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RHEOLOGICAL CHARACTERIZATION AND MICROSTRUCTURAL DEPICTION OF XANTHAN GUM AND ITS HYDROLYSATES

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In this work, xanthan gum was hydrolyzed through three different methods using acid (HCl), base (NaOH) and enzyme (cellulase) to improve its rheological properties and various other characteristics. Hydrolysis resulted decrease in moisture content, increase in protein and fiber content in case of enzyme hydrolyzed xanthan gum, ash content of acid and base hydrolyzed xanthan gum. Rheological measurements showed shear-thinning non-Newtonian behavior in all the gums with the reduction in viscosity after hydrolysis as 2.08, 1.16, and 1.90 Pa.s in acid base and enzyme hydrolyzed xanthan gum respectively as compare to native xanthan gum (2.5 Pa.s). Oscillatory properties revealed rectangular characteristic type of polymer solution curves for all the gum types. Fourier transform infrared (FTIR) spectrums depicted no major transformation in functional groups for hydrolyzed forms of xanthan gum exhibiting non-momentous change in xanthan gum basic structure due to hydrolysis. Scanning electron microscope (SEM) characterization revealed fibrous and amorphous morphology for native and enzyme hydrolyzed gum whereas, acid and base hydrolyzed gums exhibited crystalline structure. X-ray diffraction (XRD) results indicated an increase in crystallinity as a result of acid and base hydrolysis whereas enzyme hydrolysis did not alter the amorphousness as presented by native gum. Hence, xanthan gum could be modified to increase its applications without altering it integrity and basic structure.
Keywords: Xanthan gum, hydrolysis, characterization, rheology, viscosity.

INTRODUCTION

Xanthan gum (XG), an anionic hetro-polysaccharide produced by the Gram-negative bacterium *Xanthomonas campestris* through aerobic fermentation, is a high molecular weight natural exopolysaccharide. XG contains glucose, mannose and glucuronic acid in 2:2:1 ratio with different amounts of pyruvyl and *O*-acetyl residues attached on terminal mannose residue (Kennedy *et al.,* 2015). XG consists of β-(1→4) linked glucose backbone with the replacement of trisaccharide side-chains of β-(1→3)-mannose-α-(1→2)-glucuronic acid-β-(1→4)-mannose on alternative glucose units making it soluble in both cold and hot water, stable in acid base and salt conditions. The demand and production of XG from *X. campestris* has progressively increased at an annual rate of 5–10% (Kongruang *et al.,* 2005; Salah *et al.,* 2010) with the estimated production of 30000 tons per anum (Li *et al.,* 2016).

XG found its use in the food industry due to its better affinity with food ingredients, stability towards harsh conditions during processing, ability to stabilize emulsions and pseudo-plastic rheological properties. Food products containing XG have fluidity, good mouthfeel and adhesion due to abovementioned properties. These characteristics make XG a suitable candidate as thickening, stabilizing and suspending agent in many foods (Zhao *et al.,* 2009; Habibi and Khosravi-Darani, 2017).

The best conspicuous uses of XG among the applications are in salad dressing as a stabilizer, in bakery products to improve cohesion of starch particles, contributing to structure and shelf life, in cake mixes to control batter rheology, in frozen foods to prevent syneresis during freeze thaw cycles, in dairy products and desserts as a stabilizer, in beverages to improve mouth feel and in low fat products as a fat replacer (Misra *et al.,* 2018). However, addition of this charged macromolecule in dairy products is hindered due to the electrostatic interaction between casein and xanthan gum resulting in depletion flocculation and phase separation (Hemar *et al.,* 2001) unacceptable for final product.

End-use applications of XG can be improved by controlled modification with the main objective of improved dispersibility, better affinity with food system and reduced viscosity. De-polymerization of glucan backbone and hydrolyzation of trisaccharide side chain by using various chemical and enzymatic reactions have been reported (Jampala *et al.,* 2005). Ahuja and coworkers modified XG by carboxymethylation to enhance the solubility of this polymer in water and claimed a considerable reduction in XG viscosity (Ahuja *et al.,* 2012). Another work was carried out on chemical modification of XG using formaldehyde to enhance

its dissolution rate and deduced that chemically modified XG dissolves more rapidly than the unmodified one (Su *et al.,* 2003). Partially hydrolyzed xanthan gum can be used as a dietary fiber source and can easily be added to different foods without interfering the quality characteristics of end product. Partially hydrolyzed xanthan gum has reduced viscosity with low molecular weight hence, can be added to the foods gaining better acceptability (Grossi *et al.,* 2005). In another study, (Anjum *et al.,* 2015) modified the xanthan gum through hydrolysis and grafting with acrylamide to improve its functional characteristics with enhancing range of applicability. Although various studies could be found on rheological characteristics of xanthan gum but no data is found on the rheology and microstructural characterization of xanthan gum modified with chemical and enzymatic methods. Current study was designed to overcome this gap to achieve a better food additive imparting acceptable characteristics to the end product. Xanthan gum was subjected to hydrolysis using three different methods, acid (HCl), base (NaOH) and enzyme (cellulase). The effect of these hydrolysis techniques on composition mineral profile, rheological characteristics and molecular structure of XG were explored.

MATERIALS AND METHODS

Food grade XG was procured from TIC Gums (United States). Chemicals, reagents and media of different companies (Sigma Aldrich (USA), Oxoid (UK) and Merck (Germany) were bought from the local suppliers. The research work was executed in the Laboratories of National Institute of Food Science and Technology, Department of Chemistry and Biochemistry, University of Agriculture Faisalabad, Pakistan and Department of Food Science, School of Environmental and Biological Sciences, Rutgers, The State University of New Jersey, USA.

Chemical modification of xanthan gum

***Acid Hydrolysis*:** Xanthan gum was modified chemically using HCl following method of Chauhan *et al.*(2009). Xanthan gum (1 g) was dissolved in 100 mL of 0.1N HCl solution using a magnetic stirrer and placed for 2 h in a water bath at 80 ºC. After the required reaction time, the pH of the contents of the reaction mixture was neutralized using 0.1N NaOH. Hydrolyzed xanthan gum was precipitated using ethanol and then freeze dried for further studies.

***Base Hydrolysis*:** Sodium hydroxide (NaOH) was used for modification of xanthan gum according to the method of Adhikary and Singh (2004). Xanthan gum (2 g) was dissolved in 200 mL distilled water and then 100 mL of 1N NaOH was added to it. The mixture was placed in a water bath at 70ºC for 2 hours. The reaction mixture was then neutralized and precipitated using ethanol. Gum was freeze dried and used for further studies.

***Enzymatic hydrolysis*:** The enzymatic modification of xanthan gum was carried out by the method reported by Mudgil *et al.* (2014). Cellulase enzyme (Sigma Aldrich) was used at the concentration of 0.19 mg/g for hydrolysis of gum. Xanthan gum was mixed in water at 1% concentration using magnetic stirrer at 800 rpm. Before addition of xanthan gum distilled water was maintained at pH 5.6 and required amount of enzyme was added. The process of hydrolysis was carried out at 50°C for 4 h with continuous agitation at 100 rpm. The solution was then sterilized (100°C) to inactivate the enzyme, then precipitated with ethanol, filtered and freeze dried for further use.

***Chemical Analysis*:** Native xanthan gum (NXG) and its modified forms were analyzed for their chemical composition like moisture content (Method 925.09), crude fat (Method 920.39), ash (Method 923.03), crude fiber (Method 962.09) and crude protein (Method 954.01) (AOAC, 2012).

***Mineral analysis*:** Mineral contents (Na, K and Ca) in xanthan gum samples were estimated with Flame Photometer (Sherwood Scientific Ltd., Cambridge, and Model 410) and Atomic Absorption Spectrophotometer (AA240, Varian). Wet digestion method was used for sample preparation according to method No. 965.17-968.08 (AOAC, 2012). HNO_3 and $HClO_4$ with 7:3 were used for wet digestion. Organic part from the 2 g sample was removed by ignition and then wet digestion was carried out on hot plate till the contents in the digestion flask turned light green or clear. Then the sample was diluted to 25 mL by using deionized water and filtered for estimation of mineral contents on flame photometer and atomic absorption spectrophotometer (AAS). The results were stated in mg/100 g.

***Rheological studies*:** Steady shear characteristics, visco-elastic properties and effect of temperature on apparent viscosity of xanthan gum and its hydrolytic forms were studied using advance rheometric expansion system (TA instruments, New Castle, DE) fitted with a cone and plate geometry (4° cone angle, 60 mm diameter, 1.25 mm gap) with controlled shear rate (1/s) by following method stated by (Sittikijyothin *et al.,* 2010).1% xanthan gum solutions were prepared for each gum type and poured on the rheometer plate for making measurement. A linear viscoelastic range was established with strain amplitude sweep (0.01-10 %) at a fixed frequency (1 Hz). Then frequency sweep was performed to measure storage modulus G' and loss modulus G'' with a strain of 1% in the frequency range from 0.1 to 100 rad/s. Effect of temperature on xanthan gum viscosity was examined by performing temperature sweep from 25 °C to 60 °C at a heating rate of 2 °C/min with the constant frequency at 1 rad/s. The data was analyzed using Herschel-Bulkley model.

***FTIR Analysis*:** The FTIR spectra gave information regarding the vibrational frequencies of functional groups present in the polymer segments resulting from intermolecular interactions. Infra-red spectral studies were performed on FTIR spectrometer under dry air at room temperature using KBR

pellet method. Spectra were taken between 4000 and 400 cm^{-1} using Bruker Tensor 27 FT-IR as described by Faria *et al.* (2011).

***Scanning electron microscope* (*SEM*):** Xanthan gum powders were examined by SEM to obtain information about shape and size of xanthan particles. Photographic images were recorded following the method of Ahuja *et al*. (2012). 30KV Scanning Electron Microscope (JSM5910, JEOL, Japan) with SEI and EDX detectors (INCA200/Oxford Instruments, UK) was used.

***X-ray diffraction*:** Xanthan gum samples were analyzed using an X-ray diffractometer by following the method of Su *et al*. (2003). The dry sample powder was pressed into pellets. X-ray Diffractometer (JDX 3532, JEOL, Japan) with CuKα source was used. Measurements were carried out with a diffraction angle range of 10-80° and resolution of °2θ at room temperature (25 °C).

***Thermo gravimetric analysis* (*TGA*):** Thermal analysis of native and partially hydrolyzed xanthan gum was carried out with Thermo-Gravimetric and Differential Thermal Analyzer (TG/DTA) (Diamond Series TG/DTA Perkin Elmer, USA), by following the method of Mudgil*et al*. (2014). TGA measurements of NXG and hydrolyzed gums were conducted using TA instruments at heating rate of 10°C/min over a temperature range of 30-1200°C. The samples were heated at 2 steps initially, held at 30°C for 1 min and then heated at the rate of 10°C/min from 30-1200°C.

***Statistical Analysis*:** The data of each parameter was subjected to statistical analysis to determine the level of significance (Montgomery, 2008). Completely randomized design with three replicates was used to analyze the data using Statistix 8.1 software.

RESULTS AND DISCUSSION

***Compositional Analysis*:** Results for compositional parameters of native xanthan gum and its hydrolyzed forms are presented in Fig. 1. It is evident from the statistical evaluation, that hydrolysis showed non-significant effect on moisture and fat contents; significant effect on protein and nitrogen free extract (NFE); whereas a highly significant behavior was found for ash and fiber content. Ash contents depicted a remarkable change exhibiting an increase in ash content after being hydrolyzed with acid, base and enzyme. Ash contents in enzyme treated gum differed non-significantly from the native one whereas, significant increase was observed in acid and base hydrolyzed xanthan gum, respectively as depicted from Fig. 1. The results obtained are in accordance with the verdict of Mudgil *et al.*(2014) who reported an increase in ash content after partial hydrolysis of gum might be due to the attachment of minerals with the terminal mannose units. Acid and base hydrolysis impacted non-significantly on protein contents when compared with NXG whereas, enzyme hydrolysis resulted in significant increase in protein contents. The increase in protein content might be due to the addition of enzyme as enzymes are protein in nature. Similarly, fiber contents showed a remarkable increase when evaluated in all hydrolyzed forms of xanthan gums as compared to the NXG. Our findings are in concordance with the results of Anjum *et al.*(2015) who studied composition of native xanthan gum and found 79.70% of the total gum is carbohydrate content (NFE) indicating native gum contains a significant amount of xanthan gum.

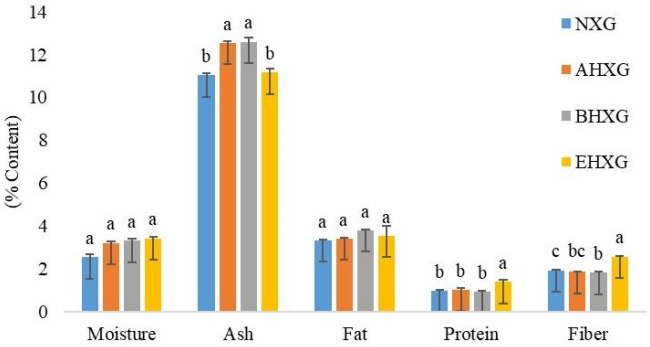

Figure 1. Composition of xanthan gum and its hydrolytic forms

***Mineral Analysis*:** Xanthan gum contains three minerals attached with its terminal glucuronic acid in the side chain. These acidic residues usually contain cations such as Na, K and Ca in different proportions (Kuppuswami, 2014). The cations mainly come from salts added in the fermentation media used for production of xanthan gum. Minerals present in xanthan gum influences its various properties specifically in solution (Klaic *et al.*, 2011).

Mean values for mineral contents (Na, K, Ca) in xanthan gum and its modified forms are presented in Table 1. The results declared that the various hydrolysis methods used for modification of xanthan gum have a highly significant impact on Na and K contents while non-significant effect was observed in case of Ca contents.

Table 1. Means for mineral content (mg/100g) of native xanthan gum and its hydrolyzed forms.

Treatments	Na	K	Ca
NXG	416±13.9^{c}	290±09.7^{a}	102.0±5.4^{a}
AHXG	520±19.5^{b}	270±10.1^{b}	98.0±3.7^{a}
BHXG	541±19.4^{a}	247±08.9^{c}	101.0±3.6^{a}
EHXG	402±16.1^{d}	286±11.4^{a}	99.8±4.0^{a}

Values are presented as means of triplicate ± standard deviation; Comparisons were made within the column for each parameter; Mean values with different letters varied significantly $p<0.05$. **NXG=** Native xanthan gum, **AHXG**= Acid hydrolyzed xanthan gum, **BHXG**= Base hydrolyzed xanthan gum, **EHXG**= Enzyme hydrolyzed xanthan gum

The Na contents were found to be increased in acid and base hydrolyzed xanthan gum when compared with NXG whereas,

in enzyme hydrolyzed xanthan gum (EHXG), statistically non-significant reduction was observed as shown Table 1. The highest Na contents were present in base hydrolyzed xanthan gum (BHXG) (541 mg/100g) followed by acid hydrolyzed xanthan gum (AHXG) (520 mg/100g), NXG (416mg/100g) and EHXG (402mg/100g). This increase in Na content in case of AXG and BXG might be due to the attachment of Na to the glucuronic acid from base (NaOH) used in the hydrolyses method as Kuppuswami (2014) stated that glucuronic acid residue in xanthan gum side chain attaches the cations from the salts present in media. Mean values for K contents in xanthan gum and its hydrolytic forms demonstrated a non-significant variation for K contents in native and EHXG with the values of 290mg/100g and 286mg/100g respectively, while AHXG and BHXG exhibited significant change in K contents showing 270 mg/100g and 247 mg/100g K contents respectively. Reduction in K contents in hydrolyzed xanthan gum fractions could be attributed to the loss of terminal glucuronic acid during hydrolysis process as the mineral is attached to it (Kuppuswami, 2014).

The findings of current research are in line with the results of Klaic *et al.* (2011) who used acid digestion method to evaluate the determination of minerals in xanthan gum. Furthermore, xanthan gum properties are influenced by the concentration and nature of added salts. The workers found an average content 550 mg/100g, 564 mg/g and 100 mg/100g for Na, K and Ca respectively (Rinaudo 2001) which also support the findings of current study.

Rheological characteristics of xanthan gum

***Steady Shear characteristics*:** Rheograms for steady shear properties of 1% solutions of xanthan gum in distilled water at 25ºC were studied and Fig 2a describes the flow behavior while Fig 2b describes the shear rate dependence of apparent viscosity of native and hydrolyzed forms of xanthan gum. Fig. 2a clearly depicted an increase in shear stress with increasing shear rate for all the xanthan gum samples. The rheogram (Fig. 2b) declared an inverse relationship between viscosity and shear rate. Aqueous solutions of xanthan gum and its hydrolytic forms exhibited a shear thinning behavior. Shear thinning behavior of xanthan gum could be explained by the fact that xanthan gum forms large aggregates when in the resting state in solution at lower or no shear rate but as the shear rate is increased the disruption in aggregates caused the decline in the viscosity of xanthan gum solutions.

Xanthan gum exhibited non-Newtonian behavior even at low shear rate as the apparent viscosity sharply decreased with the increase in the shear rate for all the gum samples. Hence, xanthan gum solutions exhibited non-Newtonian rheology and showed pseudo-plastic or shear thinning behavior, the viscosity decreases with increasing shear rate which has been proved when Chagas *et al.* (2004) conducted study on rheological studies of xanthan gum as biodegradable polymer for its used in petroleum recovery supporting our results. It has also been concluded earlier that at low shear rates stretched polysaccharide molecules interweave to form aggregates with high viscosity due to large fluid flow resistance. The increase of the shear rate disrupts the aggregates and dispersed molecules arranged along the flow direction resulting in decrease of the flow resistance of the fluid consequently leading to decrease of the apparent viscosity (Oh *et al.*, 1999; Xu *et al.*, 2013).

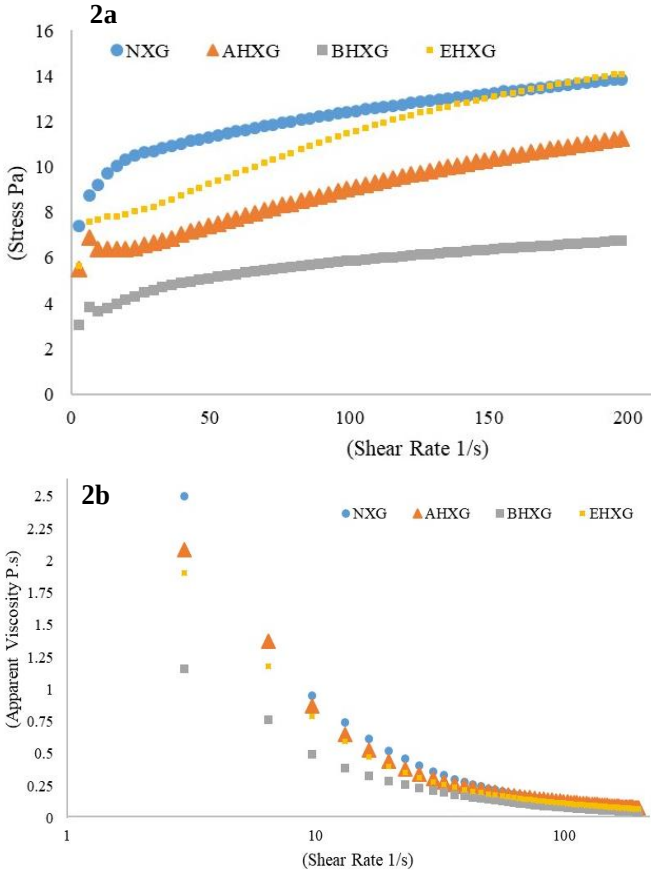

Figure 2. a) Flow behavior b) Viscosity of 1% aqueous solutions of native xanthan gum and its hydrolytic forms.

The differences among the four categories of xanthan gums under discussion showed a remarkable decrease in viscosity that could be observed after hydrolysis with various methods adopted in comparison with the NXG Fig. 2b. Highest viscosity (2.5 Pa.s) was observed for NXG followed by acid (2.08 Pa.s), enzyme (1.90 Pa.s) and base (1.16 Pa.s) hydrolyzed xanthan gum. The effect of acid and enzyme hydrolysis overlapped with respect to viscosity of xanthan gum.

The findings of current study are supported by Ahuja *et al.*(2012) who modified xanthan gum by the process of carboxymethylation to enhance solubility of the polymer in water. It was concluded that the viscosity of xanthan gum is

considerably reduced by the application of carboxymethylation process in comparison to native one.

Hussain *et al.* (2017) also modified xanthan gum to improve its rheological properties. It was established from the results that modification enhanced strength and stability of the xanthan gels. Another study conducted by Su *et al.* (2003) on xanthan gum following chemical modification using formaldehyde. The objective behind the alteration was to increase dissolution rate of the xanthan gum. It was found that chemically modified xanthan gum dissolves more rapidly than the unmodified one. The viscosity of gum solution depends upon various factors like temperature, both dissolution and measurement temperatures, the concentration of xanthan gum, salt concentration and pH. The findings were made by the study performed by Garcıa-Ochoa *et al.* (2000).

In general, viscosity of the xanthan gum samples was decreased with increase in shear stress and rate applied. The values of viscosity at low shear rates is an indication of the consistent behavior of the product as noted by Morris and Taylor (1982), while the values of viscosity at high shear rates indicates viscosity of the product as a result of processing during industrial operations (Dakia *et al.*, 2008).

***Effect of temperature on apparent viscosity*:** The aqueous solutions of native and modified xanthan gums were subjected to find apparent viscosity with different temperature conditions up to 60°C as presented in Fig. 3. The rheoghrams depicted that NXG and BHXG exhibited the typical effect of temperature increase on viscosity *i.e.* with the increase in temperature the viscosity decreases. This strong temperature dependence is attributed to a conformational transition of xanthan chains from helical at low temperatures to random coil at high temperatures (Choppe *et al.*, 2010). However, viscosity of acid and enzyme hydrolyzed xanthan gums showed reversed behavior *i.e.* an increase in viscosity was observed. This might be due to either some conformational changes in the structure of xanthan gum as per effect of hydrolysis treatment or with the increase in temperature moisture contents tends to decrease showing increase in viscosity after certain temperature.

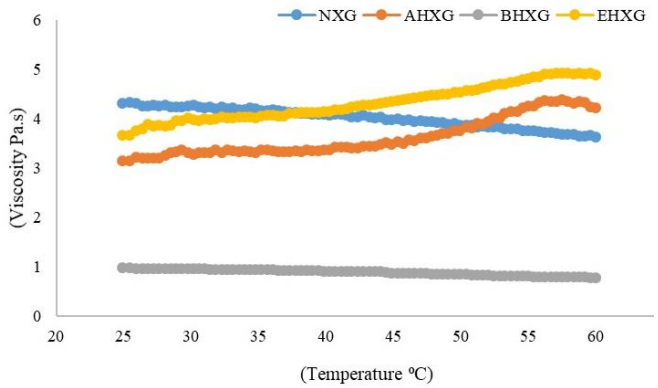

Figure 3. Temperature sweep of 1% aqueous solutions of native xanthan gum and its hydrolytic forms

The results for NXG and BHXG are in agreement with the findings of (Xu *et al.*, 2013) who studied the rheological properties of xanthan gum solutions in water. They found that temperature has a great impact on xanthan gum viscosity especially in the low shear rate (<20 s^{-1}), the apparent viscosity of xanthan gum reduces with increasing temperature. A decrease in viscosity retention rate with increase in temperature was reported by the researchers. However, at higher shear rate the change in viscosity as a function of temperature is consistent. Xanthan gum exhibits less stability in solution towards change in temperature because of its conformation changes from ordered double helix to disordered coil at higher temperature as concluded by (Jang *et al.*, 2015).

Finding of the current studies are also in concordance with the results of (Speers and Tung, 1986), who evaluated the temperature and viscosity dependency of xanthan gum dispersions and observed a decrease in viscosity with increase in temperature but with reduced temperature effect at higher concentration. Other scientists also observed the inverse relationship between temperature and viscosity (Jang *et al.*, 2015).

***Visco-elastic characteristics*:** Frequency sweeps of the storage and loss moduli in rheological measurement serve as a tool to distinguish between polymer solutions and gels (Mao *et al.*, 2012). Complex viscosities of xanthan gum and its hydrolytic forms is described in Fig. 4, while storage modulus G' and loss modulus G" of all the gum solutions is presented in Fig. 5.

It was evident from Fig. 4 that the complex viscosities of xanthan gum solutions declined with increase in frequency at constant shear rate (1/s). The complex viscosity of treated xanthan gum solutions is lower than the NXG exhibiting the effect of hydrolysis in reducing the viscosity of xanthan gum. Minimum viscosity (0.41 Pa.s) was observed in base treated xanthan gum showing maximum effect of hydrolysis as compare to NXG (7.92 Pa.s), AHXG (4.39 Pa.s) and EHXG (4.16 Pa.s).

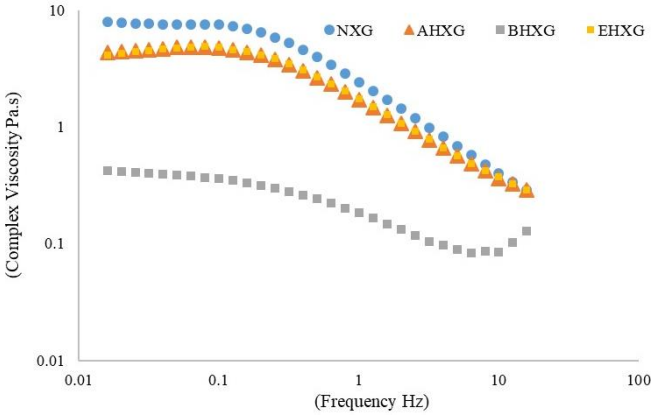

Figure 4. Complex viscosity of 1% aqueous solutions of native xanthan gum and its hydrolytic forms.

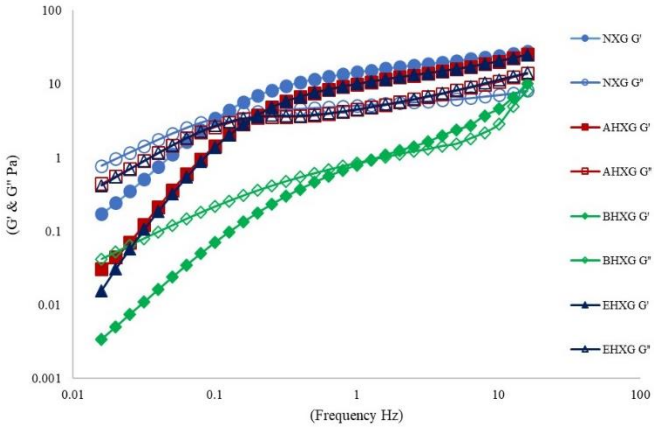

Figure 5. Dynamic properties of native xanthan gum and its hydrolytic forms.

Rheoghrams (Fig. 5) of G' and G" for all the gum samples exhibited rectangular characteristic type of polymer solution curves having higher values of G" as compared to G' at lower frequency which become inverse as the frequency increased up to crossover frequency. The values of G' and G" at lower frequencies were NXG (0.171, 0.773 Pa), AHXG (0.031, 0.437 Pa), BHXG (0.003, 0.041 Pa) and EHXG (0.015, 0.415 Pa) showing viscous properties predominating over elastic properties of 1% gum solutions. At higher frequency the results become inverse and the values for storage modulus G' and loss modulus G" were NXG (27.52, 8.18 Pa), AHXG (25.29, 13.83 Pa), BHXG (10.09, 8.05 Pa) and EHXG (24.98, 14.11 Pa) exhibiting elastic behavior dominating over viscous properties.

This phenomenon can be explained by the fact that at low frequencies there was enough time for entanglement of xanthan molecules to occur and be disrupted during the oscillation period, which allowed viscous flow to predominate over the elastic characteristics. With an increase in frequency, the time became insufficient for disruption of the entanglement and the dispersion behaved as a cross linked network with the elastic response predominating over viscous flow (G'>G") (Moraes *et al.*, 2011).

The frequency at which storage modulus G' and loss modulus G" become equal, termed as cross over frequency is higher for hydrolyzed gum as compared to native stated as NXG (0.1 Hz), AXG (0.159 Hz), BXG (0.8 Hz), EXG (0.2 Hz). This indicates that xanthan gum exhibited the polymer solution characteristics at less than 1 Hz frequency and after that the week gel like behavior was prevailed. The findings of current analysis are in line with the results of Moraes *et al.* (2011) who studied the rheological properties of xanthan gum in some fruit pulp solution and found that xanthan gum exhibited a week gel like behavior.

***FTIR Analysis*:** The FTIR spectra of acid, base and enzyme hydrolyzed xanthan gum were recorded to construe the functional groups present and structural changes occurred due to hydrolysis with different treatments within all variants of gum. The FTIR spectra of NXG and its hydrolyzed forms are presented in Fig. 6 and the characteristic functional group present in all gum samples are summarized in Table 2 along with respective wave numbers. For NXG, a strong broad vibrational peak for O-H bond was observed at 3346cm^{-1} while C=O was observed at 1641cm^{-1}. The peak observed at 650cm^{-1} was due to C=O stretching of amide group. The peaks at 1598, 1402, 1053 and 588cm^{-1} were characteristic for OH bending from water, CH stretching of methyl group, C-O-C stretching of ether depicting a glycosidic linkage and C-H bending in polysaccharide respectively.

The FTIR spectrum of AHXG, BHXG and EHXG exhibited a broad trough for OH stretching vibration at 3364cm^{-1},3372cm^{-1} and 3382cm^{-1} accordingly, indicating a slight shift as compared to native one. A new peak was observed at

Table 2 Functional groups identified from FTIR spectra of xanthan gum and its hydrolyzed derivatives

NXG		**AHXG**		**BHXG**		**EHXG**	
Vibrational frequency cm^{-1}	**Functional group**	**Vibrational frequency cm^{-1}**	**Functional group**	**Vibrational frequency cm^{-1}**	**Functional group**	**Vibrational frequency cm^{-1}**	**Functional group**
3346	OH stretch vib.	3364	OH stretch vib.	3372	OH stretch vib.	3283	OH stretch vib.
1641	C=O stretch vib.	1641	C=O stretch vib.	1658	C=O stretch Amide	1641	C=O stretch vib.
1650	C=O stretch Amide	1612	COO- stretch vib. of COOR	1641	C=O stretch vib.	1599	OH bending from water
1598	OH bending from water	1408	CH stretching of methyl group	1611	COO- stretch vib. of COOR	1402	CH stretching of methyl group
1402	CH stretching of methyl group	1054	C-O-C stretching of ether	1407	CH stretching of methyl group	1249	Skeletal C-C vib.
1053	C-O-C stretching of ether	588	C-H bending	1022	C-O-C stretching of ether	1026	C-O-C stretching of ether
588	C-H bending			556	C-H bending	591	C-H bending

NXG = Native xanthan gum, **AHXG** = Acid hydrolyzed xanthan gum, **BHXG** = Base hydrolyzed xanthan gum, **EHXG** = Enzyme hydrolyzed xanthan gum

1612cm^{-1} and 1611cm^{-1} for COO- stretch of carboxylic in case of AHXG and BHXG respectively. An additional peak was observed in case of EHXG at 1249cm^{-1} due to C-C vibration of skeletal carbon atoms. There was no major transformation of functional group in xanthan gum after hydrolysis except slight shifting in wavenumbers. The similarity in molecular structure of native and hydrolyzed xanthan gum confirms that hydrolysis treatment didn't change the molecular structure. Anjum *et al.* (2015) also observed similar results while studying hydrolysis of xanthan gum by chemical and enzymatic methods and reported only a slight change in molecular structure with no major transformation of functional groups.

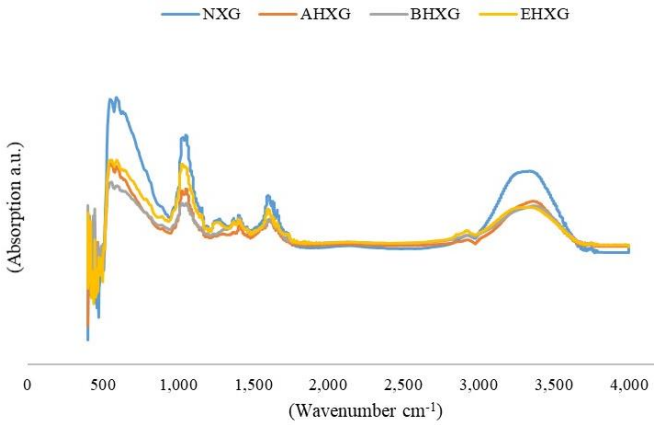

Figure 6. FTIR spectra of native xanthan gm and is hydrolytic forms

Similarly, Ahuja *et al.*(2012) studied the IR spectra of xanthan gum while working on modification of xanthan gum by carboxymethylation for medicine use. They reported that the spectrum showed a broad band at 3386 cm^{-1} that may be due to the stretching of the OH group. Absorption bands occurred at 2919 cm^{-1}, 1410 cm^{-1}, 1616 cm^{-1} and 1733 cm^{-1} owing to CH stretching of alkyl group, CH bending of methyl group, asymmetric stretching of carboxylate ion and CO stretching of alkyl esters respectively. The C-O-C stretching of ether appeared at 1060 cm^{-1}. Similar results were reported in various other studies by (Wu *et al.,* 2013; Pooja *et al.,* 2014; Li *et al.,* 2016 and Sheng *et al.,* 2016).

***Scanning electron microscope* (*SEM*):** Scanning electron microscopy was performed to investigate the detailed surface morphology of xanthan gum and its hydrolyzed derivatives to observe the effect of acid, base and enzyme treatment. The purpose was to study the effect of the treatments on surface morphology and final arrangement of gum particle after hydrolysis and drying. The results for SEM for all the gum samples are presented in Fig. 7. It depicted that NXG possesses a fibrous structure with arrangement of xanthan molecules in the amorphous structure as it is evident from the shape of particles. NXG existed in non-interlinked granular particle form which is supported by the studies conducted by Kumar *et al.* (2017) who observed fibrous nature of xanthan gum particles suggesting the amorphous nature of polysaccharide.

The AHXG and BHXG exhibited the crystalline structure as evident from Fig. 7b and c. However, the EHXG Fig. 7d exhibited the fibrous structure but these fibers are much thinner as compare to native xanthan gum showing a clear effect of hydrolysis treatments. Hydrolysis breakdown, on overall basis, exhibited that the xanthan gum was converted into smaller fractions and made the structure smoother as compared to native one. The conversion of xanthan gum from amorphous to crystalline improve its applications in food products where better dispersibility and dissolution is required.

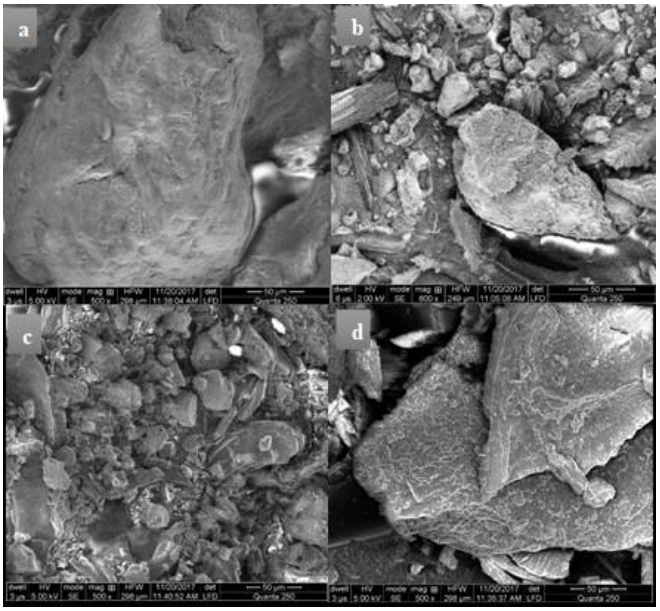

Figure 7. Scanning electron micrographs of a) NXG, b) AHXG, c) BHXG and d) EHXG

The findings of the current studies are in line with the results of Anjum *et al.* (2015), who studied the surface morphology of hydrolyzed xanthan gum by using scanning electron microscopy and deduced that native xanthan gum exhibited the fibrous morphology with lacking of clear edges and ordered structure. In hydrolyzed xanthan gum products, some fibrous morphology has been observed, indicated with reduced thickness and increased homogeneity.

***X-ray diffraction*:** Amorphous and crystalline nature of a solid matter can be described by constructive and destructive interferences of X-rays with molecular and crystalline structure of samples in X-ray diffraction phenomena (Birkholz, 2006). The X-ray diffraction pattern of xanthan gum samples are depicted in Fig. 8. It is obvious from the Fig. 8a and d that native and enzyme hydrolyzed xanthan gum exhibited the amorphous structure showing a broad peak at diffraction angle (°2θ) of 20° while another peak at 73° was

observed in case of EHXG which was not observed in NXG. The slight difference in structure variation of EHXG from native gum could be due to the hydrolysis process or particularly the enzyme used. Current findings are supported by Mudgil *et al*. (2014) who concluded that enzyme hydrolysis resulted in negligible change in X-ray diffraction curve with a slight increase in crystallinity of partially hydrolyzed gum.

The results for amorphous nature of xanthan gum are supported by the findings of Lad *et al*. (2013) who worked on X-ray diffraction pattern of xanthan gum powders. They examined xanthan gum powders with different levels of sharp patterns were superimposed by the amorphous background. Pandey and Mishra (2011) also reported the fibrous structure of xanthan gum suggesting the amorphous nature of this biopolymer.

The X-ray diffraction configuration of AHXG and BHXG exhibited crystalline nature as obvious from Fig. 8 b and c. High intensity peaks were observed at diffraction angle (°2θ) of 32° and 45° followed by low intensity peaks that were observed at angles of 46°, 67° and 75°. An additional peak was observed at an angle of 27° in case of BHXG showing slight difference in structure from the acid hydrolyzed xanthan gum that is attributed to the different methods used for hydrolysis for both the treatments. Increase in crystallinity due to modification of xanthan gum has also been reported by various scientists like Pandey and Mishra (2011) and Anjum *et al*. (2015).

***Thermo gravimetric analysis*:** Thermal stability and decomposition pattern of polymers can be analyzed by using precise and simple method of thermo-gravimetric analysis . Thermal stability of any food additive is quite important as the food may be subjected to elevated temperatures as in case of baking and sterilization (Mudgil *et al*., 2014).

TGA curves (Fig. 9) describes the method of thermal decomposition of native xanthan gum and its hydrolytic forms. Initial degradation temperature (IDT) for NXG is in the range of 30-130 °C exhibiting the initial mass loss of 11.7% that is due to the removal of lattice water. IDT for AHXG, BHXG and EHXG was 30-110 °C, 30-120 °C and 30-105 °C with the mass loss of 9.7%, 10% and 10.23%, respectively. This mass loss is due to desorption of water from xanthan structure. The difference in IDT for native xanthan gum and its hydrolysates is due to the changes in the gum after modification. The water absorption by xanthan is due to the presence of polar groups in its structure, especially the –OH grouping (Faria *et al*., 2011). Current findings for initial mass loss are supported by the results of Faria *et al*. (2011) who

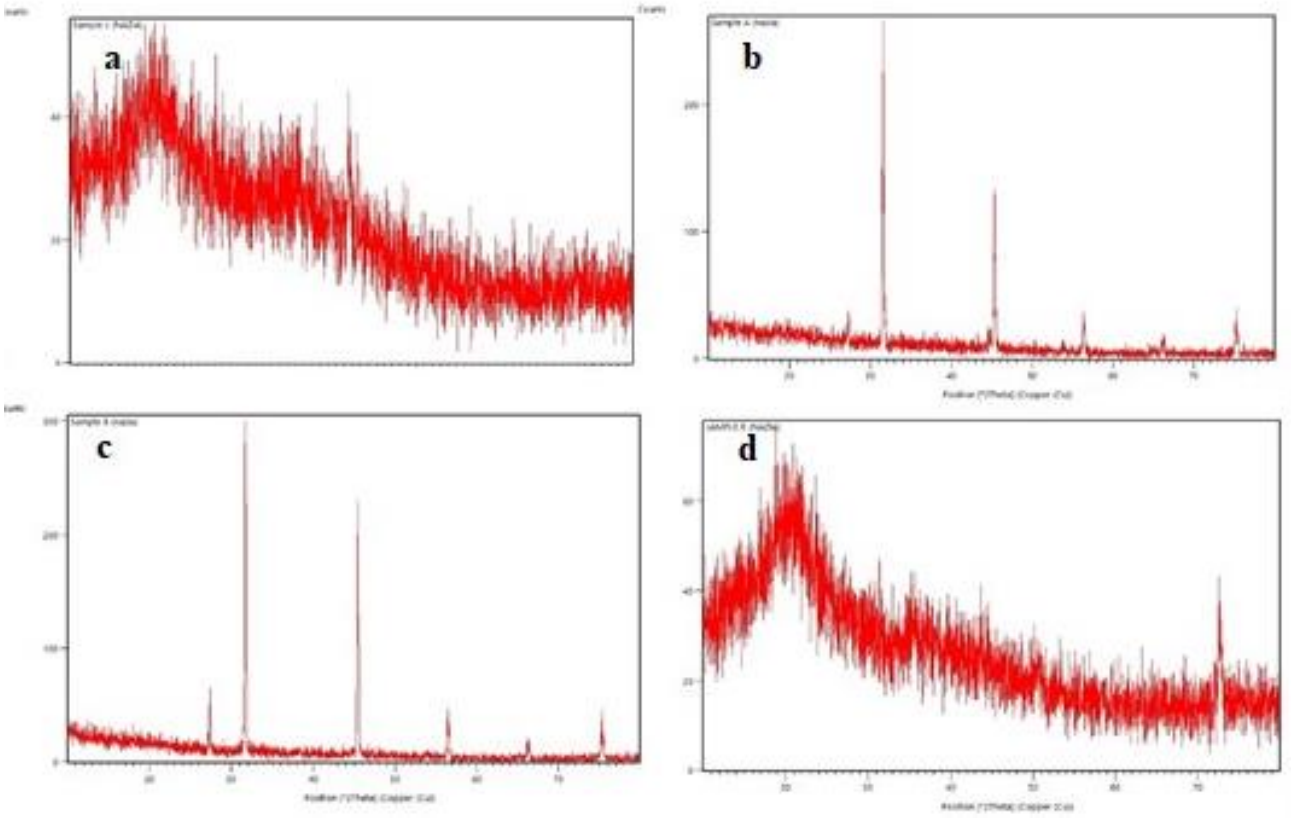

Figure 8. X-ray diffractograms of a) NXG, b) AHXG, c) BHXG and d) EHXG

reported initial mass loss in the temperature range of 30-140 °C. Similarly, Anjum *et al*. (2015) reported that initial mass loss in native and hydrolyzed xanthan gum occurred below 160 °C while acid hydrolyzed gum showed less thermal stability as the degradation started earlier.

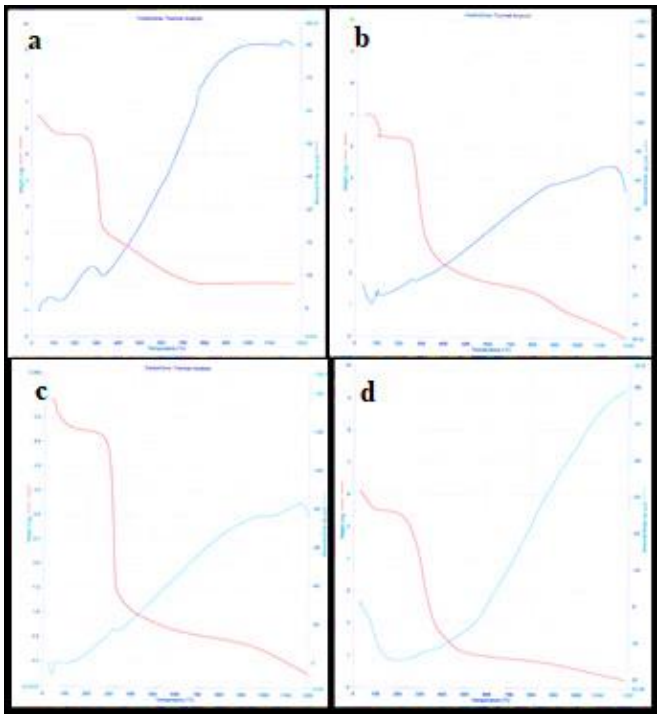

Figure 9. TGA curves of a) NXG, b) AHXG, c) BHXG and d) EHXG

Maximum rate of decomposition temperature (MRDT), the temperature during which maximum weight loss occurs, for NXG was 230-350 °C with maximum weight loss of 56% in the second zone of mass loss. MRDT for hydrolyzed xanthan gum fractions was 215-400 °C, 225-405 °C and 210-460 °C for AHXG, BHXG and EHXG with mass loss of 57, 69.5 % and 68.5%, respectively. Half of the mass loss (D1/2) occurred around 310-320 °C for all the gum samples. NXG exhibited 90% of mass loss before 700 °C while for hydrolyzed xanthan gums (AHXG, BHXG and EHXG) mass loss of 79%, 91% and 84% was observed from TGA curves. The rate of mass loss after 700 °C was very slow. The remaining products at the end were inorganic substances like minerals or ash (Zohuriaan and Shokrolahi, 2004). Findings of Jazini*et al*. (2017) are supportive to results found in our study. They reported the initial mass loss of 8 to 10 % at 35-145 °C and maximum rate of mass loss occurred at 300 °C. Although the inconsistency in the pattern has also been observed by Banerjee *et al*. (2006) who studied on synthesis and characterization of xanthan gum copolymerized with formamide.

***Conclusion*:** All the hydrolysis methods resulted in reduction of viscosity with maximum effect in base hydrolyzed xanthan gum. Hydrolysis does not cause any major transformation in the functional groups present in xanthan gum retaining its basic structure. The reduction in viscosity is beneficial for food applications most specifically in dairy products where higher viscosities resulted in negative interaction between proteins in milk and polysaccharide added. Native and enzyme hydrolyzed gum exhibits amorphous structure however the structure of xanthan gum after acid and base hydrolysis become crystalline with improved applications in food systems without interacting the integrity of final product. Further investigations are recommended through size exclusion chromatography for determination of change in molecular weight after hydrolysis for further authentication.

***Conflicts of interest*:** The authors certify that there is no conflict of interest with any financial organization or the employers regarding the views expressed in the manuscript.

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