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1 **Characterization of Rice Starch- ι-Carrageenan Biodegradable Edible Film. Effect of**
2 **Stearic Acid on the Film Properties.**

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23 **Abstract**

24 The main aim of this study was to develop rice starch (RS), ι -carrageenan (ι -car) based film.
25 Different formulations of RS (1-4%, w/w), ι -car (0.5-2%, w/w) was blended with stearic acid
26 (SA; 0.3-0.9%, w/w) and glycerol (1%, w/w) as a plasticizer. The effect of film ingredients on
27 the thickness, water vapour permeability (WVP), film solubility (FS), moisture content (MC),
28 colour, film opacity (FO), tensile strength (TS), elongation-at-break (EAB) of film was
29 examined. Interactions and miscibility of partaking components was studied by using Fourier
30 transform infrared spectroscopy (FT-IR) and X-ray diffraction (XRD). Hydrocolloid
31 suspension solution of mix polysaccharides imparted a significant impact ($p < 0.05$) on the
32 important attributes of resulting edible film. TS and EAB of film were improved significantly
33 ($p < 0.05$) when ι -car was increased in the film matrix. Formulation F1 comprising 2% ι -car, 2%
34 RS, 0.3% SA, Gly 30% w/w and 0.2% surfactant (tween®20) provided film with good
35 physical, mechanical and barrier properties. FT-IR and XRD results reveal that molecular
36 interactions between RS- ι -car have a great impact on the film properties confining the
37 compatibility and miscibility of mixed polysaccharide. Results of the study offers new
38 biodegradable formulation for application on fruit and vegetables.

39 **Keywords:** Biopolymers; Biodegradable; Edible film.

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46 **1. Introduction**

47 Edible film and coatings are the future of packaging industries. Ability of edible biopolymers
48 to reduce the serious environmental concerns by minimizing the usage of plastic packaging has
49 provided the solution for many packaging issues. Hence it is important to explore the potential
50 of natural biopolymers for the shelf life extension of fresh produce.

51 Starch is a complex polysaccharide biopolymer and a material of choice for the development
52 of edible films. Due to low cost and good film forming properties it is often used in the blending
53 combinations with other biopolymers to develop a strong film with improved properties.
54 Composite matrices with different blending combinations have been reported to have desirable
55 functional properties by combining the advantage of each component while overcoming their
56 potential limitations [1]. Combinations of polysaccharides with high molecular weight
57 compounds have provided excellent miscibility and interactions with improved mechanical
58 properties [2]. However, detailed study is required to understand the mechanism of interactions
59 influencing the physical, mechanical and barrier properties of film.

60 ι -carrageenan is water soluble biopolymers [3] extracted from certain species of red seaweed
61 of the family *Rhodophyceae*. The hydrophilic linear sulphated galactan constitutes alternating
62 galactopyranosyl units linked by β (1 \rightarrow 4) and α (1 \rightarrow 3) glycosidic bonds. Commercially ι -cars
63 are classified in to three different types: kappa (κ), iota (ι) and lambda (λ)- ι -carrageenan where
64 OSO_3^- group numbers configuration defines the rheological properties of these sulphated
65 compounds [4].

66 High flexibility and low WVP are the desired properties of edible films which are achieved by
67 using plasticizers. Glycerol is the most commonly studied plasticizer with starch based edible
68 coatings. [5]. However being hygroscopic, it affects the permeability properties of film while
69 providing it flexibility [6, 7]. Fatty acids, due to their hydrophilic and hydrophobic nature have

70 also been studied as plasticizers [8]. Hydrophobic substances (fatty acids, waxes and oils) in
71 combination with other components are helpful in controlling the permeability properties of
72 edible films. Stearic acid, palmitic acid, essential oils are most widely used plasticizers in the
73 edible coatings currently [9].

74 Other components with potential capacity to improve film properties are surfactants. These are
75 amphiphilic compounds used to improve the wettability and adhesion of film [10] and act as a
76 emulsifiers. The hydrophobic tail of a surfactant affect the mechanical properties of starch film
77 [11].

78 Improving the preservation efficiency of edible films by tailoring the mechanical and barrier
79 properties have been conducted by using different formulations of biopolymers-ingredients
80 combinations for instance, tapioca starch-protein [12], rice starch-lipid [13], pea starch-
81 cellulose [14], manioc starch-gelatine [15], and yam starch-cassava starch [16].

82 However, to the best of our knowledge no attempt has been made to study the characterisation
83 of edible film based on rice starch- ι -carrageenan formulations. The aim of this work was,
84 therefore, to develop rice starch, ι -car and steric acid based edible film, evaluate the effect of
85 interactions at different ratios of ingredients on the properties of developed film. Fourier
86 transform infrared spectroscopy (FT-IR) and X-ray diffraction (XRD) was used to highlight
87 the interactions and miscibility between polymer-polymer and polymer with other ingredients.

88 **2. Material and Methods**

89 *2.1 Materials*

90 Rice grains (*Oryza sativa*, cv Doongara) were obtained from Sunrice (Sun Rice, Leeton
91 Australia). HCl, NaOH were purchased from Merck Pty Ltd, Germany. Acetic acid, stearic
92 acid and Tween[®]20 were obtained from Sigma Aldrich, USA. Glycerol was from Ajax

93 Finechem Pty. Ltd, Australia and used as a plasticizer. The ι -car (*Chondrus crispus*) was
94 purchased from Melbourne Food Ingredient Depot, Australia.

95 *2.2 Extraction of starch*

96 Starch was extracted following a previously developed method [17] with some modifications.
97 Rice grains were steeped overnight and then grounded at low speed for 5 min using a multi
98 blender. The slurry was filtered through the muslin cloth and centrifuged at $2000 \times g$ for 10
99 min using Beckman coulter, Allegra X-15R centrifuge. The supernatant was collected and
100 washed five times with excess 0.1 M NaOH to completely remove any proteins. Resultant
101 slurry was mixed with water and filtered through 125 μ m and 63 μ m pore size sieves. The
102 mixture was dried at 50°C for 48 hrs and finally ground into powder and stored at a dry place
103 for the further experiments.

104 *2.2.1 Physiological analysis of starch*

105 Content of proteins, moisture, ash and fat of the rice starch was determined according to AOAC
106 methods AOAC (1990). Content of amylose in the rice starch was analysed using iodine
107 colorimetric reaction as reported previously [18].

108 *2.3 Polysaccharide suspension solution*

109 Polysaccharide suspension solution was prepared from rice starch, ι -car, fatty acid, glycerol
110 and tween[®]20 as shown in Table 1. Gelatinization of rice starch was obtained by mixing rice
111 starch (2-4%) with water at 85°C for 15 min. The ι -car (0.5-2%) was added and heated at 80°C
112 to a clear solution. Starch- ι -car mixture was then mixed and stirred with melted stearic acid
113 (0.3-0.9%) and 0.2% of tween[®]20. Finally, glycerol was added and stirred for 15 min. To
114 prepare the film, 20 ml of mixture solution was poured in to petri plates and dried in oven for
115 24 hrs at 35°C. Films were peeled off and conditioned at 27°C, relative humidity (RH) 60% for
116 72 hrs prior to testing.

117 *2.4 Swelling power (SP) and leached amylose (LA)*

118 SP and LA was determined by previously developed method [19] with some modifications.
119 Starch suspension solutions in different ratios (RS: 1-car -2:2%, 2.5:1.5%, 3:1%,4:0.5%, pure-
120 4 % RS) were heated in water bath at 90°C for 5-30 min. Suspension solution was cooled in
121 ice before centrifugation at 15000 rpm for 30 min. Supernatant was used for LA determination
122 using iodine colorimetric method [20]. LA was calculated by dividing the amylose content in
123 supernatant to original weight of starch. Residue was weighed and dried at 110°C for 24 hrs
124 for SP determination and calculated by ratio of wet weight of residue to its dry weight.

125 *2.5 Film Characterisation*

126 *2.5.1 Physical Properties*

127 *2.5.1.1 Film thickness (FT)*

128 FT was measured using a digital micro-meter (Mitutoyo, Co., Code No. 543-551-1, Model ID-
129 F125, 139 Japan; sensitivity= 0.001 mm). Measurements were taken randomly from 10
130 different points for individual film samples and average value was calculated. Results from
131 thickness measurement were used to assess the water vapour permeability and opacity of edible
132 film.

133 *2.5.1.2 Film solubility (FS)*

134 FS was measured according to previously reported method [21] with some modifications. Film
135 strips (15 x 50 mm) were placed in 50 ml of distilled water with subsequently shaking at 30
136 rpm for 24 hrs at room temperature. Undissolved portion was then collected and dried at 110°C
137 for 24 hrs to a constant weight. FS was determined according to Eq. 1.

138
$$FS(\%) = \frac{S_{(i)} - S_{(f)}}{S_{(i)}} \times 100 \quad (1)$$

139 $S_{(i)}$ = initial weight of film sample. $S_{(f)}$ = weight of film sample after drying.

140 2.5.1.3 Film Moisture content (MC)

141 MC of the film samples (1.5 x 4.0 cm) was determined gravimetrically by measuring water
142 removed from the initial mass. The film samples were dried at 110°C for 24 hrs to attain a
143 constant weight. MC of the films was calculated according to Eq. 2.

$$144 \quad FMC (\%) = \frac{MC_{(i)} - MC_{(f)}}{MC_{(i)}} \times 100 \quad (2)$$

145 $MC_{(i)}$ = initial weight of film sample. $MC_{(f)}$ = weight of film sample after drying

146 2.5.2 Barrier Properties

147 2.5.2.1 Water vapour permeability (WVP)

148 WVP was determined using gravimetric method, ASTM E96 procedure [22], as described in
149 previous study [21]. Briefly, permeation cells (cup containing anhydrous CaCl₂ granules with
150 0% RH) were sealed tightly by the sample film and placed under controlled RH conditions
151 (NaCl saturated solution; 75 % RH) at 25°C. Water vapour transport was determined using the
152 weight gain of the cell at a steady state of transfer. Changes in the weight of the cell were
153 recorded and plotted as a function of time. The slope of each line was evaluated by linear
154 regression ($R^2 > 0.99$), and the water vapour transmission was calculated through the slope of
155 the straight line (g/s) divided by the test area (m²). After the permeation tests, the film thickness
156 was measured and WVP (g Pa⁻¹s⁻¹m⁻¹) was calculated as:

$$157 \quad WVP = \frac{\Delta m}{A \Delta t} \frac{X}{\Delta P} \quad (3)$$

158 $\Delta m/\Delta t$ = weight of moisture gain per unit time (gs^{-1}) and can be calculated by the slope of the
159 graph. A= area of the exposed film surface (m^2), T = thickness of the film (mm), ΔP = represents
160 the water vapour pressure difference inside and outside of the film (Pa) [23].

161 2.5.3 Optical properties

162 2.5.3.1 Film opacity

163 FO measurements were performed according to previously described method [24] with some
164 modifications. Rectangular film samples were placed in the cuvette loaded to the
165 spectrophotometer (Cary 50 Bio UV-Visible spectrophotometer) and a blank cuvette was used
166 as a reference. Absorbance was recorded at 560 nm. Low value of opacity corresponds to high
167 transparency. Opacity was calculated using Eq. 4.

$$168 \quad O = \text{Abs}_{560}/x \quad (4)$$

169 O = opacity of the film, Abs_{560} = Absorbance of the film at 560 nm, x = thickness of the film
170 in mm.

171 2.5.3.2 Colour measurement

172 Film colour was determined by using Minolta colorimeter (Minolta CR-300 Japan). The colour
173 profile was expressed as L=100 (white) to L=0 (black), -a = (greenness) to +a = (redness) and
174 -b = (blueness) to +b = (yellowness). Total colour difference was calculated by the equation
175 given below [25]. Ten readings of three different replicates were recorded and mean was
176 calculated.

$$177 \quad \Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b^2)]^{1/2} \quad (5)$$

178 Where, ΔL , Δa and Δb are changes in the values of colour parameters.

179 2.5.4 Mechanical properties

180 *2.5.4.1 Tensile strength (TS) and elongation at break (EAB)*

181 Tensile strength (TS) and elongation at break (EAB) were determined by Texture Analyzer
182 (LLOYD Instrument LTD, Fareham, UK) using previously developed method [21].
183 Preconditioned (60% RH) films (15 x 40 mm) were placed in the tensile grip with initial grip
184 distance 40 mm and 1 mm/s crosshead speed. Eight samples from every formulation were
185 studied for the mechanical properties of the film. TS and EAB were expressed in MPa and
186 N/mm.

187 *2.5.5 Characterization of bio composite*

188 *2.5.5.1 Fourier transform infrared spectroscopy (FT-IR)*

189 FTIR spectra of starch- ι -car films were studied using infrared spectrometer (Thermo scientific
190 Nicolet iS10 FT-IR, USA). Spectral measurements were performed in the absorbance mode.
191 Each spectrum was recorded at the range of 400-4000 cm^{-1} with complete 32 scans at a
192 resolution of 4 cm^{-1} [2].

193 *2.5.5.2 X- ray diffraction*

194 X-ray diffraction pattern of film compositions was obtained by using x-ray diffractometer
195 (PANanalytical, X'pert PRO Multi-purpose X-ray diffractometer, Almelo, Netherland) under
196 the following instrumental conditions: 40 mA, 40 kV, angle 2θ : 5.0° and 59.9°, step size =
197 0.0130°, using $K\alpha/K\text{cu}$ radiations ($\lambda = 1.54/1.39 \text{ \AA}$). X-ray diffraction pattern for rice starch,
198 ι -car and stearic acid was also analyzed to understand the crystalline behavior of the individual
199 compounds.

200 *2.5.6 Statistical analysis*

201 Statistical analysis was performed using SPSS 23.0.0 statistical software for windows (SPSS
202 IBM, USA). One way ANOVA was used to analyse the data. The mean values were evaluated
203 using Tukey's multiple comparison test and Duncan test with $p < 0.05$ denoted statistical
204 significance.

205

206 **3. Results and discussion**

207 *3.1 Content of rice starch*

208 Amylose, proteins, ash, fat and moisture of starch was reported to affect the properties of starch
209 [26]. Starch extracted from rice showed amylose 11.33%, lipid 0.01%, protein 0.71%, moisture
210 11.64% and ash 4.9% respectively.

211 *3.2 Swelling power (SP) and Leached amylose (LA)*

212 SP is the indication of water absorption index of granules. Variations in the SP and LA are
213 shown in the Fig. 1. SP of the RS- ι -car blend increased with decrease in the starch concentration
214 and varies between 9.0-17.75 g/g and significantly affected by ι -car concentration ($p < 0.05$).
215 High concentration of starch inhibited the swelling of starch granules due to increased amylose
216 content. High absorption capacity of ι -car and low amylose content provided the formulation
217 F1 with high swelling power which decreases subsequently with increase in starch
218 concentration from F1-F4. The reason could be explained on the basis inhibition effect of
219 amylose on swelling capacity. Similar explanation is provided in the previous study based on
220 composite starch combinations [19, 26]. Furthermore, high absorption of ι -car with rice starch
221 could minimizes the chances of phase separation which thus improving the permeability and
222 mechanical properties of the film.

223 Simultaneously it is important to know the optimum time-temperature combination for
224 effective gelatinization of starch in combination with other blending material. Starch reaches

225 to its gelatinized temperature in between 80-90°C and similar range has been reported to be
226 suitable for ι-car to form a gelling mixture [19]. Findings of present study revealed that time
227 has a significant impact ($p < 0.05$) on the gelatinization mechanism as it reflects the amount of
228 leached amylose (apparent amylose). At low time-temperature combination (5min/90°C) the
229 amount of leached amylose was low (0.7-4.5 g/g) however it increased significantly when
230 formulations (F1-F4) were heated continuously for 10 minutes. It is important to note that
231 further increase in heating time reduces the amount of leached amylose content. Reason can be
232 explained on the basis of partial gelatinization in which leached out amylose prevent the further
233 amylose from leaching out. Thus the availability of free amylose reduces for interaction with
234 the carrageenan and affects the mechanical and barrier attributes of film [27].

235 *3.3 FTIR spectra*

236 Infrared spectroscopy is a powerful tool widely used for the characterization of polysaccharides
237 to study the molecular interactions characterised by the shifting of absorption bands [28].
238 Influence of different ingredient ratios on shifting of IR characteristic bands is shown in Fig.
239 2. Changes in the absorption bands stretching indicates the possible structural interactions
240 between the partaking components in the coating matrix [29]. FT-IR spectra of composite film
241 showed some common characteristics absorption bands at different wave numbers. 2851cm^{-1} -
242 3050cm^{-1} represents the C-H stretching vibrations. 2912cm^{-1} corresponds to -OH functional
243 group formed due to the vibrational stretching associated with free inter and intra molecular
244 bonds in -OH groups. The assignment of these bands are in line with previous reported results
245 with konjac-glucomannan/curdlan [30], k-carrageenan and locust bean [31], carboxymethyl-
246 gellan and pullulan [2]. It is also important to mention about smaller bands at the wavenumber
247 ranges from $700\text{-}1000\text{cm}^{-1}$ representing the fingerprinting region denoting the characteristic
248 signatures of each polysaccharide. Spectral region between $800\text{-}950\text{cm}^{-1}$ corresponds to C-O-
249 SO_3 on C_2 of 3,6-anhydrogalactosen, C-O- SO_3 on C_4 of galactose, C-O- SO_3 on C_2 of 3,6-

250 anhydrogalactose, C–O of 3,6-anhydrogalactose respectively [32]. Similar bands were
251 observed in the RS- ι -car spectra at wavenumbers 740 cm^{-1} (3, 6-anhydro-D-galactose), 800 cm^{-1}
252 1 representing anomeric configuration (α -D-galactopyranose unit). The wavenumbers, 852 cm^{-1}
253 1 (galactose-4-sulphate) and 933 cm^{-1} (3, 6- anhydro galactose or 3, 6- anhydro-galactose-2-
254 sulphate) attributed to the presence of ester sulphate groups which are the characteristics of
255 sulphated polysaccharide compounds. Film formulation F1-F4 showed bands stretching at
256 $1200\text{-}1300\text{ cm}^{-1}$, shifting of bands in this region is due to interactions between the charged
257 functional groups between the polysaccharides. The additional major peak at 1652 cm^{-1}
258 observed in the infrared spectra of RS- ι -car film could be assigned for water. Formation of
259 inter- molecular interactions in RS- ι -car film influences the interactions and miscibility
260 between these two polysaccharides can be observed in the stretched regions. Finding of this
261 work are in line with previous work based on the characterization of k-car-LBG film [31, 33].

262 3.4 X- ray diffraction

263 A semicrystalline pattern of blended film compared with pure ingredients is shown in the Fig.
264 3. XRD curve shows broad and sharp peaks comprising amorphous and crystalline region
265 respectively. Amorphous-crystalline structural transitions induced as a result of molecular
266 interactions between polysaccharides are responsible for their varying functional properties and
267 favours the amorphous structure [31]. A typical A-type polymorphic form was observed for
268 pure rice starch with unresolved peaks ($2\theta = 17^\circ$ & 18°) and separate peaks ($2\theta = 15^\circ$ & 23°). The
269 XRD pattern is similar to the previously reported results conducted on native and modified rice
270 starch [34]. Pattern showed some small peaks in the region $2\theta = 7^\circ\text{-}15^\circ$ and two broad peaks at
271 $2\theta = 22^\circ$ & 24° probably due to the presence of stearic acid crystallites. From the XRD profile
272 it is clear that miscibility of blended formulation (F1-F4) affected by the varying concentration
273 of ingredients. As it could be seen from the XRD profile that intensity of crystallite peaks

274 decreases with the increase in ι -car concentration from F4 (0.50%) to F1 (2%), indicating that
275 crystallinity of RS- ι -car-stearic acid films were strongly influenced by the presence of
276 carrageenan. The interaction mechanism between starch-carrageenan may be correlated to the
277 adsorption of carrageenan molecules on starch surface which destroy the crystalline domain of
278 the biopolymers [35]. The possible interactions between these moieties which has led to the
279 broadening of peak area, is a good indicator of miscibility between the partaking ingredients.

280 Furthermore, it is worth noting that sharp peaks at $2\theta \sim 22^\circ$ & 24° varied with stearic acid
281 content and higher for F4 formulation (0.9%) than F1 formulation (0.3%). The possible reason
282 could be due to the agglomeration of stearic acid content over the film surface after drying
283 signifies that there exists some phase separation between two biopolymers. Similar behaviour
284 regarding the accumulation of stearic acid crystals (lipid agglomeration) on the cassava starch
285 based film was also observed in previously reported by Chiumarelli and Hubinger [6].

286 In conclusion, results of XRD study explained the compatibility of starch and carrageenan in
287 combination with other components however, accurate concentrations of stearic acid is
288 necessary to improve the barrier properties of film while maintaining the other important
289 attributes.

290 *3.5 Thickness (FT)*

291 FT greatly influences the WVP and transparency of the film. Precise and accurate measurement
292 of this parameter is really important to avoid the effect to barrier properties. Thickness of rice
293 starch- ι -car films varied between 0.084 and 0.114 mm respectively. FT corresponding to
294 different formulations is summarized in Fig. 4a. Formulation F1, comprising 2% rice starch
295 and 2% ι -car with 0.3% stearic acid gives a film of minimum thickness. Increase in starch
296 concentration from the formulation F1-F4 made it possible to have a significant impact on the

297 film thickness ($p < 0.05$). Similar results were reported in the previous study where starch
298 concentration had a profound effect on the thickness of biopolymer film [36].

299 *3.6 Moisture content (MC)*

300 Interactions between the hydrocolloids affect the film affinity for water. Table 2 describes the
301 variations in the MC of composed film. Polysaccharides-lipid ratio significantly affect ($p < 0.05$)
302 the moisture content of film. To the formulation F1, higher concentration of galactans (2%)
303 minimizes the moisture content of edible film (8.48%). MC was maximum at 3% starch, 1% ι -
304 car and 0.7% stearic acid. Results of this study are consistent with the previously reported data
305 where increased concentrations of ι -car lower the moisture content of the edible film [31]. The
306 most probable reason for this behaviour could be due to the lack of interactions sites, possibly
307 because of entrapment of starch molecules in the ι -car double helices structure. Another
308 possible reason for this trend could be due to the crosslinking interactions between the
309 polymers. The availability of free OH groups diminishes as a result of anionic sulphate and
310 starch -OH group interactions at higher galactan concentration. Moreover, as the availability
311 of OH group increases polysaccharide – water interaction dominated accordingly. Similar
312 explanation was provided in the previous study based on galactans [37]. It is worth noting that
313 the further increase in stearic acid (0.7-0.9%) and starch concentration (3-4%) resulted to the
314 declines in film moisture content ($p < 0.05$). This could be due to increased fatty acid content
315 which increased the hydrophobicity of the film matrix which affect the moisture content of
316 film.

317 *3.7 Film solubility*

318 Solubility ranged from 43.35% to 63.22% which are good values for fruit applications (Fig.
319 5b). Solubility values were affected significantly ($p < 0.05$) with the increase in ι -car
320 concentration. Formulation F1 (2% rice starch: 2% ι -car: 0.3% stearic acid) showed the

321 maximum solubility value (63.22%) which decreases gradually from formulation F1 to F4. ι -
322 car undergoes structural modifications (coil to double helix) when combines with excess water
323 [38]. Moreover, these structural transitions were largely dependent on the content of ester
324 sulphate, type of carrageenan, temperature and concentration [39]. Solubility was lowest
325 ($p < 0.05$) at formulation F4 consisting of 0.5% ι -car with high starch (4%) and fatty acids
326 (0.9%) concentrations. Most probable reason associated with this trend could be due to increase
327 in the lipid content of film suspension solution from formulation F1-F4. The findings of this
328 study are in line with the previous study where increased concentration of lipids affected the
329 solubility of edible film [40].

330 *3.8 Water vapour permeability (WVP)*

331 Minimum WVP of edible films describes the better barrier properties. Polysaccharide based
332 edible coating including ι -car has low permeability values [41]. Film formulation F1,
333 containing 2% rice starch and 2% ι -car with 0.3% stearic acid showed the minimum WVP (3.55
334 $\times 10^{-11} \text{ gs}^{-1}\text{m}^{-1}\text{Pa}^{-1}$) values ($p < 0.05$) as compared to formulation F2 ($4.22 \times 10^{-11} \text{ gs}^{-1}\text{m}^{-1}\text{Pa}^{-1}$)
335 and F3 ($4.57 \times 10^{-11} \text{ gs}^{-1}\text{m}^{-1}\text{Pa}^{-1}$). Formulation F1 and F4 presented no significant statistical
336 difference ($p > 0.05$) showing better WVP values, however it was found to be statistically
337 different ($p < 0.05$) with F1 & F3 formulations (Fig.4b). Similar results were reported in the
338 previous study where WVP value substantially decreased as the concentration of ι -car was
339 increased from 0 to 100% [31]. The differences in the permeability within the formulations
340 may be due to the interactions between the film components. ι -car acts as a protector for starch
341 granules [42]. Double helical structure of carrageenan acts as a protector for the starch molecule
342 and trap it in the coiled structure (graphical abstract). Strong networking interactions with rice
343 starch gives a compact crystalline structure which strengthens the polymeric chain integrity of
344 film. Interactions study between starch- ι -car complexes using confocal scanning laser
345 microscopy provided the similar explanation for such type of behaviour of these

346 polysaccharides [35] however, comprehensive detail future study is required to understand the
347 mechanism thoroughly.

348 It is also worth mentioning that in spite of lowering the level of ι -car (0.5%), WVP
349 unexpectedly decreased at F4 with higher level of lipid content. Similar behaviour was
350 observed in the cassava starch based edible films using lipid as a hydrophobic component
351 where the rate of permeate transfer was higher at lower concentration of oleic acid and
352 decreased sequentially. [6]. This could be due to the hydrophilic-hydrophobic ratio in the film
353 structure. Fatty acid in film matrix imparts the hydrophobic character resulting in to
354 morphological alterations in the film structure [43, 44]. At low fatty acid concentration water
355 molecules permeate the hydrophilic matrix through a straight path however permeation
356 becomes difficult at high fatty acid concentration with dominated hydrophobicity of film.
357 Moreover, Low rate of permeate migration at higher starch concentration could also be
358 explained on the basis of thickness of film. Thickness of film increases ($p < 0.05$) with the
359 increase in starch concentration (2-4%) and was maximum at F4 (0.114 mm). Thickness of the
360 film matrix is straightly related to starch concentration which describes the increasing solid
361 density of film matrix and promoted the resistance to the water vapour permeability [45].

362 *3.9 Opacity and colour*

363 Opacity of film ranged from 0.5 to 1.0% (Table 2). Lowest opacity value corresponds to high
364 transparency which is prerequisite for the fruit packaging. Minimum and maximum opacity
365 values were recorded for F1 and F4 respectively ($p < 0.05$). ι -car also forms a transparent gel
366 solution on heating and gives less opacity than starch solution [43]. The RS- ι -car mixture was
367 clear for the formulation F1 and becomes turbid with the increase in starch (2-4%) and fatty
368 acid (0.3-0.9%) concentration. Results are in line with the previous study where fatty acids
369 provided the less transparency to the resulting film [46]. In term of colour there exists no

370 statistical differences in the ΔE value of F1, F2 and F4 formulations however ΔE value was
371 slightly higher in case of F3 which may be probably due to higher MC (Table 2). More MC
372 might affect the reflection pattern of light passing through the film surface thus increasing the
373 b^* value (yellow-blue shade). Similar behaviour was reported in the previous study where
374 presence of moisture affected the colour properties of film [31].

375 *3.10 Mechanical properties-TS and EAB*

376 Tensile strength describes the maximum stress developed on the film material. TS and EAB
377 values of different blends (F1-F4) of starch- ι -car film are presented in Fig. 6 (a, b) with
378 significantly different values ($p < 0.05$). Formulation F1 (2% ι -car) presented the high tensile
379 strength (116.5 N/m²) which has contributed to the formation of stronger structure. Mechanical
380 properties gradually decreased with the lowering in galactan concentration (0.5%) and was
381 minimum (31.6 N/m²) at F4 with 0.5% ι -car and 0.9% stearic acid. Finding of this study are
382 similar to previous results where mechanical properties of the film improved with the escalation
383 in ι -car concentration [19] and decreased with the increase in fatty acid concentration [47]. It
384 is interesting to note that EAB for F1 was surprisingly higher (45.60 mm) than other
385 formulations and follow the same trend as TS ($p < 0.05$). EAB for formulations F1-F4 ranged
386 from (15.7 to 45.60 mm). Formulation F4 showed the lowest value of TS and EAB ($p < 0.05$).
387 This trend finds the possible explanation on the basis of interactions and compatibility of rice
388 starch with ι -car. The ι -car helices associations in the film matrix presented a more compact
389 matrix when sample reaches to its gelation temperature and forms 3D structure as a result of
390 chain pairing with the adjacent ι -car and rice starch. Similar explanation is provided in the
391 previous study about the interaction of these biopolymers [39].

392 **Conclusion**

393 RS- ι -car blends provided the film with different varying properties. Coating formulation F1
394 composed of 2% rice starch and 2% ι -car concentration provided the film with minimum
395 thickness, better solubility, WVP and enhanced mechanical properties. However selection and
396 concentration of fatty acid is important to control the harm to film properties. FT-IR, XRD
397 study reveals that there exists compatibility and miscibility between the biopolymers and can
398 be a suitable alternative for fruits coatings applications with accurate addition of hydrophobic
399 component. Results of this study indicates that increased concentration of carrageenan with
400 low stearic acid is effective in improving the physical, mechanical and barrier properties of
401 film. However further study involving interactions between starch and carrageenan along with
402 different fatty acids will be of great interest.

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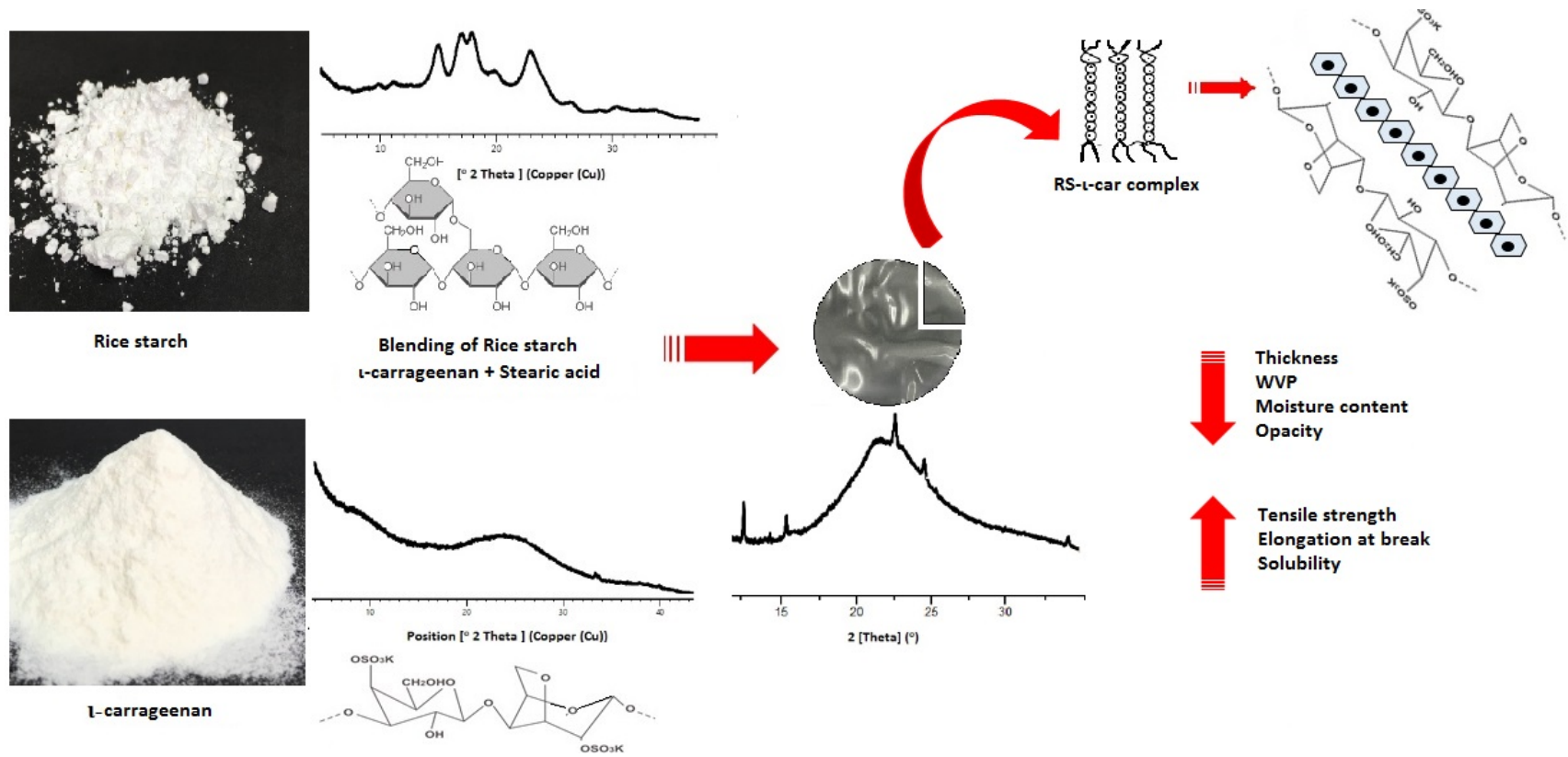
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Graphical Abstract



Graphical representation of mechanism of interaction between rice starch-carrageenan and stearic acid.

Captions

Fig. 1. Swelling power and leached amylose content of RS- ι -car formulations (F1-F4) at 90°C.

Fig. 2. FT-IR spectra of rice starch- ι -car film samples (F1-F4) in the region 400-3050 cm^{-1} .

Fig. 3. X-ray diffraction pattern of film matrix. (a) ι -carrageenan, (b) stearic acid, (c) rice starch, (d) RS- ι -car-stearic acid blended film formulation F1-F4. Where F1=RS/ ι -car/SA (2%, 2%,0.3%), F2=(2.5%,1.5%,0.5%), F3=(3%,1%,0.5%), F4=(4%,0.5%,0.7%).

Fig. 4. Thickness (a), WVP (b) of rice starch- ι -car film blended with fatty acid, glycerol and surfactant. For (b) Values are multiplied by 1×10^{-11} . Values denotes the means of replicates \pm Standard error. ^{a-c} represents the difference between mean values.

Fig. 5. Opacity (a), Solubility (b) of rice starch- ι -car film blended with fatty acid, glycerol and surfactant. Values denotes the means of replicates \pm Standard error. ^{a-c} represents the difference between mean values.

Fig. 6. Change in (a) Tensile strength (TS), (b) Elongation at break (EAB) of rice starch- ι -car film blended with fatty acid, glycerol and surfactant. Values denotes the means of replicates \pm Standard error. ^{a-c} represents the difference between mean values. The values with the same letter are not significantly different according to Duncan's multiple range test ($p > 0.05$).

Graphical abstract: Graphical representation of mechanism of interaction between rice starch-carrageenan and stearic acid.

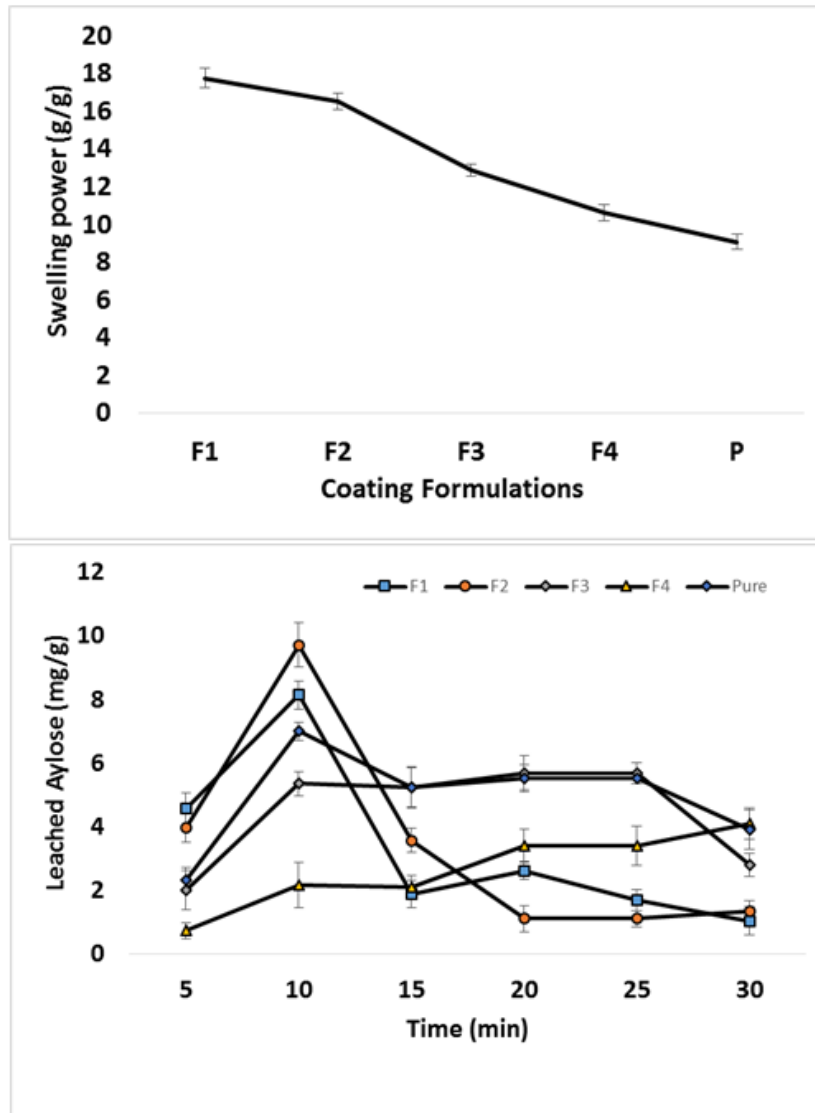


Fig. 1

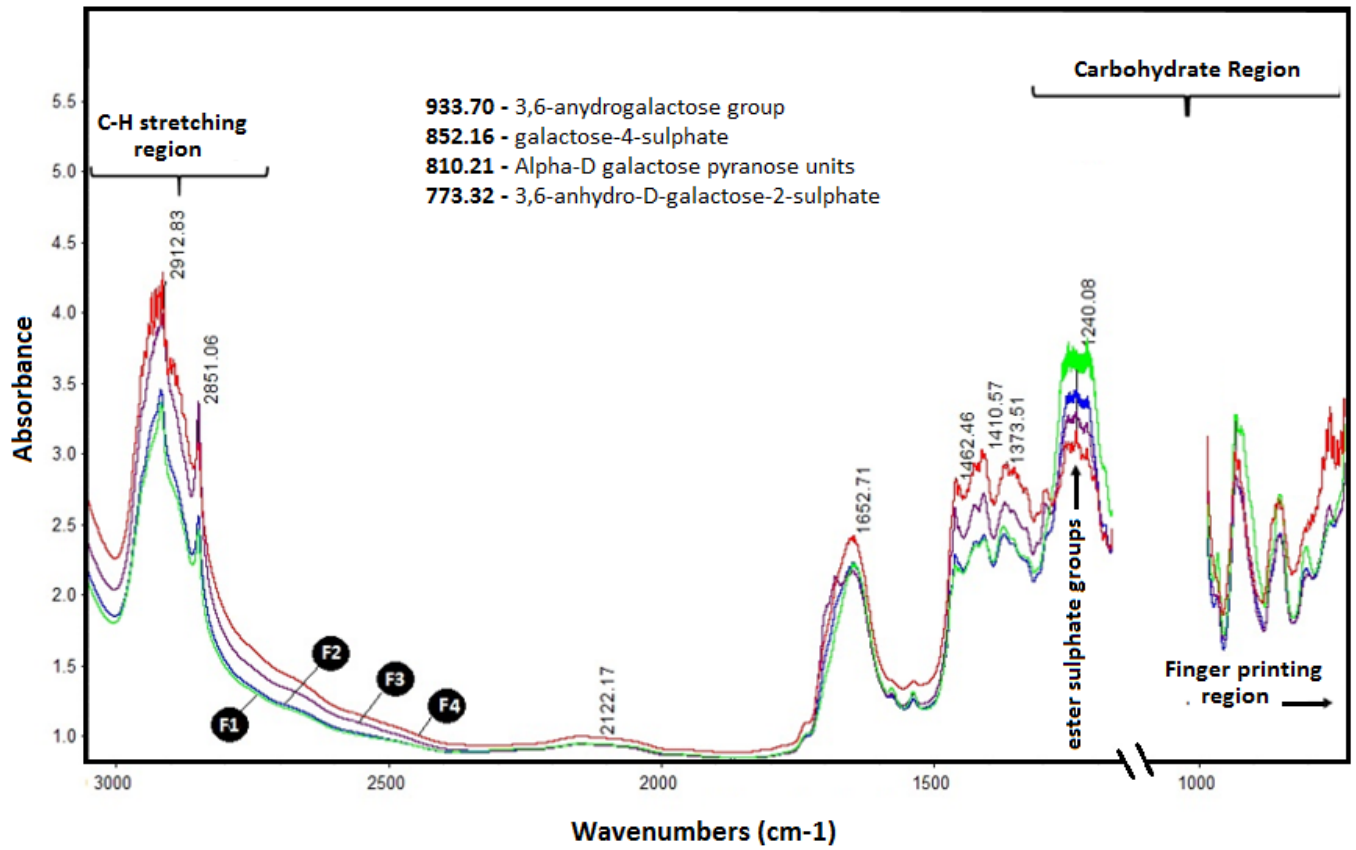


Fig. 2

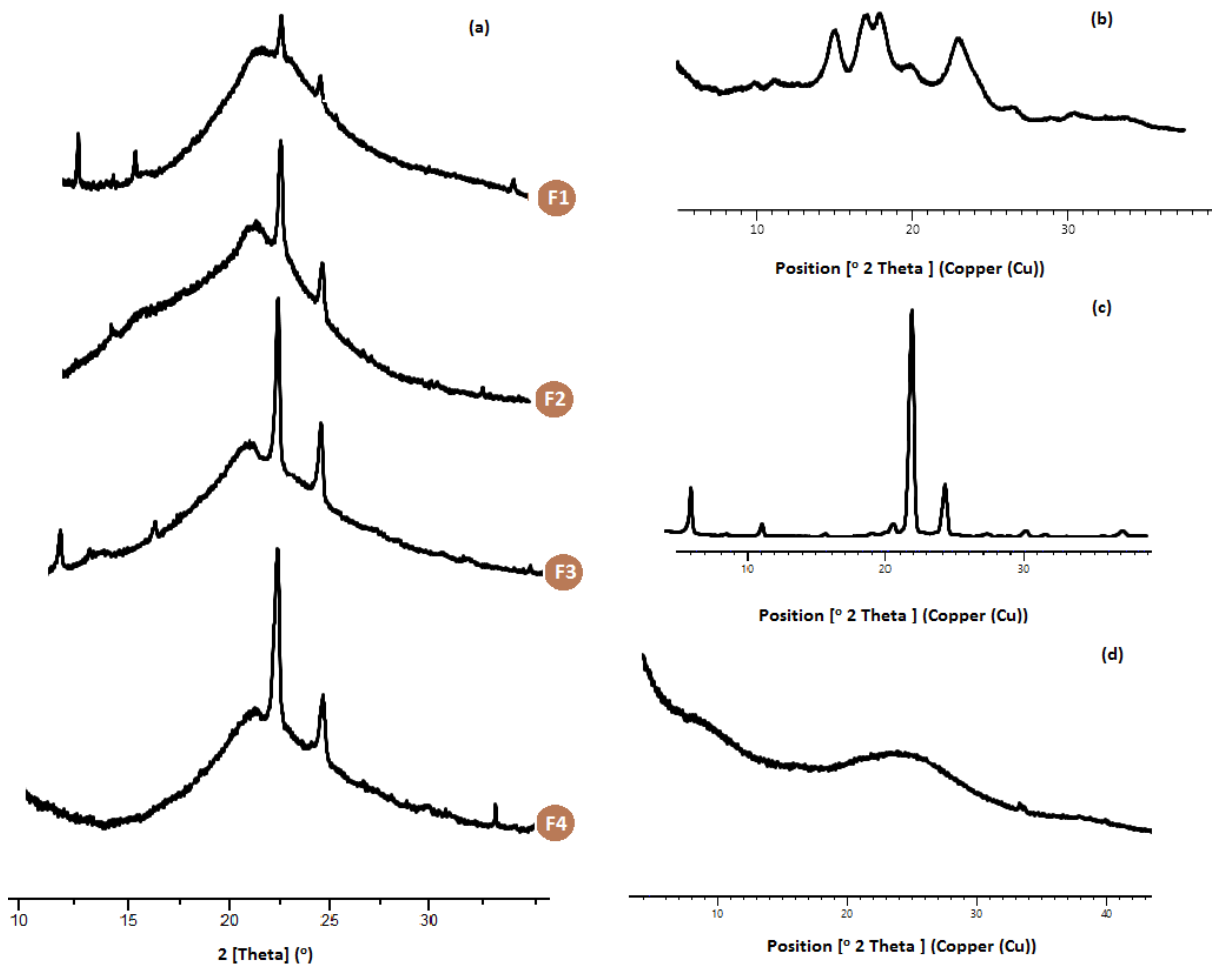


Fig. 3

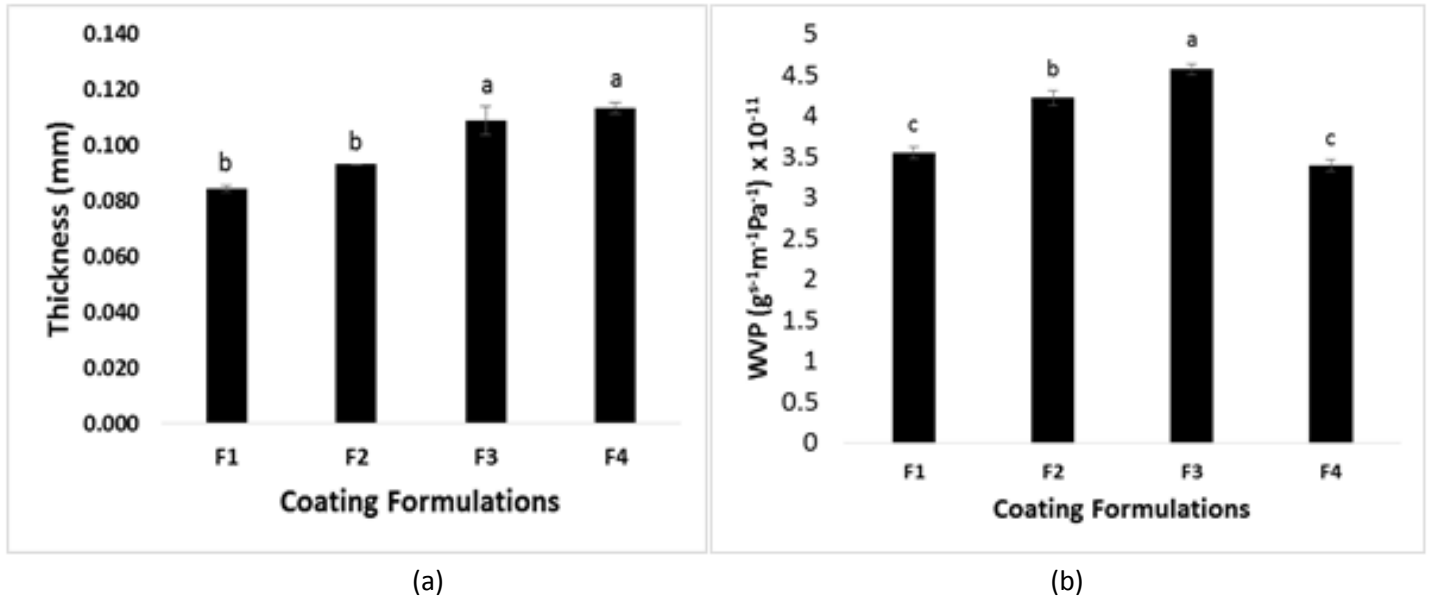


Fig. 4 (a, b)

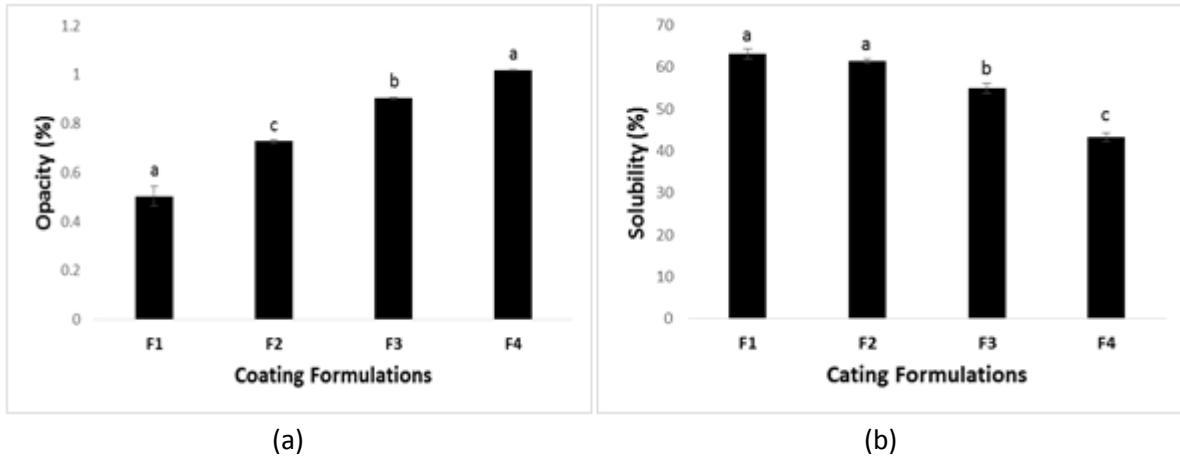


Fig. 5 (a, b)

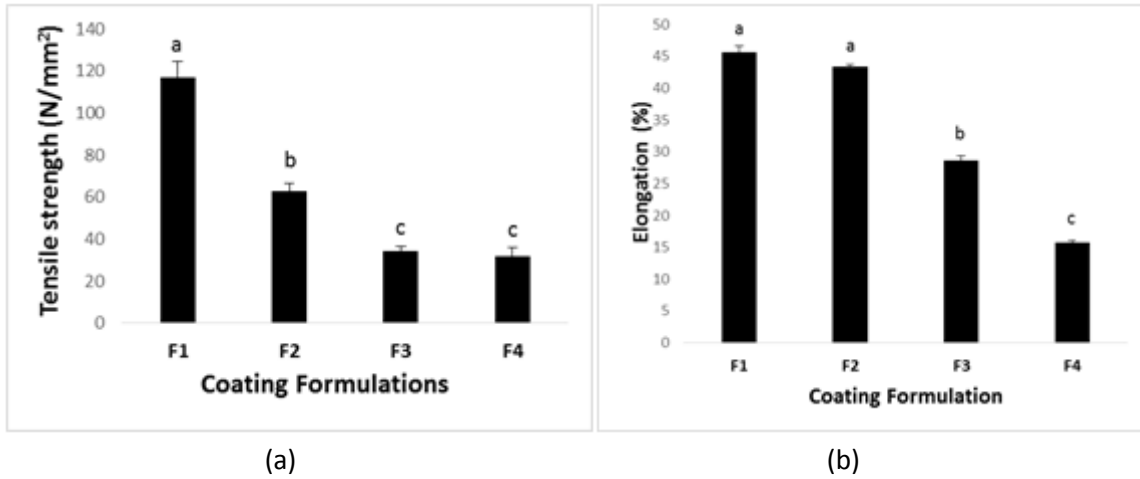


Fig. 6 (a, b)

Table 1

The different coating formulations containing rice starch (RS), ι -car (CR), stearic acid (SA), glycerol (Gly) and Tween[®]20 used for the experiments.

Variables	Formulations (F)			
	F1	F2	F3	F4
RS (% w/w)	2%	2.50%	3%	4%
CR (% w/w)	2%	1.50%	1%	0.50%
SA (% w/w)	0.30%	0.50%	0.70%	0.90%
Gly (% w/w)	30% (w/w)	30% (w/w)	30% (w/w)	30% (w/w)
Tween[®]20 (% w/w)	0.20%	0.20%	0.20%	0.20%

Table 2

Colour and moisture content of rice starch-t-car film blended with fatty acid, glycerol and surfactant.

RS: CR: FA: Gly: Tween®20 (% w/w)	L	a	b	ΔE	Moisture
Formulations					
F1	95.52±0.25 ^a	-0.31±0.00 ^b	5.03±0.25 ^b	5.33±0.2 ^a	8.48±0.571 ^b
F2	96.227±0.32 ^a	-0.16± 0.01 ^a	4.31±0.03 ^c	5.09±0.01 ^a	9.51±1.56 ^b
F3	95.448±0.40 ^a	-0.36±0.08 ^b	6.34±0.19 ^a	6.49±0.0 ^b	17.06±0.21 ^a
F4	95.897±0.06 ^a	-0.37±0.00 ^b	4.92±0.10 ^b	5.43±0.02 ^a	11.00±0.64 ^b

- Values denotes the Means of replicates ± Standard error.
- ^{a-c} represents the difference between mean values. The values with the same letter are not significantly different according to Duncan's multiple range test (p>0.05).