

# **Amylose-lipid complex as a measure of variations in physical, mechanical and barrier attributes of rice starch- $\alpha$ -carrageenan biodegradable edible film**

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1 **Amylose-lipid Complex as a Measure of Variations in Physical, Mechanical and Barrier**  
2 **Attributes of Rice Starch-  $\kappa$ -Carrageenan Biodegradable Edible Film**

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21

22 **Abstract**

23 Improvements in the hygroscopic properties of starch based films are important to strengthen  
24 their mechanical properties. The effects of different hydrophobic components-butyric acid  
25 (BA, C4:0), lauric acid (LA, C12:0), palmitic acid (PA, C16:0), oleic acid (OA, C18:1), stearic  
26 acid (SA, C18:0) and sucrose fatty acid ester (FAEs) on the rice starch (RS)- $\kappa$ -carrageenan ( $\kappa$ -  
27 car) composite films were investigated. Scanning electron microscopy (SEM), differential  
28 scanning calorimetry (DSC), thermogravimetric analysis (TGA) in combination with amylose-  
29 lipid complexing index (CI) were used to characterise the changes in structure and properties  
30 of edible films. The SEM results showed that the surface of films became smoother after the  
31 incorporation of fatty acids. Carbon-chain length was a major determinant of CI formation  
32 which further influenced the attributes of RS- $\kappa$ -car films. The addition of FAEs to RS- $\kappa$ -car  
33 improved film thickness, permeability, transparency, tensile properties (TS) and could be used  
34 to tailor biodegradable edible films with enhanced properties and future fruit coating  
35 applications.

36 **Keywords:** Carrageenan; Fatty acids; Hydrophobic; Packaging, Starch.

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## 44 **1. Introduction**

45 Edible packaging based on polysaccharides has been studied as a potential substitute for non-  
46 biodegradable synthetic materials (Cerqueira, Souza et al. 2012). Among the different  
47 polysaccharides, starch is one of the most promising candidate for the manufacturing of  
48 biodegradable edible films (Liu, Sun et al. 2016). Different starch sources have been  
49 investigated as an economical edible film material with good packaging properties (Thakur,  
50 Saberi et al. 2016).

51 Rice is one of the most important foods and is produced widely around the world. However,  
52 during harvest and packing, many rice grains crack or break resulting in a reduction of eating  
53 quality and lower prices (Bhat and Riar 2016). This lower grade underutilized material can be  
54 used as a starch source for the manufacturing of edible films and coatings. Starch functional  
55 properties are dependent on water (Wang and Copeland 2012) and like other starch sources,  
56 rice starch also has a great affinity to interact with water and this interaction has a negative  
57 impact on the films barrier and mechanical properties (González and Villanueva 2011).  
58 Moreover, lack of fragility and brittleness exhibited during starch thermoformation, limits their  
59 potential for packaging applications. Therefore, starch alone cannot form a film with  
60 satisfactory mechanical and permeability properties unless it is chemically modified, blended  
61 with other biopolymer or plasticized (Dhall 2013).

62 Applications of plasticizers for improving the limitations of starch films and coatings have led  
63 to the development of safe and novel solutions (Karbowski, Hervet et al. 2006, Kowalczyk and  
64 Baraniak 2011, Vieira, da Silva et al. 2011). Fatty acids, epoxidized triglyceride vegetable oils  
65 from soybean oil, linseed oil, castor oil, sunflower oil and fatty acid esters (FAEs) are  
66 commonly studied hydrophobic components used to improve film hygroscopic properties  
67 (Vieira, da Silva et al. 2011).

68 The incorporation of lipids into the film matrix is a strategy to improve the water barrier  
69 properties of edible film. However, their sole utilization may compromise with the film  
70 mechanical properties (Rocca-Smith, Marcuzzo et al. 2016) due to phase separation  
71 (incompatibility of materials) (Acosta, Jiménez et al. 2015). Moreover, water barrier properties  
72 of emulsion based edible films depends on the nature of lipids, chain length of the fatty acids  
73 and structure of dried film (Slavutsky and Bertuzzi 2016). Furthermore, starch functional  
74 properties and its interactions with different components, particularly with water and lipids, are  
75 of great interest to the food and packaging industries (Copeland, Blazek et al. 2009). These  
76 interactions are the basis of the selection of compatible component (s) into the film matrix. In  
77 the multi-scale structure of granules and the changes they undergo during the processing is the  
78 major determinant of starch functionality (Wang, Zhang et al. 2016). The starch granule, helical  
79 structure of amylose is occupied by the H-bonds which provide hydrophilicity to the molecule,  
80 and assist in forming complexes with the free fatty acids, fatty acids components of glycerides  
81 and iodine molecules. These complexes between amylose and lipids are generally described in  
82 terms of complexing index (CI) formation as evaluated by reduction in their iodine binding  
83 capacity (Kawai, Takato et al. 2012). According to Wang, Wang et al. (2016), these interactions  
84 varies with the fatty acid component and significantly modify the properties and functionalities  
85 of starches. Hence, it becomes more important to find out a suitable emulsion with compatible  
86 fatty acid component to the starch material to produce good film properties. To the best of our  
87 knowledge there is no such study available in the literature reporting the effect of amylose-  
88 lipid complex formation ability of different hydrophobic components on the attributes of rice  
89 starch-*t*-car edible film. Therefore, this novel study investigated the use of different fatty acids  
90 as a hydrophobic component in the rice starch/carrageenan based coating material where the  
91 objective was to investigate the influence of hydrophobic components, saturated (palmitic,  
92 stearic, lauric acid), unsaturated (oleic and butyric acid) and fatty acid ester on the

93 microstructural, physical (thickness, solubility), mechanical (tensile strength and elongation at  
94 break), barrier (water vapour permeability) and optical properties of starch-carrageenan films.

95

## 96 **2. Materials and methods**

### 97 **2.1 Materials**

98 Rice grains (*Oryza sativa* var. Reiziq) were obtained from Sunrice (Sun Rice, Leeton  
99 Australia). Stearic acid, palmitic acid, lauric acid, butyric acid, oleic acid and Tween®20 were  
100 purchased from Sigma Aldrich, USA. The ι-car (*Chondrus crispus*) was purchased from  
101 Melbourne Food ingredient depot, Australia. Sucrose fatty acid ester was from Tokyo chemical  
102 industry co. Ltd Japan. Glycerol was from Ajax Finechem Pty. Ltd, Australia. Starch isolation  
103 and characterisation of its chemical composition is described elsewhere (Thakur, Saberi et al.  
104 2016).

### 105 **2.2 Methods**

#### 106 **2.2.1 Film solution**

107 Dispersion solution was prepared according to the method described by Thakur, Saberi et al.  
108 (2016). Film solution was prepared in two steps.

109 **Step-1:** Starch solution (2%) was prepared by heating the starch-water mixture at 85°C using  
110 hot plate magnetic stirrer (180 rpm) with constant agitation for 15 min.

111 **Step-2:** The ι-carrageenan (2%) was heated in water at 80 °C with constant stirring (200 rpm)  
112 till the clear transparent solution was formed.

113 After mixing the solution in step-1 and step-2 liquid fatty acids (0.6%) were added with  
114 subsequent addition of Tween®20 (0.2%) and glycerol (1%). The final mixture was stirred for

115 20 minutes and cooled down to the room temperature before casting. 20 ml of final film  
116 solution was poured into petri plates and dried in the oven for 24h under controlled conditions  
117 (35°C, RH 50%). For evaluation films were peeled off and conditioned at 27°C, RH 60% for  
118 72h.

### 119 **2.2.2 Complexing index (CI) of starch-fatty acid mixture**

120 The CI of starch-fatty acid mixture was calculated by the reduction in the iodine binding  
121 capacity as explained by Wang, Wang et al. (2016) with minor modifications. Starches  
122 depending on grain types and varieties contain varying amount of monoacyl lipids inside the  
123 granules (Wani, Singh et al. 2012). The starch used in this experiment was defatted before use  
124 and the fat content was reported in our previous work (Thakur, Pristijono et al.). Starch-fatty  
125 acid physical mixture was prepared by dissolving 5 mmol of fatty acid in 50 ml ethanol under  
126 magnetic stirring. 5g starch was added to the mixture and stirred continuously till the complete  
127 evaporation of ethanol. 400 mg of prepared starch-fatty acid mixture was mixed with 5 ml  
128 distilled water in a 50 ml centrifuge tube. The mixture was thermally processed at 92.5°C for  
129 10 minutes to completely gelatinize the starch material. After cooling at room temperature, 25  
130 ml of water was added and tubes were vortexed for 3 minutes before 2ml of iodine solution  
131 (2.0% KI+ 1.3% I<sub>2</sub>) was added. The mixture was mixed completely by end to end rotation.  
132 Absorbance of the final solution was measured at 548 nm and CI was calculated using the  
133 equation (1). All samples were tested in triplicates and results were averaged.

$$134 \quad CI = \left( \frac{A_{(without\ fatty\ acid)} - A_{(with\ fatty\ acid)}}{A_{(without\ fatty\ acid)}} \right) * 100 \quad (1)$$

## 135 **2.3 Edible film properties**

### 136 **2.3.1 Physical properties**

137 A digital micrometer (Mitutoyo, Co., Code No. 543-551-1, Model ID-F125, 139 Japan;  
138 sensitivity=0.001 mm) was used to measure the film thickness. Average thickness value was  
139 used to measure the water vapour permeability (WVP) and opacity of film samples. Film  
140 solubility was determined according to a previously reported method (Saber, Thakur et al.  
141 2016).

## 142 **2.3.2 Permeability property**

### 143 **2.3.2.1 Water vapour permeability (WVP)**

144 WVP was measured gravimetrically using ASTM E96-92 method described by Martins,  
145 Cerqueira et al. (2012). Briefly, film samples were sealed on a permeation cell (aluminium cup  
146 containing 10gm CaCl<sub>2</sub> granules; 0%RH) and placed in a desiccator containing NaCl solution  
147 (75% RH at 25°C). Water vapour transport was determined using the weight gain of the cell at  
148 a steady state of transfer. Changes in the weight of the cell were recorded and plotted as a  
149 function of time. The slope of each line was evaluated by linear regression ( $R^2 > 0.99$ ), and the  
150 water vapour transmission was calculated through the slope of the straight line (g/s) divided by  
151 the test area (m<sup>2</sup>). After the permeation tests, the film thickness was measured and WVP (g  
152 Pa<sup>-1</sup>s<sup>-1</sup>m<sup>-1</sup>) was calculated as:

$$153 \quad \text{WVP} = \frac{\Delta m}{A \Delta t} \frac{X}{\Delta P} \quad (2)$$

154  $\Delta m/\Delta t$  = weight of moisture gain per unit time (gs<sup>-1</sup>) and can be calculated by the slope of the  
155 graph.  $A$ = area of the exposed film surface (m<sup>2</sup>),  $T$ = thickness of the film (mm),  $\Delta P$ = represents  
156 the water vapour pressure difference inside and outside of the film (Pa).

## 157 **2.3.3 Mechanical properties**

### 158 **2.3.3.1 Tensile strength (TS) and elongation at break (EAB)**



159 Tensile strength (TS) and elongation at break (EAB) was determined with Texture Analyzer  
160 (LLOYD Instrument LTD, Fareham, UK) using ASTM standard method D 882-91 (Martins,  
161 Cerqueira et al. 2012). Preconditioned (60% RH) films (15 x 40 mm) were placed between the  
162 tensile grips with 40 mm separation and 1 mm/s crosshead speed respectively. 10 film samples  
163 of each type of films were replicated. TS was expressed in Nm<sup>2</sup> and EAB was expressed in  
164 percentage.

### 165 **2.3.4 Optical and light barrier properties**

#### 166 **2.3.4.1 Film Opacity and colour**

167 Light barrier properties of film were measured by using (Cary 50 Bio UV-Visible  
168 spectrophotometer) by exposing the film samples to light absorption at wavelength 550 nm.  
169 Rectangular piece of the film samples were measured by placing into a spectrophotometer test  
170 cell directly. Transparency of film was calculated according to the following formula:

$$171 \quad T = \frac{A(550)}{X} \quad (3)$$

172 Where,  $A_{550}$  is the absorbance at 550 nm and X is the film thickness in mm. Opacity is the  
173 measure of transparency, higher opacity corresponds to the lower transparency of film samples.  
174 Colour property of film was measured using a portable chroma meter (Minolta CR-300 Japan).  
175 CIELab colour profile was used to expressed the  $L$  (whiteness) to  $-a$  (greenness) to  $+a$  (redness)  
176 and  $-b$  (blueness) to  $+b$  (yellowness).

$$177 \quad \Delta E = [(L_{film} - L_{standard})^2 + (a_{film} - a_{standard})^2 + (b_{film} - b_{standard})^2]^{1/2} \quad (4)$$

178 Ten readings of three different replicates were recorded and mean was calculated.

### 179 **2.4 Film characterisation**

180 **2.4.1. Microstructural analysis- scanning electron microscopy (SEM)**

181 Film morphology was studied by using scanning electron microscope (JEOL, JSM 6300 SEM,  
182 JEOL, and Tokyo, Japan). Film samples were stored in desiccator for 1 week to ensure the  
183 absence of water in the sample (theoretical RH in desiccator 0%). Film pieces were mounted  
184 on the copper stubs, gold coated and observed using an accelerating voltage of 10 kV under  
185 high vacuum mode.

186 **2.4.2 Differential scanning calorimetry (DSC)**

187 Thermal properties of rice starch films were determined by using differential scanning  
188 calorimeter (DSC) model 60-A, Shimadzu Corporation, Kyoto Japan, equipped with thermal  
189 analyzer (TA-60WS). The film sample were completely dried in the desiccator for one week  
190 and moisture content was <3% was observed (data not shown). For DSC analysis, film samples  
191 were contained in hermetically sealed aluminum pans and heated from 25 to 200°C at a rate of  
192 10°C/min. Changes in the phases or state and corresponding enthalpies ( $\Delta H$ ) were determined.

193 **2.4.3 Thermogravimetric analysis**

194 Thermal stability of the film samples were analyzed by thermogravimetric analyzer (TGA),  
195 model TGA-50, Shimadzu Corporation, Kyoto Japan. Samples were placed in the balance  
196 system and heated from 20°C to 500°C at a heating rate of 10 °C min<sup>-1</sup> (Martins, Cerqueira et  
197 al. 2012).

198 **2.4.4 Statistical analysis**

199 Statistical analysis was performed by using SPSS 23.0.0 statistical software for windows (IBM  
200 SPSS statistics version 24.0 Inst., Cary, North Carolina, USA). All measurements were  
201 performed in triplicate and the results reported means. One way ANOVA was used to analyse

202 the data. The mean values were evaluated using Duncan test with  $p < 0.05$  as statistical  
203 significance.

204

### 205 **3. Results and discussion**

#### 206 **3.1 Film microstructure**

207 The structural morphology of the film surfaces with different fatty acids incorporated in the  
208 RS-t-car hydrocolloidal mixture is presented in Fig 1. Discontinuous matrix with rough  
209 irregular ridged structure was observed in case of control films (without fatty acid) Fig1.f. Fatty  
210 acids treated films showed relatively smoother and uniform surface compared with pure films,  
211 manifesting a regular and homogeneous matrix with good structural integrity. More finely  
212 distribution of polymer-fatty acid mixture was observed in the surface morphology of films  
213 treated with FAEs, LA, PA and OA signifying the compatibility of ingredients in the film  
214 matrix (Fig 1. g, c, a, d). However, non-uniformity in the surface texture of films treated with  
215 SA was observed (Fig 1.b) which may be due to the presence of unevenly dispersed lipid  
216 component on the RS-t-car matrix. Similar results have been observed by Jiménez, Fabra et  
217 al. (2012) where SA was found to be clearly separated in a dispersed matrix. Moreover,  
218 presence of small discontinuous zones distributed along the network was observed where  
219 butyric acid was used in the RS-t-car mixture (Fig 1.e). The most possible explanation behind  
220 this trend may be the formation of preferential channel occurred during the drying of film.  
221 Chiumarelli and Hubinger (2014) showed that the miscibility of fatty acids with starch based  
222 films caused lipid aggregation as a result of solvent evaporation during drying. The results of  
223 SEM study showed that degree of homogeneity and heterogeneity of film matrix depended on  
224 the compatibility of the hydrophobic component and nature of lipid added into the mixture.

225

### 226 **3.2 Complexing index (CI) of starch fatty acid mixture**

227 CI describes the capacity of amylose to bind with the fatty acids and indicates the reduced  
228 availability of amylose to form an inclusion complex with iodine (Liu, Sun et al. 2016). CI  
229 complex of starch with different hydrophobic components was determined to confirm the  
230 thermal stability of starch-fatty acid complexes as influenced the functional attributes of RS-1-  
231 car edible films. The result showed that CI values were ranged from 15.6% to 41.8% (Table 1)  
232 and significant differences were exists between the fatty acids. Variation in CI has been shown  
233 to exists due to the tendency of fatty acids to bind with amylose helices thus leaving less free  
234 amylose sites to interact with iodine (Kawai, Takato et al. 2012). The reduced capacity of  
235 amylose to bind with iodine resulted into a lower absorbance than control starch sample. The  
236 data clearly shows that when a fatty acid is binding amylose, the CI values decrease as the fatty  
237 acids chain length increased indicating the greater involvement of short chain fatty acids in the  
238 complex formation. It was assumed that the shorter chain fatty acids can be better dispersed in  
239 the gelatinized mixture thus facilitating their interactions with amylose and strengthens the CI  
240 complex. These results are similar to the previously reported studies (Kawai, Takato et al. 2012,  
241 Wang, Wang et al. 2016). On the other hand Fatty acid esters also showed affinity to form  
242 complexes with amylose. However there are no studies on their dispersivity in gelatinized  
243 starch. The starch-fatty acid mixture containing unsaturated component showed lower CI  
244 values probably due to the presence of double bond hindrance as similar behaviour was  
245 observed in the previous study by [Kawai, Takato et al. \(2012\)](#). However, more detailed future  
246 study is required as only single unsaturated fatty acid was selected in this study. The concluding  
247 results demonstrate that, carbon chain length is the major determinant of the starch-fatty acid  
248 complex formation. This interaction influences in the presence of double bonds in the carbon  
249 chain of fatty acids, manifesting the interacting capacity of different fatty acids in the complex

250 formation. Moreover, complexation of amylose with lipid may impact significantly on the  
251 physical, mechanical and barrier properties of films.

252

### 253 **3.3 Thermal properties of RS-t-car -fatty acid mixture**

#### 254 **3.3.1 Differential scanning calorimetry (DSC) and Thermogravimetric analysis (TGA)**

255 The thermal properties of starch-t-car films were analysed using DSC (Fig 2) with a typical  
256 endothermic transition occurring between 75°C and 110°C illustrating the melting of amylose-  
257 amylopectin crystallites. Previous studies reported that distribution of melting temperature of  
258 starch was found to be in the range of 67.2—81.1°C (Liu, Sun et al. 2016, Wang, Wang et al.  
259 2016) which was consistent with above results. The appearance of an additional endothermic  
260 event at low temperature (29°C to 62°C) was observed in case of LA (C12:0), PA (C16:0) and  
261 FAEs films attributed to the melting of free unbounded fatty acids which were not associated  
262 in the formation of complexes with amylose (Table 2, peak I). These observations are in  
263 agreement with Liu, Sun et al. (2016). No melting peak for OA was observed due to low  
264 melting temperature (<20°C) of fatty acid. It is apparent that  $T_m$  and  $\Delta H_m$  values (Table 2, peak  
265 II) for SA (C18:0) and LA (C12:0, CI 30.89±1.53) was lower than other starch-fatty acid  
266 combinations in spite of higher complexity index. These results conclude that not all the  
267 molecules of fatty acid were involved in the complex formation and only the poorly bounded  
268 fatty acid molecule resulted into lower  $T_m$  values. So it becomes more important to consider  
269 the other DSC parameters  $T_g$  and  $\Delta H_m$  as  $T_m$  values does not appear to be a true indicator of  
270 compatibility between starch and fatty acids. Immiscibility between the different ingredients  
271 may also leads to the appearance of two  $T_g$  values, corresponding to the pure phases (Ramos,  
272 Reinas et al. 2013). However this trend was not uniform across all the formulations. For  
273 example butyric acid and oleic acid showed single endothermic transition, resulting into peak

274 convergence with starch gelatinization, signifying their interactions with starch components. A  
275 similar phenomenon of melting and gelatinization of starch-lipid mixture was observed in  
276 previous reported studies (Kawai, Takato et al. 2012, Wang, Wang et al. 2016). It is worth  
277 noting that the peak demonstrating the lipid melting was less prominent with short carbon chain  
278 length, signifying their more interactions with amylose (which leads to the formation of  
279 amylose-lipid complex). These observations may be due to the uniform dispersivity of  
280 hydrophobic components in the gelatinized starch. These DSC results (Fig 2) are in general  
281 agreement with CI complex formation that fatty acids with shorter chain can form stronger  
282 complexes with amylose (Table 1). Gelatinization parameters ( $T_m$ -melting temperature,  $T_g$ -  
283 glass transition and  $\Delta H_m$ - melting enthalpy) derived from DSC thermograms are presented in  
284 Table 2. The Fatty acids films exhibited lower  $\Delta H_m$  values than FAEs treated films, suggesting  
285 that  $\iota$ -carrageenan formed a stronger network with starch in the mixture. However, among the  
286 different fatty acids there was no uniform trend in the  $T_g$  and  $\Delta H_m$  values (Table 2). The  $\Delta H_m$   
287 is a measure of amount of energy required to break down the double helices of amylose-  
288 amylopectin crystallites (Wang and Copeland 2012) and amylose-lipid complexes. At high  $T_m$ ,  
289 the decrease in  $\Delta H_m$  shows only the melting of fatty acids and loosely bounded amylose-  
290 amylopectin components. Separate peaks (90°C-110°C) of amylose-lipid complex (1.2-  
291 21.2%) were observed in the previous studies (Liu, Sun et al. 2016), however no such peaks  
292 was observed at DSC temperatures below 200°C which may be due to strong complex  
293 formation between rice starch with different fatty acid components (15.7-36.5%) and their  
294 method of formation, thus requiring higher temperatures for their melting. This assumption can  
295 be related to the thermogravimetric event occurring at the fourth heating regime (400-600°C)  
296 of TGA study, given below.

297 TGA was performed to study the influence of thermal stability on the interaction of the film  
298 ingredients. Fig 3 shows the thermal events and corresponding weight loss of the each film

299 sample. The thermogram results show the films were stable up to 70°C. Four distinct regimes  
300 of degradation was observed. The first phase ranges of 100-200°C, which is the characteristics  
301 event of a polysaccharide with hydrophilic nature (Basiak, Lenart et al. 2017). As could be  
302 seen, films treated with OA, (C18:1), BA (C4:0), LA (C12:0), PA (C16:0) and SA (C18:0) has  
303 showed the presence of first regime of degradation in the thermograms depicting a small weight  
304 loss % (~5-10), corresponding to the presence of moisture in films. A similar effect with initial  
305 degradation curve has been reported in the previously conducted study based on the starch  
306 based composite films (Podshivalov, Zakharova et al. 2017). The second phase of the  
307 thermogram (250-275°C), represents the decomposition of glycerol rich phase and unbounded  
308 fatty acid components (Colussi, Pinto et al. 2017) where weight loss was comparatively higher  
309 (12-40%). Third thermal phase was appeared in the range of 300-350°C which may be due to  
310 the decomposition of starch crystallites. Thermal events at higher temperatures denote the  
311 strong intermolecular interactions among the amylose components and lipid moieties. Findings  
312 of TGA analysis are in line with the previously reported studies (references). The final thermal  
313 event at higher temperature (400-600°C) denotes the breakdown of strong amylose-lipid  
314 complexes, thus presented higher film stability (Cerqueira, Souza et al. 2012). It was interesting  
315 to note that weight loss % was minimum in case of FAEs and OA treated films suggesting that  
316 these films have higher thermal resistance.

317 In summary, the thermal properties of the RS-  $\alpha$ -car-lipid component films showed that, the  
318 amylose-lipid complex is a major determinant of film thermal properties. However, the DSC  
319 (fatty acids melting peak) and TGA (weight loss in the second regime) results showed that not  
320 all the fatty acids were involved in the complex formation and more detailed future studies are  
321 required to understand the profound mechanism of complex formation.

322

## 323 **3.4 Physical properties**

### 324 **3.4.1 Thickness**

325 Thickness is directly related to the barrier and optical properties of films. In this study the  
326 thickness of RS-t-car film ranged between 0.07 to 0.39 mm for the films with different fatty  
327 acids (Fig 4a). Films with FAEs (0.08mm), butyric acid (0.07mm), stearic acid (0.09mm)  
328 showed significantly lower thickness values than other films ( $p<0.05$ ). In comparison, films  
329 with oleic acid and palmitic acid were four times thicker than the control film. This may be due  
330 to the over accumulation of melted fatty acid on the film surface which increases the thickness  
331 of resultant film. However, there is little information in the literature related to the effect of  
332 fatty acid on the thickness of edible films.

333

### 334 **3.4.2 Film solubility**

335 Solubility describes the water resistance of films when applied on fresh produce (Cerqueira,  
336 Souza et al. 2012). Solubility of starch-carrageenan films with different lipid components is  
337 presented in Table 1 and shows that the solubility power of RS-t-car films decreased with the  
338 incorporation of hydrophobic components, where the values ranged from 50.3% to 82.7%.  
339 Significant differences exist in the mean values of different fatty acids and solubility vary with  
340 the type of added lipids ( $p>0.05$ ). These variations in the film solubility may be influenced by  
341 CI complex formation. Low solubility values of LA and BA could be attributed to the formation  
342 of strong stable helices formed between amylose and lipid content which formed an insoluble  
343 film on the starch granule surface and delayed the transportation of water into the granule. In  
344 addition to that fatty acids may restrict the swelling of starch granules and inhibit the leaching  
345 of amylose, which in turn causes to lower solubility of the starch films. Results of this study  
346 are similar to previously reported work where amylose-lipid complexes reduces the solubility



347 of starch composite films (Copeland, Blazek et al. 2009). The mobility of the matrix ( $T_g$ ) has  
348 also influenced the starch solubility power as water molecules can be trapped more easily in to  
349 the spaces between the helices resulting in an increase in water solubility. The control film  
350 showed lower  $T_g$  values ( $37.9^\circ\text{C}$ ) than fatty acid treated films. Lower solubility values of FAEs,  
351 may be due to the unavailability of free OH groups for the interaction with water.

352

### 353 **3.5 Barrier property-water vapour permeability (WVP)**

354 Fatty acids have been studied as a good barrier to moisture (Schmidt, Porto et al. 2013). In this  
355 experiment WVP values significantly varied between the different polymer-lipid combinations  
356 (Fig 4b). Rice starch based film with different hydrophobic components showed lower  
357 permeability values as compared to the control film (without fatty acid), demonstrating the  
358 impact of hydrophobic components on the permeability of starch based film. Lowest WVP  
359 values was observed in case of film samples with FAEs ( $1.18 \times 10^{-11} \text{ gs}^{-1}\text{m}^{-1}\text{Pa}^{-1}$ )  
360 comparatively six times lower than control film samples ( $7.56 \times 10^{-11} \text{ gs}^{-1}\text{m}^{-1}\text{Pa}^{-1}$ ) which is a  
361 promising value for potential fruit coating applications. Results of this study are consistent with  
362 the previously reported data where the addition of FAEs has been studied to improve the  
363 permeability values of films compared to fatty acids (Jongjareonrak, Benjakul et al. 2006). In  
364 this experiment the higher permeability value of the control film may be due to lack of strong  
365 network crosslinking via covalent and non-covalent bonds resulting into more interstitial  
366 spacing between molecules and as a consequence led to higher diffusion rate across the  
367 membrane. Based on the previous studies on different types of lipids with different matrices,  
368 WVP has been shown to decrease as increase in fatty acids chain length (McHugh and Krochta  
369 1994). However, in this study there was no uniform pattern of permeability based on chain  
370 length was observed. This is probably due to the differences in the fatty acids ability to forms

371 a complex with amylose and amylopectin. This reinforces the fact that there might be other  
372 bonding and interactions effects related to the structural arrangement of lipid in the film and  
373 with other ingredients which plays an important role in governing the permeability of starch  
374 gel.

375

### 376 **3.6 Optical properties-opacity**

377 Opacity of the films are directly related to the microstructural properties and affected by surface  
378 morphology and internal heterogeneity of films (Jiménez, Fabra et al. 2012). The opacity of  
379 RS-t-car film is presented in Fig 5a and shows that opacity% was lower in case of fatty acids  
380 treated films as compared with control films, signifying their higher transparency value.  
381 However, non-significant differences ( $p>0.05$ ) in the FAEs and control films values were  
382 observed and films were more transparent. The incorporation of lipids to the hydrocolloid  
383 mixture resulted a decrease in the transparency values probably due to the differences in the  
384 refractive index of the dispersed and continuous phase. The results of this study are similar to  
385 those reported previously which found that incorporation of fatty acids lowered the  
386 transparency of films (Thakur, Saberi et al. , Yang and Paulson 2000, Fernandez, de Apodaca  
387 et al. 2007).

388

### 389 **3.7 Mechanical properties- tensile strength (TS) and elongation at break (EAB)**

390 The tensile strength (TS) and elongation at break (EAB) of RS-t-car films with different fatty  
391 acids are presented in Fig 5b, c and the results shows that films prepared from OA exhibited  
392 significantly higher ( $p<0.05$ ) strength at maximum load, which possibly due to higher thickness  
393 of the OA films. Mechanical properties have been reported to impair with the addition of fatty  
394 acids (Rocca-Smith, Marcuzzo et al. 2016). However, in this experiment however, TS was

395 found to be improved, with the addition of FA in comparison to control film samples, which  
396 may be due to the interactions and compatibility of between components, further signifying the  
397 positive effect of hydrophobic plasticizer on the RS- $\alpha$ -car film. In the context of starch-fatty  
398 acid composite mixture, the improved film mechanical properties may be due to the interaction  
399 of amylose and amylopectin with lipids during the gelatinization process. It is interesting to  
400 note that BA with highest CI % (36.5) also showed lowest tensile values. The poor mechanical  
401 properties of the films may be due to the phase separation, which leads to the formation of  
402 discontinuous zones, as observed in SEM micrographs (Fig 1). The TS of stearic acid (143.23  
403 N/m) and palmitic acid (145.56N/m) had minimum or no impact on the mechanical properties  
404 of starch films, possibly due to their lower CI values 18.8%, 17.9%. Similar explanations can  
405 be provided for the films incorporated with FAEs and lauric acid. Moreover, it is worth noting  
406 that EAB values for the films plasticized with PA were significantly higher ( $p<0.05$ ) than  
407 control and films incorporated with other fatty acids. The non-covalent intermolecular  
408 crosslinking is pivotal to the development of a 3-dimensional network. Hence a likely reason  
409 for the varying tensile strength and EAB of RS- $\alpha$ -car -fatty acids films could be due to the lack  
410 of interchain associations between linear starch molecules as a consequence of inhibition of  
411 extensive exudation of amylose and amylopectin by lipids and thereby reduces the interaction.  
412 The results of this study are similar to previous reports which showed the variations in the  
413 starch mechanical properties were due to the influence of lipids on the gelatinization process  
414 (Biliaderis and Tonogai 1991). In conclusion, we showed mechanical properties are dependent  
415 on the complexing of amylose with hydrophobic ligands.

416

417 **Conclusion**

418 This study investigated the structural and permeability properties of starch film as influenced  
419 by the incorporation of fatty acids components. From the study we conclude that, different fatty  
420 acids showed varying complex formation ability with the amylose depending on the carbon  
421 chain length. The influence of these intermolecular interactions was apparently observed on  
422 the films physical, mechanical and barrier properties with short chain fatty acids showing a  
423 more positive effect presumably due to the formation of more stable complexes with short  
424 chain fatty acids. Study also confirmed that not all the fatty acids molecules participates in the  
425 complex formation as revealed by DSC and TGA, and therefore, the mechanism underlying  
426 the complex formation between amylose and lipid need further investigation. Overall, the FAEs  
427 films showed lower thickness, opacity and WVP values, which are promising indicators for a  
428 starch based composite film for fruit coating applications.

429

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437

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