Research Article

Effect of Starch Physiology, Gelatinization and Retrogradation on the Attributes of Rice Starch-ι-Carrageenan Film†

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Abstract

Edible films and coatings have been applied as the potential substitutes for conventional plastics in food packaging. However, their physical and mechanical properties still have limitations and thus require further improvement. In this study, we compared the physico-chemical properties of starches extracted from eight rice varieties and attempted predicting their promising effects on the physical (thickness and solubility), mechanical (tensile strength and elongation break), barrier (water vapour permeability) and optical properties (colour and transparency) of rice starch-ι-carrageenan films. The results showed that starch amylose content and amylose-amylopectin associations during retrogradation play a significant role in determining various properties of films., The film containing starch from 'Reiziq' variety showed minimum thickness (0.08 mm), water vapour permeability (WVP) (2.7 $gs^{-1}m^{-1}Pa^{-1}$), solubility (43.12%) opacity (0.44%) and better mechanical properties, demonstrating the importance of selection of the source of starch. The results also indicated that rice starch had compatibility with ι-carrageenan, and the blend of these two polysaccharides can be potentially used for coating fruit and vegetables.

Keywords: Edible film; Starch; Carrageenan; Amylose; Rice.

1. Introduction

There is an increasing interest in the bio-based packaging material such as biodegradable edible films. These materials can be consumed along with the food, providing additional nutrients, enhancing sensory properties and reducing the problems of rising synthetic waste [1]. A variety of polymers from renewable resources- polysaccharides, proteins, lipids, raisins, have been investigated towards the development of biodegradable packaging material [2]. Polysaccharides appear as a material for bio packaging due to their good permeability and mechanical properties. From the widely tested list of polysaccharides, starch is one of the most promising materials for manufacturing biodegradable plastics [3]. This polymer is renewable, readily available, inexpensive and able to form a continuous polymer matrix [4]. Starches from different sources have thus been investigated for their applications in the food industry.

Among all the starches, rice starch has gained the reputation as an important raw material for numerous food and non-food applications due to its, non-allergic, ease of digestibility, white colour, small granule (3-10 µm) and a wide range of amylose/amylopectin ratio [5]. Functional properties of starches are more dependent on their botanical sources, growing conditions and environmental factors [6]. Moreover, varying granular structure due to morphological differences affects the thermal profile of rice starches isolated from different sources [6] and hence the physical, mechanical and barrier properties of starch films [7]. In comparison to synthetic plastic, starch films present poor physical and mechanical properties, due to their hydrophilic nature and retrogradation mechanism which affect the film permeability (moisture) and offer inferior mechanical properties [3]. One approach to further improve these limitations has been the preparation of composite films by utilizing the intrinsic properties of the individual biopolymers. Starches from different sources have been studied either alone

or in combination with other polymers and plasticizers [3, 8-11] to improve the properties of films. Biopolymers such as chitosan [12], cellulose and cellulose derivatives [13] have been studied as compatible components with starch composite films. However, the use of high molecular weight polysaccharides such as carrageenan can be of great interest to improve the quality attributes of starch films. The ι-carrageenan is a naturally occurring anionic seaweed used in dairy industries [14] and known for its compatibility with biopolymers like starch [15]. Moreover, final properties of starch films can be modified with the addition of co-biopolymers, plasticizers and hydrophobic agents.

Glycerol has been known as a compatible plasticizer with starch-based coating materials to improve the flexibility of the polymer [12], however, use of hydrophobic compounds (e.g., fatty acids) as a plasticizing agent can be a prospective solution to improve the moisture barrier properties of starch-based films. Stearic acid, palmitic acid, and vegetable oils are mostly used lipids in the coatings for minimally processed products [16]. Starch amylose/amylopectin ratio, morphological attributes along with other biopolymers and plasticizers collectively affect the physical, barrier and mechanical properties of films. Therefore there is a need to investigate the starches differing in their attributes for their bio-degradable packaging properties. Hence, the present study was undertaken to investigate and compare the physico-chemical properties of starches extracted from eight different rice varieties and to predict their promising effects on the physical, mechanical and barrier properties of rice starch-ι-carrageenan films composed therefrom with added hydrophobic (stearic acid) plasticizer.

2. Material and Methods

2.1 Materials

Rice grains from eight different varieties *(*Langi, Reiziq, Opus, Basmati, Kyeema, Sherpa, Black Rice, and Doongara*)* were collected from a commercial company (Sun rice Ltd., Leeton, New South Wales, Australia). The ι-carrageenan (*Chondrus crispus*) was purchased from Melbourne Food ingredient depot, Victoria, Australia. The chemicals HCl and NaOH were purchased from Merck Pty Ltd, Germany and used during the starch extraction process. Stearic acid (Sigma-Aldrich, USA) and glycerol (Ajax finechem Pty. Ltd, Australia) were used as plasticizing agents in the film formulation. Tween20® was (Sigma-Aldrich, USA) used as a surfactant.

2.2 Starch extraction and physiological properties

Starch from rice was extracted by using alkaline extraction method as reported by Fabian, Ayucitra, Ismadji and Ju [17]. Starch composition (protein content, moisture, ash, fat) was determined by a standard method of AOAC (AOAC 1990).

2.3 Amylose content

Amylose content of starch was calculated by iodine colorimetric method as reported previously [18]. Briefly, rice starch was mixed with 0.5N KOH and heated in a boiling water bath for 10 min. After cooling, an aliquot of solution was mixed with 0.5 N HCl and iodine reagent (0.3% I2 + 2.0% KI). The absorbance of colour developed was measured using a spectrophotometer (Cary 50 Bio UV-Visible spectrophotometer) at 620 nm.

2.4 Film preparation

Rice starch (2%, w/w), ι-car (2%, w/w), stearic acid (0.3%, w/w), glycerol (1%, w/w) and Tween ®20 (0.2%, w/w) were mixed together in two different steps to make the film solution. The formulation was selected on the basis of our work done previously [19]

Step 1: The ι-car gelling solution was prepared by heating ι-car-H_2O mixture at 85ºC for 15 min with continuous stirring till a clear transparent gel was formed.

Step 2: Rice starch was gelatinized at 85ºC and mixed with ι-car gel (step 1) with continuous stirring. Melted stearic acid and Tween®20 mixture were added to the suspension under constant stirring. Glycerol was added to the cooled solution and mixture was stirred finally for 15 min. Film solution (20 ml) was poured into petri plates and dried in an oven for 24 h under controlled conditions (35ºC, RH 50%). For evaluation of various properties, films were peeled off and conditioned at 27ºC, RH 60% for 72 h.

2.5 Properties of rice starch-ι-car film

2.5.1 Thickness

The thickness of the film was measured using a digital micrometer (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 values were calculated to analyse WVP and optical properties.

2.5.2 Water solubility (WS) and moisture content (MC)

Water solubility was determined according to a previously reported method [11] with some modifications. The initial (S_i) dry matter content of each film was determined by drying to constant weight in a desiccator. Film samples were immersed in 50 ml of water with continuous shaking at 60 rpm at room temperature for 24 h. The pieces of film samples were taken out and dried to constant weight (S_f) in an oven at 110°C to dry the undissolved matter. The solubility of the film was calculated by Eqn.1.

$$WS\ (\%) = \frac{WS_{(i)} - WS_{(f)}}{WS_{(i)}} \times 100 \quad (1)$$

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

MC of the film samples (1.5 x 4.0 cm) was determined gravimetrically by measuring water removed from the initial mass. The film samples were dried at 110ºC for 24 h to attain a constant weight. Dried films were stored in the desiccator for two weeks to obtain uniform moisture content and calculated according to Eq. 2.

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

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

2.5.2 Water vapour permeability (WVP) measurement

The measurement of WVP was performed gravimetrically using ASTM E96 method [20], as described in the previous study [11]. Briefly, circular aluminum cups (5 × 3cm) containing anhydrous $CaCl_2$ granules with 0% RH were sealed tightly by the sample film and placed under controlled RH conditions (NaCl saturated solution; 75 % RH) at 25ºC. Water vapour transport was determined using the weight gain of the cell at a

steady state of transfer. Changes in the weight of the cell were recorded and plotted as a function of time. The slope of each line was evaluated by linear regression ($R^2 > 0.99$), and the water vapour transmission was calculated through the slope of the straight line (g/s) divided by the test area (m^2). After the permeation tests, the film thickness was measured and WVP (g $Pa^{-1}s^{-1}m^{-1}$) was calculated as:

$$\text{WVP} = \frac{\Delta m}{A\,\Delta t}\,\frac{T}{\Delta P} \qquad (3)$$

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

2.5.4 Film opacity (FO) and colour

FO measurements were performed according to the previously described method [21] using spectrophotometer (Cary 50 Bio UV-Visible spectrophotometer) at 560 nm. The low value of the opacity corresponds to the high transparency. Film colour was evaluated using a portable Chroma meter (Minolta CR-300 Japan). CIELab colour profile was used to expressed the L (whiteness) to $-a$ (greenness) to $+a$ (redness) and $-b$ (blueness) to $+b$ (yellowness). The total colour difference was calculated by the equation given below.

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

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

2.5.6 Tensile strength (TS) and elongation at break (EAB)

Tensile strength (TS) and elongation at break (EAB) were determined as described earlier [11] 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 variety were studied for the mechanical properties of the film. TS and EAB were expressed in MPa and N/mm.

2.6 Characterization of RS-ι-car bio composite film

2.6.1 Scanning electron microscopy (SEM)

Starch granule morphology was evaluated by using a scanning electron microscope ZEISS model EVO-50 at 20 KV. Starch samples were stored in a desiccator for 1 week to ensure the absence of water in the sample (theoretical RH in desiccator 0%).Granules were fixed on the copper stubs, gold coated and observed using an accelerating voltage of 10 kV.

2.6.2.1 X- ray diffraction (XRD)

X-ray diffraction pattern of film compositions was obtained by using x-ray diffractometer (PANanalytical, X'pert PRO Multi-purpose X-ray diffractometer, Almelo, Netherland) under following conditions: 40 kv/35 mA angle 2θ: 5° and 60°, step size = 0.05 °, using Kα cu radiations (λ = 1.54 A). X-ray diffraction patterns for rice starch, ι-car, and stearic acid were also analyzed to understand their crystalline behavior.

2.6.3 Differential scanning calorimetry (DSC)

Thermal properties of rice starch films were determined by using a differential scanning calorimeter (DSC) model 60-A, Shimadzu Corporation, Kyoto Japan, equipped with a thermal analyzer (TA-60WS). The samples were contained in hermetically sealed aluminum pans and heated at a rate of 10°C/min from 25 to 200°C. Changes in the phases or state and corresponding enthalpies (ΔH) were determined. Moisture content of the films prior to DSC analysis was between 17.89-19.83%.

2.7 Statistical analysis

Statistical analysis was performed by using Microsoft excel 2013 and SPSS 23.0.0 statistical software for windows (SPSS IBM, USA). One way ANOVA was used to analyse the data. The mean values were evaluated using Tukey's multiple comparison test and Duncan test with $p< 0.05$ as statistical significance.

3. Results and Discussion

3.1 Physiological analysis and morphology of starch granules

Table 1 illustrates the results of physico-chemical analysis together with functional characteristics of starches extracted from eight different rice varieties. Moisture content (7.8 to 18.31%), fat content (0.01-0.14%), protein content (0.43 to 0.71%) and ash content (4.3 to 7.3%) varied significantly among the analysed starches. Amylose content of isolated starches represents a significant difference ($p<0.05$) among the

selected varieties, the values ranged between 7.0 (black rice) and 11.33% (Reiziq var.). The variations in the amylose content of starches reported varying with the botanical sources and the growing conditions of starches during grain development [22]. Another possible reason for the varying attributes of starches could be related to the activity of enzymes involved in the biosynthesis of starch. According to Daudt, Avena-Bustillos, Williams, Wood, Külkamp-Guerreiro, Marczak and McHugh [23] and Kong, Zhu, Sui and Bao [24], starch amylose content is the major contributing factor influencing the functional properties of starches and is associated with the formation of a stronger starch gel. Fig. 1 shows the morphology of rice starch granules from different rice varieties. The granular structure (shape and size) of starches showed variations between varieties as seen in SEM micrograph (Table 1, Fig 1). Results from SEM revealed that starch granules were mostly polyhedral, hexagonal and irregular in shape with a wide range of granule size (3.9 to 6.6 µm) (Table 1). As reported in the previous studies size of the starch granules may affect the thickness of the resultant film [12, 25].

3.3 Swelling power (SP) of starches

Swelling is the absorption of water molecules inside the well-organized amorphous crystalline region of starch when the aqueous dispersion is heated. The mechanism involves the formation of hydrogen bonds with the exposed OH groups in the branch ends of amylose and amylopectin which favors the transmittance of disruptive stress from the periphery to center of the starch granule [26]. Swelling data obtained from different rice varieties are summarized in Table 1. Opus (17.3%), Sherpa (16.4%) and black rice (15.3%) showed higher swelling power and Reiziq variety (12.5%) showed the lowest value than other starches included in the study. Granule swelling is inhibited by the amylose content and primarily promoted by amylopectin which provides less stability to the structure due to availability of more

short chains thus resulting in higher swelling [19, 27]. However, findings of this study portrays a weak statistical correlation (0.46) between amylose content and swelling power, signifying that amylose ratio was not the sole basis of the inhibition of swelling capacity of starch granule. Composition of granules and the trace components such as phosphate monoesters and phospholipids may also affect the swelling of the granules due to the complex formation [28, 29].

3.5 Thermal properties of starch film

Gelatinization transition temperature (T_o, T_m, T_c) and gelatinization enthalpy (ΔH) of rice starch films from different rice varieties are presented in Table 2. Heat flow curves for all the starch composite films showed an endothermic peak between 61°C to 73°C (supplementary material). The T_o, T_m and T_c values for endothermic transition in starch composite films were ranged from 62.82°C to 66.19°C, 67.29°C to 68.26°C and 70.03 to 72.08°C respectively, which indicates the melting of starch crystallites and the fatty acid (stearic acid) respectively. Melting of starch crystallites during the gelatinization favours the interactions among the chains of amylose and amylopectin and other components in the suspension. Films were the dried product of complex matrix (biopolymers, plasticizers and fatty acid) that undergoes retrogradation during storage. Storage associated changes as a result of reorganization of broken components into crystalline segments reflects the variations in the DSC transition temperatures (T_o, T_m and T_c). The variations in the T_o, T_m and T_c values among all the starch varieties may be due to the network formed during the retrogradation phase in the amylose-amylopectin chains, amylose-lipid and among the other components in the matrix. Endothermic peak pertaining to stearic acid melting in a starch-lipid mixture was reported by Chiumarelli and Hubinger [16] at 60.48 °C. It is noteworthy to mention that only one T_m value was observed for the rice starch–ι-carrageenan-stearic acid mixture, signifying the

miscibility among the components. According to Sharma and Singh [30], if the components of the suspension (polymer-polymer, polymer-plasticizer or a blend of all these components) are not immiscible to each other, the mixture will show two or more T_m values corresponding to two or more pure phases. Table 2 illustrated the enthalpy change (ΔH) of rice starch composite films that varied significantly and ranged from 10.95 J/g to 63.28 J/g. In this case ΔH represent the amount of energy required to break down the double helices and the complex network formed during the retrogradation [31]. Moreover, the ΔH values were poorly associated with amylose content of the starch, (R^2 0.08). These observations signify the strength of RS-ι-car-stearic acid film was influenced by the aggregation that favours interactions between amylose-stearic acid or amylopectin-stearic acid components. However, detail study is required to understand the mechanism.

3.4 X-ray diffraction

The crystalline pattern of rice starch, carrageenan, stearic acid and composite films made thereof by combining different rice varieties were evaluated by XRD. It is clear from the XRD graph that mixing of ingredients significantly affected the overall crystallinity of RS-ι-car film. As observed, prepared films showed larger plateau signifying the breakdown of original crystalline structure (Fig 2). In a hydrocolloid suspension, miscible and compatible components generally reflect lower level of crystallinity as a result of molecular order disruptions during heating [32]. The differences in the relative crystallinity (1.9 to 34.11%; Table 1) of blended films showed the varying ability of starches to undergo retrogradation as a result of re-association of disrupted amylose and amylopectin into a different ordered structure. The finding of this study reveals that film relative crystallinity was directly related to the amylose content of the starch. Reiziq var. with high amylose content (17.31%) showed high crystallinity (34.11%) values that can

be attributed to the presence of strong bonding forces in amylose. Furthermore, during the drying process, in the retrogradation phase, amylose become more organised in double helices and form a crystalline network around the amylopectin [33]. These interactions during retrogradation event may influence the mechanical properties of starch films with varying amylose content as these amylose based networks are considered to provide starch films with elasticity and strength against deformation. However, the starches with low amylose content may exhibit less strength in the structure due to interruption of long range interactions with thin the films as a result of reduced availability of amylose.

3.6 Film thickness

The thickness of film affects the permeability, optical and mechanical properties of edible film. Thickness results of different films ranged from 0.08 to 0.22 mm (Fig 4a) ($p<0.05$) with Langi var. films showed higher thickness values. The varying thickness of the films was most likely related to the granule size of the starches. Different starch varieties showed variations in the granule size ranging from 3.9 to 6.6 µm. A similar correlation between starch granules size and thickness of the film have been reported by Santacruz, Rivadeneira and Castro [12] and Basiak, Lenart and Debeaufort [25]. Disruption of granular organisations as a result of hydrothermal processing that follows hydration, swelling, and solubilisation of starch granules contributes to the thickness of the films as a result of varying granule size. According to Basiak, Lenart and Debeaufort [25] lower amylose content causes the retraction of the starch gel during drying thus reduces the thickness of the film with improved homogeneity. However, no such correlation was observed in the present study signifying the involvement of other

factors for varying thickness. Moreover, it is important to mention that pouring of film matrix into petri plates, the concentration of ingredients, size of the petri plates and drying surface were carefully examined and showed no significant effect on the thickness of the film.

3.7 Solubility

Solubility indicates the integrity and affinity of film structure to interact in an aqueous system [34]. Potential applications of biodegradable films require partial solubility, primarily to maintain the product integrity and water resistance for packaging materials, however, in some cases solubility before consumption might be beneficial. Table 2 shows the water solubility data for starches from different rice varieties involved in composite film formulation. Solubility values varied from 43.12 to 69.32% and provided the evidence of interactions between water molecules and starch chains in the crystalline and amorphous regions. It can be seen that Reiziq starch films were significantly less soluble than other starches ($p<0.05$), on the contrary of that black rice variety displayed high solubility values. From the results, it is clear that solubility was profoundly affected by the presence of amylose content. The results observed for starch film solubility were in agreement with those reported in previous study [35] where the presence of amylose significantly affected the starch film solubility. Being linear, amylose possesses strong bonding forces in the starch granule and provides less OH bonding for interaction with water. Due to intact granules aggregation and strong bonding forces, the free OH groups are unavailable to interact with water, hence lowering the solubility of composed films. Formation of complexes between amylose and lipid may be accountable for the lower solubility of the films due to the formation of semi-crystalline structures. The solubility for Sherpa, black rice and Doongra variety

with lower ΔH values shows higher solubility values probably due to weak complex formation between amylose and stearic acid. However, the trend was not uniform among all the starches. According to Salman and Copeland [36] strong amylose-lipid complexation reduces the solubility, alters the rheological properties and retards the retrogradation process eventually.

3.8 Permeability and optical properties

Data pertaining to the permeability properties of rice starch films blended with ι-carrageenan and stearic acid is shown in Fig 4b. The film prepared from Langi rice starch showed significantly higher WVP values (7.77 $gs^{-1}m^{-1}Pa^{-1}$) than other rice starches (p<0.05). However, no significant difference in the WVP values of Reiziq (2.73 $gs^{-1}m^{-1}Pa^{-1}$) and basmati starch (2.71 $gs^{-1}m^{-1}Pa^{-1}$) films were observed, which were lower by 3-fold order of magnitude than Langi starch film (p>0.05). These differences in the permeability values among varieties may find the explanation in terms of tortuosity factor for mass transfer in the amorphous-crystalline structure of the film formed post thermal processing. Relative crystallinity values were higher for Reiziq (34.1%), basmati (29.3%) and Kyeema variety (28.4%) respectively (Table 1). More compact crystalline structure resists the mass transfer mechanism and makes the membrane more impermeable. Similar explanation on the relationship between crystallinity and mass transfer mechanism through the film is also provided in the previously reported studies [3, 16]. Findings of this study are also in agreement with XRD results where starch films with higher crystallinity values showed lower WVP. Additionally, a different trend of permeability values for Langi (7.76 $gs^{-1}m^{-1}Pa^{-1}$), black rice (2.89 $gs^{-1}m^{-1}Pa^{-1}$), Doongra (3.40 $gs^{-1}m^{-1}Pa^{-1}$), Sherpa (4.08 $gs^{-1}m^{-1}Pa^{-1}$) variety was observed in spite of their low crystallinity values, indicating that crystallinity was

not the only factor responsible for the variations in the WVP values of films. The other possible reason could be the irregular accumulation of stearic acid crystals at the film surface which resulted in a rough cracked surface and affected the moisture migration through the film surface. Similar behaviour regarding the accumulation of stearic acid crystals (lipid agglomeration) on the cassava starch based film was also observed in previous study as reported by Chiumarelli and Hubinger [16]. It is interesting to note that no significant effect of stearic acid was observed on the opacity of films. Opacity is the established measurement of the transparency profile of the film. Higher opacity value signifies the lower transparency. Opacity data for different rice varieties is summarized in Table 3. The opacity values of films range from 0.4 to 1.18. Significant statistical differences in the opacity values of all rice starch films were observed ($p<0.05$). Doongra and Reiziq var. rice starch films showed low opacity values disclosing their high transparency. Higher opacity values of films were observed in the case of black rice probably due to the dark colour of starch which after gelatinization retained their black colour.

3.10 Mechanical properties-TS and EAB

In order to maintain the integrity of film on the fruit surface, the film with good mechanical strength and extensibility are generally required. Table 3 shows the values of parameters used to describe the tensile strength and extensibility properties of films from different rice varieties. Langi rice starch films showed significantly higher values of TS (242.27 ± 73.09 Nm^2) and EAB (32.36±2.28 %) ($p<0.05$). The higher values of TS and EAB for Langi var. are most probably due to the strong bonding forces in the compact crystalline region formed as a result of starch retrogradation. Findings of this

study are in agreement with DSC observations that showed the high gelatinization enthalpy value was required for disruption of bonds in Langi rice starch films owing to their high mechanical resistance. TS and EAB of starch films from different rice varieties followed the order as. TS= Langi > Kyeema > Doongra > Sherpa > Opus > black rice > Reiziq > basmati rice while EAB = Langi > Opus > Kyeema > Doongra > Reiziq > Sherpa > basmati > black rice. Moreover, the differences between the TS and EAB values of different starches can be explained on the basis of interactions among starch and ι-carrageenan. Crystallinity is one factor that has been emphasized in the literature which showed the formations of order-disorder transitions resulted in the formation of a crystalline zone that improved the strength and extensibility of films [2]. However, in this study, variations in the tensile values were not in accordance with XRD results which signifying that XRD is not a true indicator of films varying mechanical properties, as crystallinity can be higher for films where stearic crystals were formed as a result of lipid agglomeration.

4. Conclusion

The study confirmed that starch- ι-carrageenan-fatty acid mixture is a suitable combination for edible film manufacturing where attributes of films are significantly affected by granule size and amylose content of rice starch. Post thermal events during retrogradation involved re-association of amylose and amylopectin content influenced the structural and functional properties of the rice starch-ι-car films. The presence of stearic acid in the suspension improved the permeability properties of the film. Among all the rice varieties Reiziq var. with higher amylose content showed the minimum thickness, WVP, solubility and presented good mechanical and optical properties and can be potentially used as a source of rice starch for the development of edible films.

Future studies are recommended to develop the best edible film formulation using Reiziq var. rice starch with other functional ingredients for coating fruit and vegetables.

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Authors declare no conflict of interest.

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Figure legends

Fig 1: SEM micrograph of starches from different varieties at 500 K magnification using SE detector. (a) Langi, (b) Reiziq, (c) Opus, (d) basmati, (e) Kyeema, (f) Sherpa, (g) black rice, (h) Doongra

Fig 2: XRD profile (x-axis (Position [°2 Theta Copper (Cu)) vs y-axis (signal intensity (cps)) of edible film with different film forming components. (a) Rice starch (b) ι-carrageenan c) stearic acid, d) basmati rice, e) Sherpa, f) black rice, g) Opus, h) Reiziq i) Kyeema, j) Langi, k) Doongra.

Fig 3: Thickness and WVP of rice starch-ι-car film blended with fatty acid, glycerol, and surfactant. Values denote the Means of three replicates ± SD. $^{a\text{-}c}$ represents the difference between mean values. The values with the same letter are not significantly different according to Duncan's multiple range test ($p<0.05$).

Supplementary material

Fig 1: Thermogram of RS-ι-car films prepared from different rice varieties. 1) Black rice, 2) Kyeema, 3) Basmati, 4) Langi, 5) Sherpa, 6) Reiziq, 7) Doongra, 8) Opus.

List of tables

Table 1: Physiological analysis, amylose content and crystallinity of starches, extracted from eight different rice varieties

	Starch granule properties							
Rice varieties	**Moisture %**	**Fat %**	**Protein %**	**Ash %**	**Amylose %**	**Granule Size (µm)**	**Granule swelling (%)**	**Granule shape**
Langi	8.50 ± 0.1^{bc}	0.14 ± 0.03^{a}	0.62 ± 0.13^{a}	5.3 ± 0.83^{ab}	11.87 ± 0.81^{b}	6.6 ± 0.35^{a}	14.2 ± 0.30^{cd}	hexagonal, polygonal
Reiziq	18.31 ± 0.14^{a}	0.01 ± 0.00^{c}	0.53 ± 0.09^{a}	7.3 ± 0.53^{a}	17.31 ± 0.42^{a}	4.3 ± 0.30^{b}	12.5 ± 0.15^{e}	irregular
Opus	12.34 ± 0.03^{b}	0.02 ± 0.005^{c}	0.43 ± 0.11^{a}	4.3 ± 0.46^{b}	10.12 ± 0.57^{bc}	4.8 ± 0.29^{b}	17.3 ± 0.54^{a}	Hexagonal, polygonal
Basmati	10.00 ± 1.28^{bc}	0.04 ± 0.008^{bc}	0.49 ± 0.09^{a}	6.1 ± 0.43^{ab}	15.1 ± 1.05^{a}	5.1 ± 0.40^{b}	13.9 ± 0.75^{cde}	Round, irregular
Kyeema	12.01 ± 1.74^{b}	0.08 ± 0.001^{b}	0.66 ± 0.09^{a}	6.2 ± 0.87^{ab}	15.43 ± 0.60^{a}	3.9 ± 0.15^{b}	13.7 ± 0.21^{de}	Irregular, polygonal
Sherpa	7.80 ± 0.96^{c}	0.01 ± 0.001^{c}	0.57 ± 0.093^{a}	6.0 ± 0.75^{ab}	9.53 ± 1.17^{bc}	4.1 ± 0.43^{b}	16.4 ± 0.66^{abc}	Polygonal
Black rice	11.70 ± 1.9^{bc}	0.01 ± 0.004^{c}	0.71 ± 0.08^{a}	5.1 ± 0.73^{ab}	7.09 ± 0.60^{ab}	4.3 ± 0.51^{b}	15.3 ± 0.56^{bc}	Hexagonal, polygonal
Doongra	11.64 ± 1.46^{bc}	0.05 ± 0.14^{bc}	0.52 ± 0.07^{a}	4.9 ± 0.51^{b}	9.05 ± 0.64^{cd}	5.1 ± 0.68^{b}	13.9 ± 0.31^{cde}	irregular polygonal

Values denote the means of replicates ± Standard error. The values with the same superscript letter are not significantly different according to Duncan's multiple range test ($p<0.05$).

Table 2: Gelatinization properties: a) gelatinization transition temperature, T_g (glass transition) and T_m (melting temperature), b) gelatinization enthalpy (ΔH) of starch-lipid complexes extracted from different rice varieties.

Rice cultivar	**Gelatinization transition temperatures**			
	To (°C)	**T_m (°C)**	**Tc (°C)**	**ΔH (J/g)**
Langi	66.19±0.03^{a}	68.26±0.03^{a}	71.19±0.03^{c}	63.28±0.02^{a}
Reiziq	64.12±0.03^{e}	67.97±0.03^{c}	71.13±0.03^{d}	46.92±0.02^{b}
Opus	62.85±0.05^{g}	67.29±0.00^{f}	70.03±0.02^{f}	38.14±0.01^{c}
Basmati	64.73±0.02^{c}	68.25±0.03^{a}	71.40±0.05^{b}	28.83±0.03^{d}
Kyeema	62.82±0.05^{g}	67.67±0.01^{e}	70.78±0.04^{e}	21.53±0.02^{g}
Sherpa	63.87±0.1^{f}	67.88±0.05^{d}	71.16±0.07^{cd}	10.95±0.02^{h}
Black rice	65.92±0.03^{b}	68.23±0.06^{a}	72.08±0.03^{a}	27.2±0.013^{e}
Doongra	64.40±0.03^{d}	68.15±0.05^{b}	71.16±0.03^{cd}	23.74±0.01^{f}

Values denote the Means of three replicates ± SD. The values with the same superscript letter are not significantly different according to Duncan's multiple range test ($p<0.05$).

Table 3: Mechanical (TS & EAB) and optical parameters (L*, a*, b* and transparency of different rice varieties films combine with ι-car and stearic acid.

Rice variety	TS (N/m)	EAB (mm)	L*	a*	b*	T (%)	Film Sol. (%)	Film relative crystallinity (%)
Langi	242.27 ± 73.09^{a}	32.36±2.28^{a}	93.55±1.59^{c}	-0.46±0.08^{c}	8.19±1.26^{b}	0.78±0.43^{a}	51.26±5.49d	18.7±0.07^{d}
Reiziq	133.6 ±17.27^{b}	18.42±2.02^{cd}	95.37±0.21^{ab}	-0.32±0.005^{b}	6.56±0.38^{cd}	0.53±0.06^{bc}	43.12±2.70a	34.1±0.05^{a}
Opus	141.7±7.87^{b}	24.86±2.39^{d}	96.0±0.30^{a}	-0.31±0.01^{b}	5.70±0.298^{d}	0.85±0.27^{bc}	56.32±1.18bc	13.4±0.06^{e}
Basmati	128.16±42.46^{b}	16.45±4.95^{cd}	95.38±0.19^{ab}	-0.31±0.01^{b}	6.33±0.194^{cd}	1.16±0.52^{bc}	49.21±5.06cd	29.3±0.05^{b}
Kyeema	162.19±14.02^{b}	22.17±0.91^{d}	94.87±0.432^{ab}	-0.32±0.02^{b}	6.84±0.427^{c}	0.46±0.10^{bc}	46.2±0.82ab	28.4±0.08^{c}
Sherpa	149.14±25.34^{b}	17.031±3.45^{cd}	94.29±0.418^{bc}	-0.34±0.005^{b}	6.66±0.143^{cd}	0.69±0.06^{b}	61.23±3.62bc	3.84±0.01^{g}
Black rice	135.01±37.05^{b}	15.24±2.61^{bc}	89.39±0.132^{d}	1.17±0.04^{a}	11.40±0.342^{a}	1.18±0.07^{c}	69.32±2.74ab	1.99±0.00^{h}
Doongra	159.8±2.49^{b}	19.799±3.62^{b}	95.23±0.366^{ab}	-0.38±0.01^{b}	6.20±0.072^{cd}	0.44±0.01^{bc}	64.36±1.62ab	4.9±0.02^{f}

Values denote the Means of three replicates ± SD. The values with the same superscript letter are not significantly different according to Duncan's multiple range test (p<0.05).

Figures

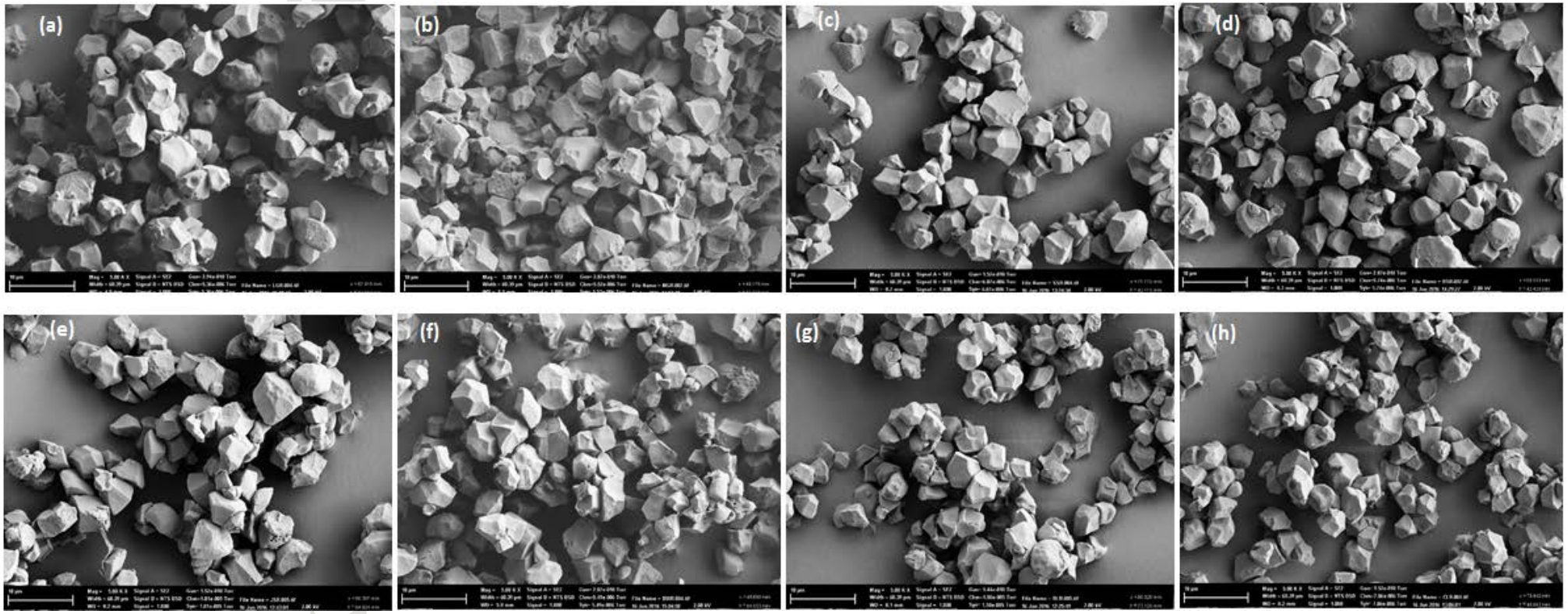

Fig 1

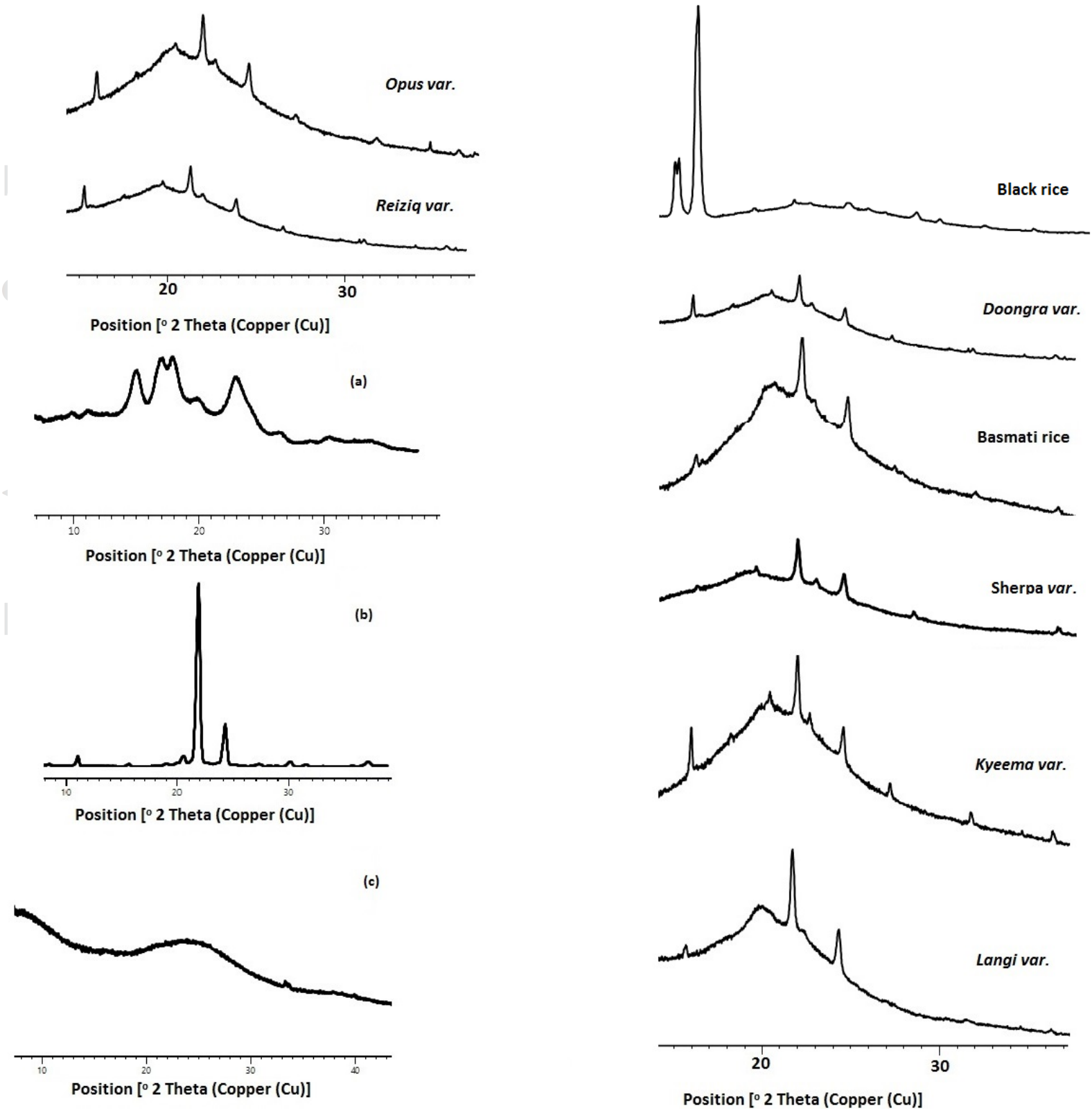
Opus var.
Reiziq var.
20
30
Position [° 2 Theta (Copper (Cu)]
(a)
10
20
30
Position [° 2 Theta (Copper (Cu)]
(b)
10
20
30
Position [° 2 Theta (Copper (Cu)]
(c)
10
20
30
40
Position [° 2 Theta (Copper (Cu)]
Black rice
Doongra var.
Basmati rice
Sherpa var.
Kyeema var.
Langi var.
20
30
Position [° 2 Theta (Copper (Cu)]

Fig 2

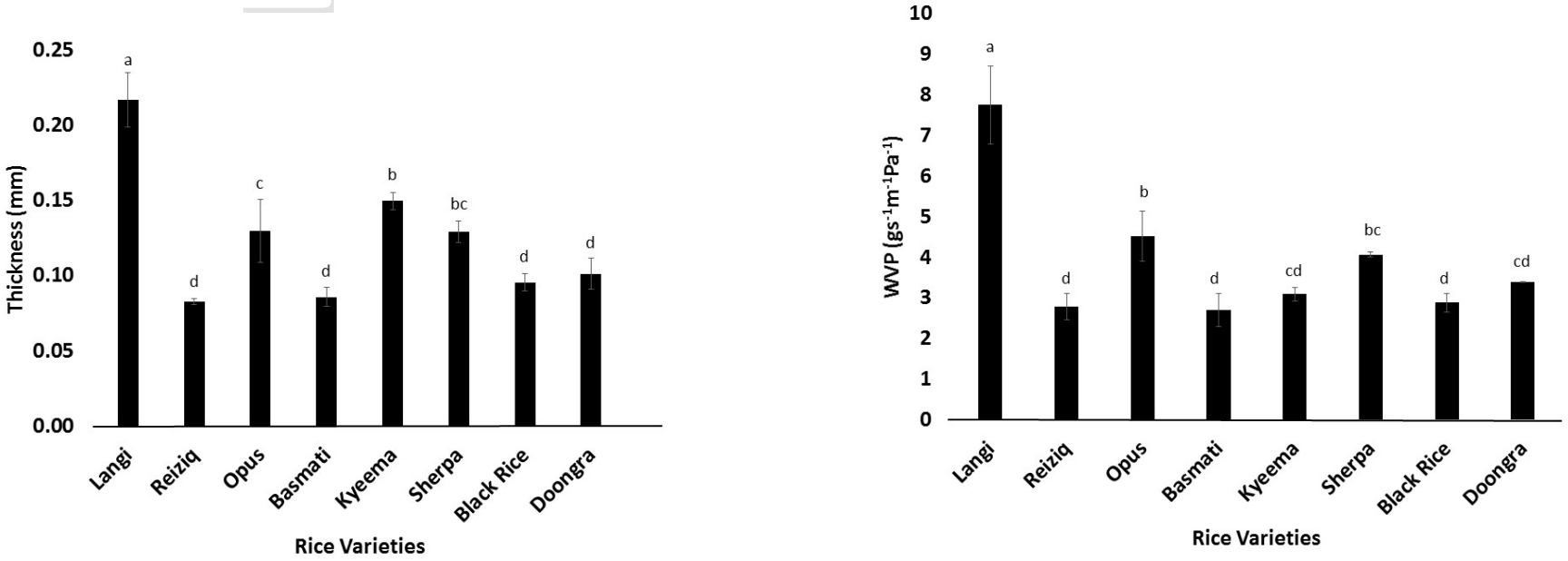

Fig 3