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RESEARCH ARTICLE

Physico-Chemical Properties of Indian Horse Chestnut (*Aesculus indica*) Starch Films as Affected by γ-Irradiation

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Abstract

Starch films were developed using starch from Indian Horse Chestnut (IHCN) and the effect of gamma-irradiations on the physico-chemical properties of films was evaluated. The starch films were irradiated at 1.25, 2.5 and 5 kGy doses. Irradiated films exhibited varied properties compared to the native IHCN starch films. The results revealed that irradiation increased the flexibility of films from 50.53 to 66.12%, water holding capacity from 84.24 to 88.55%, solubility from 25.44 to 27.85%, and transparency from 3.20 to 5.20. However, the films displayed a decrease in mechanical resistance (3.88–1.05 MPa), moisture content (10.01–8.89%), thickness (0.31–0.29 mm), swelling capacity (130.54–43.28%), and density (0.163–0.156 gcm^{-3}). An increase in water vapor permeability from 4.17 to $7.61 \times 10^{-12} gm^{-1}\ s^{-1}\ Pa^{-1}$was also observed. Scanning electron micrographs of irradiated starch films depicted less dense and more open structure with small pores than the native films which accounts for their high oxygen and water vapor permeability.

Keywords Indian Horse Chestnut · Starch films · Gamma-irradiations · Properties

Introduction

Indian Horse Chestnut-IHCN (*Aesculus indica*) also known as Himalayan chestnut belongs to family Hippocastanaceae. It is found in the temperate regions of Europe, Asia (particularly East-Asia) and North America with elevations varying from 900 to 3600 m [1]. In India, IHCN is present in hilly areas as well as in plains of Kashmir valley. Among the various constituents present in IHCN, starch is the major component constituting about 38.3% on dry weight basis.

Starch is a well-known carbohydrate and a storage glucan having anhydroglucose units: a linear chain molecule linked with α-(1–4) bonds named amylose and branched molecule with α-(1–6) branch points and linear regions of α-(1–4)-linked glucose units, amylopectin [2]. The important functional property of starch is its ability to form continuous matrix (films) in the presence of plasticizers [3]. Starch films are nontoxic to environment, eco competitive with petroleum, inexpensive, recyclable with effective barrier to gas transport (oxygen, carbon dioxide) and low polarity compounds [4]. Further, it prevents the food from the migration of packaging contaminating substances. Thus use of starch films could be used to mitigate the problems associated with the synthetic plastic packaging films. The current global consumption of plastic is more than 300 million tonnes with an annual growth of about 5%. Packaging materials based on starch have been commercialized and currently dominates the market, representing 85–90% of market's bio packaging films. As IHCN is abundant in starch, it can be used for the production of starch packaging films, thus decreasing dependence on synthetic plastic materials for commercial packaging and consumer products.

Molecular structure of starch is complex and nonlinear leading to increase in crystallinity over time (increased brittleness) due to extensive intermolecular forces. Thus to form starch film with increased chain mobility, flexibility and extensibility, chemical additives like plasticizers are added. Further improvements in starch film properties can be achieved by various modification techniques. Of these gamma irradiations are well known and very convenient

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tool for modification through degradation [5] cross-linking [6] and grafting techniques [7]. Gamma irradiations are low cost, have no dependence on any type of catalysts, require minimal sample preparation, and are an environmentally friendly method [8, 9]. Gamma irradiations can also be used to treat foods in packed form. Under such circumstances the packaging material containing foods must be able to withstand the irradiations without alteration of barrier properties. The aim of the study was to evaluate the effect of gamma-irradiations on the physico-chemical properties of IHCN starch films so as to consider the use of gamma irradiations in foods packaged in starch-based packaged material.

Materials and Methods

Materials

Indian Horse Chestnut seeds were harvested from the main campus of the University of Kashmir, Srinagar, India during the month of November, 2015. Cleaning of the seeds was done manually followed by dehulling the seeds and storing them at refrigerated temperature until further use. All the chemicals used in the study were of analytical grade.

Isolation of Indian Horse Chestnut Starch

Starch was isolated from Indian Horse Chestnut using the alkaline steeping method [10]. Seeds were manually deshelled and the cotyledons were chopped into small pieces of approximately 2 × 2 cm. The pieces were pulverized along with water for 5 min in a domestic mixer blender. The slurry obtained was then diluted to ten times (volume/volume) with distilled water, and the pH was adjusted to 9 using 0.5 M NaOH. The slurry was continuously mixed using magnetic stirrer for 1 h and then filtered through a 75 µm-mesh sieve to separate the fibre. The filtered slurry was then centrifuged at 3000×*g* for 30 min at 10 °C (5810R, Eppendorf, Hamburg, Germany). The aqueous phase obtained on centrifugation was discarded, whereas the sediment obtained was scraped off from the surface and the lower white portion was washed three times with double distilled water and recovered as starch. The starch was dried at 40 °C in a hot air oven (NSW-143; Narang Scientific Works Pvt. Ltd., New Delhi, India).

Preparation of Starch Films

The films were prepared by "casting" a suspension of 4% starch (w/w) in deionized water. The suspension was homogenized for about 30 min using magnetic stirrer and then heated at 70 °C for 40 min using stirrer cum hot plate. This was followed by addition of 0.76 g glycerol (19% w/w on starch dry basis) as a plasticizer and further heating for 15 min. Subsequently the formulation (100 g) was poured on a levelled glass slab (21 × 11 cm) and air dried at room temperature (27 °C and 45% RH) for about 10 h. The dried films were then carefully peeled off from the glass slab and stored at ambient temperature in airtight LDPE packages so as to prevent further drying or moisture ingress.

Proximate Analysis of IHCN Starch and Starch Films

Moisture (925.10), protein (920.87), crude fat (920.85) and ash (923.03) contents were determined according to the standard methods [11].

Irradiations of Starch Films

The irradiation treatments were performed at Baba Atomic Research Centre Zakura, Srinagar, J&K, India using Panoramic Batch Irradiator. Irradiation of the starch film samples (10% moisture content) was done at a dose rate of 2 kGy/h using cobalt-60 (^{60}Co) as a gamma source with absorbed doses of 1.25, 2.5 and 5 kGy at ambient temperature (21 ± 0.5 °C), 42% RH and 0.1 MPa pressure. A reference non-irradiated starch film sample was also prepared (0 kGy).

Film Characterization

Film Thickness

Screw gauge was used to determine the thickness of films. Six random positions (two in the center of the film and four around its perimeter) on each film sample were measured. Average thickness for each sample was calculated.

Film Density

Density of the starch films was determined by measuring the weight and volume ($L \times B \times H$) of the films. Density was measured using the formula:

$$\text{Density} = \frac{\text{Weight of films}}{\text{Film volume}} \quad (1)$$

Water Content

Water content of the films was measured by determining the weight loss of the film after drying using gravimetric method. Initial weight of the film samples (2 × 3 cm) was measured (M_1). Film samples were then dried at 110 °C in a laboratory oven (NSW-143, Narang Scientific Work Pvt. Ltd, New Delhi) until constant weight was obtained.

Water Solubility

Method described by Gontard et al. [12] with some modifications was used to measure the water solubility of the films. The solubility of film in water was calculated as the dry matter percent of the film solubilized. First, the film samples were cut into 2×3 cm pieces. These were dried in an oven (105 °C for 24 h) and weighed. The film pieces were placed individually in 30 mL water in beakers and maintained under shaking at 25 °C for 1 h using shaker (NT145; Novatecnica,, Brazil). Film pieces were then taken out and dried at 60 °C until constant weight was attained to determine the final weight of dry matter. Water solubility was measured using formula:

$$\text{Water solubility } (\%) = \left[\frac{(W_1 - W_2)}{W_1}\right] 100 \tag{2}$$

where W_1 and W_2 are weight of wet samples and air dried samples respectively.

Swelling Capacity

Swelling capacity was measured to determine the impact of liquid water content and absorption on starch films. Films were cut into pieces and weighed. These were then individually placed in 50-mL glass vials filled with 20-mL distilled water, capped and stored at 25 °C for 24 h. Film pieces were then taken out, and dried at 40 °C for few minutes to remove surface water and weighed. Swelling capacity was calculated as:

$$\text{Swelling capacity } (\%) = \left[\frac{(W_2 - W_1)}{W_1}\right] 100 \tag{3}$$

where, W_2 is the final weight of film after hydration and drying to remove surface water and W_1 is the initial weight of film.

Water Holding Capacity

Water holding capacity of the film was measured according to standard protocol [13]. The film samples (2×3 cm) were weighed (w_1) and then submerged in beakers individually with deionized water (25 °C±2°). The samples were removed from the beaker after 2 min and excess water was removed with filter paper. The films were weighed again (W_2) and the adsorbed water was calculated using following equation:

$$\text{Water holding capacity } (\%) = \left[\frac{(W_2 - W_1)}{W_1}\right] 100 \tag{4}$$

where W_1 and W_2 are weight of air dried and wet samples, respectively.

Mechanical Properties

The mechanical properties, tensile strength (TS, MPa), percentage elongation at break (EAB%) were measured using TA-XT2i Texture Analyzer (Stable Microsys-tems, United Kingdom). The specimens (10×2.5 cm) were cut and kept at room temperature before measurements. Tensile tests were performed using self-tightening roller grips. The initial distance between the grips was 50 mm and the initial velocity was adjusted to 5 mm/min. The force and distance were recorded during extension of film strips to the breaking point. Tensile strength was calculated using the following equation:

$$\text{TS (MPa)} = \frac{\text{Maximum force of films}}{\text{Cross sectional area of film}} \tag{5}$$

Elongation at break was measured by:

$$\text{EAB } (\%) = \frac{\text{Film elongation at rupture}}{\text{Initial gauge length of film}} \times 100 \tag{6}$$

Water Vapor Permeability

Water vapor permeability of the films were determined by placing 20 g of silica gel in aluminium cups to produce 0% RH and the films were sealed onto these cups. Using an electronic balance (Acculab, Sartorius Group, Germany), the weight gain of the cups was measured for 24 h at an interval of half an hour. For each cup, plot of weight gain (g) against time (h) was prepared. The amount of water vapor diffusion through the film per unit of time (g/h) was determined through the slope of linear portion of this plot. Water vapor diffusion per square meter (m^2) showed the water vapor transmission rate, expressed as g/m^2 h. Water vapor permeability was calculated using the formula:

$$\text{Water vapor permeability} = \frac{[(\text{WVTR} \times T)]}{\Delta P} \tag{7}$$

where WVTR is the water vapor transmission rate; T is thickness of film (mm) and ΔP is the partial water vapor pressure difference between two sides of film (4.2449 kPa at 27 °C).

Color

The color of the films was determined using portable colorimeter, Mini Scan XE (Hunter Lab-Riston, Virginia) using CIE LAB color parameters: L^*, from black (0) to white (100); a^* from green (−) to red (+); b^* from blue (−) to yellow (+). The films were placed onto a white plate which

was used as a standard background, (L_0* = 81.77, a_0* = 2.41 and b_0* = 1.25). The total difference in color (ΔE*) was calculated as:

$$\Delta E* = \sqrt{(L* - L_0*)^2 + (a* - a_0*)^2 + (b* - b_0*)^2} \quad (8)$$

Transparency

Absorbance of the films was taken at 600 nm using a UV–Visible spectrophotometer (Model UV-1800; Shimadzu, Japan). Transparency values of the films were calculated as the ratio between the absorbance and the film thickness. Greater transparency values represent lower film transparency:

$$\text{Transparency} = \frac{\text{Absorbance}}{\text{Thickness}} \quad (9)$$

Oxygen Permeability

The oxygen permeability in starch films was calculated by determining the peroxide and acid value. About 10 mL of vegetable oil was placed in a conical flask and the starch films were covered tightly over the flasks. The films were removed from the flasks after 20 days and peroxide, as well as acid value of oil was measured.

Peroxide value was determined by a modified standard method [14]. 2 mL of sample was taken. 30-mL acetic acid-chloroform was added and the sample was heated. 0.5 mL of potassium iodide solution was added and stirred. 30 mL of distilled water was added to the sample and the contents were vigorously shaken. The burette was filled with 0.1 N sodium thiosulphate and the sample was titrated using starch (0.5 mL) as an indicator. Peroxide value was calculated using the formula:

$$\text{PV (mEq/kg)} = \frac{(S - B)\text{Normality of sodium thiosulphate}}{\text{Weight of the sample}} \times 1000 \quad (10)$$

where, S and B are titre volume of sample and blank, respectively.

Acid value of the vegetable oil was measured by taking about 5 g of sample in a flask. 50 mL of 95% hot ethyl alcohol and about 1 mL of phenolphthalein indicator was added. The mixture was boiled for about 5 min and titrated while hot against standard alkali solution. Acid value was calculated as:

$$\text{Acid value} = 56.1\, VN/W \quad (11)$$

where V is volume of standard sodium hydroxide used. N is normality of sodium hydroxide solution. W is weight of sample.

Fourier Transform Infrared (FTIR) Spectroscopy

The FTIR spectra of native and irradiated IHCN starches were recorded on FT-IR Spectrophotometer (Agilent Technologies, Cary 630 FTIR, USA). Analysis was carried out at room temperature at the spectra of 7500–4000 cm^{-1} using attenuated total reflectance with Zn-Se crystal. The Spectrum Suite ES software was used for the FTIR data treatment.

Scanning Electron Microscopy (SEM)

Microstructure of the films were observed using Hitachi Scanning Electron microscope (SEM Hitachi S4160, Japan). Films with dimensions 0.5 × 1.0 cm were fixed on the bronze stubs supported with double sided adhesive tapes and sputtered with gold–palladium. The samples were placed at an angle of 90° to the surface which allowed the observation of the cross-section of the films. Films were focused up to magnifications ranging from 500× to 2000× with voltage of 4 kV.

Statistical Analysis

Analysis of variance (ANOVA) was carried out and the results were separated using the Multiple Ranges Duncan's test ($p < 0.05$) at 95% confidence level to compare all the parameters between native and irradiated IHCN starch films using statistical software of Statistical Package for Social Science 16 (SPSS Inc, Chicago, IL, USA). Tests were performed in triplicate and mean ± standard deviation values were reported.

Result and Discussion

Proximate Analysis of IHCN Starch and Films

The proximate composition of native IHCN starch revealed 11.67% moisture 0.32% protein, 0.45% ash, and 0.00% crude fat or ether extract. Lack of crude fat means that the solvent used in the extraction of the sample could not extract small amount of lipids if any present in the sample. This indicates that the isolated starch was pure and the isolation method was effective [15]. Similar results were reported for IHCN starch by authors [16].The IHCN starch films revealed 0.03% crude fat (ether extract), 0.06% ash and 0.22% proteins. The increase in ether extract percent in starch films may be due to addition of glycerol (plasticizer). A declining trend was observed in moisture content of starch films on increasing the dosage of gamma-irradiations (Table 1). Unirradiated IHCN starch films had higher moisture content than irradiated films. It may be due to cleaving and crosslinking of

starch during irradiation which may reduce the available sites for water holding. The decrease in protein content of films as compared to starch may be due to dilution of starch with the addition of glycerol.

Film Thickness and Density

All films were easily removed from the glass slab and were flexible. Film thickness ranged from 0.31 to 0.29 mm (Table 1). Among the starch films, the irradiated films (1.25, 2.5 and 5 kGy) showed lesser thickness than unirradiated films. However, the differences were insignificant ($p > 0.05$). Similar, results were observed for potato starch films [17]. Density of the films ranged from 0.163 to 0.156 gcm^{-3}. In starch films the ratio of amylose to amylopectin influences the microstructure and thus the viscosity of the film suspension. This affects the retraction of starch film network during drying and the final thickness [18]. Amylose in starch is responsible for formation of thicker, homogeneous films whereas branched amylopectin leads to transparent and thinner films [19]. Upon irradiations, decrease in amylose content of Horse chestnut starch has been reported [16]. Decrease in amylose content of starch following irradiation due to oxidative degradation in starch has also been reported [20]. Thus, the lower amylose content in irradiated films might lead to reduction in thickness. However, in native IHCN starch films starch matrix is dense and rich in inter and intra molecular interactions resulting in thicker films.

Water Solubility

Solubility determines the ability of solids to disperse in an aqueous solution (water). Water solubility of IHCN starch films increased on increasing the dosage of gamma-irradiations (Table 1). It ranged from 25.44 to 27.85%. The highest water solubility was observed in 5 kGy irradiated IHCN starch film while the lowest was present in the unirradiated starch film. Irradiations lead to the depolymerisation of starch granules causing increased levels of small molecules. These smaller molecules have high hydration affinity [21]. Further, irradiations lead to increase in short chain amylopectin proportion having greater hydration tendency than native starch [22]. This may lead to decrease in inter-chain hydrogen bonds in starch molecules and increase in hydrogen bond with water in starch films treated by gamma-irradiations. All these factors may contribute to the increased solubility. Researchers also reported similar results for solubility of maize starch films processed by gamma irradiation [23].

Swelling Power

Swelling power of starch determines the ability of starch to hold and trap water within its structure. A declining trend was observed in swelling power with the highest swelling power (130%) in native IHCN films and the lowest in 5 kGy irradiated film (43.02%). On irradiation swelling power of the films decreased (Table 1). The decline in swelling power of IHCN starch films could be due to the depolymerisation of starch and reduction in amylopectin molecules caused by gamma-irradiations. Decrease in swelling power of cowpea starch [25] and arrowhead starch [26] has been reported.

Water Holding Capacity

Water holding capacity of native and irradiated IHCN starch films ranged from 84.24 to 88.55% (Table 1). The lowest water holding capacity was observed in native starch films (84.24%) and the highest (88.55%) in 5 kGy irradiated film. The increase in water holding capacity in IHCN starch films on increasing the dose of irradiations might be due to the degradation of starch molecules due to irradiation induced damage to starch, subsequently leading to formation of smaller molecules including dextrins and other

Table 1 Moisture, thickness, density, water solubility (WS), water holding capacity (WHC), swelling power (SP) tensile strength (TS), elongation at break (EAB) and water vapor permeability (WVP) of native and irradiated IHCN starch films ($n = 3$)

Parameters	Irradiation dose			
	0 kGy	1.25 kGy	2.5 kGy	5 kGy
Moisture (%)	10.01 ± 0.51^{b}	9.61 ± 0.58^{b}	$9.49 \pm 0.51^{a,b}$	8.89 ± 0.23^{a}
Thickness (mm)	0.31 ± 0.07^{a}	0.29 ± 0.04^{a}	0.29 ± 0.02^{a}	0.29 ± 0.23^{a}
Density (g cm^{-3})	0.163 ± 0.04^{a}	0.162 ± 0.02^{a}	0.158 ± 0.02^{a}	0.156 ± 0.05^{a}
WS (%)	25.44 ± 0.67^{a}	27.08 ± 0.59^{b}	27.27 ± 0.43^{b}	27.85 ± 0.22^{b}
WHC (%)	84.24 ± 0.95^{a}	$85.42 \pm 0.76^{a,b}$	86.48 ± 0.92^{b}	88.55 ± 0.55^{c}
SP (%)	130.54 ± 0.47^{d}	63.62 ± 0.22^{c}	54.09 ± 0.50^{b}	43.28 ± 0.57^{a}
TS (MPa)	3.88 ± 0.27^{c}	3.0 ± 0.39^{b}	1.63 ± 0.41^{a}	1.05 ± 0.22^{a}
EAB (%)	50.53 ± 0.59^{a}	62.2 ± 0.73^{b}	64.1 ± 0.44^{c}	66.1 ± 0.55^{d}
WVP(10^{-12} g m^{-1} s^{-1} Pa^{-1}	4.17 ± 0.02^{a}	5.9 ± 0.16^{b}	6.1 ± 0.79^{b}	7.6 ± 0.09^{c}

Values expressed are mean ± standard deviation. Means in the row with different superscript are significantly different at $p \leq 0.05$

monosaccharides which have more affinity with water molecules as compared to starch polymer. Similar results were reported by authors in maize and bean starch [24].

Mechanical Properties

The tensile strength and elongation at break (EAB) of the films is presented in Table 1. Tensile strength was highest in native IHCN film and the lowest in 5 kGy irradiated films while as EAB was the lowest in native starch films and the highest in irradiated films. Due to the presence of higher amylose content, retrogradation rate (reassociation of amylose molecules) of native starch films would be more leading to higher mechanical resistance, more rigid and less elastic behavior than irradiated films. Besides, native films had dense polymer matrix rich in inter and intra molecular interactions and thus are more resistant to rupture [25]. Increase in irradiation doses led to the disruption of starch film structure, decreasing the rigidity of the starch structure and thus decrease in thickness and density of films. This might result in less rigidity and thus more flexibility of irradiated starch films. Similar results were reported for babassu starch films [27].

Water Vapor Permeability

Water vapor permeability of native and irradiated IHCN films is shown in Table 1. Due to hydrophilic nature of starch molecules, starch films are regarded as inefficient moisture barriers [28]. Further addition of plasticizers in starch films enhances the water vapor permeability due to interaction of hydroxyl groups with water by hydrogen bond [29]. The results showed that native starch films were more resistant to the penetration of water as compared to irradiated films. There might be two probable reasons for this: (1) hydration of starch molecules, equal to the effect of higher starch concentration in native film leading to reduction of the effective water, (2) their interactions with starch polymer chain leading to stabilization of the crystalline region of starch. Stabilization of starch crystallites leads to reduction of film permeability. Higher crystallinity in native starch [10] films may cause lower permeability of the films. Irradiation of the starch films resulted in exposure of large numbers of free hydroxyl groups [30, 31]. These hydroxyl groups readily interact with water molecules which might favor the high water vapor permeability in the irradiated films.

Color

Color is a fundamental feature in biopolymer film acceptance and depends upon various factors including plasticizer incorporation, thermal treatment, and moisture content. Color difference (ΔE) ranged from 12.31 (native films) to 8.06 (5 kGy) (Table 2). '*L**' value of the native and the irradiated starch films varied significantly from 7.34 to 5.14. The lowest '*L**' value was observed for starch film irradiated with 5 kGy while as the highest '*L**' value was observed in the native starch film. '*a**' and '*b**' color values were in the range of 1.49–4.31 and −9.84 to −5.34, respectively. '*a**' and '*b**' values showed significant ($p \leq 0.05$) variations between native and irradiated starch films. Starch films treated with 5 kGy irradiation dose showed the highest '*a**' value (more reddish). Irradiation led to decrease in moisture content of the films, which might change the reflection of light at the surface of film leading to more reddish color in film samples while in native starch films, the highest '*b**' value was observed. '*b**' value of starch films decreased on increasing the radiation dose [30, 32, 33].

Transparency

Results showed that the irradiated IHCN starch films were more transparent than the native starch films (Table 2). Transparency of starch films depends upon various factors including moisture content, thickness and amylose content. Native IHCN starch film is dense, rich in amylose and

Table 2 Color, transparency values and oxygen permeability (in terms of acid value and peroxide value of native and irradiated IHCN starch films) ($n = 3$)

Parameters	Irradiation dose			
	0 kGy	1.25 kGy	2.5 kGy	5 kGy
Colour				
*L**	7.34 ± 0.22^{b}	5.70 ± 0.21^{a}	5.67 ± 0.34^{a}	5.49 ± 0.33^{a}
*a**	0.49 ± 0.43^{b}	-0.44 ± 0.24^{a}	1.76 ± 0.13^{c}	1.37 ± 0.16^{c}
*b**	-9.84 ± 0.06^{a}	-5.23 ± 0.08^{c}	-7.85 ± 0.14^{b}	-5.34 ± 0.09^{c}
Δ*E**	12.31 ± 0.61^{d}	6.89 ± 0.15^{a}	9.89 ± 0.67^{c}	8.06 ± 0.34^{b}
Transparency	3.21 ± 0.67^{a}	$4.68 \pm 0.34^{a,b}$	5.20 ± 0.74^{b}	3.32 ± 0.36^{a}
Acid value	0.54 ± 0.02^{a}	0.69 ± 0.02^{b}	0.73 ± 0.01^{b}	1.08 ± 0.05^{c}
Peroxide value	16.66 ± 0.03a	23.33 ± 0.02a	31.83 ± 0.06a	33.33 ± 0.04a

Values are mean ± standard deviation. Means in the row with different superscript are significantly different at $p \leq 0.05$

having a compact structure. These factors prevent the passage of light through film structure resulting in less transparent films. Irradiation of the starch films caused disruption of the film structure. Inter and intra molecular interactions within starch films also got broken resulting in decrease in film thickness, moisture content and decrease in amylose content. These change the reflection of light at the film surface resulting in increase in transparency of irradiated films. Increase in transparency on irradiations was also reported in pea starch films [30, 34]. Transparency of IHCN starch films increased up to 2.5 kGy. However, further increase in irradiation dose led to decrease in transparency value. The reduction in transparency may be attributed to the modified network formed by the cross-linking (sugar bridges). On increasing, the irradiation dose hydroxyl group and molecular weight decreases between starch chains [35]. This may alter the refractive index and passage of light through starch film matrix.

Oxygen Permeability

The acid and peroxide values of oils in glass containers sealed with starch films showed higher values in oils sealed by irradiated films. This is an indirect measure of oxygen permeability and shows an increase in oxygen permeability (Table 2). Oxygen permeability is affected by water solubility, thickness and the amylose content of the starch films. Native starch films have poor solubility, high amylose content, more ordered structure and are thus less permeable to oxygen [23, 36]. Irradiation of starch films caused starch network damage leading to increase in distance between the starch molecules and porosity on the film surface. This may attribute to high diffusivity of oxygen molecules through the film structure resulting in high oxygen permeability in irradiated starch films [8, 37].

Fourier Transmission Infrared (FTIR) Spectroscopy

The FTIR spectra of native and irradiated starch films are presented in Fig. 1. Samples were analyzed to determine the effect of irradiation on various functional groups present in the starch films and the formation of new groups if any. The band absorbance in starch films were assigned and matched with the vibrations of the chemical bonds. An extremely broad band at 3265 cm^{-1} was ascribed to the O–H stretching which was followed by a sharp bend at 2924 cm^{-1} which was ascribed to CH_2. The band at 1647 cm^{-1} was attributed to H–O–H bending due to absorption of water in amorphous

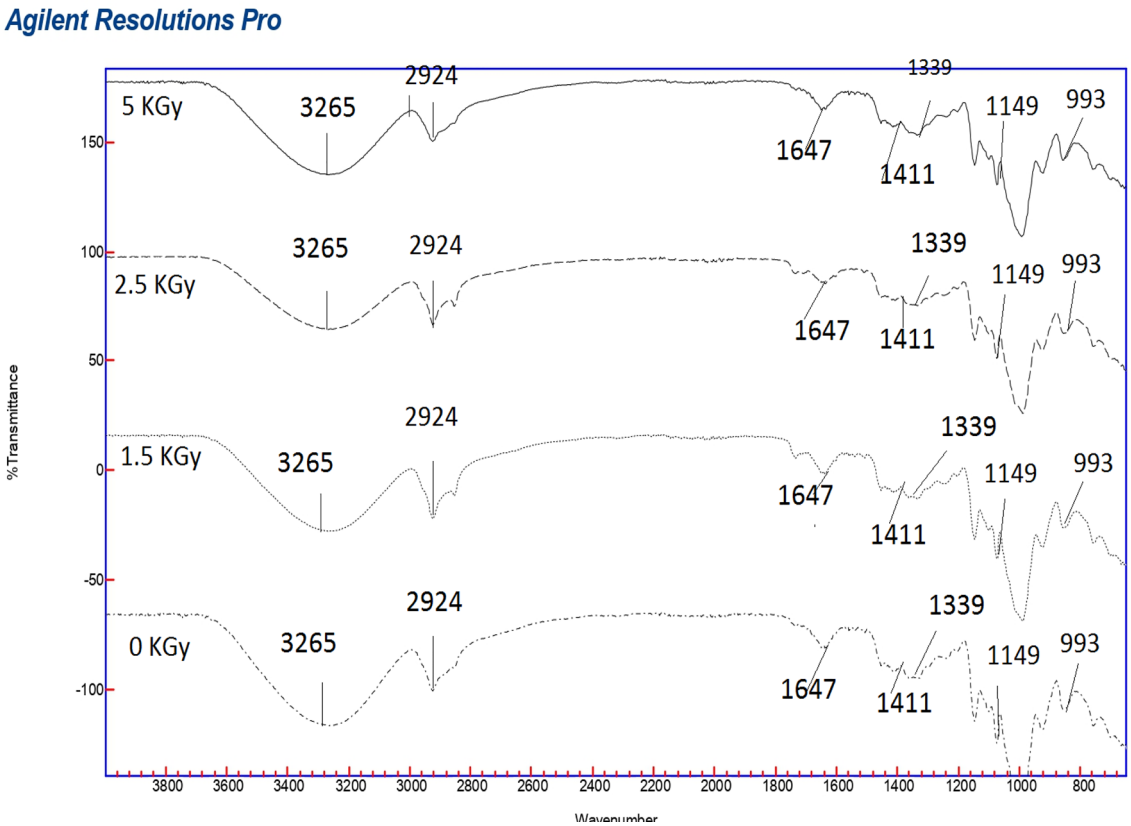

Fig. 1 FTIR spectra of native and irradiated IHCN starch films

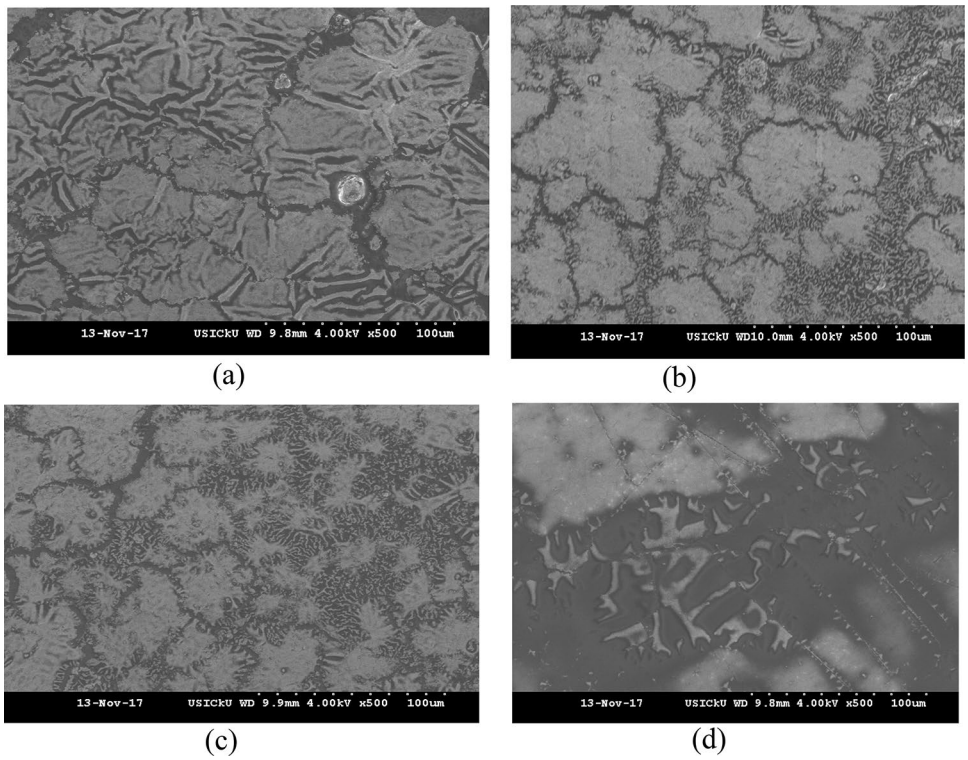

Fig. 2 SEM images of native and irradiated IHCN starch films. **a** Native, **b** 1.25 kGy, **c** 2.5 kGy, **d** 5 kGy

region of starch. The band at 1411 cm^{-1} was attributed to twisting of C–O–O and bending of CH_2 [38]. Band at 1339 and 1149 cm^{-1} has been ascribed to C–O and C–C stretching. The band at 993 cm^{-1} was attributed to C–O–H deformation and this has also been used to determine the amorphous state in starch [39]. On irradiation of the starch films, the spectral pattern of starch films did not change, no new functional groups were added but increase in the irradiation doses lead to increase in spectral intensity at 993 cm^{-1} indicating breakdown of starch into smaller molecules. Cieśla and Sartowaska [40] have also reported that after gamma irradiation, the spectral patterns of unirradiated and irradiated starches did not change but there was increase in intensities of the band at 1018 cm^{-1} caused by the high irradiation dose (10 and 15 kGy) indicating a decrease in the ordered structure of gamma irradiated starches.

Scanning Electron Microscopy (SEM)

Morphological characteristics of native and irradiated IHCN films are represented in Fig. 2. SEM micrographs of the starch films provide information about their microstructure in terms of their homogeneity, pores and cracks, and surface smoothness [41]. Micrographs of native starch films showed heterogeneities looking like fiber network. The network was open and composed of stiff rod-like strands. Similarly, the fine-rod like structural elements were observed in the cases of the films prepared based on the irradiated starch by Cieśla [42]. Gamma irradiation has been reported to have depolymerisation effect on starch polymers leading to weakening of gel structure [36, 43]. On irradiating the starch films, the cross section images for all the starches point to a homogeneous internal structure without pores, reflecting a good starch gelatinization process and the disruption of all the starch granules, as observed by other researchers [44] Besides lower amylose content was observed and this might be responsible for reduction in density of irradiated films and more smooth film surface. However, granule morphology is hardly affected at lower doses (1.25 and 2.5 kGy) with fissures on the granule surface of the starch film but at slightly higher doses of g-irradiation (5 kGy) further granule damage was seen leading to the increased homogeneity of the films, as confirmed by scanning electron microscopy (SEM). Similar results were also found in the studies by Cieśla [40]**.** Smoothing of the starch films resulting from irradiation can be related to improvement of their mechanical properties and improvement of their barrier properties against moisture and remind a similar effect observed in films by García [45].

Conclusion

Starch packaging films based on IHCN were prepared by casting techniques. Native IHCN starch yielded films with denser, more ordered and homogeneous structure. The films showed good mechanical resistance, less water

vapor permeability, most luminosity, least colorful, less oxygen permeability, less water holding capacity but the films showed less extensibility and poor solubility. Gamma irradiations induced degradation of starch network and decreased the rigidity of three-dimensional starch structures. These changes consequently altered the properties in starch films like extensibility, solubility, and water holding capacity. The films showed more oxygen and water vapor permeability with greater capacity to absorb water. Thus, it can be inferred that IHCN can be utilized for the production of starch-based edible films. However, they cannot withstand irradiation to maintain integrity for preserving the properties of products stored in it and their mechanical resistance was reduced significantly.

Declarations

Conflict of interest There is no conflict of interest.

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