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Effect of Cross-Linking Modification on Structural and Film-Forming Characteristics of Pearl Millet (*Pennisetum glaucum* L.) Starch

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Abstract: Pearl millet starch was modified using epichlorohydrin (EPI) at different concentrations (0.1%; 0.3%; 0.5%; and 0.8%) and evaluated for physicochemical, rheological, in vitro digestibility, and film-forming characteristics. The degree of cross-linking was observed at higher levels (0.5% and 0.8%) of EPI. Upon cross-linking, breakdown and setback viscosity reduced whereas pasting temperature was increased. Storage modulus (G′) and loss modulus (G″) value of cross-linked (CL) starches ranged between 2877 to 5744 Pa and 168 to 237 Pa, respectively, during the frequency sweep test. A drastic decrease was observed for steady shear (yield stress and consistency index) characteristics of CL starches. Resistant starch (RS) content was increased after starch modification, which imparts its nutritional values and starch modified at 0.8% had the highest RS content. Modifications of starch at different levels had significant effects on the moisture, opacity, solubility and mechanical properties of films. Outcomes of this study will be helpful to understand the properties of native and CL starches for their potential applications in preparation of edible films.

Keywords: pearl millet; starch; chemical modification; rheology; edible film

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1. Introduction

Pearl millet (*Pennisetum glaucum*) belongs to family *Poaceae* and it is broadly cultivated worldwide for feed and fodder. It is local to Africa and mainly cultivated in the arid and semi-arid areas of Africa and Asia. India is the leading producer (10,235,830 tons) of millets followed by Niger (3,270,453 tons) [1]. It is an underutilized crop and, due to the short of industrial applications of pearl millet, its cost is low. Isolation and modification of the starch from millet grains gives a new direction to food industries and its applications.

Native starches had a narrow range of applications due to some undesirable characteristics, such as less stability during heating, a low shear stress resistance, non-solubility in cold water, and a high syneresis rate. To increase applications and desirable characteristics, such as a high stability at high acid and shear rate, native starches are usually modified with chemical and physical treatments. Generally, starches are modified by esterification, etherification, and decomposition or physical and enzymatic methods. In the cross-linking modification, starch reacts with chemicals such as sodium trimetaphosphate (STMP), epichlorohydrin (EPI), sodium tripolyphosphate (STPP), and phosphoryl chloride

($POCl_3$) [2]. Cross-linked (CL) starch is more stable to heat, acid, and high shear resistance [3]. Cross linking shows the molecules of polymer are joined by bonding that may be covalent, ionic or hydrogen due intermolecular forces [4]. Cross-linking modification confines the interface of starch with water, resulting in structural integrity whereby starch is more stable during adverse conditions such as heat, acid, and shearing [5].

Edible films and coatings show a key role in the quality, transportation, storage, and display of a variety of processed and fresh foods [6,7]. Starch is a type of natural polysaccharide with supremacy of good renewability, good biodegradability, cheap, and wide sources; mainly, it has desirable film-forming characteristics [8]. Moreover, starch is a valuable carrier of different functional additives and ingredients, i.e., antimicrobial agents, pigments, vitamins, and antioxidants; therefore, films prepared from starches enhance the value of food products [9]. Films prepared from native starches have some limitations such as high-water vapor permeability and poor mechanical properties [10]; to improve these characteristics of films, physical and chemical modifications of starches are usually performed [11,12]. Limited information is available on cross-linking modification of pearl millet starch and its utilization in different food applications. Therefore, this study was conducted to evaluate the pasting, rheological, and film-forming properties of CL pearl millet starches.

2. Materials and Methods

2.1. Materials

Pearl millet variety (HC-10) was purchased from Chaudhary Charan Singh Haryana Agriculture University (CCS HAU), Hisar, Haryana, India. All chemicals were used of analytical grade.

2.2. Methods

2.2.1. Starch Isolation and Method for Preparation of CL Starch

Isolation of starch was carried out by following the method of Sandhu and Singh [13]. To isolate the starch, pearl millet grains were steeped in distilled water containing sodium metabisulphite (0.1%) for (18–20 h). Steeped grains were grinded in a laboratory grinder and the content was sieved through different sieves (0.250, 0.150, 0.100, 0.075, and 0.045 mm). To remove non-starch portion, slurry was centrifuged at 3000 rpm for 10 min and, the upper non-white layer was scrapped off. To remove the impurities, the white layer was re-suspended in water and centrifuged. This process was repeated 4–5 times. Starch was dried in universal oven (45 °C, 12 h). The Wurtzburg [14] method was used to modify the pearl millet starch. Firstly, starch was added in 0.5% sodium hydroxide solution and mixed properly. Then, different concentrations of EPI (0.1%, 0.3%, 0.5%, and 0.8% v/w, (dry weight basis) were used to modify the starch. Reaction time was 5 h and after 5 h, reaction was stopped using IM HCl by adjustment pH of suspension 5.0. Excess acid and alkali were removed by washing step using distilled water and suspension was centrifuged (3000 rpm, 10 min). At last, starch was dried in a conventional oven (45 °C, 12 h).

2.2.2. Degree of Cross-Linking (DC)

Relative DC of starches was evaluated using the method of Chatakanonda et al. [15]. Starch suspension (10%) were heated from 50 to 95 °C at a heating rate of 11 °C/min (after an equilibration time of 1 min at 50 °C), held for 2 min, cooled to 50 °C at the same rate, and again held at 50 °C for 2 min. The relative DC was analyzed using following formula:

$$\mathrm{DC} = \frac{A - B}{A} \times 100$$

A-Peak viscosity of control sample; *B*-Peak viscosity of CL-starch

2.2.3. Physicochemical Properties

The method described by Williams et al. [16] was used for the determination of amylose content (AC). Swelling power (SP) and solubility of starches were evaluated by using the method of Leach et al. [17]. Starch (1%) solution was prepared by using distilled water and heated in a water bath at 90 °C for 30 min. The content was then cooled and transferred to pre-weighted centrifuge tubes and centrifuged at 3000 rpm for 10 min. Sediments were used to calculate SP while supernatant was transferred to pre-weighed petri dishes and dried at 100 °C for 12 h. After cooling, it was re-weighed to calculate the solubility power.

2.2.4. Pasting Properties

Pasting properties of native and modified starches were evaluated using the starch cell of Rheometer (MCR-52, Anton Paar, Graz, Austria). Starch suspension (10%) was used for determination of pasting properties. For evaluating pasting properties, starch suspension was heated at 50 °C for 1 min, then was heated from 50 to 95 °C at a rate of 11 °C/min, and cooled to 50 °C at the same rate after holding for 2 min at 95 °C. The sample was then kept at 50 °C (2 min). Different pasting parameters were calculated from the pasting graph.

2.2.5. Rheological Properties

Dynamic Properties

Rheological properties of starches were analyzed using Rheometer (MCR-52) equipped with parallel plate system. The diameter (4 cm) and gap size (1000 μm) were maintained throughout the study. Strain 2% was used for the all determinations. Strain was selected from linear visco-elastic range. For preparing the starch paste (15%), starch suspension was heated at 85 °C in a water bath for 3 min. Starch paste was cooled at room temperature and then transferred on ram of rheometer. A frequency sweep test was performed from 0.1 to 100 rad/s at 25 °C. Storage modulus (G'), loss-modulus (G''), and tanδ values were calculated from the graph.

Steady Shear Properties

Process described by Park et al. [18] was adopted to determine the steady shear characteristics of starch pastes. For preparing the sample, the method of frequency sweep was opted. Starch (10%) was used, and the sample was measured from 1 to 1000 s^{-1}. The Herschel–Bulkley model was used for determination of steady shear properties.

2.2.6. Morphological Characteristics

Scanning electron microscopy (Model EVO-LS10 ZEISS, Oberkochen, Germany) was used to determine the morphological characteristics of starches. To prepare the sample, starch was suspended in ethanol solution (1 g/100 g). The sample was loaded on an aluminum stub and was coated with gold–palladium (60:40). During micrography, an acceleration potential of 5 kV was used.

2.2.7. In Vitro Starch Digestibility

In vitro starch digestibility was analysed by following the method described by Englyst et al. [19] and modified by Chung et al. [20]. Porcine pancreatic alpha-amylase (No. 7545, Sigma-Aldrich, St. Louis, MO, USA), and amyloglucosidase (No. 9913, Sigma-Aldrich, St. Louis, MO, USA) (3.89 g) were used for the analysis. The glucose content was measured using glucose oxidase and peroxidase assay kits (No. GAGO-20, Sigma-Aldrich, St. Louis, MO, USA). On the basis of hydrolysis time, starches were classified as rapidly digestible starch (RDS) (20 min), slowly digestible starch (SDS) (20 to 120 min) and resistant starch (RS) (not hydrolysed within 120 min).

2.2.8. Film Formation

To prepare the films, a method described by da Rosa Zavareze et al. [11] with minor modifications was used. Starch suspension (4%) was heated using a water bath (90 °C; 10 min) and, after cooling the sample, 1% glycerol was transferred into the gelatinized sample; it was stirred at 150 rpm for 20 min. The content was strained through a muslin cloth; it was transferred on baking trays and dried in the oven (50 °C; 16–20 h). Films were removed from the tray after cooling at room temperature and stored at 25 °C and 53% RH for 48–50 h by using $Mg(NO_3)_2$ solution.

Properties of Films

The moisture of films was calculated using the method of Galus et al. [21]. The Vernier Calliper method was used for determination the thickness of films [22]. Thickness of films were analysed at 10 random locations on the films, and their mean values in mm were calculated. The solubility of films was analysed by keeping the films in water (24 h) [23]. The films were collected from the water and transferred in a desiccator to achieve the final dry weight, and the difference between the two weights of the films was determined as their solubility (%). The opacity of films was calculated using a spectrophotometer (Thermo Scientific, G1OS UV-Vis, Shanghai, China). The pre-conditioned films were cut into rectangular shape (2mm × 7mm), and then attached onto the surface of a cuvette. An empty cuvette was used as a reference. Opacity of films was measured at 600 nm [24].

Tensile Strength (TS) and Elongation at Break Point (EAB)

TS and EAB were measured with a texture analyser (TA XT Plus Connect, Stable Micro Systems, Godalming, UK). Filmstrips of 1 cm × 7 cm were affixed to a pair of grips on the AT/G probe. Initial grip separation and cross-head speed were set at 50 mm and 1 mm/s, respectively.

TS was calculated by dividing the maximum load (N) by the cross-sectional area (m^2) as follows:

$$\text{TS (MPa)} = \frac{P}{b \times d}$$

where P is maximum load (N); b is sample width (mm); and d is film thickness (mm).

The percentage of EAB was calculated as follows:

$$\text{EAB (\%)} = \frac{l\text{max}}{l\text{o}} \times 100$$

where l_{max} is film elongation (mm) at the moment of rupture; and lo is the initial grip length (mm) of each sample. All final determinations were recorded as the mean of three measurements.

2.2.9. Statistical Analysis

Statistical analyses of mean of triplicate values data were carried out using Minitab Statistical Software version 14 (Minitab Inc, State College, PA, USA).

3. Results and Discussion

3.1. DC and Physicochemical Characteristics

DC of CL starches are tabulated in Table 1. DC was found at higher levels (0.5% and 0.8%) of cross-linking reagent concentrations. DC was observed 40.8% and 74.6%, and the starch modified with 0.8% EPI concentration had the highest value. Sandhu et al. [25] also observed similar observations, DC was observed only at high concentration of EPI (0.5% and 1%). AC, SP, and solubility of starches are shown in the Table 1. AC, SP, and solubility of native starch were observed 10.04%, 14.2 g/g and 14.1%, respectively. Siroha et al. [26] reported AC, SP, and solubility 11.57–21.93%, 11.11 g/g –17.91 g/g, and 12.20–15.20%, respectively, for different pearl millet starches. AC of CL starches ranged between 6.5% to 9.8%; the largest value for starch 0.1% EPI sample, while smallest value for 0.8 EPI

sample was observed. It was observed that AC of starches decreased after the modification. Sandhu et al. [25] observed a decrease in the AC with an increase in the DC. SP and solubility of CL starches ranged from 7.8 g/g to 18.1 g/g and 7.1% to 16.5%, respectively, and the smallest value was observed for 0.8% EPI starch. SP and solubility values were decreased for CL starches in comparison to native starch. Singh and Nath [27] observed lower SP of CL starches due to development of more gelly mass which restricted the access of water into starch. CL starch is less disintegrated due to high density which is responsible for decrease in solubility of CL starches [3].

Table 1. Degree of cross-linking and physicochemical properties of native and modified starches.

Samples	DC (%)	Amylose Content (%)	Swelling Power (g/g)	Solubility (%)
Native	ND	10.0 ± 0.5^{d}	14.2 ± 0.3^{c}	14.1 ± 0.1^{c}
0.1 EPI	ND	9.8 ± 0.3^{c}	18.1 ± 0.2^{e}	16.5 ± 0.2^{e}
0.3 EPI	ND	9.5 ± 0.3^{c}	15.2 ± 0.2^{d}	14.9 ± 0.1^{d}
0.5 EPI	40.8 ± 0.3^{a}	9.0 ± 0.1^{b}	8.5 ± 0.1^{b}	7.5 ± 0.2^{b}
0.8 EPI	74.6 ± 0.5^{b}	6.5 ± 0.4^{a}	7.8 ± 0.2^{a}	7.1 ± 0.2^{a}

Data of triplicate shown as average ± SD and difference were not significant ($p < 0.05$) for similar superscripts in a column. Native starch; 0.1; 0.3; 0.5; and 0.8 (EPI): Starch modified with 0.1%; 0.3%; 0.5% and 0.8% EPI. ND-Not detectable.

3.2. Pasting Characteristics

The pasting characteristics of the native and CL starches are shown in Table 2. PV of starches ranged from 471 to 2588 cP, starches treated with 0.3% EPI concentration had the highest value. Significant ($p < 0.05$) effects of DC were observed on the pasting properties of the modified starches. Peak viscosity (PV) of CL starches increased at lower concentrations (0.1% and 0.3%), whereas it decreased at higher concentrations (0.5% and 0.8%) in comparison to native starch. Kaur et al. [28] evaluated that potato starches showed higher SP when treated with a lower concentration of the cross-linking reagent while SP decreased when starch was treated with higher concentrations. Breakdown viscosity (BV) indicates the stability of starch granule during shear at high temperature [29]. BV ranged between 83 to 618 cP for CL starches, and the smallest value was observed for starch treated with 0.8% EPI concentration. Ackar et al. [30] observed a significant lowering of BV when wheat starches were treated with EPI. Final viscosity (FV) of CL starches were observed 2397, 2180, 906, and 452 cP, respectively. Setback viscosity (SV) shows the ability of starch for retrogradation and varied from 64 to 524 cP, respectively; the largest and the smallest values were observed at 0.1% and 0.8% EPI concentrations. Ackar et al. [30] observed that SV of modified starches were significantly lower than native counterpart starches. PV, trough viscosity (TV), and BV were observed the highest for starch treated with 0.3% EPI concentration, while starch modified at 0.8% EPI concentration had the lowest value for PV, TV, BV, SV, and FV.

Table 2. Pasting characteristics of native and modified starches.

Samples	PV (cP)	TV (cP)	BV (cP)	SV (cP)	FV (cP)	P T (°C)
Native	1860 ± 22^{c}	839 ± 11^{c}	1021 ± 12^{e}	591 ± 14^{e}	1430 ± 15^{c}	88.5 ± 0.1^{a}
0.1 EPI	2247 ± 25^{d}	1873 ± 15^{d}	374 ± 9^{c}	524 ± 12^{d}	2397 ± 18^{e}	88.3 ± 0.1^{a}
0.3 EPI	2588 ± 19^{a}	1970 ± 21^{a}	618 ± 8^{d}	210 ± 9^{c}	2180 ± 21^{d}	89.1 ± 0.2^{b}
0.5 EPI	1101 ± 15^{b}	803 ± 14^{b}	298 ± 6^{b}	103 ± 8^{b}	906 ± 16^{b}	89.6 ± 0.1^{b}
0.8 EPI	471 ± 09^{a}	388 ± 09^{a}	83 ± 4^{a}	64 ± 4^{a}	452 ± 11^{a}	90.2 ± 0.2^{c}

Data of triplicate shown as average ± SD and difference were not significant ($p < 0.05$) for similar superscripts in a column. Native starch; 0.1; 0.3; 0.5 and 0.8 (EPI): Starch modified with 0.1%; 0.3%; 0.5%; and 0.8% EPI.

3.3. Rheological Properties

3.3.1. Dynamic Shear Properties

G′, G″, and tanδ values calculated at 6.28 rad/s and 25 °C from frequency sweep tests (Table 3 and Figure 1A–C). G′ and G″ values ranged between 2877 to 5744 Pa and

168 to 237 Pa, respectively, for CL starches. The highest G′ value was observed for 0.1% EPI sample, while the lowest value was observed for 0.8% EPI sample. G′ and G″ values slightly increase with increase in the magnitude of angular frequency, and a greater increase was observed for G″ as compared to G′. After the modification value of G′ was decreased for CL starches at higher concentrations of EPI (0.3%, 0.5%, and 0.8%), whereas an increase was found for 0.1% EPI sample as compared to native starch. Two moduli (G′ and G″) do not cross each other in the observed frequency range (0.1–100 rad/s), showing the stability of starch pastes in this frequency range. Sandhu and Siroha [31] reported G′ and G″ values 997 to 1871 Pa and 67 to 107 Pa, respectively, for the pearl millet starches. G′ and G″ values observed in this study are higher as reported by Sandhu and Siroha [31]. This may be due to the higher concentration of sample used in this study. Sandhu et al. [25] found lesser value for G′ when starch was modified with a higher level of EPI.

Table 3. G′, G″ and tanδ value of native and modified starches.

Samples	G′ (Pa)	G″ (Pa)	tan δ
Native	5541 ± 25 ^{d}	228 ± 9 ^{d}	0.04
0.1 EPI	5744 ± 23 ^{e}	221 ± 8 ^{c}	0.03
0.3 EPI	5263 ± 21 ^{c}	209 ± 6 ^{b}	0.03
0.5 EPI	3558 ± 18 ^{b}	168 ± 8 ^{a}	0.04
0.8 EPI	2877 ± 19 ^{a}	237 ± 9 ^{e}	0.08

Data of triplicate shown as average ± SD and difference were not significant ($p < 0.05$) for similar superscripts in a column. Native starch; 0.1; 0.3; 0.5; and 0.8 (EPI): Starch modified with 0.1; 0.3; 0.5; and 0.8% EPI.

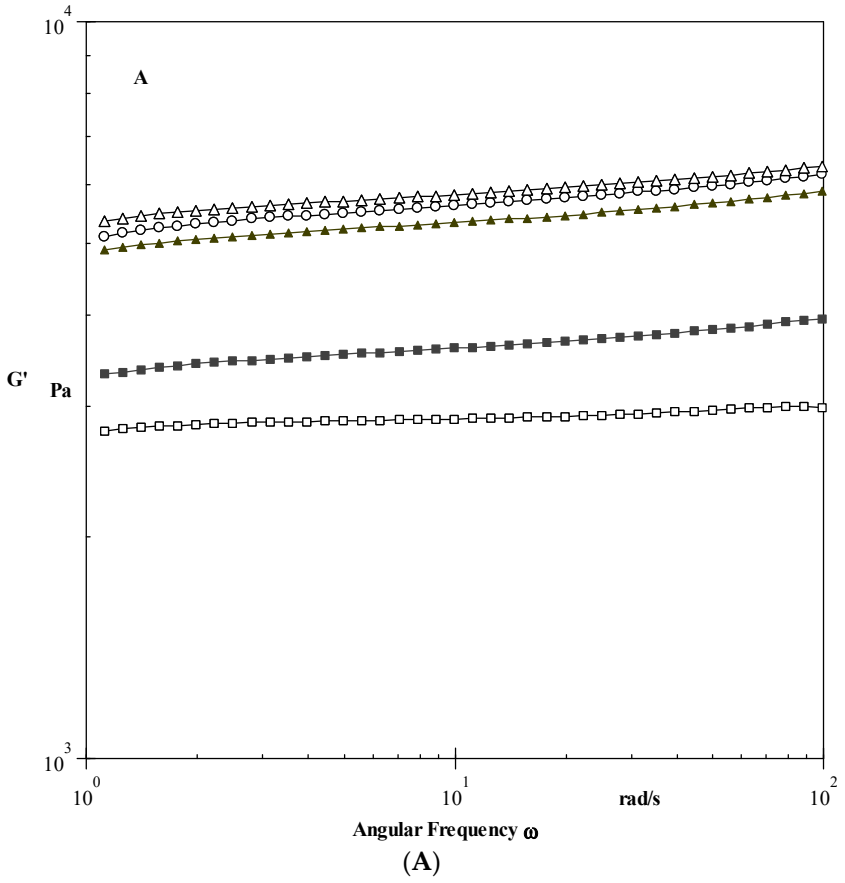
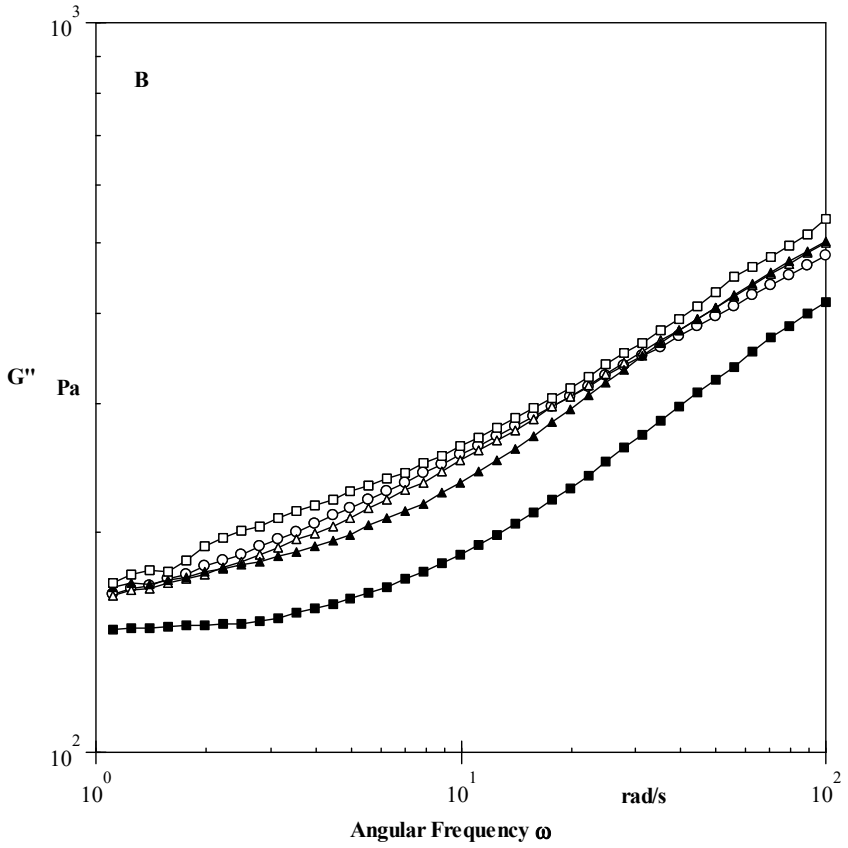


Figure 1. *Cont.*



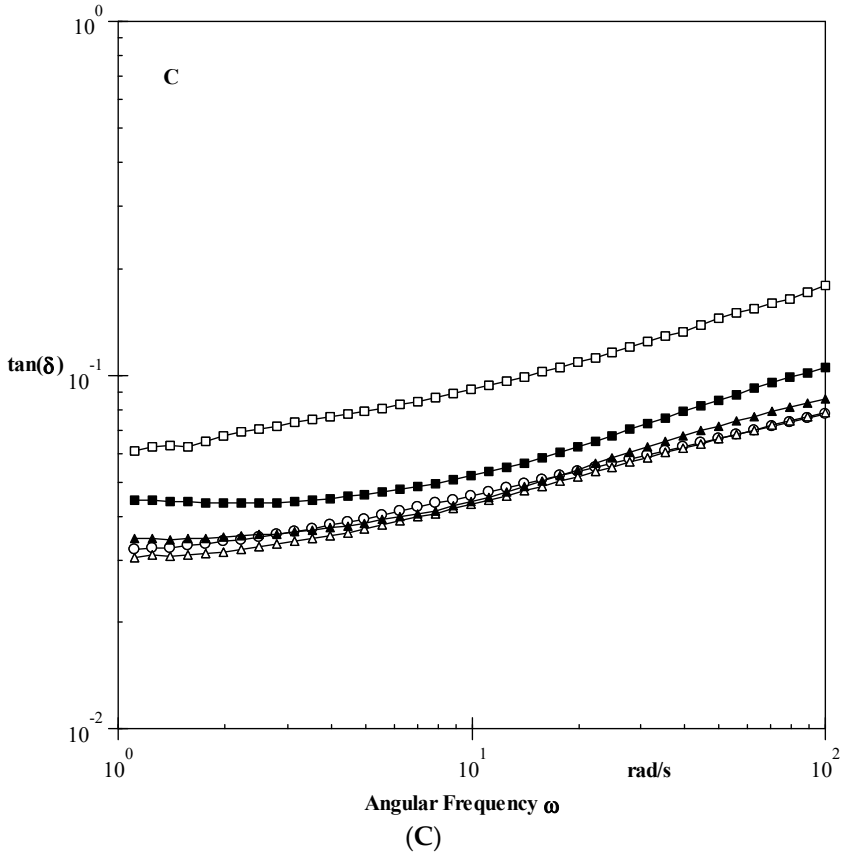


Figure 1. (**A**–**C**) G′, G″ and tanδ of native and modified starches. Samples are denoted by o-Native; Δ-(Cross-linking at 0.1%); ▲-(Cross-linking at 0.3%); ■-(Cross-linking at 0.5%); □-(Cross-linking at 0.8%).

3.3.2. Steady Shear Characteristics

Steady shear characteristics of native and CL starch pastes at 25 °C are tabulated in Table 4 and Figure 2A,B. The Herschel–Bulkley model equation was used to explain the steady shear characteristics of starches. The Herschel–Bulkley model shows the good coefficients values (R^2 = 0.98–0.99) for evaluation of steady shear characteristics. The flow behavior index (n) values ranged from 0.32 to 0.87. Chan et al. [32] stated that n values less than 1 showed that starch gel structure is disrupted due to applied shear stress which shows the shear thinning property of starch pastes. The yield stress (σ_o) value of modified starch pastes varied from 2.3 to 4.64 Pa; starch modified using 0.1% EPI concentration had the highest value. The consistency index (K) value varied from 0.08 to 49.29 (Pa·s); the largest and the smallest value was found for starches modified at 0.1% and 0.8% EPI concentrations. The reduced value of K and σ_o of CL starches in comparison to native starch may be due to a reduced SP and solubility power of modified starches [33]. The relationship between shear rate and viscosity is shown Figure 2B. The viscosity of starch pastes decreased with the increase in the shear rate.

3.4. In Vitro Digestibility

RDS, SDS, and RS contents of native and modified starches are tabulated in Table 5. RDS content of CL starches ranged from 45.9% to 49.6%; the largest value for starch treated with 0.3% EPI and the smallest value was found for starch modified at 0.8% EPI. The SDS content varied from 35.1% to 37.8% for modified starches, which was less than the native starch. The RS content increase significantly ($p < 0.05$) with an increase in the amount of cross-linking agent. When cross-linking modification is performed at a high level, the water binding capacity of CL starches is reduces, which reduces the availability of amylase enzyme to starch molecules [34]. Huber and BeMiller [35] stated that cross-linking modification blocks all porous channels that help the α-amylase enzyme to enter in the molecules of starches. Jyothi et al. [3] observed that DC affects the digestibility of starches, starch which have lower DC showed the higher digestibility while reverse was observed for higher DC.

Table 4. Steady shear characteristics of native and modified starches.

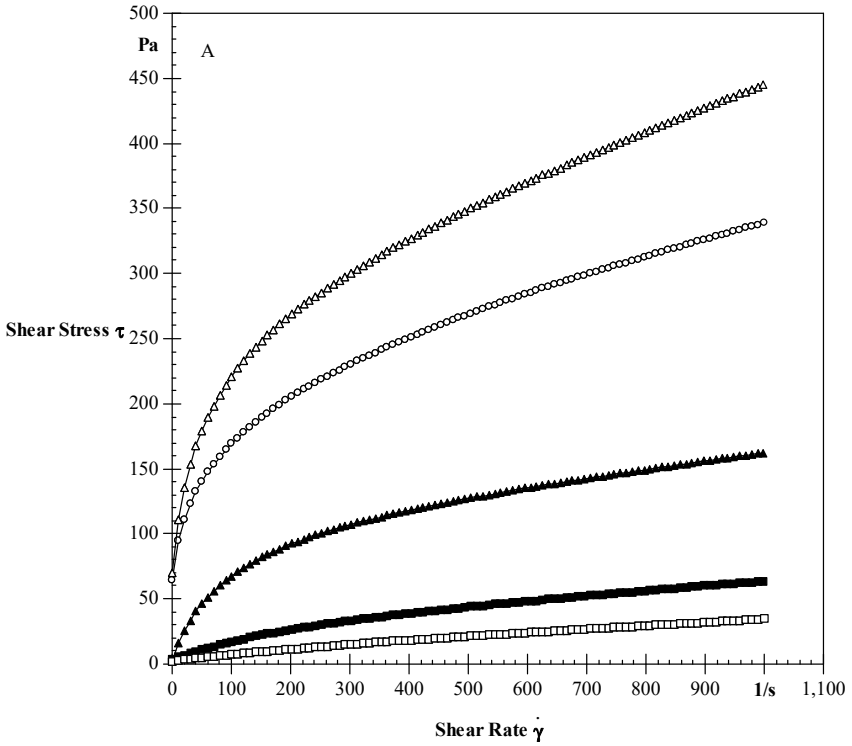
Samples	σ_o (Pa)	K (Pa·s)	n	R^2
Native	25.59 ± 2 ^{e}	31.38 ± 2 ^{d}	0.32 ± 0.007 ^{a}	0.999
0.1% EPI	4.64 ± 0.9 ^{d}	49.29 ± 3 ^{e}	0.32 ± 0.005 ^{a}	0.999
0.3% EPI	4.33 ± 0.7 ^{c}	1.46 ± 0.3 ^{c}	0.77 ± 0.003 ^{b}	0.985
0.5% EPI	3.22 ± 0.5 ^{b}	0.27 ± 0.02 ^{b}	0.85 ± 0.005 ^{c}	0.999
0.8% EPI	2.3 ± 0.5 ^{a}	0.08 ± 0.01 ^{a}	0.87 ± 0.009 ^{c}	0.995

Data of triplicate shown as average ± SD and difference were not significant ($p < 0.05$) for similar superscripts in a column. Native starch; 0.1; 0.3; 0.5; and 0.8 (EPI): Starch modified with 0.1; 0.3; 0.5; and 0.8% EPI.

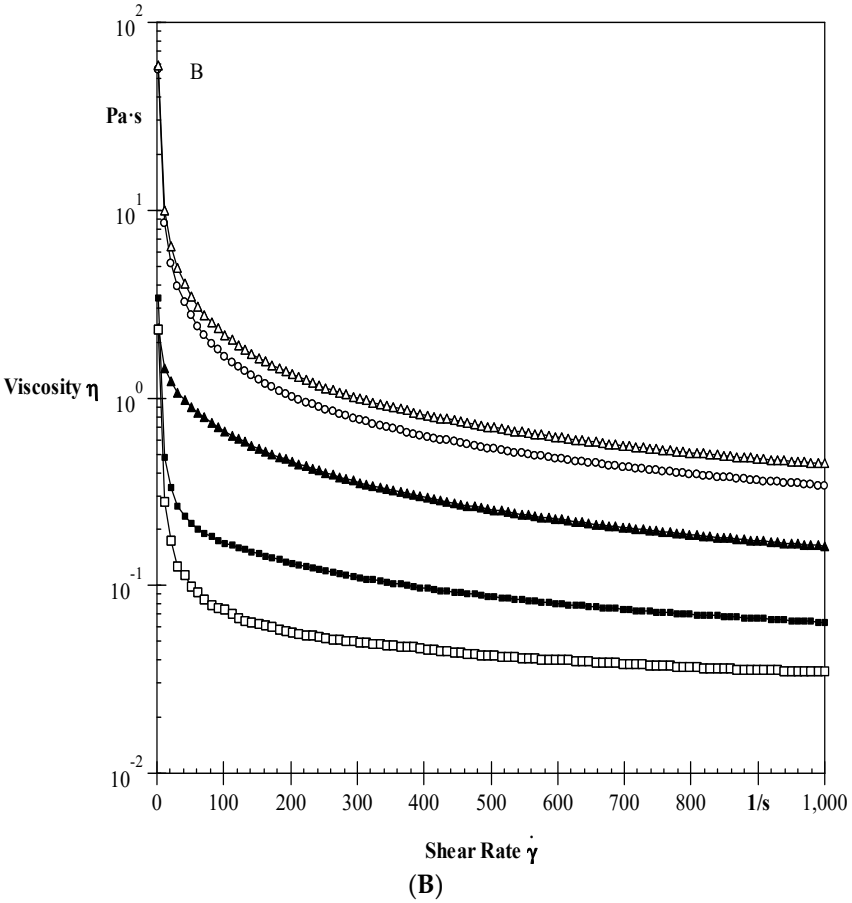
Table 5. In vitro digestibility of native and modified starches.

Samples	RDS (%)	SDS (%)	RS (%)
Native	49.9 ± 0.9 ^{d}	38.1 ± 0.6 ^{d}	12.0 ± 0.5 ^{a}
0.1% EPI	49.6 ± 0.6 ^{c}	37.8 ± 0.5 ^{c}	12.6 ± 0.3 ^{b}
0.3% EPI	49.9 ± 0.8 ^{d}	36.0 ± 0.8 ^{b}	14.1 ± 0.8 ^{c}
0.5% EPI	48.3 ± 0.9 ^{b}	36.4 ± 0.7 ^{b}	15.3 ± 0.7 ^{d}
0.8% EPI	45.9 ± 0.6 ^{a}	35.1 ± 0.7 ^{a}	19.0 ± 0.8 ^{e}

Data of triplicate shown as average ± SD and difference were not significant ($p < 0.05$) for similar superscripts in a column. Native starch; 0.1; 0.3; 0.5; and 0.8 (EPI): Starch modified with 0.1%; 0.3%; 0.5%; and 0.8% EPI.



(**A**)



(**B**)

Figure 2. (**A**,**B**) Steady shear properties of native and modified starches. Samples are denoted by o-Native; Δ-(Cross-linking at 0.1%); ▲-(Cross-linking at 0.3%); ■-Cross-linking at 0.5%); □-(Cross-linking at 0.8%).

3.5. Morphological Properties

A scanning electron micrograph of native and CL starches is shown in Figure 3. The SEM of native starch showed varying in shape (spherical and polygonal) and size (small to large), and changes were observed in comparison to native starch. CL starches showed rough surfaces and small cavities on the surface of granules. Cross-linking modification slightly affects the starch granule as compared to native starch [36,37]. Native pearl millet starches were polygonal in shape and had well defined edges, while a slightly rough surface and a black zone on the surface were observed for CL starches [37]. Mirmoghtadaie et al. [38] reported that oat starch granules were not affected by cross-linking modification. Jafari et al. [39] stated that particle size of starches affects the various physicochemical properties of starches; it was also observed that particle size affects the encapsulation efficiency of the starch.

3.6. Properties of Starch Films

Characteristics of film prepared by native and CL starches are shown in Table 6. The moisture content of films ranged between 20.09% to 28.11%, and films prepared from CL starches showed lesser moisture content in comparison to native starch films except film prepared from 0.1% EPI starch. Thickness of film is an essential parameter that shows the mechanical power and permeability characteristics of films to gases and water vapors. Determining the thickness of films is also important to evaluate the smoothness of the films [40]. Thickness of films varied from 0.094 to 0.105 mm, and film prepared from 0.8% EPI starch had the lowest value. Solubility is one of the important factors for food packaging materials, and, on the basis of characteristics of diverse foods, there is a requirement to select the packaging content with different solubility. Lesser soluble packaging materials are required for the packaging of high moisture material [41]. Solubility of films ranged between 28.36% to 39.10% and, after modification, the solubility content was decreased except the film formed with 0.1% EPI starch. This may be due to reduced solubility power of CL starches in comparison to native starch. Transparent packaging material is preferred by consumers as food materials are visible through this packaging. Opacity is inversely associated with transparency and varied from 1.679% to 2.730%, respectively. Bangar et al. [42] observed opacity 2.191% to 2.812% for films prepared from different modified starches.

Mechanical characteristics of films are determined in terms of TS and EAB. TS refer to the highest tensile stress endured by the sample during the tension test. TS of films varied from 6.80 to 9.60 MPa; the lowest value was found for native starch film. The TS value of modified starch films increased in comparison to native starch and its value increase with increase in the level of DC. EAB is an indication of the film's flexibility and extensibility, and ranged between 47.5% to 62.8%. Its value decreased as the concentration of EPI increased to modify the starch. Bangar et al. [43] observed TS value for millet starches from 3.79 to 6.95 MPa, and the EAB values at 53.4% to 73.2%, respectively. Bruni et al. [44] observed an increase in the TS value for films prepared from CL wheat starch in comparison to native starch. Due to cross-linking modification, the starch molecules are interconnected, which results in an increase in molecular weight and inter molecular interactions of the starches, resulting in better TS [45]. Gonzalez et al. [46] reported the effect of plasticizers on strength of films, and it was observed that D-isosorbide presented the highest strength values, followed by those with glycerol, while those containing 1,3-propanediol showed the weakest behaviour. Mitrea et al. [47] conducted the research on colour of films; to produce more natural products, the colour pigments were extracted from tomato by-products.

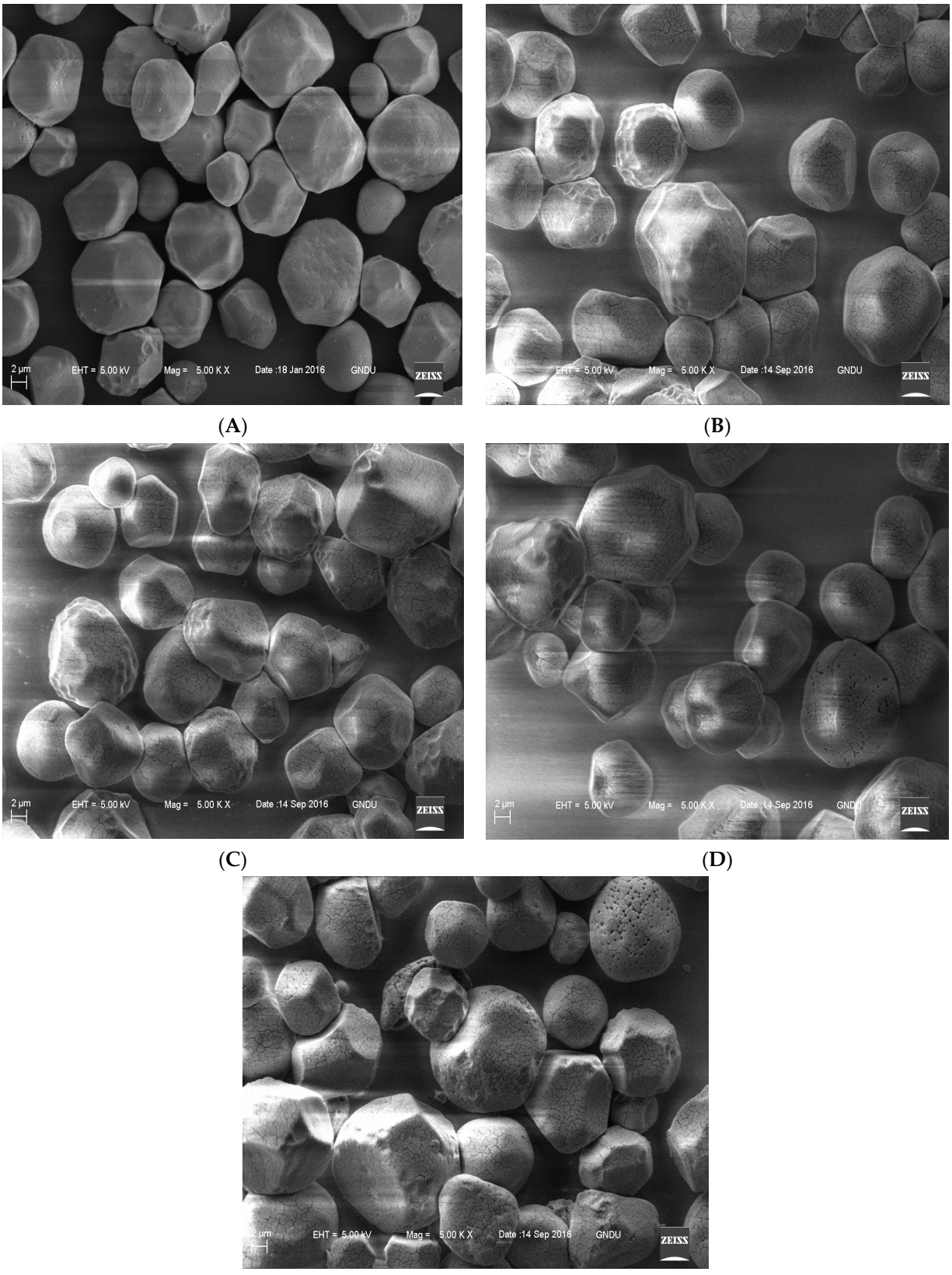


Figure 3. Morphological properties of native and modified starches. (**A**)-Native; (**B**) (Cross-linking at 0.1%); (**C**) (Cross-linking at 0.3%); (**D**) (Cross-linking at 0.5%); (**E**) (Cross-linking at 0.8%).

Table 6. Properties of films.

Samples	Moisture Content (%)	Thickness (mm)	Water Solubility (%)	Opacity (%)	Tensile Strength (MPa)	Elongation at Break (%)
Native	26.08 ± 1.9 ^{d}	0.103 ± 0.004	38.34 ± 2.5 ^{d}	1.679 ± 00 ^{b}	6.80 ± 0.25 ^{a}	62.8 ± 1.9 ^{e}
0.1 EPI	28.11 ± 2.1 ^{e}	0.105 ± 0.001	39.10 ± 2.1 ^{e}	1.695 ± 00 ^{a}	7.35 ± 0.30 ^{b}	58.7 ± 1.7 ^{d}
0.3 EPI	25.01 ± 2.2 ^{c}	0.102 ± 0.002	36.14 ± 2.4 ^{c}	2.656 ± 00 ^{c}	7.91 ± 0.11 ^{c}	56.9 ± 1.1 ^{c}
0.5 EPI	22.88 ± 1.4 ^{b}	0.099 ± 0.001	33.11 ± 1.9 ^{b}	2.711 ± 00 ^{d}	8.11 ± 0.22 ^{d}	52.1 ± 1.5 ^{b}
0.8 EPI	20.09 ± 1.7 ^{a}	0.094 ± 0.005	28.36 ± 2.5 ^{a}	2.730 ± 00 ^{e}	9.60 ± 0.14 ^{e}	47.5 ± 2.1 ^{a}

Data of triplicate shown as average ± SD and difference were not significant ($p < 0.05$) for similar superscripts in a column. Native starch; 0.1; 0.3; 0.5; and 0.8 (EPI): Starch modified with 0.1%; 0.3%; 0.5%; and 0.8% EPI.

4. Conclusions

Starch modified with cross-linking reagent at different levels of EPI caused significant changes in structural, in vitro digestibility, and film-forming characteristics. AC, SP and solubility were reduced successively with increase in concentration of EPI. CL starch showed an increase in PV and FV at low concentration (0.1% and 0.3% EPI) while the reverse was observed at higher concentrations (0.5% and 0.8% EPI) in comparison to native starch. G′ value increased at 0.1% EPI, after successive increases in EPI concentration decrease for G′ values were observed during the frequency test. During measurement of steady shear properties, yield stress values were decreased while flow behavior index values were increased. SDS content was decreased while the reverse was observed for RS as compared to native starch. CL starches showed rough surfaces and small cavities on the surface of granules. Films prepared by CL starch showed lesser moisture and solubility content as compared to native starch and higher percentage of opacity. More research should be conducted to increase strength and the application of CL starches to prepare starch films.

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