determination of total polyphenolic content (TPC). Briefly, diluted juice sample (1 ml of fruit juice in 50 mL of water) was mixed with 5 mL of Folin-Ciocalteu reagent and 4 mL of 7.5% Na_2CO_3 solution. The mixture was left at room temperature for 1h to complete the reaction. The optical absorbance was measured at 765nm using UV-spectrophotometer (Varian Australia Pty. Ltd., Victoria, Australia). A calibration curve (R^2 0.998) was constructed with ten points using Gallic acid as a pure standard. The results expressed as gallic acid equivalents (GAE) mg GAE mL^{-1} of fresh fruit juice sample.

2.6.2 Total flavonoids content (TFC)

The total flavonoid content was measured by $AlCl_3$ colorimetric assay as described by Šamec *et al.* (2016) with some modifications. Briefly, to 0.5 mL of diluted sample 2 mL of H_2O and 0.15 mL of 5% (w/v) $NaNO_2$ were added and left at RT for 6 minutes. Then, 0.15 mL of 10% (w/v) $AlCl_3$ was added and left at RT for another 6 minutes. It was followed by the addition of 2 mL of 4% (w/v) NaOH and 0.7 mL of H_2O with the final solution being mixed well and left at RT for a further 15 minutes before the absorbance was measured at 510 nm using a UV

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

2. 7 Determination of antioxidant capacity

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

The DPPH (1,1-diphenyl-2-2picrylhydrazyl) radical scavenging activity and the antioxidant capacity of the plum fruit samples was determined using the assays described previously (Bhuyan et al., 2015; Jatoi *et al.*, 2017). Briefly, 1 mL of 10mM $CuCl_2.2H_2O$ was mixed with 1 mL of 7.5mM neocuproine solution and 1 M NH_4CH_3COO solution. Filtered juice sample (0.5 mL) was added to the above solution and final volume was completed to 4.1 mL with pure distilled water. The solution was let to stand at room temperature for 1.5h to achieve equilibrium. Absorbance measurements of the resulting cuprous-neocuproine complex was measured at 450 nm against a reagent blank. Trolox was used as standard and results expressed as millimole Trolox equivalent (mg TE mL^{-1} juice sample).

2.8 Statistical analysis

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

3. Results and discussion

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

The results showed that the final casted film has an average thickness of 0.07mm, tensile strength 253.5 N/m^2, EAB 35 mm and WVP 2.8 x 10^{-11} $gs^{-1}m^{-1}Pa^{-1}$ respectively. Compared to the properties of a standalone film, actual coating performance is affected by coating distribution over the fruit surface for example whether it forms a continuous uniform layer over the fruit surface (Fagundes *et al.*, 2015). Therefore, film morphology becomes more important aspect of the analyses of film surface features. SEM images of the manufactured films showed no solid granule remnants or aggregates within their structure, indicating high miscibility of the formulation ingredients (Fig 1). A recent study by Huc et al. (2014) reported favorable miscibility between polysaccharides and carrageenan molecules to result from the formation of a strong networking complex arising from the incorporation of carrageenan strands into the helical structures of amylose and amylopectin. RS-ι-car-FAEs films showed smooth surfaces, free of defect (pores or cracks) and no sign of phase separation. The smooth surface further reflects the stronger inter- and intra- molecular interactions between the components. In summary, these morphological observations confirm

254 that rice starch-ι-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-ι-
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 x 10^{-11} $gs^{-1}m^{-1}Pa^{-1}$). 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 handing 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

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

3.1.2 Ethylene production rate

Endogenous ethylene production is a primary characteristic of ripening in climacteric fruit. Fig 3 shows the rate of ethylene production for uncoated and coated fruit during the three weeks storage period at ambient temperature (20°C). A significant increase (p<0.05) in the ethylene production was observed from the first week (from 0.03 to 9.76 µL C_2H_2/Kg/h) which was 8.08 µL C_2H_2/Kg/h higher than coated fruit at the end of storage. These effects were similar to those obtained with other edible coatings (Martínez-Romero *et al.*, 2017; Pan et al., 2016). Biosynthesis of ethylene occurs as ripening progresses in the fruit and is regulated by ripening enzyme 1-aminocyclopropane-1-carboxylic acid (ACC) synthase and oxidase. ACC synthase convert the ACC into S-adenosyl-methionine (SAM) to ACC which is subsequently converted to ethylene via the action of a second enzyme - ACC oxidase. (Wills and Golding, 2016). The decreased levels of ethylene expressed by the coated fruit signify that coating has provided an effective gas barrier between the fruit and the surrounding atmosphere. The semi anaerobic conditions formed inside the fruit might have decreased the catalytic activity of ACC oxidase thus the ethylene production was effectively maintained by the coated fruit during the storage (Both *et al.*, 2016; Deng *et al.*, 2017).

3.1.3 Firmness

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

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

3.1.4 Respiration rate

Atmospheric gases, particularly O_2, serve as a crucial substrate of many biochemical reactions in the fruit (Dongen and Licausi, 2015). The respiration rate for control and coated fruit is presented in Fig 5 and shows that the treatment apparently suppressed the respiration rate during storage. In general fruit metabolic process, higher the energy metabolism rate (respiration), more quickly will be the consumption of nutrients and faster the ripening rate. Differences in the respiration rate of the fruit reveal that coating was a sensitive indicator for the gas exchange abilities of edible coating. Permeability of gases is a function of Fick's law of diffusion and Henry's law of solubility and can be used to express the steady state permeability of a permeate through a non-porous barrier of known thinness, hence the need to design films critically with the thickness as low as possible (Thakur *et al.*, 2017). An impermeable coating will prevent the fruit respiration process and cause anaerobic conditions that leads to the accumulation of off-flavor volatiles (Arnon *et al.*, 2015). On the other hand, a film with high permeability will not sufficiently modify the atmosphere to retard the respiration (Baldwin *et al.*, 1999). Respiration rate was lower than control fruit throughout the storage period however no statistical difference was observed until 18D ($p>0.05$). The possible fluctuations in the respiration graph could be due to the fact that true equilibrium of gases between system (fruit) and surrounding was hard to achieve since the fruit were continuously ripening. It is interesting to note that control and coated fruit undergo an abrupt increase during the 3rd week of storage from 24.99 to 45.09 $mLCO_2Kg^{-1}h^{-1}$ and 22.06 to 30.85 $mLCO_2Kg^{-1}h^{-1}$ and a significant difference was observed in the control and coated fruit ($p<0.05$). The most possible reason for this trend could be the widening of stomatal pores due to the rapid process of ripening leading the fruit to consume more oxygen. However, no such information related to this event is available in the literature hence further study is recommended to understand the behavior of plum respiration rate under the conditions

experimented in this experiment. In summary, the slow rate of fruit respiration combined with relatively low concentration of CO_2 was observed due to the modified atmosphere created by the coating over the fruit surface.

3.1.5 SSC & TA

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

3.1.6 Color

In the process of ripening and senescence, plum fruit color changes from light red to dark red due to the biosynthesis of anthocyanins. The variations in fruit skin color as represented by the hue angle and shown in Fig 7. Starch coating delayed the synthesis of anthocyanin in control and coated fruit without any significant differences between them (p>0.05). The possible reason for the lower value of hue^{o} in the coated fruit could be the suppressed 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

edible coating has not promoted the biosynthesis of theses secondary metabolites during storage.

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

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

Conclusion

Results presented in this study demonstrated that RS-ι-car-FAEs delayed the increase in respiration rate and inhibiting the ethylene production. Control fruit lost marketability within two weeks of storage due to loss of firmness while coated plums remained firm with good color for up to three weeks at room temperature for coated plums. The delay in ripening was 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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