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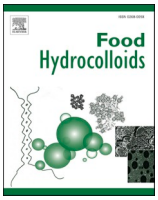

Ascorbic acid mediated hydrolysis of galactomannans

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A B S T R A C T

Ascorbic acid (AA) is an antioxidant widely used in the food industry to prevent colour fade and spoilage. This study assesses the effect of 0.02 wt% AA on the rheology of common food thickeners – galactomannans (GM). GMs immediately exhibit a significant reduction in solution viscosity upon AA addition: guar gum (−67 % ± 7 %), locust bean gum (−47 % ± 5 %), and cassia gum (−58 % ± 4 %). Other food acids at 0.02 wt% showed no decline in viscosity, nor did another reducing agent, potassium iodide. GMs were then mixed with xanthan gum (XG) ± low acyl gellan gum (LAG) and AA's impact was assessed using small amplitude oscillatory shear rheology. As the temperature decreased, the storage modulus decreased in the presence of AA compared to without. The molecular weight (*MW*) of the GMs ± AA, was assessed using size exclusion chromatography – multi angle light scattering. The reduction in *MW*, was between 6 and 8 times for each GM, and was supported with analytical ultracentrifugation. This established the hydrolytic decomposition of GMs by AA, leading to a decrease in function due to a reduction in *MW*. This hydrolytic effect was observed regardless of pH, showing that acid hydrolysis isn't the primary mechanism. This study shows, for the first time, that AA causes extensive degradation of galactomannans, affecting their viscoelastic characteristics. These findings could affect many products; informing decisions on their quality and shelf life, as well as their cost-effectiveness and environmental life cycle.

1. Introduction

Ascorbic acid (AA) is widely used across various industries including food, pharmaceuticals, agriculture and microbiology. Also known as vitamin C, it is a water-soluble, naturally occurring compound that cannot be synthesised by humans owing to the absence of L-gulono-lactone oxidase an enzyme used in its biosynthesis (Smirnoff, 2001). This makes AA essential to the healthy functioning of the human body, and without continuous ingestion poor health and eventually death will occur. The primary uses of ascorbic acid are related to its antioxidant capacity, and thus ability to scavenge reactive oxidant species (Ames et al., 1993). Since these species can cause degradation of DNA, lipids, proteins and carbohydrates, as well as other types of materials, it is important to prevent them from acting on these materials (Choe & Min, 2006). In the food industry AA is widely deployed to fortify foods with an essential vitamin, as well as a preservative to prevent colour fade, spoilage and acting as a radiation protector (Aliste & Del Mastro, 2004). AA is a preferred ingredient in the food industry owing to a relatively low cost, ready availability and 'clean label' status, with many synthetic antioxidants starting to fall out of favour due to issues with consumer perception (Mesías et al., 2021). While AA is known to act as a reducing agent, under certain circumstances such as the presence of transition metals, AA can actually act as pro-oxidant (Buettner & Jurkiewicz, 1996; Yen et al., 2002). AA has been shown to cause the breakdown of starches (Majzoobi et al., 2012; Vallès-Pàmies et al., 1997) and other polysaccharides such as xyloglucan and pectin (Zou et al., 2020).

Galactomannans are a group of plant-derived polysaccharides that consist of a mannose backbone with galactose side chains. The mannose backbone, owing to its similarity to cellulose, is insoluble in water but the galactose side chains interfere with the interchain mannose association and impart a degree of water solubility (Prajapati et al., 2013). The most commonly used galactomannans are locust bean gum (LBG), guar gum (GG), cassia gum (CG) and tara seed gum. The differences between them are based on the ratio of galactose to mannose, and a lot of their function is derived from their molecular weight and side chain interactions (Prajapati et al., 2013). Galactomannans have multiple applications in food systems including as thickening agents to replace fat in low-fat products or to thicken sauces and dressings to prevent sedimentation and separation (Singh et al., 2018). Galactomannans undergo synergistic interactions with other polysaccharides including xanthan

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gum (XG), kappa-carrageenan and agar. With XG, galactomannans see an increase in viscosity greater than either of the two gums can achieve alone. In addition to this, while neither forms a self-supporting gel alone, together they form a soft, elastic gel (Copetti et al., 1997). With agar and kappa-carrageenan, galactomannans cause an increase in gel strength, and a decrease in Young's modulus, indicating a reduction in brittleness (Dunstan et al., 2001).

In this study, the effect of AA on the structure and function of three galactomannans is examined: LBG, CG and GG. AA was added at 0.02 wt % to single galactomannan systems at 2 wt% and the subsequent changes in viscosity were measured via rotational rheometery. This was compared to other food acids at the same concentration, and potassium iodide, another small species reducing agent. Following this, systems containing a galactomannan, XG and low acyl gellan gum (LAG) were examined by small amplitude oscillatory shear rheology in order to assess whether AA affected the function of galactomannans in combination with other hydrocolloid gelling agents. XG and LAG were tested alone with AA, to demonstrate that it is specifically the galactomannan degradation that was responsible for the observed rheological changes. Back extrusion texture profile analysis was used to examine the effects of AA on the bulk structure of the gel systems consisting of CG, XG and LAG. Then to investigate the mechanism of action, the molecular weight of the galactomannans with and without AA was scrutinised by size exclusion chromatography – multiple angle laser light scattering (SEC-MALLS) and this was confirmed with analytical ultracentrifugation (AUC).

2. Materials and methods

2.1. Materials

Cassia gum and GG were purchased from Daymer ingredients (UK). AA, XG, LAG, Potassium iodide, malic acid and fumaric acid were purchased from VWR (UK). Citric acid and tri-potassium citrate were purchased from Sigma-Aldrich (UK). Deionised water was used for all sample preparation. All materials were used as received with no further modification or purification.

2.2. Preparation of hydrocolloid solutions

2 wt% solutions of LBG, CG and GG were prepared by heating deionised water to 65 °C, then shearing it on a Silverson L5M for 3 min at 6000 rpm and adding the 2 wt% gums slowly, until they dispersed and there were no clumps of material. To prepare 2 wt% galactomannan solutions with 0.02 wt% AA, a common concentration used in food applications, the same approach was implemented and then the AA was added to the galactomannan solution and mixed at 3000 rpm on the Silverson L5M for 1 min. For preparation of galactomannan solutions with 0.02 wt% of other food acids the same approach was used and then the malic, citric and tartaric acids were added to the galactomannan solutions and allowed to mix at 3000 rpm on the Silverson L5M for 1 min. For the preparation of 2 wt% cassia gum solutions with 0.02 wt% potassium iodide the same procedure was followed, but with 0.02 wt% potassium iodide added alongside the cassia gum.

0.25 wt% XG solutions were prepared by heating deionised water to 65 °C, then shearing it on a Silverson L5M for 3 min at 3000 rpm and adding the 0.25 wt% xanthan gum slowly, until it dispersed and there were no clumps of material. To test with AA, 0.02 wt% AA was added with the xanthan gum and then they were sheared together until dispersed.

0.2 wt% LAG solutions buffered to pH 3.5 were prepared to test the effect on AA on LAG. 0.2 wt% LAG and 0.16 wt% tri-potassium citrate were added to water heated to 65 °C and mixed in the Silverson L5M for 3 min at 3000 rpm. Following this, 0.42 wt% citric acid was added and mixed for another 3 min. After this it was heated to 85 °C and held for 15 min to allow the LAG to hydrate. To test the impact of AA, the same procedure was followed, with 0.02 wt% AA added at the same stage as the citric acid.

For solutions containing galactomannans and xanthan gum, 0.09 % wt galactomannan solution and 0.21 wt% XG were added to deionised water heated to 65 °C and then mixed in the Silverson L5M for 3 min at 3000 rpm. If AA was included, this was added after the 3 min mixing stage and allowed to mix for another 1 min at 3000 rpm.

For solutions containing xanthan gum, a galactomannan and low acyl gellan gum, a typical dessert gel formulation was used. This involved 0.08 wt% LAG, 0.09 wt% galactomannan, 0.21 wt% XG, and 0.16 wt% tri-potassium citrate added to deionised water heated to 65 °C and mixed for 3 min on the Silverson L5M. Then, 0.42 wt% citric acid was added, and another 3 min of mixing occurred. When 0.02 wt% AA was incorporated, this was alongside the citric acid.

2.3. Rotational and small amplitude oscillatory shear rheology

Shear rate ramps were performed using a modular compact rheometer 92 (Anton Paar, Austria) using a C-CC27/T200/XL/AL 50 mL measuring cup geometry with a diameter of 28.920 mm and a rotating vane with four blades, each 40 mm in length and 10 mm in diameter. The rheometer was set to 50 °C and the sample was loaded into the cup. Sunflower oil was placed on top of the sample to prevent evaporation during testing. Samples were left to equilibrate for 10 min. The shear rate ramp ran from 0.1 to 100 s^{-1}. This gave data for the viscosity as a function of shear rate. Samples were prepared and tested in triplicate. Amplitude sweeps were carried out at a fixed frequency of 1 Hz and the strain amplitude was varied from 1 to 100 % which has been shown to be suitable for these systems (Jo & Yoo, 2019). These were carried out at 20 °C and samples were left for 10 min to equilibrate. This gave data for the linear viscoelastic region (LVER) which was defined as the range of strain values which showed no significant change (±5 %) in the value of the storage modulus (G'). Data was also obtained for (G′) and loss modulus (G″). Samples were prepared and tested in triplicate.

2.4. Size exclusion chromatography – multiple angle laser light scattering

Weight average molar masses (*Mw*) were estimated by SEC-MALLS where the concentrations were 2 mL of 0.2 wt% for galactomannans and 0.05 wt% for ascorbic acid, within a PBS buffer at pH 6.5 with an ionic strength of 0.2. The solvent/buffer was pumped at a steady flow rate of 0.5 mL/min through a column (Shodex LB-805), which was protected by a guard column (Shodex LB-G6B), coupled on-line to MALS (Dawn Heleos-II), an 87 differential pressure viscometer (ViscoStar-II) and refractive index (Optilab rEX) detectors (Wyatt Technology, Santa Barbara, CA, USA). After being filtered through a 0.2 μm syringe filter (Whatman, Maidstone, England), the solutions were injected into the size exclusion system using the SparkHolland Marathon Basic autosampler. ASTRATM (Version 6.2) software (Wyatt Technology, Santa Barbara, CA, USA) was used to analyse the data. The apparent weight average molar mass (*Mw*, app) was calculated by using a linear fit to the Zimm model (Zimm, 1948):

$$\frac{Kc}{R(\theta)} = \frac{1}{M_w}\left[1 + \frac{16\pi^2 n_0^2 R_g^2}{3\lambda^2}\sin^2\left(\theta/2\right)\right] + 2Bc \quad [1]$$

where K is an experimental constant dependent on the wavelength of the light (λ) and the refractive increment of the polysaccharide $\left(\frac{dn}{dc}\right)$. The $R(\theta)$ is the Rayleigh ratio used to determine the ratio of the intensity of light scattered by a macromolecule at an angle θ to that of the incident radiation, B is the second virial coefficient ($m^3 \cdot mol/g^2$), R_g is the radius of gyration (m), n_0 is the refractive index of the medium, and c is the solute concentration (Dinu et al., 2019).

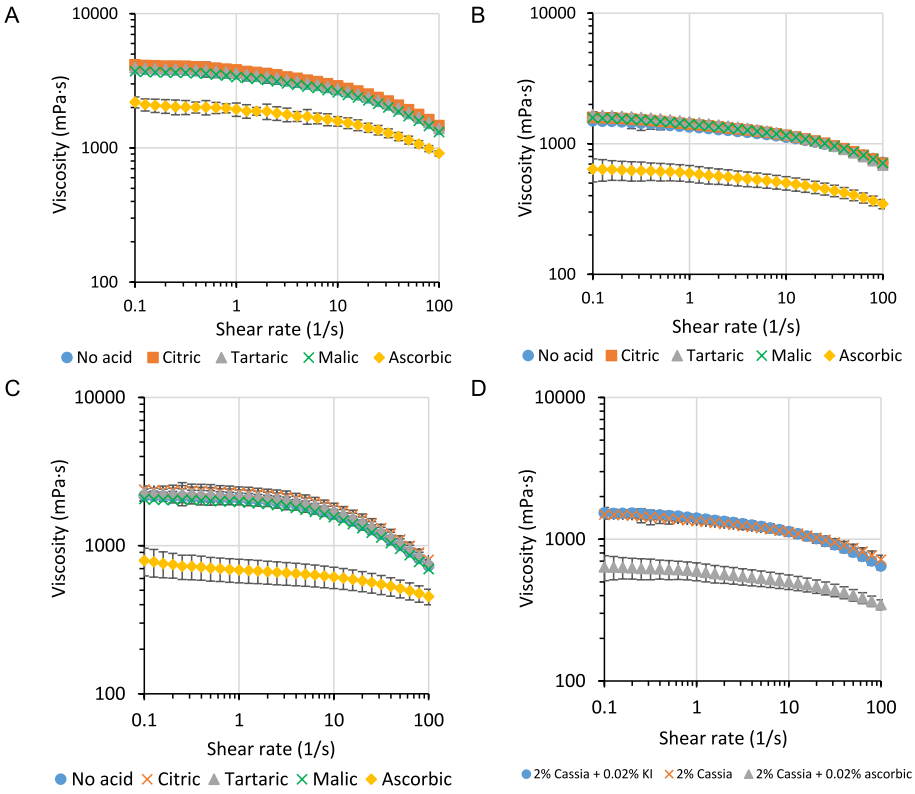

Fig. 1. Shear rate ramp data for 2 wt% A) locust bean gum, B) cassia gum and C) guar gum alone, and with 0.02 wt% of various food acids. D) Shows 2 wt% cassia gum with 0.02 wt% potassium iodide (KI) and ascorbic acid.

2.5. Analytical ultracentrifugation

Sedimentation coefficient distributions were determined using the optimal XL-I analytical ultracentrifuge (Beckman Instruments, Palo Alto, CA, USA) with Rayleigh interference optics. Reference solvent (0.2 M PBS) and samples (400 μL) at 0.1 wt% and 0.05 wt% CG with and without 0.02 wt% AA were injected into channels of 12 mm, double-sectored cells with sapphire windows. Then these cells were aligned and loaded into an eight-hole rotor and centrifuged at a rotor speed of 45,000 rpm at a temperature of 20.0 °C for a run time of ≈24 h (Channell et al., 2018; Dinu et al., 2020). The data was analysed using the SEDFIT algorithm which gives the sedimentation coefficient distribution, $g^*(s)$ versus $s_{T,b}$, where s is the sedimentation coefficient measured at temperature T in the buffer b (Dam & Schuck, 2004). The s distributions were corrected to standard solvent conditions (density and viscosity of water at 20.0 °C) to produce $s_{20,w}$ using the equation:

$$s_{20,w} = s_{T,b}\left(\frac{1-\bar{\nu}\rho_{20,w}}{1-\bar{\nu}\rho_{T,b}}\right)\left(\frac{\eta_{T,b}}{\eta_{20,w}}\right) \quad [2]$$

where $\bar{\nu}$ was 0.5 mL/g.

2.6. High performance anion exchange chromatography analysis of free sugars

Monosaccharide analysis (as galactose and mannose) in samples were quantified using a Dionex ICS 6000 (Thermo Scientific, UK) equipped with a CarboPac PA210 column (250 × 4 mm) and guard column (50 × 4 mm) using a gold electrode PAD and triple-pulsed amperometry calibrated for carbohydrate analysis. Data analysis was carried out using Chromeleon 7.2 software (Thermo Fisher Scientific, UK). Mobile phase eluent composition for monosaccharide analysis has been previously detailed (Reid et al., 2025). Eluents are degassed using sonication and stored under positive pressure nitrogen.

Samples (0.10 g) were diluted into 10 mM NaOH (10.0 mL. Solutions were vortexed to ensure complete dispersion before subsequent dilution (100 μL in 10.0 mL 10 mM NaOH). Samples were then filtered using 0.45 μm PES filters into 2 mL chromatography vials with split septum caps.

Quantitation was done using a calibration curve of standards. Galactose and mannose were

First prepared in a single solution each at 1.0 g/L in ultrapure MQ water. The solution was then diluted using 10 mM NaOH to final concentrations of 2.000, 1.000, 0.500, 0.250 and 0.125 mg/L. Concentrations of detected sugars are reported as percentage from total galactose or mannose expected (i.e. assuming a galactose:mannose ratio of 1:2 for guar gum, 1:4 for locust bean gum and 1:5 for cassia gum) (Hallagan et al., 1997).

2.7. Atomic force microscopy (AFM)

Microscopic characterization of cassia gum (with and without ascorbic acid) was conducted in air in intermittent contact mode using JPK NanoWizard V AFM (JPK, Bruker, Berlin, Germany). A 5 μL drop of the dilute, 0.01 wt%, solution in double-deionised was of the gum was spread on a freshly cleaved muscovite mica (SPI Supplies, PA) and dried under gentle nitrogen stream with ultra-filter to avoid any contamination. Prior to solution preparation, the gum and gum with ascorbic acid samples were dialysed to remove any contamination and for the sample with ascorbic acid to remove any freely dissolved ascorbic acid. This procedure enabled for gum molecules to get trapped on the mica surface. During initial stages of method development, we evaluated other deposition approaches such as passive adsorption followed by rinsing with ultra-pure water, as well as the use of $NiCl_2$ and poly-L-lysine to enhance attachment. None of these approaches were effective.

For intermittent contact imaging we were using RTESP-300 cantilevers with the rectangular pyramid probe with the nominal tip radius of

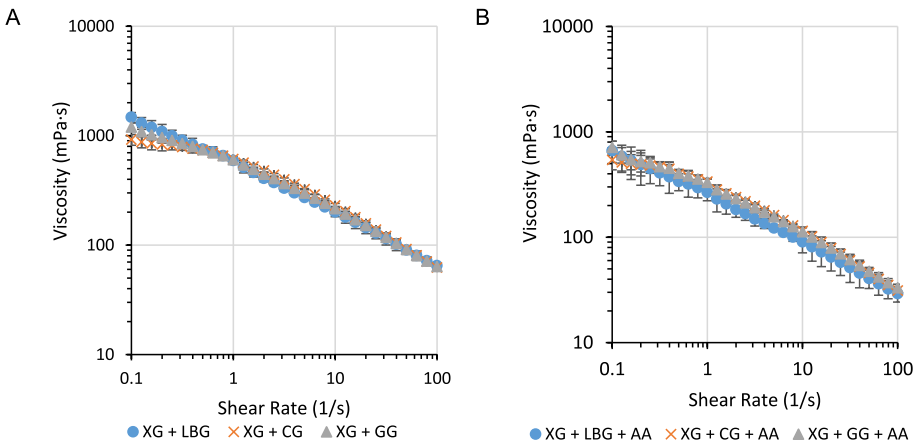

Fig. 2. Shear rate ramp data for A) 0.21 wt% XG (xanthan gum) with 0.09 wt% galactomannans (LBG (locust bean gum), CG (cassia gum) and GG (guar gum)) and B) 0.21 wt% XG with 0.09 wt% galactomannans and 0.02 wt% AA.

8 nm (nominal frequency 300 kHz, nominal spring constant 40 N/m) (Bruker AFM Probes, UK). The probes were used without further modification. The data contained within the figures are based on two batches of gum preparation and at least three technical replicates. Data analysis was conducted using JPK processing software using simple linear line fitting correction to remove the residual sample tilt.

2.8. Statistical analysis

The data contained within the figures encompasses the average of at least three measurements. The error bars represent the standard deviation of the mean. Where mean values of data are compared between samples, analysis of variance (ANOVA) tests were conducted to determine significant differences. Data analysis was conducted with the analysis toolpack in Microsoft Excel. Confidence levels were set at 95 %, ergo if $P < 0.05$, the two sets of data have different means, otherwise the two means have no significant difference.

3. Results and discussion

3.1. Viscometry of single thickener systems

To establish the effects of AA on the function of galactomannans, shear rate ramps were run on unbuffered systems to establish the viscosity values with and without 0.02 wt% AA. Data is presented in Fig. 1.

As can be observed from the above figures, AA causes a significant decrease in the viscosity of galactomannans that is not observed by other food acids at 0.02 wt%. It has been established that the molecular weight of galactomannans correlates with their functionality (Robinson et al., 1982). This relationship is known to be logarithmic, with a doubling in concentration equating to more than double viscosity for tested solutions (Wu et al., 2009; Xuewu et al., 1996). This indicates that AA has an outsized effect on galactomannans, causing rapid and extensive hydrolysis in a short period of time. The large error bar values for tests with AA represent the continued degradation of the galactomannans, while being held at 50 °C, over a relatively short period of time (30 min). While pH dependent hydrolysis is an established mechanism, the unbuffered systems all had pH values between 6 and 7. It has been established that galactomannans tend to break down under high temperature and extreme pH, neither of these conditions was present under the testing conditions (Liu et al., 2020; Wang et al., 2000). This further indicates that there is a specific mechanism of the degradation of galactomannan by AA.

To assess whether this phenomenon was caused by a reduction reaction, another small species reducing agent, potassium iodide was tested. Fig. 1D shows that there is no significant difference between 2 wt % CG and 2 wt% CG with 0.02 wt% potassium iodide. This indicated that potassium iodide did not cause any breakdown of the CG, and so a reduction effect is not suspected to be the driver of the galactomannan degradation. Indeed, previous studies have shown that other antioxidants (sodium sulphite and propyl gallate) protect galactomannans from degradation (Kök, 2010). This again, indicates that there is something specific to AA that causes breakdown of galactomannans. The other galactomannans were tested, but with the same effect observed, the data is not presented here.

Xanthan gum, another typical food thickening agent was also tested. Since XG is also susceptible to acid hydrolysis, it provided a control to see if the effects of AA are limited to galactomannans, or extend to other polysaccharides (Phillips & Williams, 2009). Shear rate ramp data for 0.25 wt% XG with and without AA is presented in Fig. S1. The concentration of 0.25 wt% XG was chosen as this had a similar starting viscosity value to the galactomannans at 2 wt%.

Fig. S1 shows that there was no significant difference observed in the presence of 0.02 wt% AA. This further signifies that the hydrolytic effect is specific between AA and galactomannans. Fig. S1 makes clear that any reduction in viscosity observed in the mixed systems will be due to breakdown of the galactomannans, rather than the XG.

3.2. Viscometry of mixed thickener systems

It is typical for galactomannans to be mixed with XG, as their synergistic interaction is far more cost effective than utilising single thickener systems (Williams et al., 1991). Therefore, it was prudent to test whether the hydrolysing effects on AA on galactomannans were seen when they were interacting with XG, thus potentially affecting their conformation and potential sites for interaction (Tako et al., 2010). The data for mixed galactomannan and XG systems is in Fig. 2.

The data from the mixed galactomannan solutions shows that even when mixed with another hydrocolloid, AA can still cause the breakdown of the galactomannans. Fig. S1 confirmed that XG is unaffected by the hydrolytic effect of AA, so the data in Fig. 2B shows that the degradation of the galactomannan affects the viscosity of the mixed systems on average by 55 % ± 5 % for LBG, 48 % ± 7 % for CG and 47 % ± 10 % for GG. This is consistent with hydrolysis leading to a decrease in molecular weight. This will in turn, interfere with the interaction between XG and the galactomannan present (Grisel et al., 2015).

3.3. Oscillatory rheology

With the viscometry highlighting the clear effect of AA on the viscoelastic properties of galactomannan solutions, both alone and with XG, it was also important to assess its effect on the viscoelastic properties

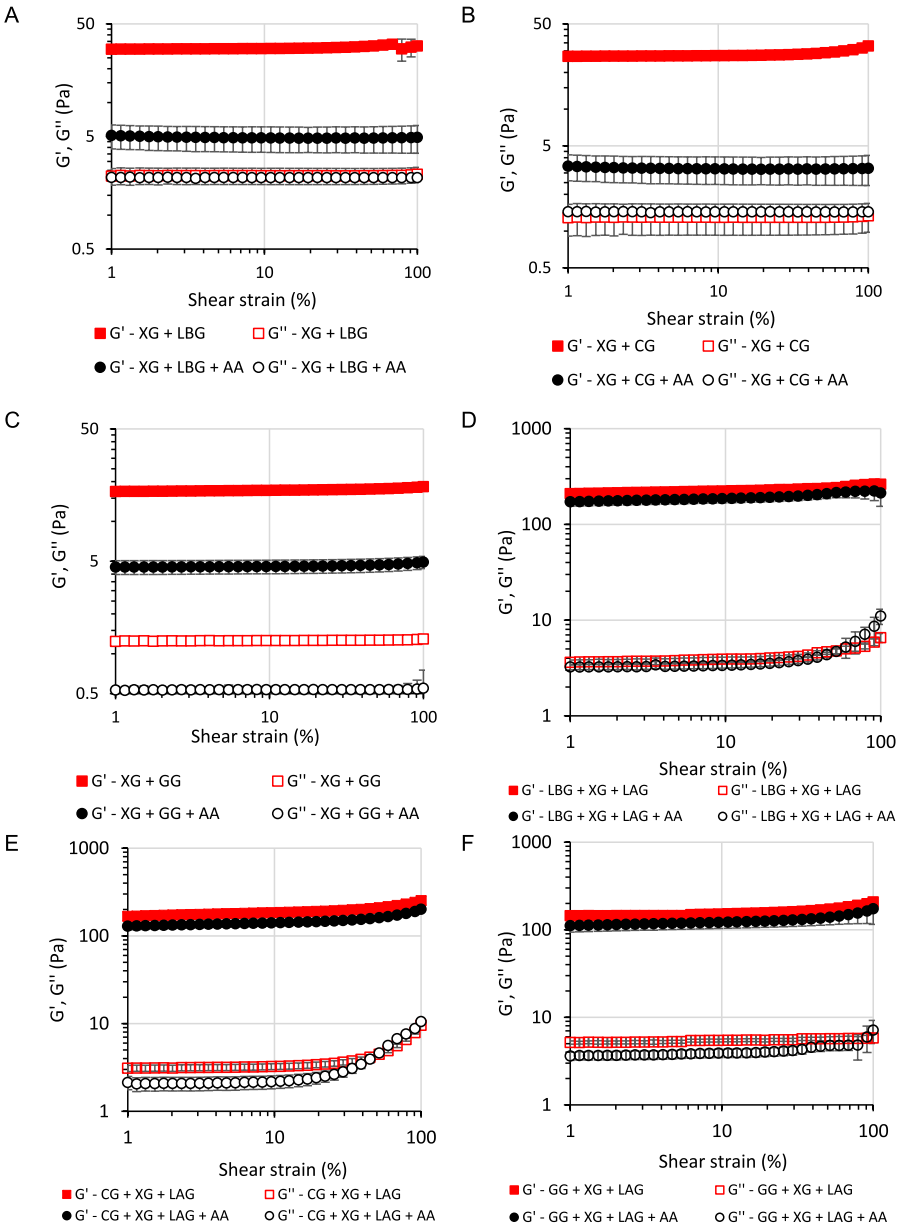

Fig. 3. Amplitude sweep data for 0.21 wt% XG (xanthan gum) with and without 0.02 wt% AA in addition to 0.09 wt% A) LBG, B) CG and C) GG *and* gelling systems based on typical dessert gels, containing LAG (low acyl gellan), citric acid, tri-potassium citrate and *D*) LBG (locust bean gum), *E*) CG (cassia gum) and *F*) GG (guar gum) with and without 0.02 wt% AA (ascorbic acid).

of gelled systems comprising galactomannans. This was carried out by mixing them with XG and then lowering the temperature to 20 °C allowing them to gel within the rheometer. Following this, a full dessert jelly formulation comprising a galactomannan, XG and LAG, buffered to pH 3.5 with citric acid and tripotassium citrate was produced (Damasio et al., 1997). Then amplitude sweeps were carried out, and the data for this is presented in Fig. 3.

Amplitude sweeps showed a very large LVER, with shear strain values of 51 % for LBG, 33.9 % for CG and 51 % for GG. This is typical for low modulus gels (Ross-Murphy & Shatwell, 1993). GG's lower G′ compared to LBG and CG is due to the GG's lower mannose:galactose ratio of roughly 2:1 compared to 4:1 for LBG and 5:1 for CG (Iijima et al., 2013; Phillips & Williams, 2009). This data also supports the viscometry data, with AA causing a marked decrease in the functional properties of galactomannans. LBG saw a decrease in G′ from 29 to 5, CG from 27 to 3.4 and GG from 16 to 4.5. Since the molecular conformation of galactomannans change upon formation of a gel with XG, this shows that even following these changes AA can still target galactomannans and impeded their functionality (Cheetham & Mashimba, 1988). This highlights that the use of AA will impact the galactomannans even when they are in mixed systems.

Fig. 3F, for the GG showed that without the AA, GG systems had the largest LVER of 6.25 % compared to systems with LBG (4.09 %) and CG (3.5 %), indicating that they were less stiff, less brittle gels that were more resistant to shear strain. This is probably due to GG's galactose: mannose ratio of 2:1 giving weaker gels, which was confirmed by the lower G′ value. LBG three-phase gel systems had the highest G′, possibly due to a higher purity for the LBG gels, as indicated by their clarity compared to observed turbidity in CG and GG gels. The presence of AA had a smaller difference in the three-phase systems as a total value, because it was mostly derived from LAG which was unaffected as seen in Fig. S2. However, in Fig. 3, there is consistent decrease in G′ in the presence of 0.02 wt% AA compared to without. This value is similar to that of the galactomannan:XG gel systems, indicating that the

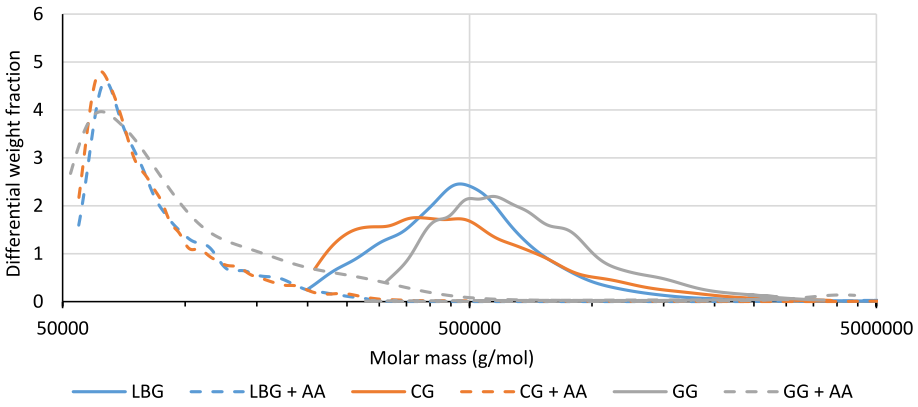

Fig. 4. Data for SEC-MALLS of analysis of 0.2 wt% galactomannans with and without 0.05 wt% AA (ascorbic acid).

breakdown of the galactomannans, impeding the gelation process could also be seen in the three-phase gel system.

Another possibility was that AA was also capable of degrading the LAG. Therefore, LAG gel systems were produced with and without AA and amplitude sweeps were carried out on them as well. This is shown in Fig. S2 which shows that AA has no discernible impact on the viscoelastic properties of 0.2 wt% LAG gels. (The concentration of 0.2 wt% was chosen to match the G′ of the formulated 'model dessert' tertiary system CG:XG:LAG, 0.09:0.21:0.08 wt%) There was no significant difference observed between LAG gels and LAG gels with AA present, in terms of G′ and G''. This, in combination with the data from Fig. 1, indicates that any loss of viscosity or G′ in the mixed systems is primarily, if not entirely caused by the degeneration of the galactomannan within the tertiary system. The data from the oscillatory rheology correlates with the viscometry. The AA appears to degrade galactomannans, while having little to no impact on XG and LAG, despite these also being polysaccharides. It is also a possibility that the AA is binding to the galactomannans, or another change that impacts their functionality.

3.4. Analysis of galactomannan molecular weight

The rheological data strongly indicates that AA specifically affects galactomannans and negatively impacts their functional properties. To further understand the mechanism causing these effects, the molecular weight of these galactomannans with and without AA was assessed using SEC-MALLS. The data for this is presented in Fig. 4.

The data in Fig. 4 shows that the molar mass of the galactomannans without AA are all in a similar range, with values for each being detected between 200,000 and 1,200,000 g/mol but with the average value of around 400,000 g/mol. This value is slightly higher than galactomannan values reported in literature, which are typically around 300,000 g/mol (Carvalho et al., 2021). One reason for this observation could be that the samples used were commercial samples and therefore were not highly refined. This means that there were other species present including proteins, fats and other carbohydrates such as cellulose and lignin. In addition to this galactomannans, as natural ingredients are typically present as a range of molecular weights (BeMiller, 2017). Finally, it has been observed that analysis by SEC-MALLS can overestimate molecular weight values owing to molecular association leading to molecular

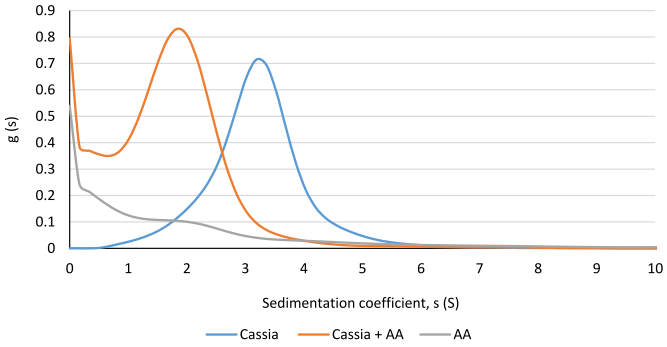

Fig. 5. Data from analytical ultracentrifugation of 0.1 wt% CG (cassia gum) with and without 0.05 wt% AA (ascorbic acid). The data are should up to 10 Svedberg units to demonstrate the absence of larger aggregates.

complexes being detected as single molecules (Dinu et al., 2019; Shakun et al., 2013).

Once AA is introduced into the samples, there is a clear decrease in average molecular weight, from around 400,000 g/mol to approximately 60,000 g/mol. While the different galactomannans displayed different peaks in the SEC-MALLS data, following treatment with AA, they appeared a lot more uniform. With the wider peak for GG + AA, indicative of a larger average molecular weight, which tallies with the peak observed for GG alone, which trends to a higher average molecular weight than LBG and CG. Despite this, according to Fig. 1, LBG had the highest viscosity values. This suggests other factors could have had an impact on solution viscosity such as structural differences between the galactomannans and the presence of non-galactomannan molecules that don't impact the viscoelastic properties of the solution. The data from the SEC-MALLS shows the clear impact of AA on galactomannans, with a marked decrease in detected molecular weight. This correlates with the rheological data and allows conclusions to be drawn about the nature of AA's interaction on galactomannans. It appears that AA causes hydrolysis of galactomannans, even when present at very low concentrations. Since this occurred in Fig. 1, even with pH readings in Table 1, this is mechanism is believed to be specific to AA negatively affecting the galactomannans' functionality. Even though 0.02 wt% AA was added,

Table 1
Data for pH values recorded for 0.02 wt% of several food acids and 2 wt% LBG, CG and GG.
pH values were recorded for each food acid and can be seen in Table 1.

	Locust bean gum					Cassia gum					Guar gum				
Acid	None	Citric	Tartaric	Ascorbic	Malic	None	Citric	Tartaric	Ascorbic	Malic	None	Citric	Tartaric	Ascorbic	Malic
pH	6.9 ± 0.1	6.5 ± 0.2	6.1 ± 0.1	6.7 ± 0.1	6.6 ± 0.1	6.9 ± 0.0	6.6 ± 0.1	6.2 ± 0.1	6.8 ± 0.1	6.6 ± 0.1	6.8 ± 0.1	6.5 ± 0.1	6.3 ± 0.2	6.7 ± 0.1	6.4 ± 0.1

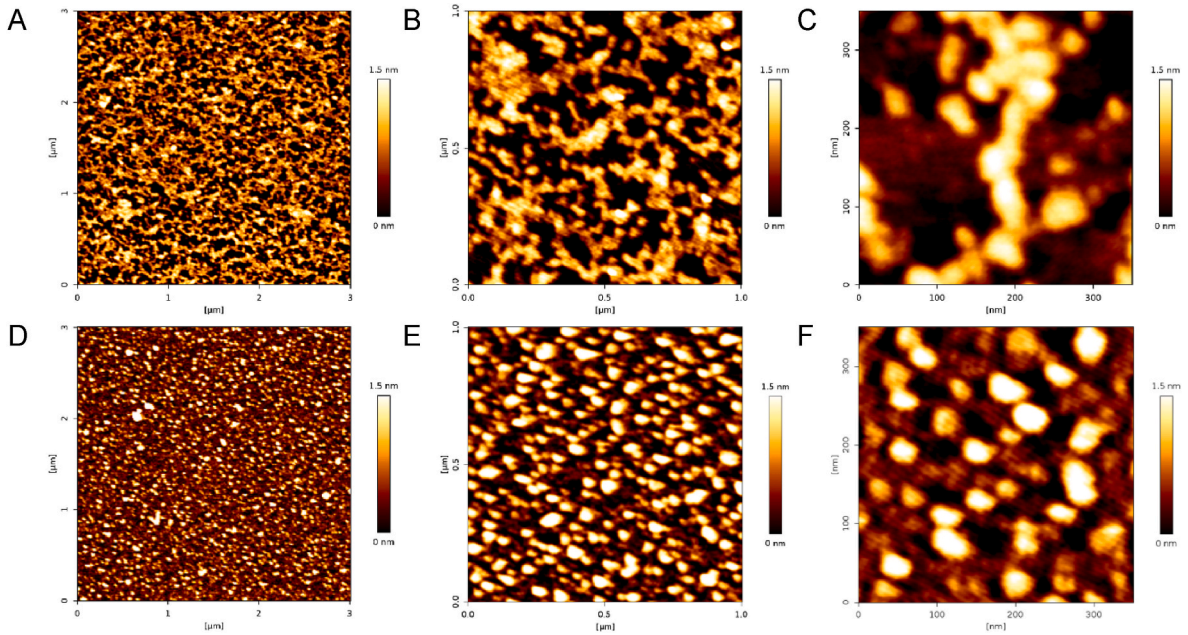

Fig. 6. AFM (atomic force microscopy) intermittent contact mode images of CG (cassia gum) (A, B, C) and CG with AA (ascorbic acid) (D, E, F) shown at three scan areas of 3 µm, 1 µm, and 350 nm. For the CG with AA, distinct globular features are observed with the sizes between 14 and 24 nm (which correspond to the radii of 7 and 12 nm, respectively).

AA is known to degrade during heat-based processes, meaning that even at the low concentrations added, it is possible even less remains intact to carry out the hydrolysing effect on the galactomannans (Abbas et al., 2012).

To reinforce the findings from the SEC-MALLS analysis, AUC and AFM analysis was carried on CG with and without AA. The results of the AUC analysis are presented in Fig. 5, which shows a distinct single peak for pure CG with a maximum at 3.2 S. The sedimentation curve for CG in the presence of AA is somewhat more complex. The position of the main peak shifts to a markedly lower value of s, 1.8 S, which is in line with the SEC-MALLS data and hypothesised reduction in the molecular weight. The shoulder part of the sedimentation curve for $s < 0.8$ S is likely to be associated with the influence of AA present in the solution. The sedimentation curve for pure AA also exhibits a similar shoulder with a small feature around 0.3 S, which translates into a *Mw* in the range of 165–185 g/mol which is consistent with the molecular weight of AA 176 g/mol. The absence of this shoulder in the pure CG solution also appears to be consistent with this observation.

The effect of AA on CG can also be observed in AFM images. Due to a neutral charge of CG, it does not readily adsorb on the mica surface. Therefore, AFM images were taken by arresting CG molecules on the surface of mica by drying a 5 µL drop of a dilute, 0.01 wt% CG solution (with and without AA) to effectively trap CG molecules on the surface. The resulting images are shown in Fig. 6. For CG without AA, we see a marked overlap between molecules, whilst for CG with AA, we see individual globular features that appear to show little overlap. The radius of the globular features was found to be in the range from 7 to 12 nm, which is consistent with a crude estimate for the end-to-end radius $R \sim a\sqrt{DP} \approx 10.6$ nm (Flory & Volkenstein, 1969) of CG chain treated with AA, where a is the unit length of a hexose residue (~0.55 nm), and DP is the degree of polymerisation that is estimated to be ~370, based on the molecular weight from SEC-MALLS of 60,000 g/mol and the molecular weight of monosaccharide hexose residue of 162 g/mol (i.e. the *Mw* of a hexose (180 g/mol) minus *Mw* of water molecule (18 g/mol) that is lost in the process of formation of the glycosidic bond).

Although the hypothesis that favours hydrolytic cleavage of galactomannans by AA find strong experimental support from SEC-MALLS, AUC, and to an extent AFM data. Below we discuss other possible scenarios that can explain our observations. Several reports, such as by Hjerde et al. (1994) and Fry (1998) attribute the AA activity to the generation of free radicals in the presence of free metal ions that causes oxidative breakdown of polymeric chains (Buettner and Jurkiewicz, 1996; Majzoobi et al., 2012). These effects have been observed in such polysaccharides as pectin, κC, starch, and carboxymethylcellulose (Fry, 1998; Hjerde et al., 1994). However, in these studies the free metal ions have been purposefully incorporated free into solution and strong oxidative agents such as hydrogen peroxide, or ascorbate at far higher concentrations than tested in this study have been used. However, this mechanism should affect any polysaccharides, which contradicts our own observations that LAG and XG are seemingly unaffected by AA, which appears to have a specific effect on galactomannans.

Another potential mechanism, based on the AUC and, in particular, AFM data could be that the AA causes a severe contraction in the galactomannan polymer chains through for example intra-chain cross-linking. Such cross-linking would reduce the size of the coil, making it smaller in comparison with a random-like coil assumed for galactomannans. In this case the oxidative effect of AA results in the permanent conformational change, that shows as the reduction of sedimentation coefficient, *s*, and the apparent changes in molecular morphology observed in AFM (Dinu et al., 2019). This scenario is supported by the fact that the peak shape in the SEC-MALLS becomes less broad, indicating that the polydispersity decreases because of AA treatment. If random cleavage of glycosidic bonds is assumed, then the hydrolysis products would have much broader distribution of molecular weights characterised by the presence of lower molecular weight species. AFM observations shown in Fig. 6C indicate that CG appears to have a necklace-like structure with globular domains interlinked by fibrous elements. If the structural characteristic of galactomannans are highly 'blocky', with two or more structural elements being present in the molecule, it is possible to suggest that the cleavage of the fibrous elements would result in liberation of globular domains, which appear to have narrow distribution of molecular weights.

3.5. High performance anion exchange chromatography

While the AUC and SEC-MALS data indicate that a considerable

Table 2
Data for HPAEC (High performance anion exchange chromatography) detection of free sugars for the galactomannans with and without the presence of AA (ascorbic acid) (ND: not detected).

Locust bean gum				Cassia gum				Guar gum			
Alone		With AA		Alone		With AA		Alone		With AA	
Free galactose (%)	Free mannose (%)	Free galactose (%)	Free mannose (%)	Free galactose (%)	Free mannose (%)	Free galactose (%)	Free mannose (%)	Free galactose (%)	Free mannose (%)	Free galactose (%)	Free mannose (%)
3.3	ND	5.2	ND	ND	ND	ND	ND	1.2	ND	3.7	ND

decrease in molecular weight occurs, they do not indicate the nature of the breakdown of the galactomannan structure. Therefore, each galactomannan was tested alone and with ascorbic acid to assess whether considerable quantities of galactose was liberated, indicating that the hydrolysis was preferential to the side chains or whether mannose was released indicating preferential breakdown of the mannose backbone. The amount of galactose and mannose released per galactomannan is shown below, with the concentrations given as a percentage of the total sugars expected to be present for the mass of galactomannan tested.

The HPAEC data from Table 2 shows that a small amount of galactose is released from the galactomannans even in the presence of ascorbic acid. The presence of galactose in LBG and GG samples without AA are due to the manufacturer standardising the gums with galactose in order to ensure a constant viscosity, whereas the manufacturer for the CG does not do this. LBG and GG showed a small amount of galactose released after treatment with AA, whereas CG showed none. All three galactomannans had no detectable free mannose regardless of the presence of AA. This suggests a hydrolysis mechanism that is not specific to either the galactose side chains or the mannan backbone. This is supported by the oscillatory and rotational rheometry data in Sections 3.1-3.3 as well as the SEC-MALLS data in Section 3.4. With the decrease in the order of magnitude of the molecular weight indicating that the mannan backbone has been cleaved at single points. This would also explain the absence of any detected free mannose as this would require two consecutive mannose-mannose linkages to be cleaved and in a random reaction this is a very low probability event, so even if small amounts of free mannose were released, this would likely be below the limit of detection for the HPAEC instrument. Whereas for the release of free galactose, only one linkage would have to be broken at a time to liberate galactose from the mannan backbone, hence the increases seen in both LBG and GG.

4. Conclusions

This study uncovers a previously unreported interaction between galactomannans and AA. Rheological data shows that small quantities of AA have severe disruptions on the function of galactomannans, alone and in multi-phase gel systems, with decreases in solution viscosity and gel storage modulus. These disruptions were found to not be due to any disruption of XG or LAG, with their functionality not found to be affected by the presence of AA. Analysis of the molecular weight showed that introduction of AA to the galactomannans caused a decrease in detected molecular weight of more than 70 %, and this would have had a vastly detrimental effect on their functionality. This was shown to occur without any release of free mannose and a small quantity of free galactose, indicating that it is a non-specific hydrolysis mechanism rather than targeted to the mannan backbone or galactose sidechains. This was observed by AFM imagery as well, with the captured images of the cassia gum chains being shown to align with the theoretical dimensions calculated based on molecular weight observed from SEC-MALLS. Considering the widespread use of both in the food industry, these findings are of particular interest, as they have the potential to impact a wide range of products, influencing their quality and storage stability, as well as having broader repercussion on the cost-effectiveness and environmental life cycle of food ingredients. Future studies could focus more on the exact delineation of the mechanism of the breakdown of the galactomannans by AA.

CRediT authorship contribution statement

Michael-Alex Kamlow: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Julian Marks:** Writing – review & editing, Supervision, Methodology, Funding acquisition. **Joshua E.S.J. Reid:** Writing – review & editing, Investigation, Formal analysis. **Vlad Dinu:** Writing – review & editing, Investigation, Formal analysis. **Ian D. Fisk:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Gleb E. Yakubov:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of generative AI and AI-assisted technologies in the writing process

Authors have utilised Grammarly AI (GPT 3.5) during the writing of this article to amend grammatical errors and ensure sentence coherence.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodhyd.2025.111996.

Data availability

Data will be made available on request.

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