



## RHEOLOGICAL, PASTING, THERMAL AND GEL PERMEATION CHROMATOGRAPHIC CHARACTERISTICS OF GUAR GUM ACID HYDROLYSATES

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### ABSTRACT

Guar gum was partially hydrolyzed with HCl for 30 (GGH<sub>30</sub>) and 60 (GGH<sub>60</sub>) min to obtain its acid hydrolysates. Guar gum and its acid hydrolysates were studied for hydration, pasting, rheological and thermal properties. Acid hydrolysis significantly lowered the intrinsic viscosity and molar mass of the hydrolysates. The steady shear properties of guar gum and its hydrolysate (GGH<sub>30</sub>) studied at 25°C revealed a shear thinning behavior while GGH<sub>60</sub> displayed a nearly Newtonian behavior. Dynamic measurements revealed a typical biopolymer viscoelastic gel behavior for NGG and GGH<sub>30</sub>. The FT-IR study indicated no structural changes in the hydrolysates as no additional functional groups were added in the molecular structure after acid hydrolysis of the intact guar gum. The differential scanning calorimetry results displayed lowered thermal stability of the hydrolysates relative to its native counterpart. The present study suggested that partial hydrolysates could be used suitably in different food products as a soluble dietary ingredient.

### 1. Introduction

Guar gum is a high molar mass polymer comprising of a linear backbone of (1→4) linked β-D- mannopyranosyl units linked with (1→6) α-D-galactopyranosyl side chain residues having mannose and galactose units in 1:2 ratio. The key features such as molar mass, mannose/galactose ratio (M/G) and the distribution of galactose units over the mannose backbone influence the technical applications of the polysaccharide. Guar gum has been employed as a thickening, stabilizing agent and also as a dietary fiber in food industry due to its viscous nature which in turn maintains the rheology of food systems (Ellis *et al.*, 2001).

However, guar gum has some drawbacks such as high-water insoluble content, low dissolution rate, tough to regulate consistency and vulnerable to microbial growth (Hongbo *et al.*, 2013). The sensorial attributes of foods containing guar gum also tend to be poor

because of its high viscosity and conjointly limits its addition in foods at high concentrations (Cui *et al.*, 2007). To overcome these drawbacks, guar gum needs to be modified to attain desired characteristics like increased water solubility, solution clarity, and ionic character and increased shelf life depending on the specific application. Degradation of guar gum is vital for several food applications with an objective to raise the dietary fiber amount in foods as low molar mass or partially hydrolyzed gum is regarded as ample source of dietary fiber and has remarkable health implications (Yoon *et al.*, 2008). Partially hydrolyzed guar gum is considered as a prebiotic ingredient which can play beneficial role in improving the gastrointestinal health (Li and Hu, 2018). The guar gum especially partial degraded guar gum finds its food applications in juice, ice creams, sauces and salad dressing etc. (Krishnaiah *et al.*, 1998;

Tuohy *et al.*, 2001). Prebiotic potential of guar gum hydrolysate has been reported in biscuits (Tuohy *et al.*, 2001). Acid hydrolysis is considered as a convenient method for the degradation of guar gum into various low molar mass units allowing its addition in liquid food such as beverages at a concentration effective enough to provide the beneficial health effects of guar gum.

Rheology has been employed to investigate the potential applications of guar gum in food products. However, still the rheological characterization of guar gum is unclear due to its complicated nature that cannot be described by a single model (Wientjes *et al.*, 2000). Also, there has been too little studies discussing about the pasting profiles of gum solutions. RVA has been mainly associated with the starch, the primary reason behind the less applicability of RVA as an analytical tool for gum solutions. Mainly controlled shear stress devices have been used to analyze the rheological behavior of gum solutions but RVA can be used as a powerful tool to distinguish gums on basis of their viscous behavior in respect of the temperature. So, an attempt has been made to analyze the pasting, rheological and thermal behavior of guar gum and its acid hydrolysates to expand its applicability in various food products.

## 2. Materials and methods

### 2.1. Materials

Food grade guar gum was procured from Hindustan Gums & Chemicals Ltd., Bhiwani and other reagents and chemicals in the study were of analytical grade.

### 2.2. Acid hydrolysis of guar gum

Guar gum was depolymerized by the procedure as proposed by Chauhan *et al.* (2009) with minor changes. 10g guar gum was dispersed in 80% methanol (200ml) consisting HCl (5% w/v). The reaction mixture was then heated for 30 and 60 min separately, at 65°C. The gum hydrolysis was terminated by neutralization with NaOH (1N) and

hydrolysates were filtered, washed with absolute ethanol, freeze dried and milled.

### 2.3. Intrinsic Viscosity

Intrinsic viscosity was analyzed at ambient temperature (25° C) with the aid of an Ostwald's viscometer. Gum solutions (0.02-0.1% w/v) were prepared by sprinkling gum in a vortex with water and left overnight for complete dissolution. Relative viscosity was determined using the following equation:

$$\eta_r = \frac{t}{t_0} \quad (1)$$

where t is the flow time of gum solution and t<sub>0</sub> is the flow time of pure solvent. Specific viscosity was determined from relative viscosity using the equation

$$\eta_{sp} = \eta_r - 1 \quad (2)$$

Reduced viscosity was then obtained from specific viscosity using the correlation

$$\eta_{red} = \frac{\eta_{sp}}{C} \quad (3)$$

Intrinsic viscosity ( $\eta$ ) was analyzed by measuring reduced viscosity at different concentrations and extrapolating to concentration C = 0.

### 2.4. Molar mass determination

The molar mass was analyzed from the viscometry results as well as chromatographic results.

#### 2.4.1. Molar mass determination with viscometry

Viscosity average molar mass ( $M_v$ ) was measured from intrinsic viscosity using Mark-Houwink's equation,

$$[\eta] = KM_v^\alpha \quad (4)$$

where K= 5.13 x 10<sup>4</sup> and  $\alpha$ = 0.72 (Beer *et al.*, 1999).

#### 2.4.2. Gel permeation chromatography

Number average molar mass ( $M_n$ ), weight average molar mass ( $M_w$ ) and polydispersity index (PDI) ( $M_w/M_n$ ) of guar gum and its

hydrolysates were assessed using a gel permeation chromatography system (Viscotek GPC max, Malvern, UK) with an aqueous column (A6000, 300x8 mm) connected to a RI detector. Guar gum and its hydrolysates (0.5 mg/ml) were dissolved in NaNO<sub>3</sub> (0.05 M), filtered through 0.22 µm nylon filter prior injection and eluted at a flow rate of 0.7 ml/min at 35° C. The GPC was calibrated with the known molar mass standards of pullulan (342 - 7,10,000 Da, Fluka, USA). Molar mass was analyzed using linear regression equation obtained for pullulan standards.

## 2.5. Degree of Polymerization

Viscosity average molar mass of gums was used to determine the degree of polymerization using the following equation:

$$\text{Average DP} = \frac{\text{molecular weight of polymer}}{\text{molecular weight of monomer}} \quad (5)$$

Molar mass of guar gum monomer used was 270 (Mahammad *et al.*, 2006).

## 2.6. Hydration properties of gums

Water binding capacity (WBC) of guar gum and its hydrolysates was measured according to the standard AACC method (2010) using 0.1 g sample. The WBC was determined in g/g as the amount of water bound to per gram of the sample. Swelling power and solubility of gums were determined by the procedure described by Bae *et al.* (2009) by using 0.1 g sample. Swelling power was calculated as the ratio of the wet precipitate weight to the dry sample weight whilst solubility was measured as the dry supernatant weight to dry sample weight.

## 2.7. Rheological properties of gums

### 2.7.1. Solution preparation

The appropriate amount of gum sample was dissolved in deionized water to get the final concentration of 1, 2 and 3%. The dispersions were vigorously mixed for 1 h at room temperature and subsequently heated at 80° C in a shaking water bath for 30 min to achieve complete hydration.

### 2.7.2. Dynamic and steady shear rheological properties

Dynamic and steady flow measurements of aqueous gum solutions were performed at 25° C with a dynamic rheometer (MCR 102, Anton Paar GmbH, Germany) equipped with a cone and plate geometry (40 mm diameter, 0.08 mm gap and 1° cone angle). Frequency sweep measurements were conducted in the range (0.1-100 rad/s) at 1% constant strain within the linear viscoelastic range as observed by amplitude sweep test.

Steady shear tests were also conducted at 25° C to obtain flow curve data with the increasing shear rates from 0 to 100s<sup>-1</sup>. The data obtained was fitted to the following models:

Power law model,

$$\tau = K\gamma^n \quad (6)$$

Herschel-Bulkley model,

$$\tau = \sigma_0 + K\gamma^n \quad (7)$$

where  $\tau$  is the shear stress (Pa),  $\sigma_0$  is the yield stress (Pa),  $\gamma$  is the shear rate (s<sup>-1</sup>),  $n$  is the flow behavior index (dimensionless) and  $K$  is the consistency coefficient (Pa s<sup>n</sup>).

## 2.8. Rapid visco analysis

The viscosity profiles of gums were studied by Rapid Visco Analyzer (RVA). Sample dispersions (1 and 2% w/w) were prepared in a canister and positioned in the instrument. In the beginning of the test, samples were stirred at 960 rpm for 1 min succeeded by 160 rpm all over the test. The temperature was held at 80°C for 2 min and subsequently cooled to 30°C at a wage of 4°C/min.

## 2.9. FT-IR spectroscopy

The structural variations among guar gum and its hydrolysates were analyzed using FT-IR spectroscopy. The guar gum samples were directly placed on to sampling unit and spectra were recorded between 4000 and 400cm<sup>-1</sup> with a FT-IR spectrophotometer (Bruker Alpha, Platinum ATR, Germany).

### 2.10. Thermal properties

The thermal properties of guar gum and its hydrolysates were assessed with a differential scanning calorimeter (DSC 25 TA instruments, USA). The powder samples (3.5 mg±0.1) were weighed in an aluminum pan and loaded in the instrument with a blank pan as a standard. The scans were performed in the temperature range from 50-450°C with a wage of 10° C/min and different thermal parameters including onset temperature ( $T_o$ ), peak temperature ( $T_p$ ), conclusion temperature ( $T_c$ ) and enthalpy ( $\Delta H$ ) were measured.

### 2.11. Statistical analysis

The statistical variations were assessed by one-way Analysis of Variance (ANOVA) using SPSS version 19.0 with a significant level ( $p < 0.05$ ). All determinations were made in triplicates and the data was expressed as mean values ± standard deviation.

## 3. Results and discussion

### 3.1. Intrinsic viscosity and viscosity average molar mass of gums

Intrinsic viscosity measures the hydrodynamic size engaged by the molecules present in the solution and is directly linked to their molar mass and shape (Gupta *et al.*, 2015). Acid hydrolysis led to the cleavage of glycosidic bonds of the polymer chain and molar mass of guar gum leading to a drop in the intrinsic viscosity. The  $\eta$  of the NGG was found to be greater (11.99 dL/g) in comparison to its hydrolysates, GGH<sub>30</sub> (4.98 dL/g) and GGH<sub>60</sub> (2.01 dL/g) respectively (Table 1). High molar mass guar gum chains were disintegrated into low molar mass fragments which indicated weakened aggregations in hydrolyzed gums and thus low molar mass fractions can be obtained to achieve specific applications (Cheng *et al.*, 2002). Molar mass was observed to be a function of duration period of acid hydrolysis as it decreased from  $1.06 \times 10^6$  Da to  $3.19 \times 10^5$  Da and  $9.02 \times 10^4$  Da after 30 and 60 min duration of acid hydrolysis, respectively (Table 1).

**Table 1.** Molar mass and intrinsic viscosity of guar gum and its hydrolysates

Sample	$\eta$ (dL/g)	$M_v$ (Da)	DP	$M_w$ (Da)	$M_n$ (Da)	PDI
NGG	11.99± 0.02 <sup>c</sup>	$1.06 \times 10^6 \pm$ 0.01 <sup>c</sup>	3925.92± 6.11 <sup>c</sup>	$1.02 \times 10^7$ ±0.02 <sup>c</sup>	$2.39 \times 10^6$ ±0.01 <sup>c</sup>	4.26± 0.01 <sup>c</sup>
GGH <sub>30</sub>	4.98± 0.03 <sup>b</sup>	$3.19 \times 10^5 \pm$ 0.01 <sup>b</sup>	1181.48± 3.51 <sup>b</sup>	$3.11 \times 10^6$ ±0.01 <sup>b</sup>	$8.16 \times 10^5$ ±0.02 <sup>b</sup>	3.80± 0.02 <sup>b</sup>
GGH <sub>60</sub>	2.01± 0.01 <sup>a</sup>	$9.02 \times 10^4 \pm$ 0.02 <sup>a</sup>	334.26± 3.02 <sup>a</sup>	$2.79 \times 10^5$ ±0.01 <sup>a</sup>	$1.05 \times 10^5$ ±0.01 <sup>a</sup>	2.64± 0.01 <sup>a</sup>

NGG - Native guar gum, GGH - Guar gum hydrolysate,  $\eta$  - intrinsic viscosity,  $M_v$  - viscosity average molar mass, DP - degree of polymerization,  $M_w$  - weight average molar mass,  $M_n$  - number average molar mass, PDI - polydispersity index

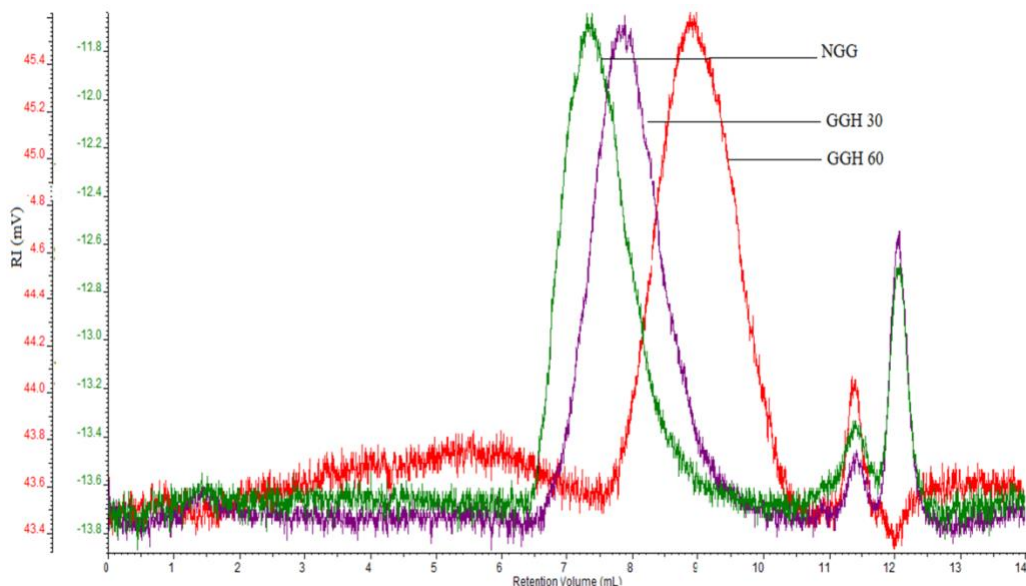
### 3.2. Gel permeation chromatography (GPC)

The GPC chromatograms of guar gum and its acid hydrolysates are shown in Figure 1. The GPC profiles of acid hydrolysates were moved towards the right side in retention time as compared to NGG revealing their low molar masses. The weight average molar mass ( $M_w$ ) and number average molar mass ( $M_n$ ) were observed to be higher for NGG ( $1.02 \times 10^7$  and  $2.39 \times 10^6$  Da) when compared to its acid hydrolysates, GGH<sub>30</sub> ( $3.11 \times 10^6$  and  $8.16 \times 10^5$

Da) and GGH<sub>60</sub> ( $2.79 \times 10^5$  and  $1.05 \times 10^5$ ) respectively (Table 1). The value for molar mass determined by GPC were on the higher side in comparison to those obtained from intrinsic viscosity molar weight. Similar observations have been observed for hydrolysed galactomannan from *C. pulcherrima* (Buriti *et al.*, 2014). Polydispersity index was found to be lower for GGH<sub>30</sub> (3.80) and GGH<sub>60</sub> (2.64) in comparison to NGG (4.26) indicating a high proportion of high

molar mass molecules in native guar gum. The hydrolysates showed narrow molar mass distribution (MWD) reflecting the homogeneity of low molar mass molecules. Acid hydrolysis might have disrupted the glycosidic bonds resulting into a decrease in the molecule weight of gum molecules. The lower degree of polymerization of hydrolysates in contrast to its native gum also confirmed that acid hydrolysis

had significantly broken the bonds between mannose units leading to decrease in molar mass and hence lower degree of polymerization. Molar mass and concentration are the key factors that affect the functional properties of polysaccharides, and the above results indicate that guar gum can be degraded to various  $M_w$  with narrow MWD to achieve desired characteristics.



**Figure 1.** GPC chromatograms of guar gum and its acid hydrolysates.

**Table 2.** Hydration properties of guar gum and its acid hydrolysates

Sample	Water binding capacity (g/g)	Swelling power (g/g)	Solubility (%)
NGG	$21.44 \pm 1.42^c$	$66.52 \pm 2.13^c$	$30.86 \pm 4.16^a$
GGH <sub>30</sub>	$6.81 \pm 0.48^b$	$20.42 \pm 3.71^b$	$74.64 \pm 3.79^b$
GGH <sub>60</sub>	$3.56 \pm 0.29^a$	$4.21 \pm 0.25^a$	$87.86 \pm 0.14^c$

Values are mean  $\pm$  S.D of triplicates.

Values in the same column with different letters are significantly different ( $p < 0.05$ )

### 3.3. Hydration properties

Hydration properties of food components are very crucial in food industry as it influences the overall product quality, stability, and shelf life of food products. Highest water binding capacity was observed for NGG, followed by GGH<sub>30</sub> and GGH<sub>60</sub>, respectively (Table 2). The greater affinity of native guar gum towards water is directly related to its molecular structure owing greater number of OH groups allowing more water interactions via hydrogen

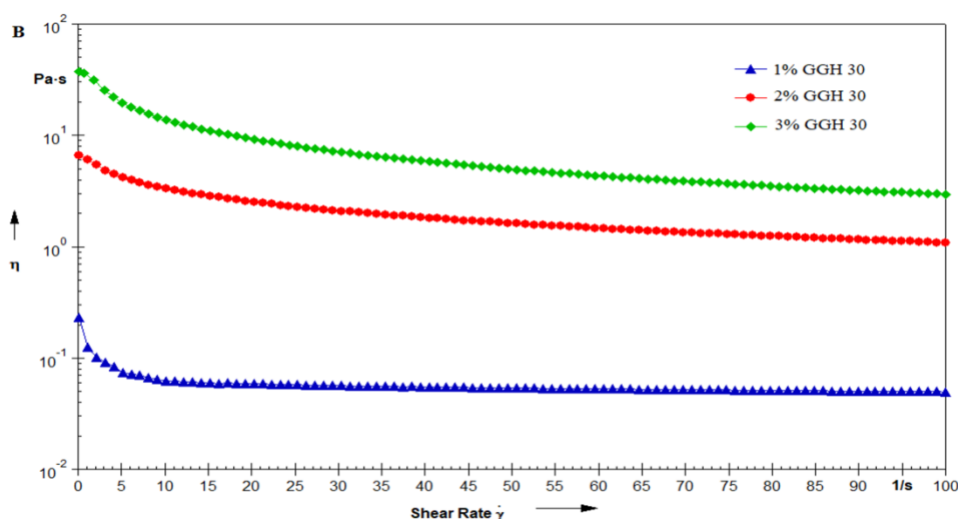
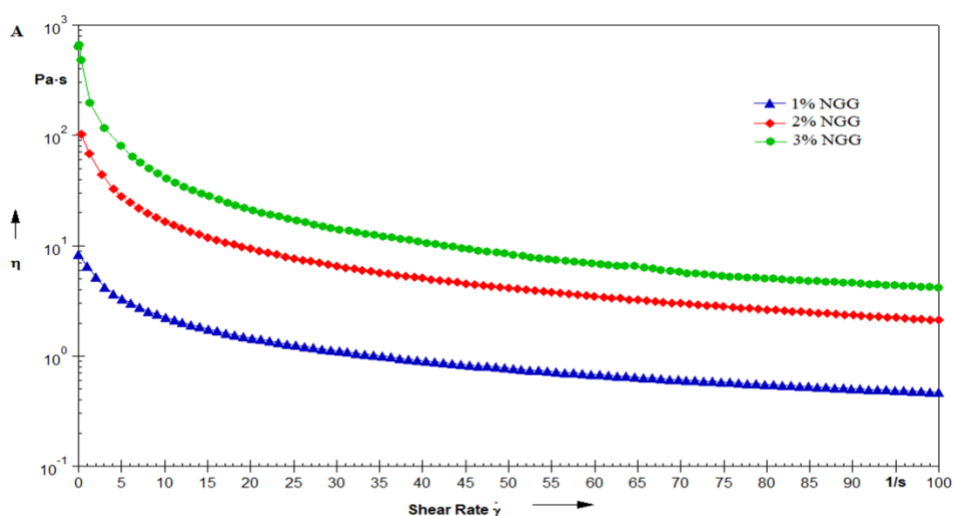
bonding. The hydrolysates showed less affinity towards water in comparison to NGG because of a smaller number of hydroxyl groups resulting from the reduction in chain length during acid hydrolysis. Similarly, swelling power was also significantly ( $p < 0.05$ ) higher in case of NGG in contrast to its acid hydrolysates (Table 2). Molecular structure plays a crucial part in regulating the functionality of gums in foods. Higher the swelling power, lesser will be the commercial

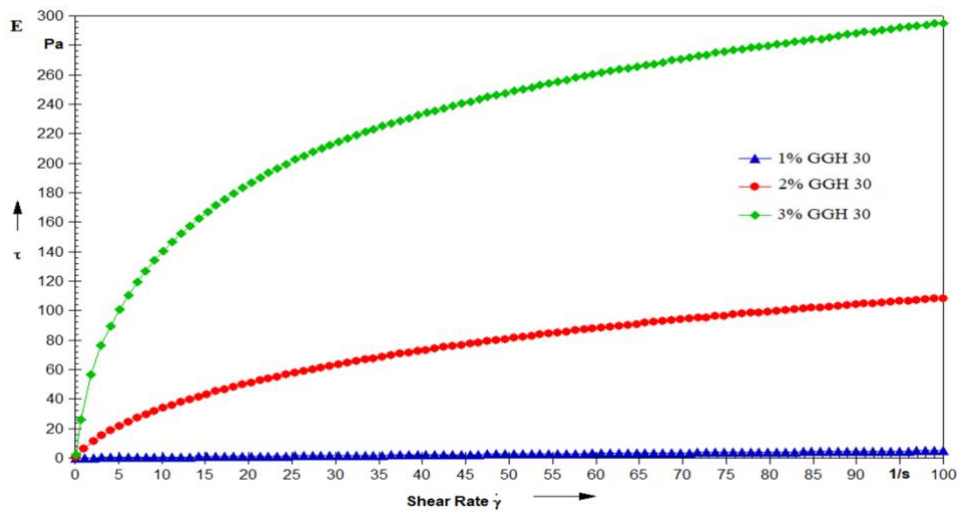
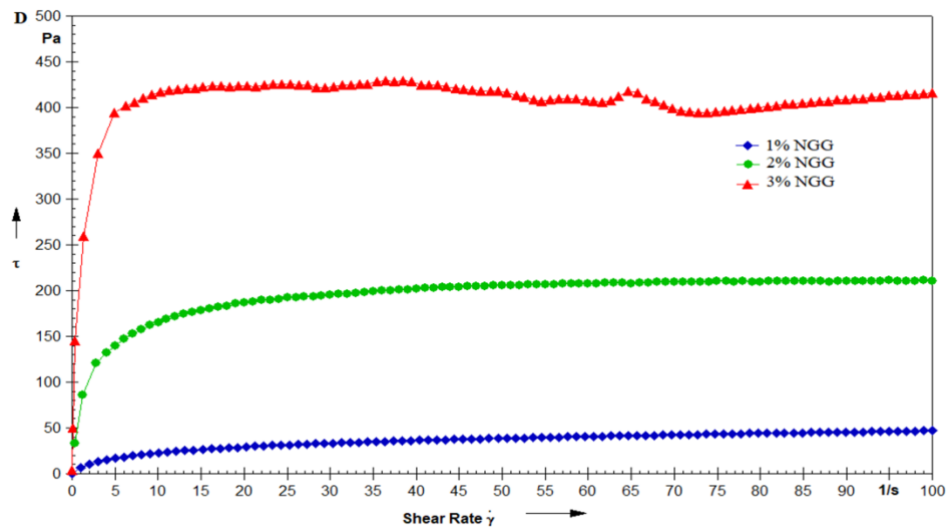
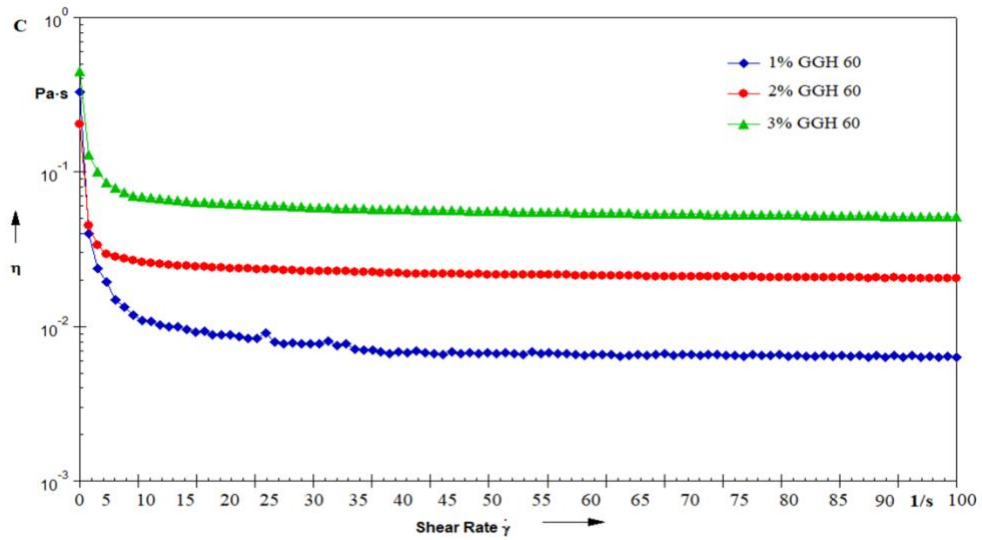
applications (Dodi et al., 2011). So, acid hydrolysis can be used to increase the potential applications of guar gum in foods. A significant increment ( $p < 0.05$ ) in solubility was noticed for hydrolysates, being maximum for GGH<sub>60</sub> (87.86%) and minimum for NGG (30.86%). Solubility increased by 141% after 30 min hydrolysis and by 184% after 60 min hydrolysis. Formation of low molar mass fragments through disintegration and depolymerization of guar chains after acid hydrolysis might be responsible for the increased solubility of hydrolysates as small monosaccharaides have higher hydration ability as compared to native gum. These results implied that guar gum hydrolysates can be used a soluble dietary ingredient in food products.

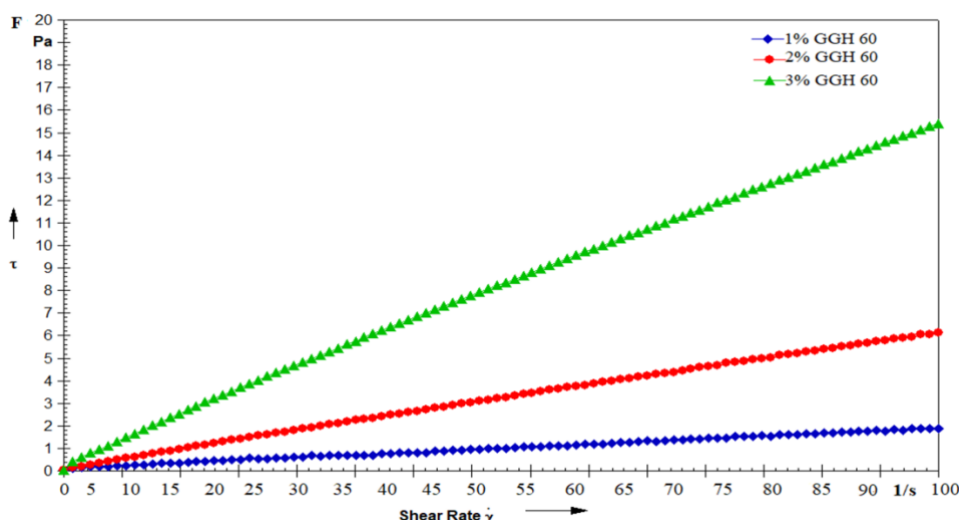
### 3.4. Rheological properties of gums

#### 3.4.1. Steady shear properties

Rheology illustrates the flow behavior of polysaccharides and determine their potential applications in foods. The solutions of guar gum and its acid hydrolysates prepared with different concentrations of 1, 2 and 3% exhibited a non-Newtonian shear-thinning behavior in the shear range from 0-100s<sup>-1</sup>. As shown in viscosity curves given in Figure 2 (a, b and c), viscosity decreased with the growing shear rates, which could be attributed to the reason that disruption dominated over the formation of new entanglements with increasing shear rates and molecules aligned in the direction of flow resulting into lowering of the viscosity (Hussain *et al.*, 2015).







**Figure 2.** Viscosity curves (a, b, c) and flow curves (d, e, f) of NGG (native guar gum), GGH<sub>30</sub> and GGH<sub>60</sub> (guar gum hydrolysate) at different concentrations.

These outcomes also reflected the concentration dependency of viscosity of the guar gum and its hydrolysate which increased with increasing concentrations. Guar gum and its hydrolysates have hydroxyl groups within their chemical structure due to which entanglement occurs at higher concentrations resulting in higher viscosities (Gong *et al.*, 2012).

The flow curves shown in Figure 2 (d, e and f) depicts the flow properties of gum solutions with different concentrations at 25° C. The flow behavior as studied by using Power law and Herschel-Bulkley model also indicated a non-Newtonian pseudoplastic behavior. Significant variations were observed ( $p < 0.05$ ) in the values of ‘ $\sigma$ ’, ‘ $n$ ’ and ‘ $K$ ’ for NGG and its acid hydrolysates (Table 3).

**Table 3.** Flow parameters of guar gum and its acid hydrolysates determined at different concentrations.

Sample	Power $n$	Law $K$ (Pa.s <sup><math>n</math></sup> )	Model $R^2$	Herschel $\sigma$ (Pa)	Bulkley $n$	Model $K$ (Pa.s <sup><math>n</math></sup> )	$R^2$
1% NGG	0.31±0.01 <sup>a</sup>	10.48±0.32 <sup>b</sup>	0.97	5.75±0.58 <sup>d</sup>	0.12±0.01 <sup>c</sup>	46.73±4.34 <sup>c</sup>	0.98
2% NGG	0.25±0.06 <sup>a</sup>	75.63±18.6 <sup>d</sup>	0.74	32.53±1.16 <sup>c</sup>	0.06±0.01 <sup>b</sup>	548.78±58.93 <sup>e</sup>	0.94
3% NGG	0.24±0.03 <sup>a</sup>	127.48±4.46 <sup>e</sup>	0.00	48.81±2.04 <sup>f</sup>	0.01±0.00 <sup>a</sup>	2701.56±425.08 <sup>f</sup>	0.00
1% GGH <sub>30</sub>	0.80±0.02 <sup>d</sup>	0.11±0.15 <sup>a</sup>	0.98	0.08±0.02 <sup>b</sup>	0.86±0.02 <sup>f</sup>	0.09±0.03 <sup>a</sup>	0.99
2% GGH <sub>30</sub>	0.61±0.01 <sup>d</sup>	7.53±0.66 <sup>b</sup>	0.93	0.74±0.11 <sup>b</sup>	0.52±0.01 <sup>e</sup>	11.30±1.14 <sup>b</sup>	0.98
3% GGH <sub>30</sub>	0.48±0.03 <sup>b</sup>	36.09±0.12 <sup>c</sup>	0.82	2.36±0.43 <sup>c</sup>	0.34±0.02 <sup>d</sup>	70.61±1.30 <sup>d</sup>	0.97
1% GGH <sub>60</sub>	0.62±0.01 <sup>c</sup>	0.04±0.01 <sup>a</sup>	0.93	0.03±0.02 <sup>a</sup>	0.88±0.02 <sup>f</sup>	0.04±0.02 <sup>a</sup>	0.98
2% GGH <sub>60</sub>	0.77±0.03 <sup>c</sup>	0.06±0.01 <sup>a</sup>	0.97	0.05±0.03 <sup>a</sup>	0.94±0.01 <sup>g</sup>	0.08±0.01 <sup>a</sup>	0.99
3% GGH <sub>60</sub>	0.81±0.01 <sup>c</sup>	0.13±0.01 <sup>a</sup>	0.98	0.12±0.04 <sup>a</sup>	0.92±0.002 <sup>g</sup>	0.10±0.04 <sup>a</sup>	0.99

Values are mean ± S.D of triplicates.

Values with different letters in the same column are significantly different ( $p < 0.05$ )

Highest yield stress was observed for 3% NGG (48.81) while lowest for 1% GGH<sub>60</sub> (0.03). This was indicative of the presence of some entangled networks in the samples which should be disintegrated to initiate the flow and it also reflected the strong associated network

of NGG in comparison to its hydrolysates. Acid hydrolysis imparted a drastic change in the flow properties of guar gum as the magnitude of shear stress values for GGH were much lower than that of the NGG. Also, a decrease in the pseudoplasticity was observed with increase



in the degree of hydrolysis. The flow behavior index ( $n$ ) shows the Newtonian or non-Newtonian behavior of the polymers and ' $n$ ' value near to 1 indicates the Newtonian behavior while ' $n$ ' values less than 1 reflects the pseudoplastic behavior of the polymers. The ' $n$ ' values decreased with rising concentration for NGG and GGH<sub>30</sub> indicating that the pseudoplastic behavior enhanced at higher concentrations while an opposite trend was found for GGH<sub>60</sub> where ' $n$ ' values increased with increasing concentration suggesting increasing concentrations led to the Newtonian like behavior of GGH<sub>60</sub>. The consistency index for hydrolysates was much lower in comparison to NGG suggesting a much drop in viscosity due to decrease in the molar mass and chain length by acid hydrolysis. A near Newtonian behavior was observed for GGH<sub>60</sub> and almost negligible viscosity values were obtained. It can be concluded from the flow results that guar gum hydrolysates can be used in food products like beverages to enhance their dietary fibre content without modifying their viscosity. The flow results were also suggestive of the fact that NGG should be added upto 2% concentration in the food products, as the concentration beyond this may deteriorate the textural properties of foods. Evidently, NGG at 3% concentration showed a very high consistency and no regression coefficient was found which suggested a very high viscosity making it unsuitable for the textural properties of foods. Also an interrupted flow was achieved which might be due to the high viscosity of the system.

### 3.4.2. Oscillatory experiments

The viscoelastic characteristics of guar gum and its acid hydrolysates at different concentrations were analyzed by performing frequency sweep tests which determines the storage ( $G'$ ) and loss modulus ( $G''$ ). The dynamic mechanical spectra of guar gum and its acid hydrolysates analyzed at 25° C are shown in Figure 3 (a and b). Dynamic measurements were only conducted on the NGG and GGH<sub>30</sub> because GGH<sub>60</sub> showed a

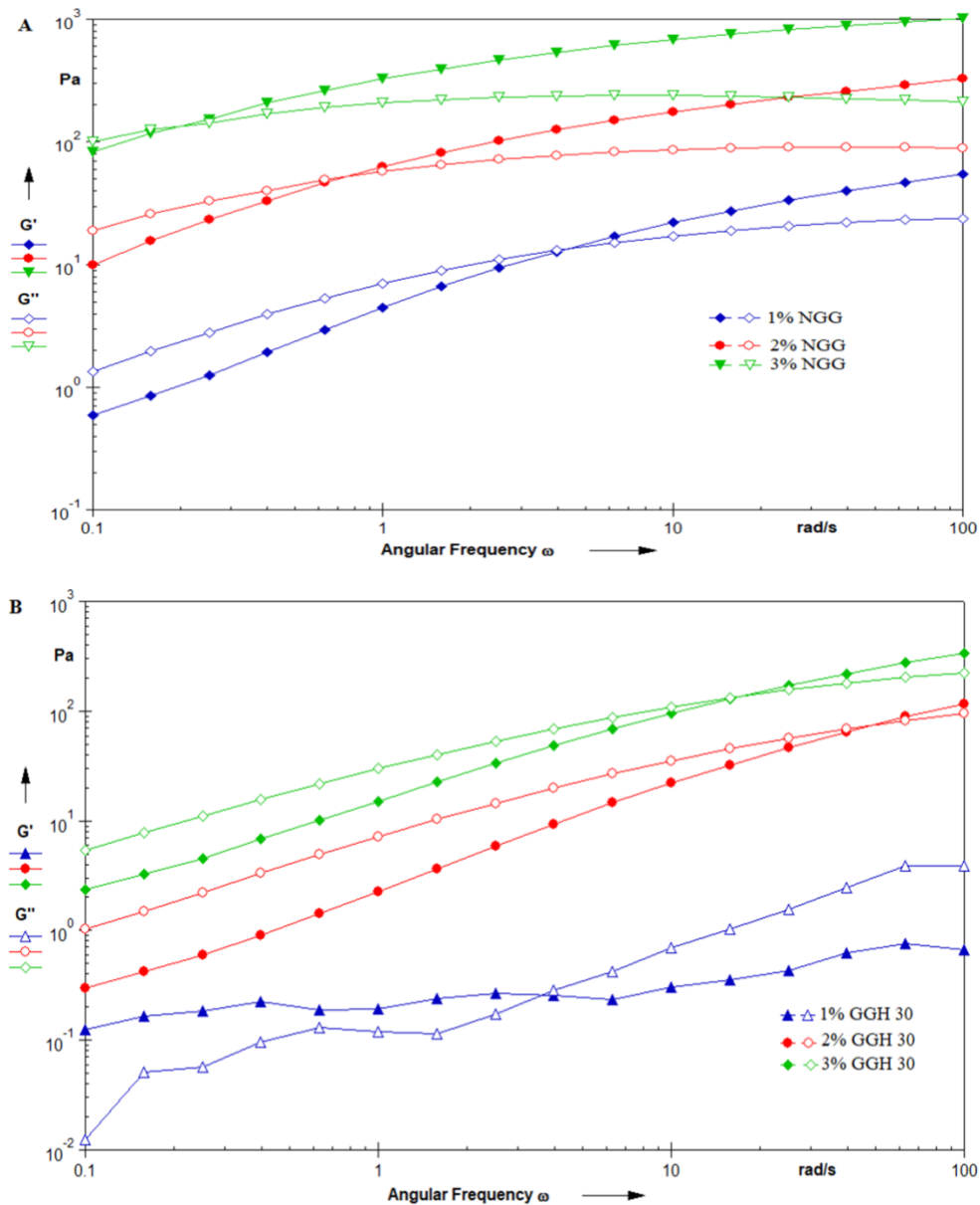
near Newtonian behavior and was having very less viscosity even at the highest concentration used and the LVE range could not be reached as the strain value was too low to be determined by the instrument. Therefore, only flow properties were analyzed for GGH<sub>60</sub>. The rheograms for NGG and GGH<sub>30</sub> revealed that values of both  $G'$  and  $G''$  shifted upwards with rising frequency ( $\omega$ ). The frequency sweep for various gum solutions showed the classic characteristics of macromolecular solutions where  $G''$  was higher than  $G'$  at low frequencies while at higher frequency range,  $G'$  was predominant. Similar behavior for various galactomannans and disordered random-coil polysaccharides have reported by different researchers (Bourbon *et al.*, 2010; Oblonsek *et al.*, 2003).

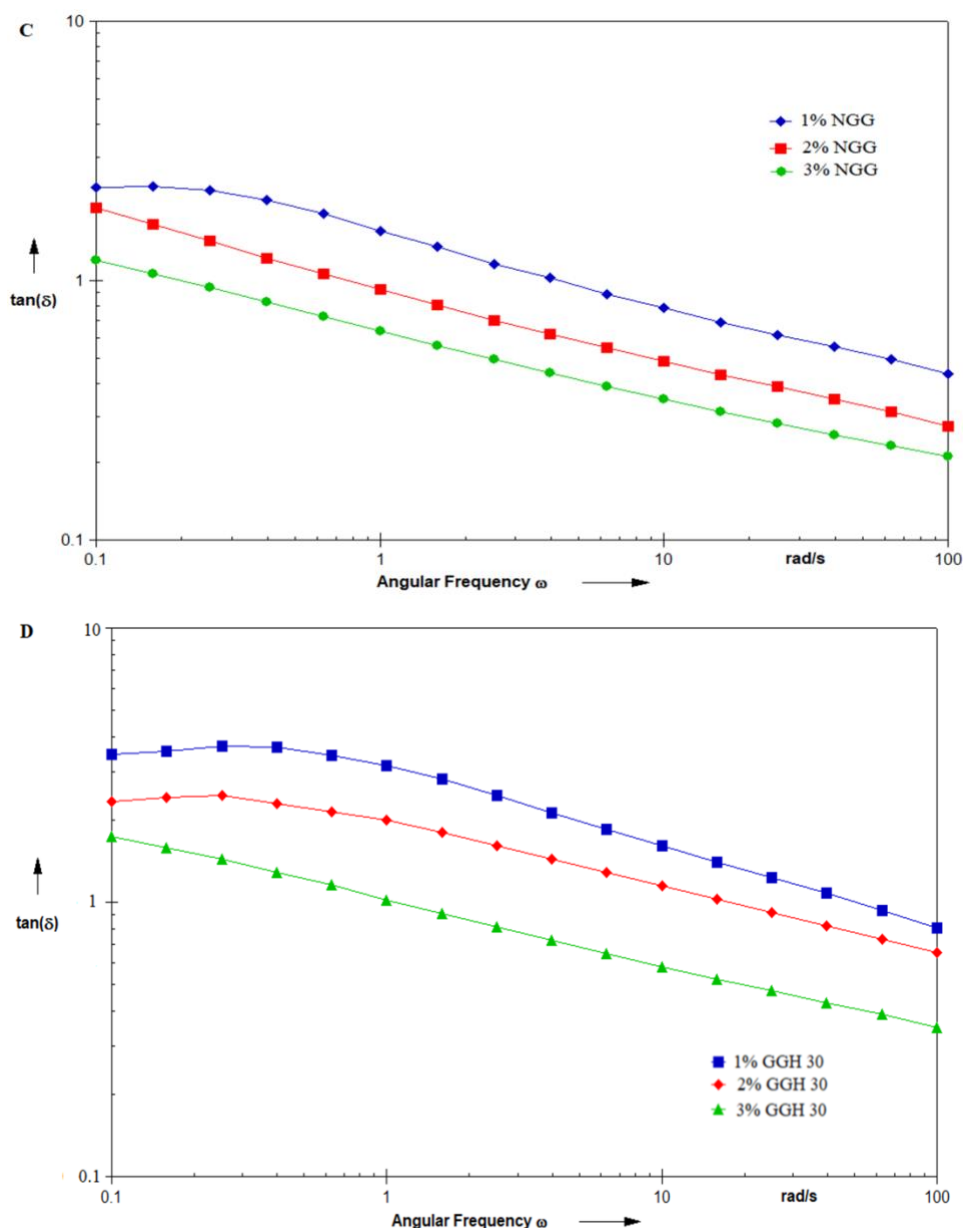
The extent of  $G'$  and  $G''$  was found to be greater for NGG as compared to GGH<sub>30</sub> which increased with increasing gum concentrations. These results revealed that the solutions showed a liquid like character till crossover occurred after which elasticity prevailed due to highly structured nature of the polysaccharide. It was found that GGH<sub>30</sub> showed more liquid like behavior while NGG exhibited more entangled behavior as  $G'$  dominated over the  $G''$  at higher frequencies. Similar findings were also reported by Pollard *et al.* (2010) and Thombre and Gide (2013). It was observed that the crossover frequency decreased as the concentration of NGG was increased, which might be a consequence of increased relaxation times resulting into entanglement of polymeric chains in solution (Torres *et al.*, 2014). However, in case of GGH<sub>30</sub>, the crossover frequency increased as the concentration increased from 1-2% but it decreased at a concentration of 3%. These results indicated that elasticity improved with increasing concentrations indicating the strong associations among the molecules in the polymer solutions.

Tan  $\delta$  distinguishes polymers based on their viscoelastic character which is the ratio of  $G''$  versus  $G'$ . Tan  $\delta > 1$  shows the prominence of viscous nature while tan  $\delta < 1$  reflects the

elastic nature. As can be seen from Figure 3 (c and d) NGG displayed an elastic characteristic at higher frequencies while viscous characteristics at lower frequencies as  $\tan \delta$  values increased in the lower frequency range and were beyond 1. Similar trend was revealed by GGH<sub>30</sub> but NGG presented more elasticity in comparison to its acid hydrolysate which was obviously due to highly structured network of the native gum because of its greater branching and molar mass.  $\tan \delta$  values decreased with increasing gum concentrations

in both NGG and GGH<sub>30</sub>. Low  $\tan \delta$  values suggested that higher stress values are needed to the break the intermolecular associations between gum molecules. These results are also consistent with the flow behavior as described above reflecting higher yield stress values in case of NGG in comparison to its hydrolysates. Overall, these dynamic results showed that guar gum and its hydrolysates had elastic behavior at high concentrations and viscous behavior at low concentrations.





**Figure 3.** Dynamic rheological properties (a, b) mechanical spectra and (c, d) loss tangent ( $\tan \delta$ ) of NGG and GGH<sub>30</sub> at different concentrations measured at 25°C.

### 3.5. Rapid visco analysis

The viscosity profiles of guar gum and its hydrolysates were studied at two levels (1 and 2%) with the aid of RVA. Guar gum is a cold-water soluble polymer whose final viscosity relies on its molar mass and dissolution temperature. As can be seen from Figure 4 the decrease in viscosity continued till temperature persisted at 80°C. However, as the temperature dropped, the viscosity began to rise reaching the final viscosity. This behavior reflects that

viscosity was reliant on temperature and gum amount. It has been reported that gum molecules move faster at higher temperatures due to enhanced thermal vibrations and eventually results into lower viscosities, while at low temperatures, molecules associate to make entanglements via intra molecular hydrogen bonding due to reduced mobility of gum molecules (Morris, 1990). Significant difference ( $p < 0.05$ ) was observed between the viscosities of guar gum and its acid

hydrolysates. Highest final viscosity was observed for 2% NGG (3265cp) while lowest for 1% GGH<sub>60</sub> (22cp). 3-fold reduction in viscosity was observed when guar gum was hydrolyzed for 30 min succeeded by 64-fold reduction in 60 min hydrolysis at 2% level. Guar gum and its hydrolysates revealed their specific cooling profile which was obvious due

to changes in the molar mass of gums after acid hydrolysis. Viscosity is often related to molar mass and chain length; greater the molecular weight, greater will be the viscosity. These results suggest that RVA can be used to distinguish the hydrocolloids on basis of their viscosity and to assess their suitability for food purposes.

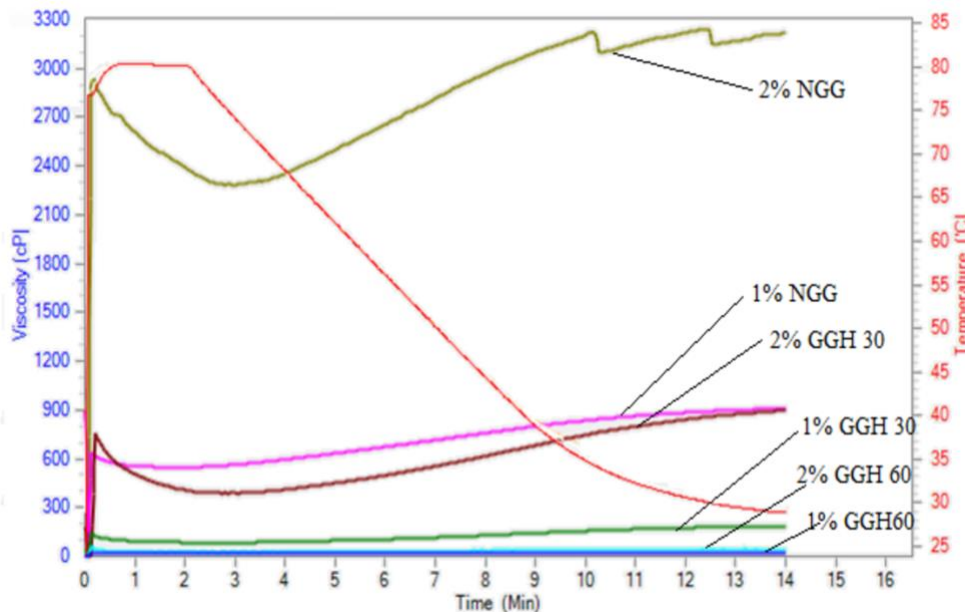


Figure 4. RVA profiles of guar gum and its hydrolysates at different concentrations.

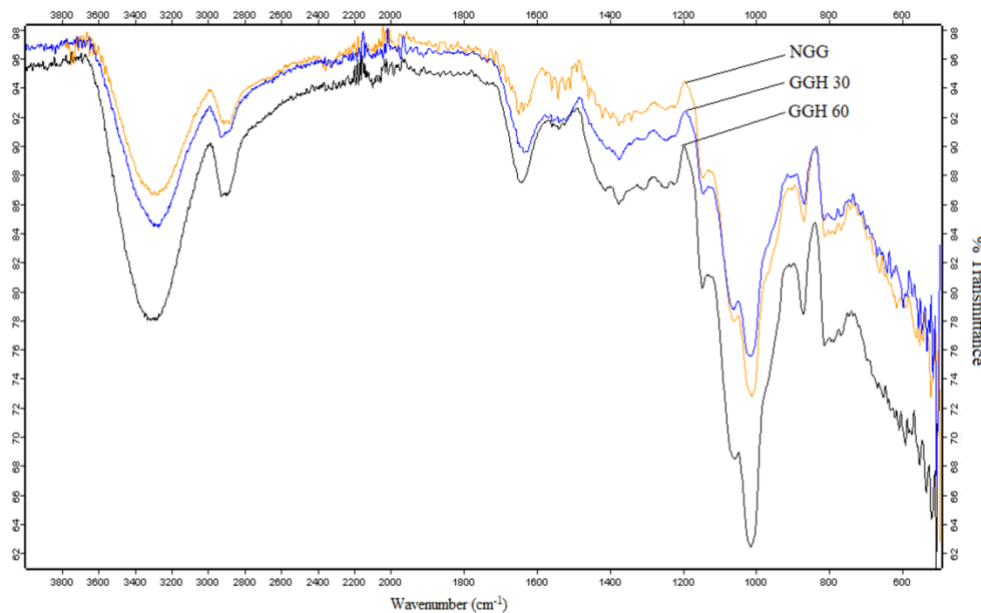


Figure 5. FT-IR spectra of guar gum and its acid hydrolysates.

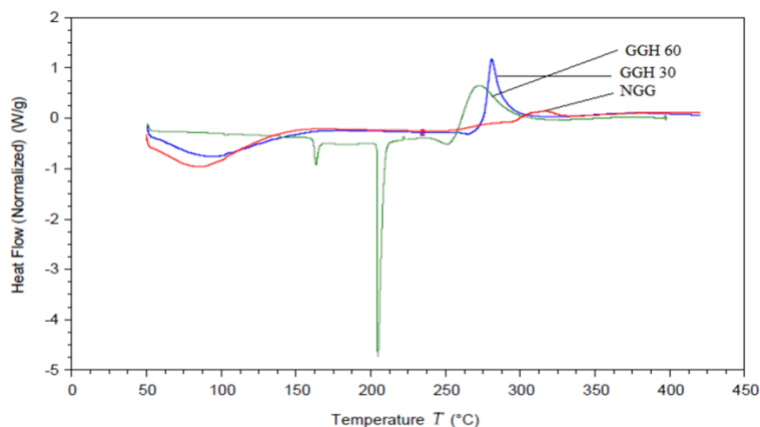
### 3.6. FT-IR

Acid hydrolysis might have brought some chemical changes in the structure of guar gum due to the disintegration of glycosidic bonds. So, FT-IR was performed to analyze the structural variations in the guar gum after acidic depolymerization. It can be seen that both hydrolysates had the similar superimposable spectra to that of NGG, which indicated that no structural changes occurred on account of acid hydrolysis (Figure 5). FT-IR results also revealed that no functional groups were induced and the depolymerization of polymer was basically due to cleavage of glycosidic bonds between the guar molecules. Peaks in the spectral region of  $3200\text{--}3400\text{ cm}^{-1}$  are attributed to O-H stretching of the polymer and water involved in hydrogen bonding and peaks in the region near  $2900\text{ cm}^{-1}$  corresponds to  $\text{--C-H}$  stretching mode (Shobha *et al.*, 2005). The bands near 800 and 1200 is due to the stretching vibrations of  $\text{C--C--O}$ ,  $\text{C--OH}$  and  $\text{C--O--C}$  polysaccharide backbone respectively. The peak around 1600 is attributed to the bound water and in the spectral region around 1400 corresponds to  $\text{--CH}_2$  bending. Earlier studies also reported no structural changes after partial hydrolysis of galactomannans (Mudgil *et al.*, 2012; Prajapat *et al.*, 2015).

### 3.7. Thermal properties

Thermal stability of gums is considerable in determining the applicability of polymers in

foods where food is thermally processed such as baking, sterilization, and pasteurization etc. (Hussain *et al.*, 2018). Two distinct peaks were observed for NGG and GGH<sub>30</sub> while three peaks were observed for GGH<sub>60</sub>. The first peak at temperature around  $100^\circ\text{C}$  for NGG and GGH<sub>30</sub> and around  $150^\circ\text{C}$  for GGH<sub>60</sub> corresponds to the early endothermic events associated with the dehydration of water. The second endothermic peak observed for GGH<sub>60</sub> at around  $200^\circ\text{C}$  could be due to the early disintegration of mannose and galactose units from the guar gum backbone resulting from the acidic depolymerization. The presence of low molecular fragments in the acid hydrolysate, GGH<sub>60</sub> could be responsible for the early decomposition of the guar molecules. The later peaks correspond to the exothermic transitions showing the commencement of combustion due to the random breakdown of glycosidic bonds arising from dehydration, pyrolytic decomposition and vaporization and elimination of volatile substances at higher temperatures (Cerqueira *et al.*, 2011; Mudgil *et al.*, 2012). Wider peaks were observed in case of native guar gum when compared to its hydrolysates. The presence of wider peaks in case of intact guar gum was indicative of broad range of molar mass distribution in comparison to hydrolysates. These observations were consistent with the observations on the polydispersity index made during the GPC study.



**Figure 6.** Thermal scans of native guar gum and its acid hydrolysates

Thermograms of guar gum and its hydrolysates are shown in Figure 6. The onset, peak and conclusion temperature shows the temperature range in which thermal transitions occur in the gum molecules. Onset temperature describes the initiation of thermal transitions while conclusion temperature denotes the termination of the transitions occurring in the polymer molecules. In first endothermic event, there was a shift in onset, peak and conclusion temperatures towards higher side while  $\Delta H$  decreased for acid hydrolysates in comparison to NGG suggesting that molecular structure of guar gum particles was destroyed due to acid hydrolysis (Hongbo *et al.*, 2013). However, in the second exothermic event an opposite trend was found where  $T_o$ ,  $T_p$  and  $T_c$  shifted towards lower temperature side and enthalpy increased for GGH<sub>30</sub> and GGH<sub>60</sub>. The exothermic peak temperatures decreased by 11 and 14% for GGH<sub>30</sub> and GGH<sub>60</sub>, respectively. The acid hydrolysis caused a decrease in the decomposition temperatures of hydrolysates as long guar gum chains were degraded to shorter chains which was also evidenced by molar mass determination indicating less thermal stability than native gum. Similar results have been earlier reported for partially hydrolyzed guar gums (Min *et al.*, 2013).

#### 4. Conclusions

NGG was observed to be more viscous having high molar mass in contrast to GGH<sub>30</sub> and GGH<sub>60</sub>. Acid hydrolysis substantially declined the intrinsic viscosity and molar mass in a time dependent manner. Water binding capacity and swelling power decreased but solubility increased after acid hydrolysis. FT-IR results indicated that no structural variations after depolymerization and thermal stability was reduced consequent upon acid hydrolysis of guar gum. Flow behavior results revealed a pseudoplastic behavior for NGG and GGH<sub>30</sub> whereas a near Newtonian behavior for GGH<sub>60</sub> suggesting the applicability of GGH<sub>60</sub> especially in liquid food products. Dynamic results showed an elastic behavior at higher frequencies while viscous nature at lower

frequencies depicting a viscoelastic behavior. It could be proposed from this study that acidic depolymerization provides an easy approach to prepare hydrolysates of various  $M_w$  having specific applications.

#### 5. References

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