PHYSICO-CHEMICAL CHARACTERISTICS OF OSA-STARCH ISOLATED FROM BASMATI RICE AND ITS UTILIZATION IN ETHYLENE GLYCOL PLASTICIZED PVA BLEND FILMS

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In the current research, basmati rice starch (BRS) has been modified with octenyl succinic anhydride (OSA). The properties of BRS and modified basmati rice starch (MBRS) were evaluated, and subsequently, the effect of their addition to polyvinyl alcohol (PVA) in two different ratios (70/30 and 30/70) to prepare blend films was studied. The degree of substitution of MBRS was 0.00483%, which was found within the permitted range of Food and Drug Administration guidelines. The pH (6.1 vs. 5.8) and viscosity average molecular weight (1.5×10^4 and 1.275×10^4 Da) were found to be lower for MBRS, solubility (11.52 vs. 13.60%), swelling power (11.5 vs. 13.60 g/g), and oil absorption (2.4 vs. 3.2 g/g) capacities were higher. FTIR and XRD studies revealed minor differences in the MBRS spectra owing to the low substitution. The blend films cast with PVA and MBRS showed higher film thickness, hydration characteristics, transparency, and UV-blocking efficiency.

Keywords: basmati rice starch, color analysis, depolymerization, hydrophobic properties, hydration properties, ethylene glycol plasticized films

INTRODUCTION

Rice (*Oryza sativa*) is a well-known agricultural cash crop in Pakistan, and after meeting the domestic demand, Pakistan exports 4 million tons of rice, accounting for approximately 10% of global trade.[1,2] The rice plants need plenty of sunshine and water to grow, and the climate of Pakistan offers such potential. As a result, different varieties, such as basmati, IRRI, and bold grain are grown. Basmati rice is aromatic, extra-long, and whiter, which makes it a first-class product on the market. The Hindi translation of basmati is the queen of fragrance.[3]

The main component of rice is starch, which makes up about 90% of its total weight. It is a semi-crystalline polymer, with amylose and amylopectin as its main constituents. Apart from the primary constituents, starch contains a small amount of non-carbohydrate elements, such as proteins, lipids, and minerals, which influence its activity.[4] It has distinguishing characteristics, for instance, a flavorless taste, hypoallergenic properties, a small granular size, whiteness, and digestibility. These properties allow starch to be used as an emulsifier, defoaming agent, encapsulating agent, sizing agent, and film-forming agent in industrial goods.[5] Native starches generally bring some physico-chemical changes (texture and appearance) to the food due to their high hydrophilicity, tendency to retrograde, and syneresis. These changes vary as a function of the starch-water interaction, fat content, time, temperature, mechanical force, and biological activity of the native starches.[6]

To avoid this, native starch is modified with various physical (thermal and non-thermal,[7] radiation[8]) and chemical methods (esterification, etherification, acidolysis, cross-linking, oxidation, enzymatic), or by combinations of these as a modification process.[9] Modifications of starch can improve its functionality and broaden its

application.[10] Chemical modification is an approach that typically entails adding new functional groups to starch.[11] Among various chemical methods, the most commonly employed are esterification and etherification. In the esterification process, researchers[12–16] utilized octenyl succinic anhydride (OSA) under mild alkaline conditions. The esterification equation is shown in Figure 1.

Due to the modification, the starch achieved an amphiphilic and interfacial character,[17] which improved its basic properties, making it an excellent candidate for food and non-food applications,[18] pharmaceutical industry,[19] cosmetics,[20] film formation,[21] encapsulation,[22] stabilizers, and emulsification.[23] The FDA (Food and Drug Administration) in the United States tested OSA for toxicity and carcinogenicity, and concluded that it is safe.[17]

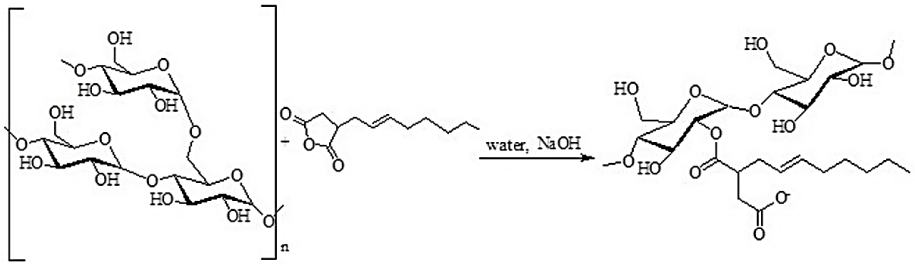

Figure 1: Esterification reaction of octenyl succinic anhydride with starch

In our everyday lives, we use plastic-based materials because they are easy to handle, durable, and flexible. However, most of them are made from petroleum resources, which are non-renewable, non-degradable, and costly. The global annual production of waste plastics is approximately 10 million tons. Hence, plastics are becoming the chief reason for pollution and the depletion of petroleum resources. As an alternative, researchers have developed biodegradable plastics made from various biomass resources, as they are renewable, low-cost, and widely available.[24]

Native and modified starch films are promising potential replacements for petroleum-based plastics. However, compared to available stretchable plastic films, these films have certain flaws, including brittleness and poor mechanical and water barrier properties.[25] Numerous authors have attempted to overcome these flaws by blending them with a synthetic biodegradable polymer, such as polyvinyl alcohol (PVA). PVA is a man-made, biodegradable, non-toxic, and water-soluble polymer. PVA and starch have excellent compatibility, which arises from the hydrogen bonding interactions among the available hydroxyl (OH) groups.[26] Phattarateera *et al*.[27] worked on PVA/starch blends of different ratios, and developed a blend of pre-gelatinized and hydrolyzed starch with PVA.

The present study aimed to isolate starch from basmati rice, modify it with OSA, and evaluate the functional properties of the starches, such as retrogradation, solubility, pH, oil and water absorption, color, and viscosity, and structural characterization with Fourier-transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD) analysis. Then, the native and modified basmati rice starches were utilized in preparing blend films with PVA. The effects of their addition to the film formulation on the physical, optical, and barrier properties of the blend films were studied.

EXPERIMENTAL

Chemicals

Kernel basmati rice (1120) was picked up from a market in Karachi, Pakistan, and stored at room temperature after cleaning. OSA (octenyl succinic anhydride) and NaOH (sodium hydroxide) were provided by Merck Chemicals Ltd. (Darmstadt, Germany). HCl (hydrochloric acid), I_2 (iodine), KI (potassium iodide), and KOH (potassium hydroxide) were delivered by Sigma Aldrich. PVA was purchased from Avon Chem. (UK), the degree of hydrolysis was 99%, and the molecular weight was 13158.77 Da, determined through the Mark-Houwink relation. C_6H_6 (n-Hexane), CH_3Cl (chloroform), ethylene glycol, $Mg(NO_3)_2$ (magnesium nitrate), and NaCl (sodium chloride) were supplied by BDH Laboratories (China). These reagents were 99.99% pure, and used without any further purification.

Basmati rice starch isolation

A laboratory grinder (Philips Mixture Grinder, model no. HL7699, China) was used to prepare basmati rice flour. Rice flour (200 g) and NaOH (500

mL, 0.1 wt%) were mixed with constant stirring and stored at 4 °C overnight. After decanting, the protein layer was stripped out, and NaOH was added to the solid phase and stirred again. After repeating this procedure twice, deionized water was added to the filtrate to adjust the pH (6.0-6.5). The residual was dehydrated in the oven (Binder ED53, Germany) at 40 °C for 48 h. The basmati rice starch (BRS) obtained was stored at 4 °C for further use.[28]

Basmati rice starch modification

A 30% suspension of BRS was prepared by maintaining a pH of 8.5 with NaOH. Modified basmati rice starch (MBRS) was collected by introducing the OSA reagent (3%) that was previously prepared in 1:3 v/v ethanol/water and added in the form of droplets with frequent stirring and retained a pH of 8-8.5. After 24 h, the reaction was ceased by lowering the pH (6.5) of the system with HCl (3% v/v). The MBRS slurry was dried at 45 °C for 48 h after being washed with distilled water thrice. The obtained MBRS was stored at 4 °C for further use.[12]

Preparation of PVA/BRS and PVA/MBRS blend films

The starches (BRS or MBRS) were gelatinized separately in a magnetic stirring bath at 80 °C. After cooling to room temperature, ethylene glycol (EG) was added (25% w/w of the total solid content, 3 g to the mixture), and further stirred for 10 min. The plasticizer amount was selected in light of initial research findings to create films free of cracks. Finally, the plasticized starch solutions were blended with predissolved PVA (in water at 90 °C) with continuous stirring. The resulting film-forming solution (FFS) was poured into poly-acrylic trays and placed in an oven at 60 °C, for 18 h. The films were peeled off and stored in plastic bags for successive experimental analyses.[26] In the investigation, several formulations with various weight ratios of PVA to starches (BRS and MBRS) were made; however, two ratios – one representing higher and lower PVA and starch (BRS and MBRS) concentrations – were chosen for further study. The two selected ratios account for the least transparent and the most transparent blend films. The film formulations and codes are listed in Table 1.

Table 1
Film formulation codes and the amounts taken to cast the blend films

Film formulation codes	PVA (g)	BRS (g)	MBRS (g)	EG (g)
30/70	0.9	2.1	-	0.75
70/30	2.1	0.9	-	
30/70	0.9	--	2.1	
70/30	2.1		0.9	

Physico-chemical properties of BRS and MBRS

Color assessments of starches

An easy to access Color Analysis app was employed to assess the color of the starches. The CIE Lab parameters of BRS and MBRS were determined with photographs taken on white paper. The color difference (ΔE), chromaticity (ΔC), and whitening index (WI) were evaluated using Equations (1)-(3):

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \quad (1)$$

$$\Delta C = \sqrt{\Delta a^2 + \Delta b^2} \quad (2)$$

$$WI = 100 - \sqrt{(100 - L^*)^2 + a*^2 + b*^2} \quad (3)$$

where ΔL, Δa, and Δb are the differences between the color parameters of the samples and the color parameters of the white standard (L* = 92.82, a* = -1.21, b* = 0.45).

pH Measurements

A suspension containing one gram of starches in 30 mL of deionized water was agitated for 10 min and left to settle for an hour. The pH was taken in water fractions with a pH meter (Orion, 520A, USA).[28]

Degree of substitution

A gram of starches was dissolved in 75% v/v ethanol in a stirring water bath at 50 °C. After cooling the samples to room temperature, NaOH (0.5 mol/L, 40 mL) was added. The flasks were covered with aluminum foil and left for three days. The flasks were titrated with HCl (0.5 mol/L) using pH titration. The degree of substitution (DS) was determined in two steps, as follows:[12]

%OSA Substitution =

$$(V_{BRS} - V_{MBRS}) \times 0.1 \times M_{HCl} / W \times 100 \quad (4)$$

DS =

$$162 \times \%OSA\ Substitution / 2100 - (209 \times \%OSA\ substitution) \quad (5)$$

where V_{BRS} is the volume of HCl consumed for BRS, V_{MBRS} is for HCl and w is the weight of BRS and MBRS taken.

Wettability test

The amphiphilicity was determined by suspending 10 mL of starches (BRS and MBRS) in two organic solvents (hexane and chloroform).[29]

Swelling power and solubility determination

For the determination of swelling power (*SP*) and solubility (*SOL*), 0.5 g of starches (BRS and MBRS) were gelatinized at 90 °C in 20 mL of distilled water. The gelatinized solutions were cooled to room temperature and centrifuged at 3000×g rpm for 15 min. The supernatant was removed cautiously and vaporized at 100 °C for 4 h. The *SP* and *SOL* were evaluated by:

$$SP\ (g/g) = \frac{\text{weight of starches after centrifuged} - \text{initial weight of starches}}{\text{initial weight of starches}} \quad (6)$$

$$SOL\ (\%) = \frac{\text{weight of starches after centrifuged}}{\text{initial weight of starches}} \times 100 \quad (7)$$

Oil and water absorption capacity

One gram of both starches (BRS and MBRS) was transfused with 10 mL of vegetable oil in a mixer (Heidolph Reax 2000, Germany). The tubes were centrifuged for 20 min at 4000 ×g rpm with an electronic centrifuge (80-1 m, China). The supernatant volume was measured by a graduated cylinder. The oil absorption capacity (*OAC*) evaluated by:[30]

$$\textit{Oil or Water absorption} = \frac{\text{volume of Oil absorbed} \times \text{density of Oil}}{\text{weight of starch}} \quad (8)$$

Depolymerization of BRS by the viscosity method

A series of solutions (0.1-0.6 g/dL) of BRS and MBRS were prepared in 1M KOH. The flow times were measured with an Ostwald viscometer (Techniconomial constant 0.05 Cs/s, England) at 25 °C. The intrinsic viscosity for BRS and MBRS was determined graphically.[31] The data were further used to estimate the molecular weight of BRS and MBRS through Staudinger-Mark Houwink's relation:

$$[\eta] = KM^{\alpha} \quad (9)$$

where $[\eta]$ is the intrinsic viscosity, K and α are the Mark-Houwink constants, and their values for BRS are 1.18×10^{-3} and 0.89, and for MBRS are 1.4×10^{-4} and 0.73774 at 25 °C.

Retrogradation test

One percent (w/v) gelatinized solution of starches (BRS and MBRS) was placed into a graduated cylinder of 50 mL. The supernatant volumes were recorded during the storage period of 180 h. The retrogradation rate was calculated by using the relation:[32]

$$\text{Supernatant rate} = \frac{\text{volume of supernat}}{\text{volume of paste}} \times 100\% \quad (10)$$

Measurements of iodine absorption spectrum

The test specimens were prepared by dissolving 200 mg of starches in KOH (0.5 mol/L). The pH of a 10 mL aliquot was adjusted to 3 with HCl (0.5 mol/L). A quantity of 0.5 mL of 0.2% I_2 in 2% KI was added to this mixture and the final volume was adjusted to 50 mL. The absorbance of the samples was measured with a UV-visible spectrophotometer (Beckman Coulter, DU 730, USA) in the range between 380-800 nm.[33]

Fourier transform infrared (FTIR) spectroscopy analysis of BRS and MBRS

The FTIR spectra of BRS and MBRS were recorded in the solid phase, with an FTIR spectrophotometer (Shimadzu, Japan) in the range of 400 and 4000 cm^{-1} at the resolution of 8 cm^{-1}.

X-ray diffraction (XRD) assessments

The X-ray diffraction of BRS and MBRS was performed at room temperature using an X-ray diffractometer (XPERT-PRO, model PW3064, Philips, Japan), having anode material, CuKα radiation (λ = 1.5405°A) in a wide range of 2θ = 10° to 80° at a scan speed of 0.6/min. The operating voltage and current were 40 kV and 30 mA, respectively.

Characterization of polymer blended films

Film thickness

The film thickness (d) data were collected by employing a digital micrometer (Mitutoyo-Co, Japan), with the sensitivity of ±0.001 mm, from eight different random positions, and an average value was calculated.[34]

Light transparency properties

The transparency (T) data were collected for 1 cm × 3 cm pieces of the blended films at two wavelengths (200 and 600 nm) by inserting the film directly into the spectrophotometer (Beckman Coulter, DU 730).[35]

Water absorption, water content and solubility of blend films

The water absorption, water content, and solubility were determined for the blend films. A 30 mm^2 piece was calibrated at 57% RH for two days and weighed. It was then dried in an oven (Binder ED53, Germany) at 60 °C. After cooling to room temperature, the films were again weighed and inserted in a glass vial containing 20 mL of deionized water. After 24 h, the blend film samples were removed and the extra water from the film surface was wiped off with filter paper, and the films were weighed again. These blend film specimens were dried at the same temperature and weighed until constant weights were achieved. The water absorption, moisture content, and solubility were determined as follows:

$$Water\ Absorption = \frac{m_s - m}{m} \quad (11)$$

$$Solubility = \frac{m - m_c}{m_c} \times 100\% \quad (12)$$

$$Moisture\ content = \frac{m - m_d}{m_d} \times 100\% \quad (13)$$

where m is the mass of the film calibrated at 57% RH, m_d is the mass of the dried film specimen, m_s is the

mass after immersing the film piece in water, and m_c is the mass after soaking and drying.

Statistical analysis

All the tests were conducted in triads to get reproducibility. The t-test was used to analyze the samples through IBM-SPSS (9.0) software. Differences were considered as significant at 95% ($p<0.05$).

RESULTS AND DISCUSSION

Color assessments of starches

The spectrophotometric and colorimetric techniques are the predominant instruments used to evaluate color. The polyphenol compounds, carotene, ascorbic acid, and any form of pigmentation reaction used to make the final product are responsible for the color of starch. The acceptability of starch for certain applications is dependent on its color and whiteness. The color characteristics of BRS and MBRS are presented in Table 2. The modification imparted an improvement in coordinates *L*, *a*, *b*. The *L* (black to white) value of MBRS was significantly higher than that of BRS. Besides this, it has lowered chromaticity. In general, consumer preference is based on high values for brightness, low chromaticity, and a high whitening index. The color shifts of the MBRS in the +a direction depicted a shift toward red, while the –b specified shifts toward blue. The result showed that BRS is yellowish due to a significantly higher +b value. This is because, during the modifying treatment with OSA, the used NaOH caused a decrease in the enzymatic browning reaction.[36]

pH and degree of substitution for MBRS

The acidity values of the BRS and MBRS are shown in Table 2. BRS has a higher pH value as compared to MBRS. The MBRS is a weak polyelectrolyte species. The carboxyl group (O=C-O-) imparts an acidic character to the starch granules.[37] Anchondo-Trejo *et al*.[38] have also reported a decrease in pH for the modified rice starch. The acidic values of the MBRS favor the breaking of the hydrogen bonding network in the starch entity, make it easier to solubilize, and lower the viscosity.

The alkaline saponification method was utilized to evaluate the DS, and a value of 0.00483 ±0.0002 was observed for MBRS. The result fits in the FDA recommendation ranges (3%). The DS is the average number of hydroxyl groups substituted per glucose unit, which is consistently used as an indicator to evaluate the effectiveness of OSA modification.[39] The esters or ether groups are known to bind with starch in the range of 0.002-0.2. Consequently, one substituent is likely to be found in every 500 glucopyranosyl units. The esterification process replaces the carbon atoms in positions 2, 3 and 6 as they have less steric hindrance. Thus, DS leads to an increase in the active binding sites among the substituted MBRS.[40] The DS for BRS is influenced by the botanical origin, geographical location, sodium hydroxide concentration, pH, and mechanical activation time.

Wettability test

Wettability is a convenient and qualitative test that confirms the amphiphilic nature of MBRS. It depends on the adhesive and cohesive forces of the system.[41] When an amphiphilic compound is added to immiscible solvents, the polar heads connect with the polar ends, while the lipophilic chain will move in a non-polar liquid. The hydrophilic heads of the amphiphilic chains stay in an aqueous phase, whereas the hydrophobic tail inhibits direct contact with water. Here, two solvent systems were tested: distilled water/chloroform and distilled water/hexane. The solvents water, chloroform, and hexane have polarities of 10.2, 4.1 and 0.1 and densities of 1, 1.49 and 0.66 g/cm^3, respectively.

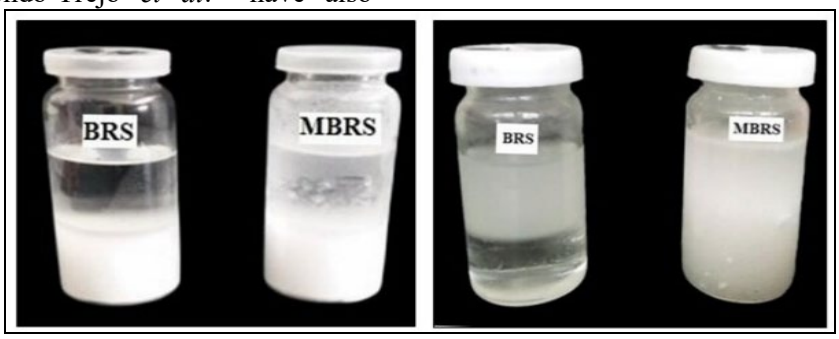

Figure 2: BRS and MBRS in hexane (left) and chloroform (right)

As shown in Figure 2, the MBRS enters the organic phase, while the BRS remains in water, which confirms its amphiphilic nature.[42] A food system is an aggregate of hydrophilic (sugars and amino acids) and hydrophobic (fats and oils) molecules. It was thus concluded that MBRS has the readiness to interact with both hydrophilic and hydrophobic molecules and can stabilize both types of molecules.[43]

Swelling power and solubility for BRS and MBRS

The swelling power (*SP*) of starch specifies the extent of water absorption by starch granules, and the solubility (*SOL*) reveals the dissolution of starch in the process. These two properties give information about the non-covalent interactions between the guest water molecule and the host starch molecule. When starch is heated over water, the semi-crystalline structure is disrupted, resulting in the formation of a hydrogen bond between water and the available hydroxyl groups of starch (particularly with amylose and amylopectin). As a result, the starch granules swell, increasing their size and solubility. It has been reported[44] that amylopectin facilitates *SP*, whereas amylose, lipids, and proteins obstruct it.

The *SP* and *SOL* of BRS and MBRS are listed in Table 2. MBRS has significantly higher *SP* and *SOL* than BRS, which is due to an increase in polarity gained by MBRS by the insertion of the OSA molecule.[45] The accumulation of negative charge induced inter-particle repulsion, which resulted in the breakdown of the hydrogen network and the glycosidic linkages in starch molecules. This made water penetration inside the granules easier, and thus the *SP* and *SOL* increased. Similar findings have been confirmed by Naseri *et al*.[13] for sago starches. High *SP* starches are the preferred choice for pharmaceuticals, since they can quickly disintegrate to release the active medicinal ingredient.

Oil absorption capacity of BRS and MBRS

Oil absorption capacity (*OAC*) represents the interaction of starch with oil, which takes place through the capillary attraction mode.[44] As shown in Table 2, MBRS has a higher *OAC* than BRS. This is because BRS is a hydrophilic substance and thus has a weaker tendency to interact with oil. The modification with OSA imparted hydrophobic channels in the native BRS, making it a good oil absorber. Generally, hydrophobic ligands, such as oil, iodine, fatty acids, and surfactants, tend to build inclusion complexes with the linear amylose molecule. Since MBRS has an amphiphilic character, its lipophilic channels allow the oil to penetrate and thus construct an inclusion complex easily.[46,47] It can be said that MBRS is probably advantageous in food structure interaction, particularly in flavor persistence, food quality improvement, and extending the storage period, thus prolonging the shelf life of the product.

Starch depolymerization studies with intrinsic viscosity

The simplest and least expensive way to evaluate the average molecular weight of polymers is to measure their intrinsic viscosity, and the molecular weight obtained through this method is called the viscosity average molecular weight (VM_{avg}).[48] However, the foremost condition for determining molecular weight by this method is the mono-dispersed nature of the solution. Because starch solutions are poly-dispersed in water, collecting intrinsic viscosity data is difficult. To eliminate such effects, the starch solution was made in KOH, and its transmittance was checked before passing through a viscometer. All the samples showed transmittance greater than 95%.[49] The intrinsic viscosities of BRS and MBRS were 6.2 and 0.25 dL/g, respectively. The lower value of MBRS specifies the chain scission in the BRS backbone, where the degradation process happens in the KOH solutions. It is believed that when the BRS molecules are treated in strongly alkaline conditions, the protons dissociation leaves negative charges on the BRS. This develops an ion-solvent repulsion among the negative charges of BRS and OH groups, which swells the BRS granules. It brought tension to the adjacent crystallite of BRS, led to the uncoiling or detachment of the double helices region, broke up starch granules, and decreased VM_{avg}.[50] The VM_{avg} of BRS and MBRS was 1.512×10^4 and 1.275×10^4 Da, respectively. Takizawa *et al*.[51] showed a decrease in the average molecular weight of various starches after treatment with potassium permanganate and lactic acid. It has been found that modified starch with a lower molecular weight has increased encapsulation efficacy and capacity.[52]

Retrogradation

Retrogradation is the restoration of amylose and amylopectin molecules when gelatinized starch is cooled. It can be either short-term or long-term. The amylose molecule reconstruction is short-term; whereas the amylopectin reconstruction is long-term.[32] Figure 3 shows the retrogradation rates of BRS and MBRS stored at room temperature for seven days. There was a comparable difference in the retrogradation rate; in BRS, it increased enormously during the first 24 h, while in MBRS, there was no noticeable change. The curve represents a constant plateau after 120 h for both starches (BRS and MBRS). The amylose association occurs early, and the smaller, unbranched amylose molecules reassemble quickly in BRS. However, hydrophobic sites in MBRS render these reconstructions fast, and hence, they increase steeply.[53] Subsequently, the amylopectin association occurs in a far longer process that takes several days.[54] Amylopectin reconstruction leads to the transformation of the starch into a hard gel. This phenomenon is worthwhile because starch is frequently employed as a food constituent in numerous products, and the quality of the food product diminishes over time. It makes the products objectionable to the consumer and contributes to waste. It depends on storage temperature, the composition of food contents (sugars, lipids, and water content), amylose content, and the average chain length of starch.[5]

Table 2
Physico-chemical characteristics of BRS and MBRS

Starch	L*	a*	b*	WI	ΔE	ΔC	pH	*SP* (g/g)	*SOL* (%)	*OAC* (g/g)	(1022/995)
BRS	72.5 ±0.21^{b}	-1.3 ±0.0a	+7.0 ±0.1^{b}	71.5 ±0.1^{a}	21.084 ±0.002^{b}	6.551 ±0.002^{b}	6.10 ±0.10^{b}	11.525 ±0.003^{a}	8.288 ±0.005^{a}	2.465 ±0.004^{a}	4.193 ±0.007^{a}
MBRS	78.9 ±0.12^{a}	+0.2 ±0.0^{b}	+3.4 ±0.0^{a}	78.6 ±0.2^{b}	14.606 ±0.001^{a}	3.153 ±0.001^{a}	5.87 ±0.01^{a}	13.606 ±0.002^{b}	11.589 ±0.004^{b}	3.276 ±0.008^{b}	2.704 ±0.002^{b}

Values are the mean of three tests. Different superscript letters within columns are significantly different at $p<0.05$ by using a t-test

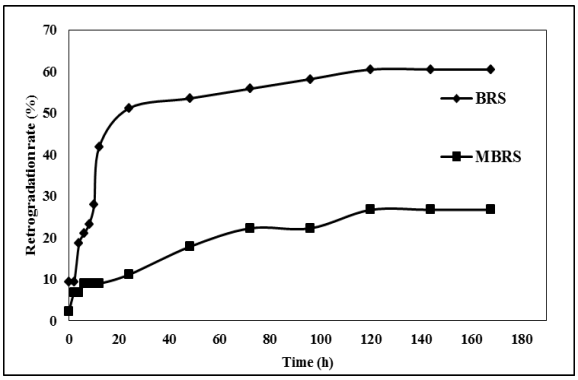

Figure 3: Retrogradation behaviors of BRS and MBRS

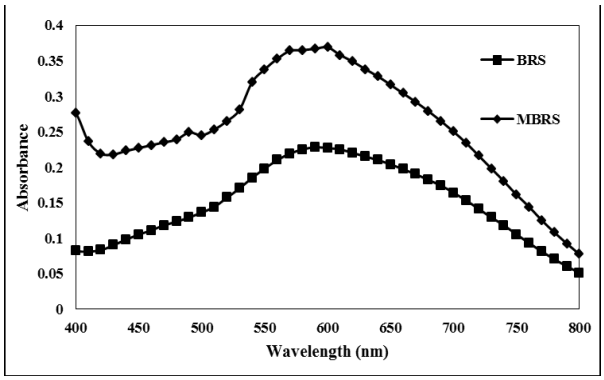

Figure 4: Iodine absorption spectra for BRS and MBRS

Iodine absorption spectrum

Iodine produces non-covalent, left-handed, single-helical, colorful inclusion complexes with amylose in starch. As a result, it can be used as an indicator to reflect the amount of linear fraction contained in starch. Molecular iodine acts as a Lewis acid, combining with electron-rich substances to form charge transfer complexes.[55] The iodine inclusion complex is shown in the following equations:

$$I_2 + H_2O \leftrightharpoons H^+ + I^- + HOI \quad (14)$$

$$I^- + I_2 \leftrightharpoons I_3^- \quad (15)$$

$$I_3^- + starch \leftrightharpoons I_3^- - starch\ complex \quad (16)$$

The iodine-absorption spectra of BRS and MBRS after iodine exposure are shown in Figure 4. It could be observed that the $\lambda_{(max)}$ of the two starches (BRS and MBRS) was around 600 nm, which indicated that the amylose content of the two starches (BRS and MBRS) was equivalent. Our results are further confirmed by the study by Singh *et al.*,[56] who showed that modification of

different cereal starches with strong and weak acids did not appreciably change the iodine binding capacities. It could also be observed from Figure 4 that the iodine absorbance values of MBRS are higher, which is due to the presence of protein in BRS. It has been reported[57] that the presence of proteins in starch decreased its capacity to bind iodine and had no effect on the $\lambda_{(max)}$ values. Thus, our results indicate that the existence of starch-protein interactions in BRS influences the establishment of starch-iodine complexes, altering the mobility and unit chain of the polymer chains accessible to complex with iodine because the iodine absorption value is reliant on chain mobility and $\lambda_{(max)}$ is highly dependent on the degree of polymerization.

Fourier transform infrared (FTIR) spectroscopy analysis of BRS and MBRS

FTIR was used to confirm the structural amendment of MBRS. Figure 5 depicts the combined FTIR spectra of BRS and MBRS. The FTIR spectra of BRS and MBRS were similar, indicating that OSA modification does not influence the structural skeleton of the starch.

The existence of absorption bands in the two spectra at roughly 3300-3600, 2900, 1150, and 1000-1100 cm^{-1} revealed that both starches (BRS and MBRS) have OH, C-H, C-O-C, and CO functional groups, respectively. It further emphasized that no new functional group is formed as a result of OSA modification; therefore, the two spectra appear to be identical. However, there is a change in the vibrational frequencies and intensities due to BRS modification with OSA. The peak at 1640 cm^{-1} is due to H_2O bending vibrations, which result from bound water in starch granules.[15,58,59]

The IR spectra peak around 800-1200 cm^{-1} could be used to investigate the crystalline structure and short-range organization of starch granules, and the peak ratio of 1022/995 cm^{-1} indicates a change in the short-range molecular order of double helices in starch. As illustrated in Table 2, MBRS has a higher value at 1022/995 cm^{-1}, which suggests a significant reduction in the short-range order of MBRS, indicating that OSA groups were grafted on the surface of the starch granules.

The fingerprint region shows C-O stretching in the region of 1000-1200 cm^{-1}. BRS and MBRS presented similar infrared spectra. The similarity in the FTIR spectra for BRS and MBRS indicated no structural changes. It has been reported in the literature[60] that the introduction of the carboxyl group in the starch is confirmed through a peak at 1722 cm^{-1}, which was not seen in the current study owing to the lower DS (0.00045).

XRD examination

X-ray diffraction (XRD) computes the crystallinity of the system. Starch was categorized as a semi-crystalline substance after the pioneering work of Katz[61] in 1930. There are three types of crystal patterns presented: A, B, and C, which are sorted based on their botanical origin. Type A starch is isolated from cereals (rice, wheat, pulses), type B starch – from maize, potato, and canna, and type C – from sago and arrowroot. The recorded XRD spectra of BRS and MBRS are shown in Figure 6. The diffraction peaks in BRS were marked strongly at 2θ = 15.11, 19.9, 23.01, and 44.63°, which corresponds to the amylopectin portion that establishes the granules of starch.

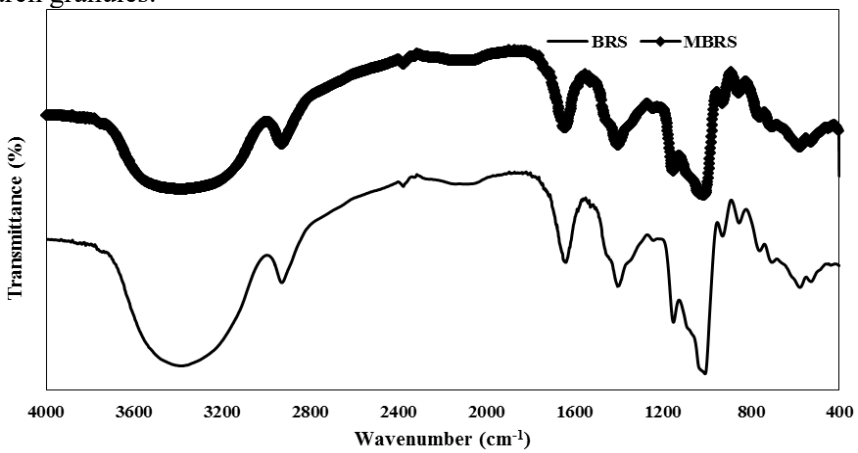

Figure 5: FTIR spectra of BRS and MBRS

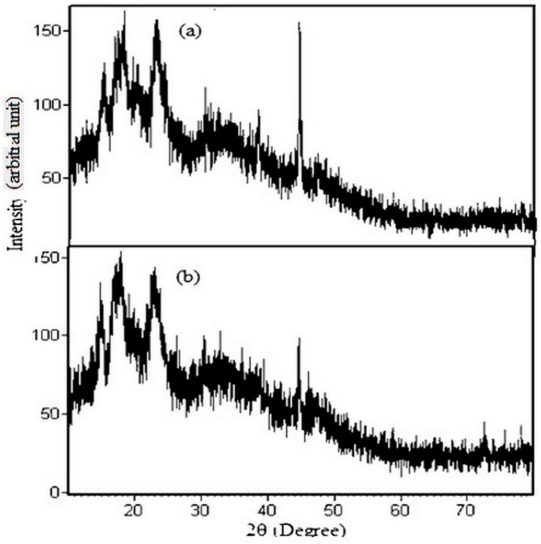

Figure 6: XRD patterns of (a) BRS and (b) MBRS

The observed peaks corresponded to inter-planar distances of 5.86, 5.03, 3.86 and 2.03 Å, respectively. The results show that BRS belongs to the family of type A starches.[62] A-type starch shows compact packing arrangements in such a way that water molecules are present between the double helix assembly.[58] The XRD diffractogram of MBRS was similar to that of BRS, suggesting no structural changes have occurred; the overall modification takes place in amorphous regions. The differences in the peak intensities were due to changes in crystallinity. Similar results were also reported by other researchers,[63] who had previously worked on waxy maize starch, with a low degree of substitution (0.049).

Characterizations of PVA/BRS and PVA/MBRS blend films

Film thickness

It could be observed from Table 3 that, regardless of the starch type (BRS or MBRS), the films with greater starch concentrations (70/30) have higher thickness values than the films with lower starch concentrations. It has been reported in the literature[64] that films with linear polymer chains are thinner, while those with branched polymer chains are thicker. Rice starch is a mixture of linear (amylose) and branched (amylopectin) molecules; on the other hand, PVA is a linear chain polymer. As a result, when blend films were formed, the ones with a lower starch percentage (70/30) had a more ordered linear chain than the 30/70 films.

It could also be observed that, despite using the same volume of FFS in the blend film formation process, the PVA/MBRS blend films were thicker than the PVA/BRS blend films, which is due to the presence of the OSA group, which promotes greater spacing between MBRS chains, and hence PVA/MBRS has higher film thickness values.[34]

Transparency of blend films

The packaging industry generally has a demand for transparent and UV-protecting films. The T_{200nm} denotes protection from UV radiation, whereas the T_{600nm} describes their transparency. The higher the transmittances in this region, the better the product visualization.[65] The transmittances at T_{200nm} and T_{600nm} are listed in Table 3, which indicates that the blend films have UV-blocking efficiency. It is thus concluded that the casted blend films are helpful for UV-protecting devices (spectacles, gloves), drugs and foods. Certain types of chromophore groups have the capability of absorbing UV radiation. In BRS and MBRS, the chromophore group is the carbonyl (–C=O) responsible for blocking UV radiations.[67]

The T_{600nm} was found to be influenced by starch concentration. Blend film 30/70, with the maximum concentration of starches, had the lowest T_{600nm} or vice versa. The drop in transmittance at greater concentrations of starches is attributed to the polarizable amylose and amylopectin components inside starches, which can oscillate visible light. Hence, reinforcement in obstructing light transmittance occurs, which induces opaqueness.[68]

The T_{600nm} values were lowered for the PVA/BRS blended film. A reason for this was linked to the protein in BRS, which brought a yellow color to the film. Additionally, in MBRS, the attached OSA groups would cause repulsions between the chains of amylose and amylopectin, which would prevent their accumulation and result in high transparency.[67]

Water absorption, water content and solubility of blend films

The most appropriate film material for packing should prevent environmental contaminants, such as moisture, from penetrating, since this water could set a breeding ground for pathogens, making it crucial for the food preservation.[70] The water absorption (*WA*), water content (*WC*), and solubility (*SOL*) of the PVA/BRS and PVA/MBRS are shown in Table 3. PVA and starch are hydrophilic, which means they readily absorb water. However, *WA*, *WC*, and *SOL* were lower for lower starch concentration blend films (70/30), which shows that the complex formation between the three -OH groups of starch and PVA resulted in a highly rigid three-dimensional network structure and did not facilitate *WA*, *WC* and *SOL*.[71]

It could also be observed that PVA/MBRS have significantly ($p<0.05$) higher *WA*, *WC* and *SOL*. It was predicted that the hydrophobic effect of OSA would promote reductions in these parameters; however, contrary results were observed. The OSA molecule acts as a plasticizer that destroys the intermolecular hydrogen bonds between starch chains, increasing their mobility among starch chains and thereby increasing *WA*, *WC* and *SOL*. This property of PVA/MBRS blend films may be advantageous for wrapping fresh food items like fruits and vegetables, but it may be disadvantageous for items like bread and cereals, which are dry. The improved hygroscopic nature also favors film hydro-biodegradation. Similar findings have been reported by Naseri *et al*.,[13] who worked on the properties of OSA-modified sago starch films.

The analysis of *WC* at two different RH conditions revealed that the blend films stored at 75% RH contained more moisture. This is because the water molecules are adsorbed onto the films faster in high RH environments.[70]

Table 3
Film thickness, light barrier and water absorption, content, and solubility of PVA/BRS and PVA/MBRS blend films

Property		PVA/BRS		PVA/MBRS	
		30/70	70/30	30/70	70/30
d (mm)		0.092±0.0005a	0.072±0.001a	0.102±0.001b	0.083±0.002b
T (%)	200 nm	0.0±0.0577b	0.0±0.000a	0.0±0.000a	0.0±0.000a
	600 nm	40.8±0.457b	80.6±0.208b	45.2±0.208a	82.2±0.200a
Water absorption		2.456±0.001b	1.993±0.005a	3.452±0.005a	2.176±0.001b
Water solubility (%)		35.212±0.001a	42.131±0.001a	51.213±0.002b	45.102±0.003b
Water content	57%RH	2.310±0.0577b	1.810±0.003a	4.710±0.0333a	3.330±0.005b
	75%RH	8.242±0.003a	6.340±0.003a	12.407±0.008b	9.136±0.005b

Values are the means of three tests. Different superscript letters within columns are significantly different at $p<0.05$ by using a T-test

CONCLUSION

The present studies focused on modifying Pakistani basmati rice starch (BRS) with octenyl succinic anhydride (OSA). The DS of the modified basmati rice starch (MBRS) was 0.00485. The pH value of MBRS was lower than that of BRS due to the insertion of a carbonyl group upon modification. The color parameters showed that MBRS is whiter than BRS due to the use of NaOH in the modification process, which suppresses the enzymatic browning reaction. The intrinsic viscosity and molecular weight were lower in MBRS, which supports better emulsification properties. Due to the increase in amphiphilic character, the oil absorption characteristics of MBRS improved. The swelling power and solubility increase, whereas the retrogradation rates become lower for MBRS due to the replacement of -OH groups with OSA. The wettability studies confirmed the amphiphilic nature of MBRS. The FTIR and XRD studies revealed that no structural changes had been brought about in the proximity of BRS owing to low substitution.

BRS and MBRS were utilized to prepare PVA blend films by using solution casting techniques

for a systematic evaluation of their physical, optical, and hydration properties. It was concluded in the study that the film cast with PVA and MBRS had a higher thickness and strongly blocked UV radiations, while being highly transparent to visible light. The studied hydration properties showed that the PVA/MBRS film was more resistant to water absorption, absorbed less moisture, and displayed higher solubility. According to the findings of the study, MBRS exhibited superior qualities, compared to BRS, and may be utilized in substitution of the latter and the casted films may find use in the food packaging sector.

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