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Structure conformation, physicochemical and rheological properties of flaxseed gums extracted under alkaline and acidic conditions

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ABSTRACT

The present work aimed at investigating an extraction protocol based on consecutive steps of isoelectric point (pH ~ 4.25) mediated gum swelling and deproteinisation as an alternative method to produce flaxseed gum extracts of enhanced techno-functional characteristics. The osidic and proximate composition, structure conformation, flow behaviour, dynamic rheological and thermal properties of gums isolated from brown and golden flaxseeds were assessed. Gum extraction under near-to-isoelectric point conditions did not impair the extraction yield, residual protein and ash content, whilst it resulted in minor changes in the sugar composition of the flaxseed gum extracts. The deconvolution of the GPC/SEC chromatographs revealed the presence of four major polysaccharidic populations corresponding to arabinoxylans, rhamnogalacturonan–I and two AX-RG-I composite fractions. The latter appeared to minimise the intra- and interchain polymer non-covalent interactions (hydrogen bonding) leading to a better solvation affinity in water and lyotropic solvents. Golden flaxseed gums exerted higher molecular weight (M_w = 1.34–1.15 × 10^6 Da) and intrinsic viscosities (6.63–5.13 dL g^{-1}) as well as better thickening and viscoelastic performance than the brown flaxseed gum exemplars. Golden flaxseed gums exhibited a better thermal stability compared to the brown flaxseed counterparts and therefore, they are suitable for product applications involving severe heat treatments.

1. Introduction

Flax (*Linum usitatissimum L.*) is one of the most important industrial crops globally, cultivated for its fibres (used extensively in the textile and bio-composite industry) and seeds [1]. Owing to its macronutrient-rich profile, i.e. essential lipids, proteins, bioactive peptides (orbitides), and soluble and insoluble dietary fibre, flaxseed has shown promising potential for food and nutraceutical industry applications [2]. The dietary fibre content of flaxseed accounts for 22–28% of its overall weight, with the outermost (epidermal) seed layer containing about 8% wt of mucilaginous gum [3]. Flaxseed mucilage and its derivatives (deproteinised or fractionated) have been extensively investigated over the last decade, mainly due to their food industry relevant inherent techno-functional (thickening, gelling, interface-stabilising and film-forming) properties [4]. In addition, flaxseed hull polysaccharides and their hydrolysates (FGOS) have antioxidant [5], immune-stimulatory [6], body fat mass and weight controlling aspects [7], as well as other significant health benefits, including the suppression of acute postprandial glycaemic response and glucose diffusion [8–10] and the regulation of gut microbiota [11,12]. In the latter case, flaxseed gum exhibited a modulatory role in gut microbial ecosystem diversity, as dictated by the alteration of the *Firmicutes* to *Bacteroidetes* ratio and the increase in the relative abundancies of *Prevotella*, *Phascolarctobacterium, Clostridrium, Megamonas, Lactobacilli* and *Bifidobacteria* [11,12].

The structural elucidation of crude (non-fractionated) flaxseed mucilage demonstrated the *co*-existence of two major polysaccharide

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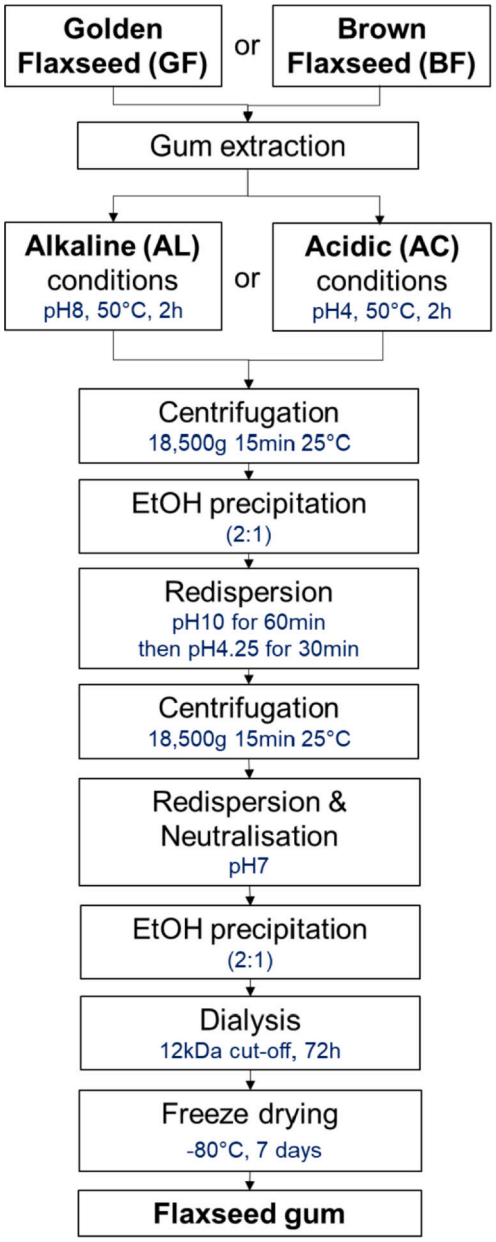

Fig. 1. Extraction flowchart illustrating the implemented procedure to extract, isolate and purify the flaxseed gums .

fractions: an acidic arabinan rich rhamnogalacturonan-I (RG-I) fraction and an arabinoxylan with a β-D-(1 → 4)-xylan backbone neutral fraction [13,14]. The chemical structure and molecular properties of flaxseed hull polysaccharides are inextricably associated with the flaxseed genotype and origin. Cui et al. [15] found that yellow flaxseed cultivars exhibited a lower content in rhamnose and galacturonic acid and a higher arabinoxylan content than brown flaxseed, which was associated with a more pronounced shear-thinning and weak gel-forming capacity of the extracted gums. In two successive studies [16,17], the techno-functionality (thickening and swelling power, emulsifying and foaming capacity) of the gum extracts, provided by the flaxseed cultivar and origin endowed was clearly dictated. Interestingly, the umami, bitter and sweet sensory modalities, strongly driven by cultivar type, were identified as the primary determinants of flaxseed gum acceptability [16].

The practices implemented for extracting, isolating, purifying and fractionating the polysaccharide matter from the flaxseed hulls are essential drivers of the chemical structure molecular characteristics and techno-functionality of flaxseed gum [3,18]. Water extraction-associated parameters, such as the temperature, pH, ionic strength of the aqueous medium, and seed-to-water ratio are known as the major factors affecting the extraction yield and the chemical composition of the final gum [4]. As a common rule, flaxseed mucilage is extracted under neutral or mildly alkaline conditions (pH = 6–8) at a temperature of 20 to 90 °C for 1 to 24 h, maintaining a flaxseed-to-water ratio of 1:5–1:40 [19]. Increasing the extraction temperature results in higher extraction yields (up to 16–20% of the defatted flaxseed weight) but compromises the purity i.e., protein and ash residual content of the final gum [20,21]. On the other hand, the impact of the seed-to-water ratio on the gum extraction yield appears to be less important than temperature, whereas it becomes negligible when it exceeds the ratio of 1:20 to 1:25 [18]. The protein residual matter of flaxseed gum can be substantially reduced by acidic precipitation (e.g., acetic, trichloroacetic, etc.), salts (e.g., calcium chloride, sodium chloride etc.), proteolytic enzymes, ultrafiltration, or ion exchange chromatography [19,22–24]. Moreover, enzymatic – ultrasound-assisted extraction can reduce not only the amount of residual protein but may also enhance the techno-functional properties of the gum extracts [25].

In a recent study [26], a procedure based on isoelectric point (4–4.5) gum swelling and extraction followed by an alkaline protein solubilisation (at pH = 10) and isoelectric precipitation allowed the preparation of highly pure (≈ 1% wt protein residual) galactomannan extracts. The present work explores the aforementioned extraction protocol in conjunction with the flaxseed phenotype (brown vs. golden), based on the compositional, structure conformational, physicochemical and rheological properties of the gum extracts obtained.

2. Materials and methods

2.1. Extraction and isolation of flaxseed polysaccharides

Brown (Delhaize, Belgium) and golden (Priméal, France) flaxseeds were purchased from the local branches of the abovementioned supermarkets. The non-adherent mucilage was extracted from the flaxseed coat layer, as previously described in Soukoulis et al. [27] with minor modifications (Fig. 1). In particular, flaxseeds were soaked in deionised water (18.2 mΩ, Millipore Inc., US) at 50 °C for 2 h under gentle magnetic stirring (IKA GmbH, Staufen, Germany). The pH of the seed suspension was adjusted at either pH = 8 or pH = 4 with 1 M NaOH or 1 M HCl, respectively. To facilitate the hydration of the seed coat layer, the flaxseed-to-water ratio was adjusted to 1:10. The flaxseed suspension was vacuum filtered using a nylon mesh (100 μm, VWR, Leuven, Belgium) and the crude mucilage solution obtained was centrifuged at 18500*g* for 15 min (Multifuge X3R, Fiberlite F14–6, ThermoFisher, Belgium) to remove the insoluble impurities. Then, the supernatant was mixed 1:2 with absolute ethanol (VWR, Leuven, Belgium) and kept under mechanical stirring for 1 h to allow sufficient aggregation of the polysaccharide fraction. The ethanolic suspension was centrifuged at 18500*g* for 10 min, and the polysaccharide pellets collected were reconstituted into MilliQ water adjusted to pH = 10 with 1 M NaOH solution. Following the complete dissolution of the gum pellet, the pH of the gum solution was adjusted at the isoelectric point of the flaxseed proteins (pI ~4.25), kept under stirring for 1 h and finally centrifuged at 18500*g* for 30 min. The supernatants were neutralised with 1 M NaOH and dialysed against MilliQ water for 72 h until no remarkable changes in the conductivity of the intermittently renewed (every 12 h) dialysate were recorded. Following lyophilisation at −80 °C for 96 h (Christ, Alpha 1-2LD Plus, Germany), flaxseed gums were finely powdered using a knife mill (IKA, Staufen, Germany) and stored under controlled temperature and relative humidity conditions (a_w = 0.11, 25 °C) until further use.

2.2. Proximate, sugar monomers and uronic acid composition analyses

The moisture and ash content of the flaxseed gum extracts were gravimetrically determined according to the standard AOAC methods. The protein content was determined according to the Dumas method (protein converting factor = 5.41 [28]) using a CHNS analyser (Elementar Vario Cube, Langensenbold, Germany). The total carbohydrate content was measured using an enzymatic assay kit (Megazyme, K-ACHDF 08/16), whilst the total lipid content was measured via gravimetric determination of the n-hexane extracted (3-fold) liposoluble residue.

The sugar monomer composition of the flaxseed gum extracts was determined as detailed in Hellebois et al. [26].

2.3. Gel permeation size-exclusion chromatography (GPC/SEC)

A 1200 Infinity gel permeation chromatography (GPC, Agilent Technologies) was used to determine the number-average (M_n), weight-average (M_w) and *Z*-average (M_z) molecular weight and dispersity (Đ) of the flaxseed gum extracts. The chromatograph was equipped with an integrated IR detector, two columns (PL aquagel-OH MIXED-H and PL aquagel-OH MIXED-M) and a PL aquagel-OH guard column (Agilent Technologies). 0.1 M $NaNO_3$ aqueous solution containing 0.02% wt of NaN_3 was used as an eluent, the flow rate was maintained at 1.0 mL min^{-1} and the measurements were performed at 50 °C. Pullulan standards (ReadyCal-Kit Pullulan high, PSS Polymer Standards Service GmbH, M_p = 180–1530 × 10^3 Da) were used to perform calibration. All the samples were filtered through a 0.2 μm Teflon filter before injection.

Peak deconvolution of the GPC chromatograms was conducted with Origin software (OriginPro v.2019b, OriginLab, USA) using the quadratic Savitzky-Golay method.

2.4. Intrinsic viscosity measurements

The intrinsic viscosity [η] of flaxseed gum solutions (0.02–0.1% wt) in 0.1 M $NaNO_3$ aqueous solution containing 0.02% wt NaN_3 was measured using a 0C Ubbelohde capillary viscometer (Paragon Scientific, United Kingdom) at 25 ± 0.1 °C. The intrinsic viscosity was determined as the intercepts of the Huggins (Eq. (1)) and Kraemer (Eq. (2)) equations after extrapolating them to an infinite dilute system:

$$\frac{\eta_{sp}}{c} = [\eta] + k_H[\eta]^2 c \quad (1)$$

$$\frac{ln\eta_{rel}}{c} = [\eta] + k_K[\eta]^2 c \quad (2)$$

where: η_{sp} and η_{rel} denote the specific and relative viscosity, respectively, c is the flaxseed gum concentration, and k_H and k_K are the Huggins and Kraemer coefficients.

2.5. Dynamic light scattering and zeta-potential determination

The hydrodynamic diameter and zeta potential of flaxseed gums was recorded using dynamic light scattering (Zetasizer Nano, Malvern Instruments, Worcestershire, UK). To determine the hydrodynamic diameter of the gum particles, 10 mg of gum was dissolved in 10 mL of 0.1 M $NaNO_3$ aqueous solution and filtered through a cellulose acetate filter (cut-off 0.2 μm) prior analysis.

The zeta-potential of the gums was determined by dissolving flaxseed gum in MilliQ water to obtain a final concentration of 0.25% wt. The pH of the solutions obtained was adjusted from 7 to 2 using 0.1 M HCl and NaOH solutions and filtered through a 0.2 μm cellulose acetate filter (VWR, Leuven, Belgium).

2.6. Steady-state and dynamic rheological measurements

Aqueous solutions (0.1, 0.2, 0.25, 0.375, 0.5, 0.625, 0.75, 0.875, 1, 1.25, 1.5, 2, 2.5, 3, 4 and 5% wt) of flaxseed gum in either MilliQ water (adjusted at pH = 7 using 0.1 M NaOH) or $NaNO_3$ 0.1 M were prepared for carrying out the rheological measurements. All rheological analyses were performed in an Anton-Paar oscillatory rheometer (MCR 302, Graz, Austria) equipped with either a concentric cylinder (steady-state rheological measurements – 0.1 to 2.5% wt) or cone plate geometry (dynamic rheological measurements – 1 to 5% wt).

2.6.1. Flow behaviour

Aliquots of the flaxseed gum solutions (ca. 15 mL) were transferred to the measuring geometry and tempered at ambient temperature (25 ± 0.05 °C) for 10 min before the analyses. Due to the time-dependent flow behaviour of the solutions, the gum solutions were pre-sheared at 200 s^{-1} for 5 min and left to settle for 5 min before measurement. Upward shear rate sweeps in the range of 0.01 to 1000 s^{-1} were performed. The shear stress τ (Pa) – shear rate $\dot{\gamma}$ (s^{-1}) data obtained were fitted into the Ostwald-de Waele (power) model (Eq. (3)) as follows:

$$\tau = K\,\dot{\gamma}^n \quad (3)$$

where K (Pa s^{-n}) and n (dimensionless) denote the consistency coefficient and rheological behaviour index, respectively.

2.6.2. Zero shear viscosity measurements

To define the concentration where the biopolymer chain entanglement takes place, the limiting viscosity of flaxseed gum aqueous solutions at zero shear rate (η_0) in the concentration range of 0.1 to 2.5% wt was measured. For this purpose, the Williamson-Cross model (Eq. (4)), was fitted to the viscosity – shear rate (0.01 to 1000 s^{-1}) data obtained:

$$\eta = \eta_\infty + \frac{\eta_0 - \eta_\infty}{1 + C\cdot\dot{\gamma}^m} \quad (4)$$

where η_0 and η_∞ represent the zero and infinite shear viscosity, and C, m are the Cross time and rate constants, respectively.

2.6.3. Amplitude and frequency sweep measurements

For the dynamic rheological measurements, the flaxseed gum extracts were dispersed in MilliQ in the concentration range of 1 to 5% wt. Amplitude sweeps were conducted to determine the linear viscoelastic (LVE) region under controlled shear stress conditions (1 Hz and 25 °C). From the amplitude sweep rheological spectra obtained, the viscoelastic moduli (G'_{LVE} and G''_{LVE}) in the LVE region, the yield stress at the limit of LVE region (τ_y) and the stress and elastic modulus (G′) at the flow-point (G′ = G″) were calculated using the RheoCompass analysis software (Anton-Paar, Graz, Austria).

Frequency sweeps (0.1 to 100 Hz) within the LVE region (strain = 0.5%) were performed at 25 °C to evaluate the viscoelastic profile of the flaxseed gum solution profile. The slope of the double logarithmic storage modulus (G′) –frequency (f) curves and the complex viscosity η* at f = 1 Hz was calculated using the Rheocompass software (Anton Paar, Graz, Austria). Furthermore, the frequency (f) at which the crossover of the viscoelastic moduli (G′ = G″) takes place was calculated using Solver (Excel, Microsoft Inc).

2.7. Thermal analyses

Thermal gravimetric analysis (TGA) was carried out in air on a TGA2 STARe System (Mettler Toledo, Zurich, Switzerland), applying a heating rate of 5 °C min^{-1}. The onset weight loss temperature (T_{onset}) was determined as the point in the TGA curve at which a significant deviation from the horizontal was observed.

Differential scanning calorimetry (DSC) analysis was performed on a DSC3+ STARe System (Mettler Toledo, Zurich, Switzerland).

Table 1
Proximate and sugar monomer composition of gums extracted from brown and golden flaxseed under acidic and alkaline conditions.

	Golden alkaline	Golden acid	Brown alkaline	Brown acid
Proximate composition[1] (g 100 g^{-1} of dry matter)				
Total carbohydrates	87.1 ± 2.5^{a}	87.7 ± 1.9^{a}	84.0 ± 1.1^{a}	84.4 ± 0.8^{a}
Protein	7.2 ± 2.3^{a}	6.5 ± 1.1^{a}	8.6 ± 0.2^{a}	9.2 ± 0.1^{a}
Ash	5.7 ± 0.2^{a}	5.8 ± 0.8^{a}	7.4 ± 0.6^{b}	6.4 ± 1.0^{a}
Sugar monomer composition (g 100 g^{-1} of total carbohydrate matter)				
Fucose	4.2 ± 0.2^{bc}	4.4 ± 0.4^{c}	3.2 ± 0.4^{ab}	3.0 ± 0.5^{a}
Rhamnose	24.2 ± 0.8^{b}	22.3 ± 1.2^{b}	18.0 ± 1.1^{a}	18.8 ± 0.5^{a}
Arabinose	8.4 ± 0.8^{a}	8.5 ± 0.6^{a}	11.1 ± 0.9^{b}	10.6 ± 0.5^{b}
Galactose	14.3 ± 0.7^{a}	14.0 ± 0.9^{a}	13.5 ± 0.8^{a}	14.3 ± 0.4^{a}
Glucose	1.4 ± 1.2^{a}	3.7 ± 0.9^{b}	5.8 ± 0.4^{c}	7.9 ± 0.1^{d}
Xylose	25.3 ± 2.2^{a}	25.0 ± 2.1^{a}	31.8 ± 1.4^{c}	29.0 ± 2.4^{bc}
Galacturonic acid	22.3 ± 1.1^{b}	22.1 ± 1.6^{b}	16.7 ± 1.3^{a}	16.4 ± 0.6^{a}
Rhamnose/xylose	0.96^{b}	0.89^{b}	0.57^{a}	0.65^{a}
Arabinose/xylose	0.33^{a}	0.34^{ab}	0.35^{bc}	0.37^{c}

$^{a\text{-}d}$Different letters between the columnss indicate a significant difference ($p < 0.05$) according to Tukey's post hoc means comparison test.
[1] Lipid matter was detected in traces.

Approximately 5 mg of flaxseed gum extract were placed in aluminium pans and hermetically sealed inside the argon-filled glove-box (MBRAUN MB-Labstar, H_2O and O_2 content <0.5 ppm). To determine the thermal behaviour of the flaxseed gum extracts the following thermal scanning protocol was applied: 1) isothermal hold at 25 °C for 5 min, 2) cooling to −80 °C at 5 °C min^{-1}, 3) isothermal hold at −80 °C for 5 min, 4) heating to 65 °C at 5 °C min^{-1} and annealing at the same temperature for 10 min, 5) cooling to −80 °C as previously mentioned, 6) heating to 180 °C at 5 °C min^{-1}. A second cooling – heating scan between −80 and 200 °C was repeated for measuring the glass transition temperature of the gum extracts.

2.8. Statistical analyses

The normal distribution of the data was verified employing the Shapiro-Wilk test and Q-Q plot representation. Also, the equality of variance among the variables was verified using Levene's test. To determine the significance of the gum concentration on the physico-chemical and rheological properties, multifactorial (two or three-way) ANOVA was performed. Tukey's multiple range test was used to separate means of data when significant differences ($p < 0.05$) were detected. Hierarchical cluster analysis was carried out using Ward's agglomeration method to cluster, based on rows (flaxseed gum extract) and columns (physico-chemical, rheological and structure conformational main properties). All statistical analyses were conducted using Origin software (OriginPro v.2019b, OriginLab, USA).

3. Results and discussion

3.1. Proximate and sugar monomer composition

The flaxseed gum extraction yields were estimated at 2.51, 2.47, 2.02 and 2.57% wt for alkaline (GFAL) and acidic (GFAC) extracted gum from golden flaxseed and alkaline (BFAL) and acidic (BFAC) extracted gum from brown flaxseed, respectively. These data are in keeping with the findings of Kaewmanee et al. [16] and Moczkowska et al. [25], yet are lower than the gum extraction yields achieved in other studies e.g. 4 to 8% wt [15,23,28]. The variations in gum extraction yields have been ascribed to several parameters, including flaxseed genotype, extraction temperature and pH, seed-to-water ratio, use of intact seeds or seed meal, etc. [15,16,20,23,28].

In Table 1, the proximate (on dry basis) composition of the flaxseed gum extracts is given. The protein and ash residual in the gum obtained ranged from 6.5 to 9.5 g 100 g^{-1} and 5.7 to 7.4 g 100 g^{-1}, respectively. The residual protein and inorganic matter (ash) content in flaxseed gum is inextricably associated with the extraction conditions i.e., temperature, extraction duration, pH, and ionic strength [20,29]. Enzymatic (protease) hydrolysis, acid or salt-induced protein precipitation, ultracentrifugation and ion exchange chromatography may result in a significant reduction in the proteinaceous residue of flaxseed gum [19,23,29]. In our study, an alternative route based on acidic mucilage extraction (at conditions close to the pI of flaxseed proteins i.e., 4–4.5) followed by a pH shifting deproteinisation step, was investigated. The approach was previously proven to be a very effective deproteinisation strategy for non-ionic polysaccharide isolates i.e., galactomannans [26]. As for flaxseed gum, the protein depletion effectiveness of the extraction method was dependent ($p < 0.05$) on the flaxseed type (6.85 and 8.9 g 100 g^{-1}, for golden and brown flaxseed, respectively), whilst no significant impact of the pH of the aqueous extracting medium on protein was detected. According to literature findings, the protein content in flaxseed gums varies from 6 to 22 g 100 g^{-1} [20,20–22,25,30], which is comparably higher than that reported for other industrially relevant gums such as galactomannans (3–10%), xanthan gum (0.8–4.7%), gellan gum (0–3%), and seaweed polysaccharides (0–1.5%) [31–34]. A substantial reduction in the proteinaceous matter can be achieved when additional purification steps (e.g. proteolytic attack, ultracentrifugation, membrane or chromatographic separation etc.) are implemented [16,23]. Moreover, chemical processing aids (acids and salts) may be employed in gum purification, yet undesirable losses in the carbohydrate matter, due to the partial hydrolytic depolymerisation of the gum, may be induced under highly acidic conditions [19].

Corroborating the literature data [3,28], the osidic composition of the flaxseed gum (Table 1) confirmed the presence of neutral and acidic polysaccharide fractions. The flaxseed gums were composed of xylose (25–31.8%), rhamnose (18–24.2%), galactose (13.5 - 14.3%), arabinose (8.4–11.1%), glucose (1.4–7.9%), fucose (3.0–4.4%) and galacturonic acid (16.4–22.3%). The mean rhamnose/xylose ratio was estimated at 0.9 and 0.6 for golden and brown flaxseed gums, respectively. In their studies, Cui et al. [15] and Oomah et al. [28] demonstrated that the rhamnose/xylose mass fraction may vary significantly among brown and golden flaxseed cultivars i.e., 0.3–2.2. The rhamnose/xylose ratio is considered an indicator of the proportion of the pectic-like to hemicellulose matter [28] and therefore, it can be deduced that only the flaxseed phenotype affected the major polysaccharide fraction composition of the gum extracts. The average arabinose/xylose ratio, indicative of the branching degree of the polymer chains [28,35], was estimated at 0.33, 0.34, 0.35 and 0.37 for GFAL, GFAC, BFAL and BFAC, respectively. Both flaxseed phenotype and extraction conditions were significant ($p < 0.05$). Although the quantification of most of the sugar monomers was in keeping with the existing literature data [28], glucose quantity was remarkably ($p < 0.001$) low. Wannerberger [35] and Fedeniuk and Biliaderis [36] demonstrated that the presence of buffer salt in the aqueous extraction medium leads to the preferential isolation of glucose. The absence of buffer salts in the extracting medium (MilliQ water) may explain the remarkably low glucose levels. Interestingly, the acidic gum extracts had higher glucose levels (5.8 g 100 g^{-1}) than their alkaline extracted counterparts (3.6 g 100 g^{-1}). This may be ascribed to the buffering effect of sodium chloride formed during the neutralisation process prior to the pH shifting deproteinisation step.

3.2. Structure conformation and physicochemical properties

To determine the molecular properties of the flaxseed gum extracts, GPC/SEC analysis using 0.1 M $NaNO_3$ as the eluent, was used. The use of nitrate solution instead of water as the mobile phase aimed at preventing the aggregation of the polysaccharide molecules due to intermolecular

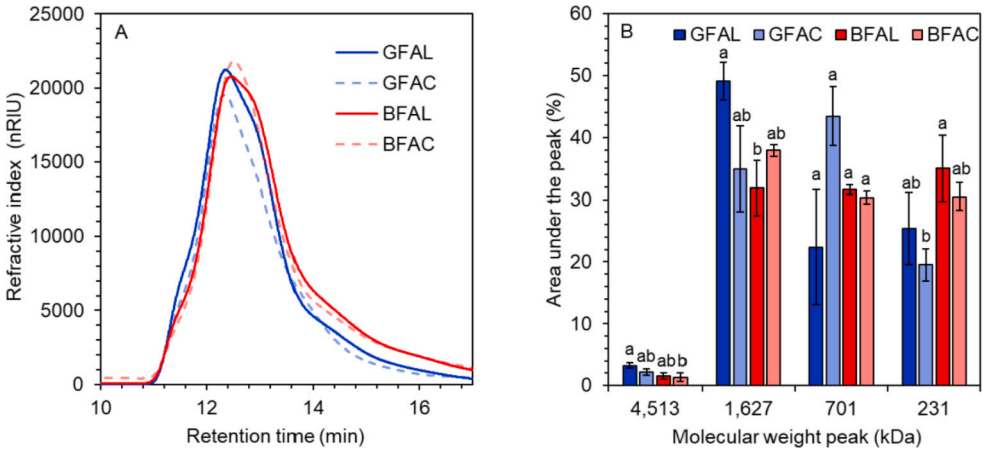

Fig. 2. A) Gel permeation size-exclusion chromatograms (GPC/SEC) and B) occurrence of the four polysaccharidic populations as detected by peak deconvolution of the GPC/SEC chromatograms of the gums extracted from golden (GF–) and brown (BF–) flaxseed under acidic (–AC) or alkaline (–AL) conditions.

Table 2
Comparison of structure conformational characteristics of the four flaxseed gum extracts.

	Golden alkaline	Golden acid	Brown alkaline	Brown acid
$\overline{Mn}$ ($\times 10^5$ Da)	5.65 ± 0.64^{ab}	6.50 ± 0.99^{a}	3.34 ± 0.86^{c}	4.26 ± 0.40^{bc}
$\overline{Mw}$ ($\times 10^5$ Da)	13.33 ± 0.10^{a}	13.50 ± 1.16^{a}	11.48 ± 0.49^{b}	11.45 ± 0.42^{b}
$\overline{Mz}$ ($\times 10^5$ Da)	21.90 ± 0.18^{a}	21.69 ± 1.34^{a}	21.57 ± 1.56^{a}	19.68 ± 0.55^{a}
Đ	2.40 ± 0.26^{ab}	2.11 ± 0.14^{a}	3.82 ± 1.17^{b}	2.71 ± 0.16^{ab}
$\overline{DP_n}$	2959 ± 337^{ab}	3406 ± 517^{a}	1741 ± 446^{c}	2220 ± 208^{bc}
[η] (dL g^{-1})	6.52 ± 0.11^{a}	6.74 ± 0.08^{a}	5.01 ± 0.40^{b}	5.24 ± 0.35^{b}
k_H	0.62 ± 0.02^{b}	0.55 ± 0.01^{a}	0.62 ± 0.07^{ab}	0.56 ± 0.03^{a}
c* (g 100 g^{-1})	0.55 ± 0.02^{b}	0.50 ± 0.01^{a}	0.72 ± 0.06^{c}	0.79 ± 0.04^{c}
z-diameter (nm)	97.8 ± 5.9^{a}	93.7 ± 4.2^{a}	109.7 ± 4.3^{a}	101.2 ± 10.5^{a}

$^{a-d}$ Different letters between the columns indicate significant differences ($p < 0.05$) according to Tukey's post hoc means comparison test. Symbols and abbreviations used: number-average molecular weight (Mn), weight-average molecular weight (Mw), z-average molecular weight (Mz), dispersity (Đ), number-average degree of polymerisation (DP_n), intrinsic viscosity calculated based on average of Huggins and Kraemer intercepts ([η]), critical coil overlap concentration (c*).

non-covalent interactions such as electrostatic, van der Waals attraction or hydrogen bonding [13,21]. The molecular weight distribution profiles of the flaxseed gum extracts are illustrated in Fig. 2. Regardless of the extraction method or the botanical origin of the flaxseed, the molecular weight distribution of the gum extracts was broad with the dispersity index (Đ) ranging from 2.11 to 3.82 (Table 2). In general, the average M_w and DP_n values of the golden flaxseed extracts were higher than the brown flaxseed ones but the direct impact of the extraction method remained unclear. The deconvolution of the GPC/SEC chromatograms allowed a satisfactory separation of the peaks corresponding to the different polysaccharide fractions (Fig. 2B). In all flaxseed gum systems, four polysaccharide populations were identified, corresponding to 4513 (less than 3%), 1627 (31–48%), 701 (22–43%) and 231 (19–35%) kDa, which is in keeping with the findings of Qian et al. [23] and Elboutachfaiti et al. [22]. Studies on fractionated flaxseed gum using ion exchange chromatography have demonstrated its polysaccharidic complexity, as due to neutral (arabinoxylan-rich – M_w up to 5500 kDa), acidic (rhamnogalacturonan–I rich – M_w < 500 kDa) and composite AX-RG-I fractions (1700 < M_w < 700 kDa) co-existing [22,23,37,38]. It is generally accepted that high molar mass arabinoxylans contribute mostly to the inherent viscosity of flaxseed gum, whilst the pectic fractions are considered rather small to significantly alter its thickening potential [23,37]. On the other hand, Naran et al. [38] and Qian et al. [23] reported that the thickening capacity of flaxseed gum did not comply with the ideal mixture law (i.e., viscosity does not develop proportionally to the AX and RG-I mass fractions). Conversely,

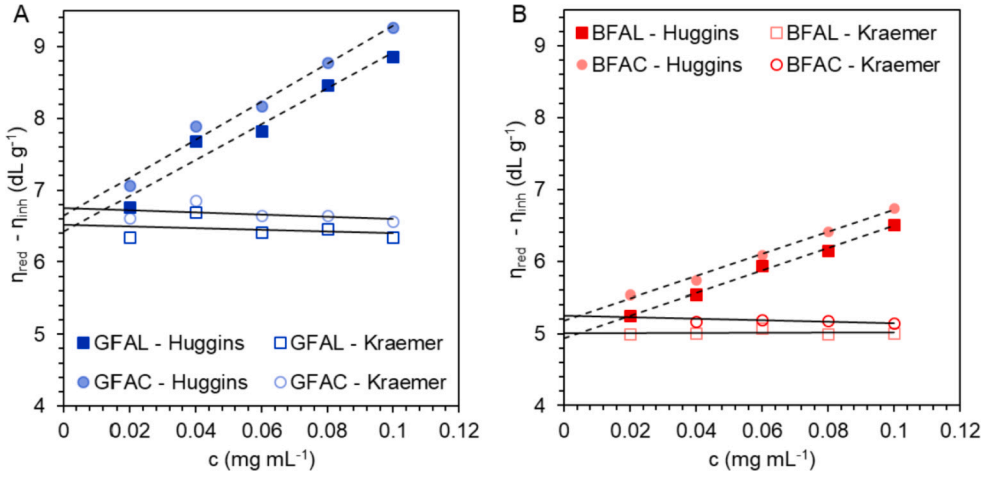

Fig. 3. Inherent (Kraemer) and relative (Huggins) viscosity as a function of A) golden flaxseed and B) brown flaxseed gum concentration at 25 °C.

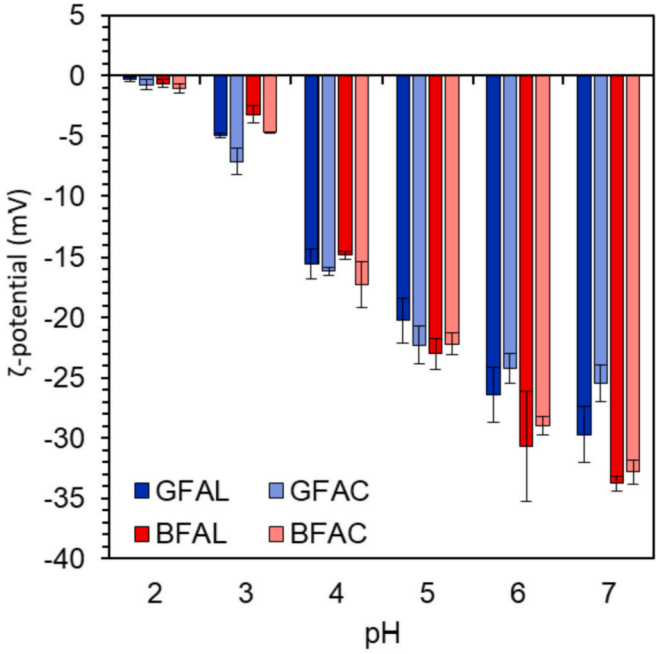

Fig. 4. Surface charge density of golden (GF–) and brown (BF–) flaxseed gums extracted under alkaline (–AL) and acid (–AC) conditions measured in Milli-Q water at 25 °C.

the composite AX-RG-I fractions may result in a significant contribution to the overall viscosity of flaxseed gum solutions, which is ascribed most probably to non-covalent intermolecular interactions between the two polysaccharide species [38].

Dynamic light scattering measurements (Table 2) confirmed that golden flaxseed gum solutions were less prone ($p < 0.05$) to polymer-polymer chain aggregation than brown flaxseed gum. The acidic extracts were characterised by smaller hydrodynamic diameters compared to their alkaline extract counterparts; nevertheless, the differences were not significant. In agreement with the GPC-SEC measurements, the polymer-polymer aggregative phenomena were substantially stronger in MilliQ water than lyotropic solvents (0.1 M $NaNO_3$) i.e., $d_H = 216–262$ and 94–110 nm, respectively.

To diminish the polyelectrolyte effects observed in MilliQ water, the intrinsic viscosities [η] of the flaxseed gum were measured in 0.1 M $NaNO_3$. As a general trend, the golden flaxseed gum extracts exhibited significantly ($p < 0.001$) higher intrinsic viscosities than their brown flaxseed analogues i.e. 6.52–6.74 vs 5.01–5.24 dL g^{-1} (Fig. 3, Table 2). Similar to the M_w observations, the pH of the aqueous extraction media did not significantly modify the intrinsic viscosities of the gums. The intrinsic viscosity is considered an inherent characteristic of biopolymers, which is directly associated with the molecular weight and the rigidity of the polymer chains as well as the solvent affinity [39]. The average Huggins constants (k_H) were 0.55 and 0.62 ($p < 0.05$) for the acidic and alkaline extracted gums, respectively, but no differences were identified concerning the flaxseed phenotype . The k_H is a measure of the polymer – polymer and polymer – solvent interactions as influenced by the molecular conformation of the polymer. In general, the k_H received values of around 0.35 for flexible biopolymers with extended coil conformation in good solvents, whereas higher values ranging from 0.5 to 0.8 were observed in theta solvents [40]. Based on our findings, it can be deduced that the solvent did not have a net effect on the polymer conformational state, with the acidic fraction showing a higher degree of polymer chain flexibility than its alkaline counterparts.

The surface charge density of the flaxseed gum solutions as a function of pH is illustrated in Fig. 4. The reduction of the pH resulted in a progressive decrease ($p < 0.05$) in the net (negative) charge of the gum solutions due to the partial protonation of the carboxylate groups of the RG-I fraction [41], whereas the isoionic points (pK_a) were estimated at pH = 1.8–2. The surface charge of the polymers was rapidly depleted for pH < 4, which may be attributed to the increase in the positive charge density of the residual proteins [42].

Thermal gravimetric analysis was performed to study the thermal stability and decomposition pattern of the flaxseed gum extracts. As seen in Fig. 5, a weight loss accounting for approx. 16% of the total gum weight was observed in the temperature range from 29.9 to 134.6 °C, which was ascribed to the evaporation of the monolayer (hydrogen bond bound) moisture content. A second thermal event related with the thermal decomposition of the gum extracts (i.e. degradation of the saccharide rings and disintegration of the polymer chains) was detected in the temperature range from 202.2 to 380.0 °C. Similar thermal decomposition patterns have been also reported for other flaxseed gum extracts [24,43]. It should be noted that neither the extraction conditions nor the flaxseed phenotype impacted the onset, midpoint and endpoint temperatures of the recorded thermal events. To gain a better insight into the thermal stability of the flaxseed gum extracts the integral procedure decomposition temperature (IPDT) was determined as detailed in Doyle [44]. The calculated IPDT values were 304.2, 416.7, 368.2 and 276.9 °C, for GFAL, GFAC, BFAL and BFAC, respectively. This implies that golden flaxseed gum extracts exert higher thermal stability compared to the brown flaxseed exemplars, and therefore, they are more suitable in product applications involving severe heat treatments such as baking, sterilisation etc.

According to the acquired DSC thermographs (Suppl. Fig. 1), dinstict temperature regions where changes in the heat capacity took place were detected only during the first heating step (at temperatures well below

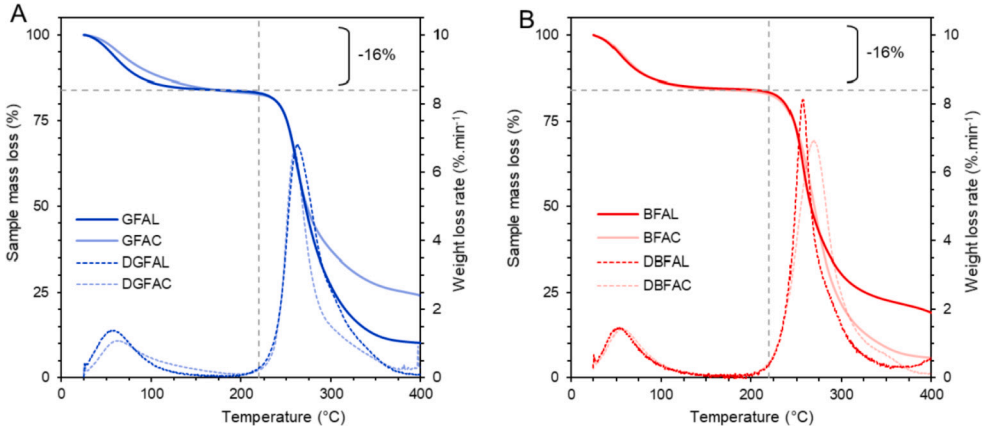

Fig. 5. TGA (continuous lines) and DTGA (dashed lines) thermographs of A) golden and B) brown flaxseed gum extracts.

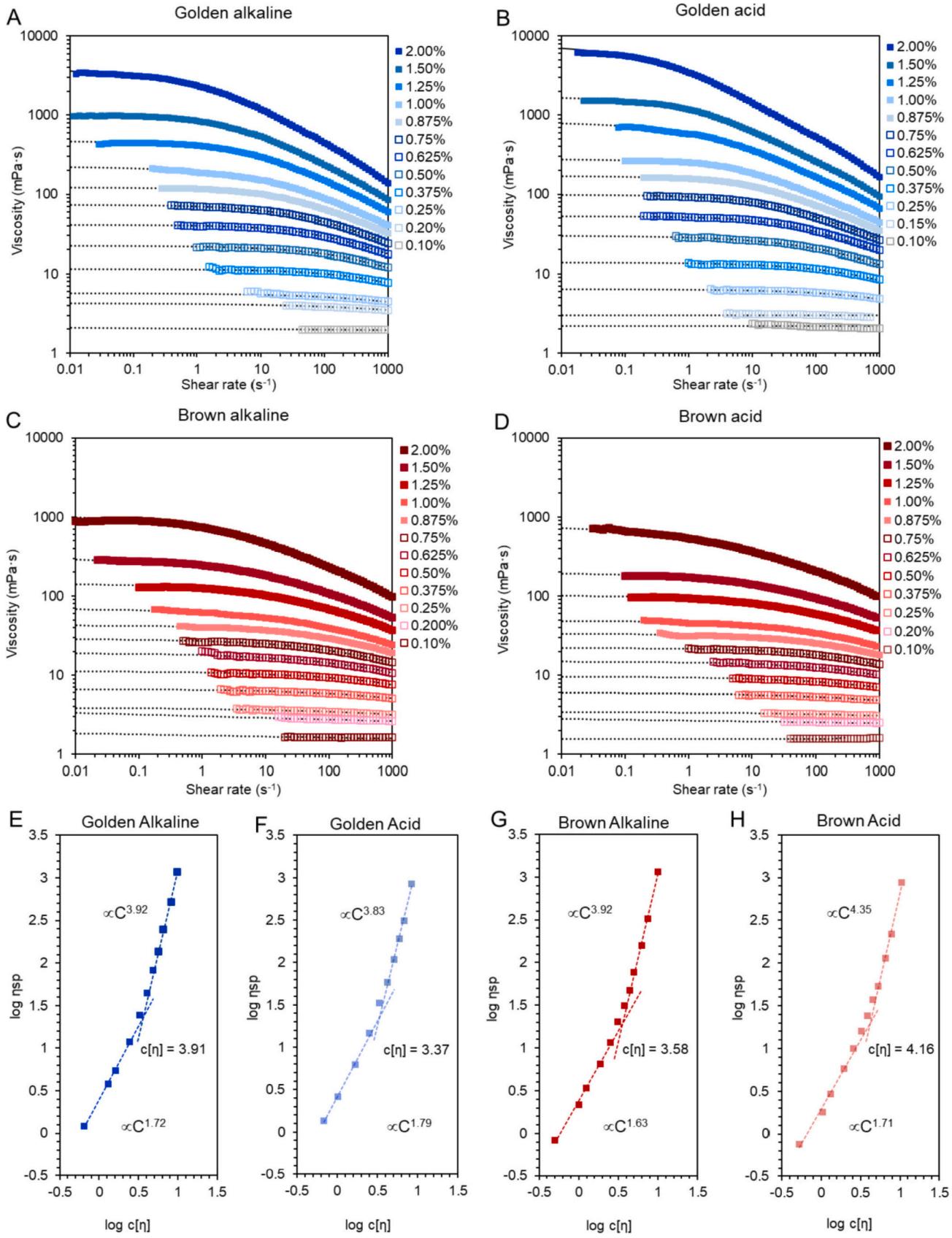

Fig. 6. Flow behaviour curves of flaxseed gum solutions as influenced by gum concentration (A-D); double logarithmic plot of specific viscosity at zero shear rate ($\eta sp,_0$) as a function of coil overlap parameter c[η] at 25 °C (*E*-H).

Table 3
Steady flow characteristics of golden and brown flaxseed acid and alkaline-extracted gum solutions for c > c* (0.625 to 2% wt in 0.1 M $NaNO_3$), calculated according to the Cross - Williamson model.

Gum concentration	τ				$\dot{\gamma}_{crit}$				m			
(g 100 g^{-1})	(s)				(s^{-1})							
	GFAL	GFAC	BFAL	BFAC	GFAL	GFAC	BFAL	BFAC	GFAL	GFAC	BFAL	BFAC
0.625	0.004	0.008	–	–	246.71	119.09	–	–	0.027	0.038	–	–
0.75	0.007	0.015	–	–	148.47	67.47	–	–	0.052	0.067	–	–
0.875	0.012	0.026	0.003	0.0007	81.87	37.86	291.46	1368.19	0.057	0.104	0.063	0.047
1	0.023	0.042	0.005	0.001	42.79	23.8	203.42	840.46	0.163	0.138	0.127	0.067
1.25	0.048	0.178	0.016	0.009	20.75	5.62	60.79	116.87	0.169	0.414	0.157	0.104
1.5	0.099	0.263	0.051	0.017	10.05	3.8	19.55	57.34	0.251	0.464	0.222	0.150
2	0.381	1.451	0.156	0.145	2.62	0.69	6.4	6.92	0.583	1.220	0.412	0.453

Symbols and abbreviations used: Gum extracted from golden flaxseed (GF–) or brown flaxseed (BF–) under alkaline (–AL) or acidic (–AC) conditions; τ: relaxation time; $\dot{\gamma}_{crit}$: critical shear rate; m: Cross constant.

the water evaporation peak). In order to confirm the T_g values obtained from the first heating step, the samples underwent Thermal Mechanical Anaylsis under inert atmosphere (He) using a DIL 402 select Expedis dilatometer (NETZSCH, Germany) at a heating rate of 5 °C min^{-1} and a constant load of 0.3 N in the range from −140 to 200 °C (Suppl. Fig. 1). The thermomechanical spectra confirmed the presence of a physical state change event ($T_{g,\ onset} \sim 42.4$ °C, $T_{g,midpoint} \sim 56.5$ °C), which is generally in accordance with the DSC findings. However, it should be noted that no measurable changes in the heat capacity were observed in the DSC thermographs obtained during the second and third heating step. Instead, the presence of a broad endothermic midpoint peak ranging from 101.1 to 112 °C, which is comparable to the values reported for other mucilages such as tamarind [45], cactus [46], chia seed [47], *Albizia stipulata* gum exudate [48], and nettle galactomannan [49], was observed. Although the endothermic thermal event was not recorded during the third step, the acquired thermograph did not allow to evidence any glassy to rubbery state phase transition event. Such a behaviour has been previously reported for several polysaccharides including dextran, pullulan, xanthan, pectin, alginate and galactomannans [50]. Appelqivst et al. [51] attributed such a phenomenon (observed in low moisture content polysaccharides) to a type of structural melting or phase transition phenomena involving water molecules and the relatively immobilised biopolymer. On this occasion, intermolecular associations between water and polymer residues can be still energetically formed or disrupted instead of achieving a purely glassy state (entropically driven), where the molecular mobility of the polymer backbone is hindered.

3.3. Steady state rheological behaviour

The steady state flow curves of the flaxseed solutions can be seen in Fig. 6A-D. To diminish the impact of polyelectrolyte effects, the flaxseed gum extracts were dispersed into a 0.1 M $NaNO_3$ solution. The steady flow rheological data (viscosity vs. shear rate) were fitted into the Cross-Williamson model (Eq. (4)). Table 3 reports the Cross-Williamson parameters i.e., the time constant τ, the rate constant m and the critical shear rate $\dot{\gamma}_{crit}$ at which $\eta = \frac{\eta_0 - \eta_\infty}{2}$. For the entire range of concentrations, the flaxseed gum solutions exhibited a shear-thinning flow behaviour, with the intensity of the pseudoplasticity (as expressed by m value) increasing proportionally to flaxseed gum concentration. In addition, the golden flaxseed gum solutions were more pseudoplastic than their brown flaxseed counterparts, which is attributed to their structure conformational and AX to RG-I fraction proportional differences. On the other hand, the impact of extraction conditions on the pseudoplasticity of the gum solutions was governed by the type of the flaxseed phenotype i.e., acidic gum extraction favoured the shear-thinning character of golden flaxseed gum solutions but reduced the pseudoplasticity of the brown flaxseed gum analogues. A similar pattern concerning the impact of extraction conditions was observed also for the τ and $\dot{\gamma}_{crit}$ parameters.

Employing MilliQ water as the biopolymer solvation medium did not modify the rheological behaviour of the gum solutions regarding the impact of the gum extraction and flaxseed phenotype conditions. Thus, the consistency coefficient and pseudoplasticity of the aqueous gum solutions was reduced proportionally to the gum concentration. The extraction conditions were impactful on the consistency coefficient only in the case of the brown flaxseed gum extracts (Table 4). Previous studies have demonstrated that the flow behaviour of flaxseed gum may be highly diversified based on its genotype and phenotype, as well as on the extraction, fractionation and purification conditions [15,23,25,30,52,53].

Morris et al. [54] demonstrated that for random coil polysaccharides, the specific zero-shear viscosity ($\eta_{sp} = (\eta_0 - \eta_s)/\eta_s$, where $\eta_s = 0.885$ mPa.s) as a function of the coil overlap parameter c[η] can be described by a power law relationship as follows (Eq. (5)):

Table 4
Steady flow characteristics of golden and brown flaxseed acid and alkaline extracted gum aqueous solutions (0.25 to 2.5% wt), calculated according to the Ostwald-de Waele model.

Gum concentration	K				n			
(g 100 g^{-1})	(Pa s^{-n})							
	GFAL	GFAC	BFAL	BFAC	GFAL	GFAC	BFAL	BFAC
0.25	$0.02^{A,a}$	$0.04^{A,a}$	$0.04^{A,a}$	$0.02^{A,a}$	$0.881^{A,a}$	$0.839^{A,a}$	$0.857^{A,a}$	$0.903^{A,a}$
0.5	$0.07^{A,a}$	$0.10^{B,a}$	$0.12^{B,a}$	$0.05^{A,ab}$	$0.789^{A,a}$	$0.766^{A,ab}$	$0.784^{A,ab}$	$0.851^{A,ab}$
0.75	$0.12^{A,a}$	$0.13^{A,a}$	$0.33^{B,ab}$	$0.12^{A,abc}$	$0.771^{A,ab}$	$0.753^{A,abc}$	$0.720^{A,abc}$	$0.796^{A,abc}$
1	$0.28^{A,a}$	$0.58^{B,a}$	$0.64^{B,b}$	$0.25^{A,c}$	$0.700^{A,abc}$	$0.651^{A,bcd}$	$0.678^{A,abc}$	$0.751^{A,abcd}$
1.5	$1.55^{B,b}$	$1.74^{B,b}$	$1.80^{B,c}$	$0.70^{A,d}$	$0.584^{A,bcd}$	$0.582^{A,bcd}$	$0.608^{A,bc}$	$0.685^{A,bcd}$
2	$4.11^{B,c}$	$4.32^{B,c}$	$2.30^{B,d}$	$1.52^{A,e}$	$0.519^{A,cd}$	$0.517^{A,d}$	$0.634^{B,bc}$	$0.624^{B,cd}$
2.5	$8.80^{B,d}$	$9.70^{B,d}$	$3.03^{A,e}$	$2.65^{A,f}$	$0.467^{A,d}$	$0.466^{A,d}$	$0.573^{B,c}$	$0.600^{B,d}$

$^{A\text{-}B,a\text{-}e}$Different letters between the flaxseed gum extracts (uppercase) or concentrations (lowercase) for each rheological property indicate a significant difference ($p < 0.05$) according to Tukey's post hoc means comparison test. Abbreviations used: gum extracted from golden flaxseed (GF–) or brown flaxseed (BF–) under alkaline (–AL) or acidic (–AC) conditions; K: consistency coefficient; n: flow behaviour index.

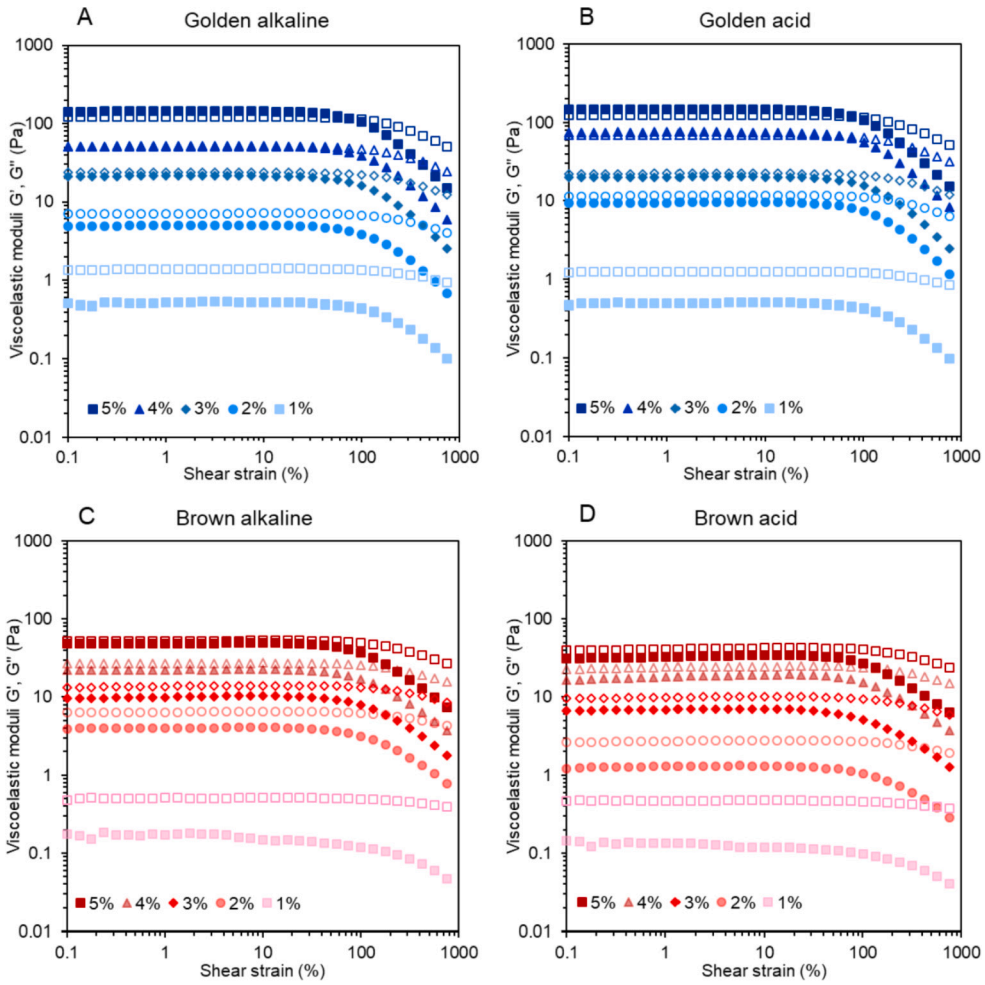

Fig. 7. Amplitude sweep rheological spectra of flaxseed gum aqueous solutions as a function of concentration measured at 25 °C. The closed symbols denote the storage modulus (G′) and the open symbols the loss modulus (G″).

$$\eta_{sp} = f(c\cdot[\eta])^{b} \quad (5)$$

According to the constructed double logarithmic plots of η_{sp} and $c[\eta]$ (master curves) (Fig. 6E-H), the critical coil overlap parameter $c\bullet[\eta]_{crit}$ values varied from 3.37, 3.91, 3.58 and 4.16 for GFAL, GFAC, BFAL and BFAC solutions, respectively. The $c[\eta]_{crit}$ is considered as the boundary between the dilute and semi-dilute solution state. This implies that at $c[\eta]_{crit}$ or c^* ($c^* = c_{crit}$), mark the onset of the transition from the dilute solution state where the polymer coils are isolated to a more concentrated (semi-dilute) state in which the hydrodynamic volume occupied by the polymer chains is higher than the solvent-free volume. Morris et al. [54] reported that the $c[\eta]_{crit} = 4$ for polysaccharides exerting a random coil structure conformation, whilst lower values of $c[\eta]_{crit}$ (~ 2.5 to 3.3) have been reported for polysaccharides undergoing specific intermolecular associations (known as hyperentanglement), such as galactomannans [26,54,55]. The $c[\eta]_{crit}$ values calculated here are generally in keeping with the previous findings of Qian et al. [23] and Repin et al. [52] i.e., $c[\eta]_{crit} \sim 2.16$ to 4.12. However, it should be noted that the degree of space occupancy appears to be highly dependent not only on the flaxseed cultivar but also on the adopted gum extraction, fractionation and purification practices [23,52]. As seen in Table 2, the c^* values of the gum extracts were estimated at 0.50, 0.55, 0.72 and 0.79 for GFAL, GFAC, BFAL and BFAC, respectively. As an inherent polysaccharide property, c^* is inextricably associated with the structure conformational and compositional characteristics of flaxseed gum, such as the molar mass, degree of polymer branching, the mass fraction of AX, AX-RG-I and RG-I components etc.[23]. Indeed, our data showed significant correlations between the c^* and molar mass ($r = -0.75$, $p < 0.01$), degree of branching ($r = 0.85$, $p < 0.001$), the hydrodynamic diameter values ($r = 0.66$, $p < 0.05$) as well as the total mass fraction of the AX-RG-I composite ($r = -0.66$, $p < 0.05$).

3.4. Dynamic rheological behaviour

Small amplitude oscillatory shear tests were performed on flaxseed gum solutions using MilliQ water (instead of $NaNO_3$) as the solvent, in order to achieve a better understanding of the physical state and the maximal structuring performance of the gums in conditions relevant to food colloids. Amplitude sweep tests (Fig. 7A-D) were carried out to define the boundary of the linear viscoelastic regime (LVE) of the flaxseed gum solutions (1–5% wt). As seen in Table 5, the increase of the flaxseed gum concentration was accompanied by a progressive ($p < 0.001$) increase of the viscoelastic moduli at the offset of the LVE region (G'_{LVE} and G''_{LVE}) and the yield stress (τ_y). Based on the responsiveness of the τ_y to flaxseed gum type and concentration, it can be deduced that golden flaxseed gum systems required an at least two-fold higher energy input to induce the irreversible (plastic) deformation of the polymeric structures of the semi-dilute (1% wt) or concentrated ($c > 2.5\%$ wt; non-obeying to the Cox-Merz rule) aqueous systems. On the other hand, the impact of the extraction conditions on the dynamic rheological behaviour of the flaxseed gum aqueous solutions remained unclear. In general, no remarkable differences between GFAL and GFAC solutions were observed, whereas in the case of brown flaxseed, the acidic counterparts were characterised by significantly lower viscoelastic moduli and yield

Table 5
Viscoelastic properties (strain sweeps with controlled shear deformation at 1 Hz and frequency sweeps (within the LVE region)) of the flaxseed gum solutions (1 to 5% wt, pH = 7) at 25 °C.

Gum concentration	G'_{LVE}		G''_{LVE}		Yield stress, τ_y		Flow point, τ_f		G'_f		Complex viscosity η^*		Crossover frequency f_c		Slope G′ - f	
(g 100 g^{-1})	(Pa)		(Pa)		(Pa)		(Pa)		(Pa)		(Pa s)		(Hz)			
	GFAL	GFAC	GFAL	GFAC	GFAL	GFAC	GFAL	GFAC	GFAL	GFAC	GFAL	GFAC	GFAL	GFAC	GFAL	GFAC
1	$0.36^{B,a}$	$0.40^{B,a}$	$1.04^{B,a}$	$0.91^{B,a}$	$0.79^{B,a}$	$0.69^{B,a}$	nd	nd	nd	nd	$0.17^{B,a}$	$0.15^{B,a}$	nd	nd	$1.26^{B,c}$	$1.00^{A,d}$
2	$4.7^{B,b}$	$5.1^{B,b}$	$6.5^{B,b}$	$6.4^{B,b}$	$3.8^{B,b}$	$3.4^{B,b}$	nd	nd	nd	nd	$1.3^{B,b}$	$1.3^{B,b}$	nd	nd	$0.91^{A,b}$	$0.90^{A,cd}$
3	$19.5^{B,c}$	$21.1^{B,c}$	$21.4^{B,c}$	$21.3^{B,c}$	$13.5^{B,c}$	$12.5^{B,c}$	nd	nd	nd	nd	$4.8^{C,c}$	$4.8^{C,c}$	28.3a	nd	$0.76^{A,ab}$	$0.72^{A,bc}$
4	$52.4^{B,d}$	$59.4^{B,d}$	$50.6^{B,d}$	$51.8^{B,d}$	$30.6^{B,d}$	$31.8^{B,d}$	$42.5^{A,a}$	$56.9^{B,a}$	$45.6^{A,a}$	$40.70^{A,a}$	$11.7^{B,d}$	$12.1^{B,d}$	$2.26^{A,b}$	$8.15^{B,a}$	$0.67^{A,a}$	$0.58^{A,ab}$
5	$138.2^{C,e}$	$147.1^{C,e}$	$118.2^{C,e}$	$116.2^{C,e}$	$76.5^{C,e}$	$74.7^{C,e}$	$116.2^{A,b}$	$128.0^{A,b}$	$111.9^{A,b}$	$108.35^{A,b}$	$29.3^{C,e}$	$37.5^{C,e}$	$0.42^{A,c}$	$0.59^{A,b}$	$0.56^{A,a}$	$0.50^{A,a}$
	BFAL	BFAC	BFAL	BFAC	BFAL	BFAC	BFAL	BFAC	BFAL	BFAC	BFAL	BFAC	BFAL	BFAC	BFAL	BFAC
1	$0.15^{A,a}$	$0.10^{A,a}$	$0.5^{A,a}$	$0.4^{A,a}$	$0.3^{A,a}$	$0.8^{B,a}$	nd	nd	nd	nd	$0.24^{C,a}$	$0.07^{A,a}$	nd	nd	$1.27^{B,c}$	$1.28^{B,c}$
2	$4.10^{B,ab}$	$1.3^{A,a}$	$6.6^{B,ab}$	$2.8^{A,a}$	$3.4^{B,a}$	$1.3^{A,a}$	nd	nd	nd	nd	$1.38^{B,ab}$	$0.53^{A,ab}$	nd	nd	$0.79^{A,b}$	$0.74^{A,a}$
3	$10.2^{B,b}$	$6.9^{A,b}$	$14.0^{B,b}$	$10.3^{A,b}$	$7.9^{A,b}$	$5.5^{A,b}$	nd	nd	nd	nd	$2.78^{B,b}$	$2.01^{A,b}$	nd	nd	$0.70^{A,ab}$	$0.59^{A,a}$
4	$22.9^{A,c}$	$18.6^{A,c}$	$27.5^{A,c}$	$24.7^{A,c}$	$16.1^{A,c}$	$14.5^{A,c}$	nd	nd	nd	nd	$5.75^{A,c}$	$5.09^{A,c}$	nd	nd	$0.60^{A,ab}$	$0.58^{A,a}$
5	$49.7^{B,d}$	$33.4^{A,d}$	$53.2^{B,d}$	$41.7^{A,d}$	$32.4^{B,d}$	$25.0^{A,d}$	nd	nd	nd	nd	$11.90^{B,d}$	$8.65^{A,d}$	6.06^{B}	17.0^{C}	$0.52^{A,a}$	$0.58^{A,a}$

$^{A-C,a-e}$Different letters between the flaxseed gum extracts (uppercase) or concentrations (lowercase) for each rheological property indicate a significant difference ($p < 0.05$) according to Tukey's post hoc means comparison test. Abbreviations used: gum extracted from golden flaxseed (GF–) or brown flaxseed (BF–) under alkaline (–AL) or acidic (–AC) conditions. *nd = not detected.

stress values.

The frequency sweep tests (Fig. 8A-D) were carried out within the LVE region (strain 0.5%) at 25 °C. For all systems, the increase in the flaxseed gum concentration was followed by a progressive transition from the pronouncedly viscous behaviour to the viscoelastic ($G' \approx G''$) or weak gel-like ($G' > G''$) behaviour. In general, the aqueous solutions of golden flaxseed gum exerted significantly higher ($p < 0.05$) complex viscosities than the brown flaxseed gum analogues for the entire range of concentration, which is in keeping with the observations of Cui et al. [15]. It should be noted that G′ - f slope values (Table 5) for all viscoelastic gum solutions remained quite high i.e., 0.95 < G′ - f slope < 0.45, which indicates that contrary to other types of random coil polysaccharides, e.g. galactomannans, carrageenans etc., the gel forming ability of flaxseed gum remains very weak even at concentrations as high as 5% wt. The insufficient capacity of flaxseed gum to undergo true gel formation ($\tan\delta << 0.1$) was observed even in super-concentrated systems containing up to 8% wt of gum solids (data not shown).

Using the Time – Concentration – Superimposition (TCS) principle, the data illustrated in Fig. 8A-D, were collapsed against the arbitrarily chosen aqueous solutions containing 2% wt of flaxseed gum extract (Fig. 8E-H). According to the obtained master curves, the viscoelastic spectra were satisfactorily reduced to single curves for G′ (ω_r) and G″ (ω_r). As a general trend, all the extracts had a viscous-like behaviour for low frequency range whereas only golden flaxseed extract exhibited a clear viscoelastic moduli crossover point ($G' = G''$) at higher frequencies. The horizontal (a_c) and vertical (b_c) superimposition shift parameters, respectively, were calculated. The changes in the b_c shift factor remained under one order magnitude (0.2–0.3 and 0.3–0.9 for golden and brown flaxseed gum, respectively), which is in agreement with the literature [56,57]. However, a remarkably higher variation in the frequency shift factor a_c values was observed, ranging from 3.2–3.4 to 3.9–4.3 orders for golden and brown flaxseed, respectively (Fig. 8I – L). The shift factor - power law dependency, calculated as $a_c \propto C^n$, was estimated at $\propto C^{4.5}$, $C^{4.7}$, $C^{5.6}$ and $C^{5.8}$ for GFAC, GFAL, BFAC and BFAL, respectively. These values were lower than throse reported for xanthan gum ($\propto C^{6.9}$ - $C^{7.4}$) [57,58] but higher than the *Sphingomonas* exopolysaccharide ($\propto C^{3.3}$) [59] and welan gum ($\propto C^{4.0}$) [60] exemplars.

3.5. Hierarchical cluster analysis

To gain a deeper insight into the techno-functional affinities of the flaxseed gum extracts, the generated data were subjected to a hierarchical cluster analysis based on rows (flaxseed gum extracts) and columns (molecular, physicochemical and rheological properties) using Ward's agglomeration and the Euclidean distance criteria (Fig. 9). As illustrated in Fig. 9, gum extracts of the same botanical origin were clustered together without exerting any clear individualisation. Three major clusters i.e., I, III, IV identified the disparities between the gum extracts associated with phenotypes. According to the agglomeration pattern of the variables (columns), it can be deduced that the steady and dynamic rheological properties (cluster I and IV), as well as the inherent molecular characteristics (molecular weight, intrinsic viscosity, degree of polymerisation, critical concentration) and physicochemical aspects (surface charge density and hydrodynamic diameter), were the discriminating factors of the gums based on their phenotype origin. On the other hand, the classification of the gum extracts based on the pH conditions deployed was primarily achieved using the variables found in cluster III. Specifically, seed hydration and gum extraction at pH ≈ pI favoured the isolation of intermediate molar mass polysaccharides (significant reduction of the polysaccharide fractions at 231 and 4513 kDa) leading to a lower surface charge density and hydrodynamic diameter of the polymeric chains. On the other hand, the pI-mediated extraction led to gum isolates with a higher water solvation affinity, which implies that the AX-RG-I composite polymer has a better ability to interact with water via hydrogen bonding, leading to less pronounced polymer-polymer aggregative phenomena. Concerning the variable

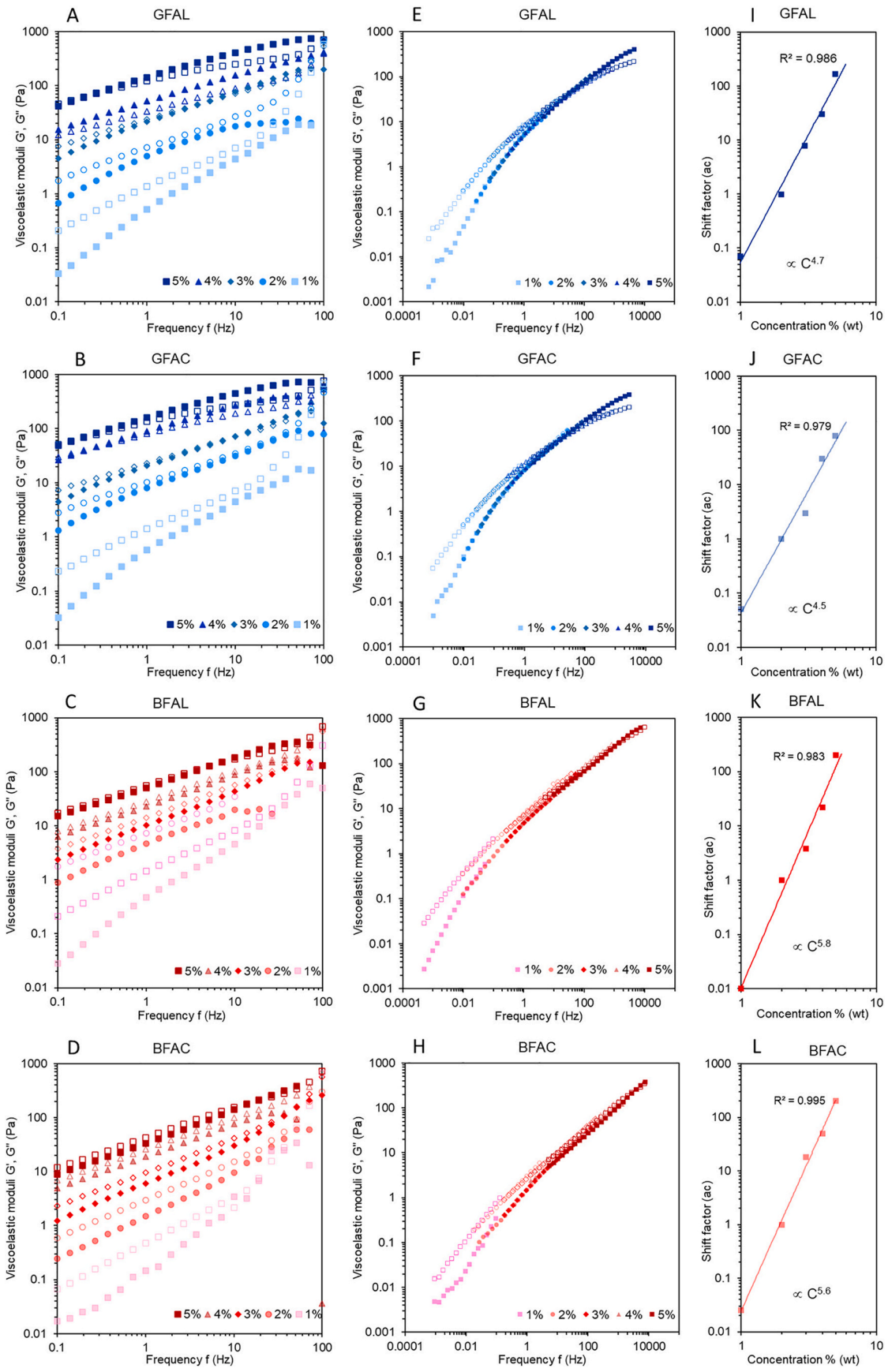

Fig. 8. Frequency sweeps rheological spectra (A–D), frequency – concentration master curves (E–H) and frequency shift factor values (a_c; I–L) as influenced by the concentration of the flaxseed gum extracts.

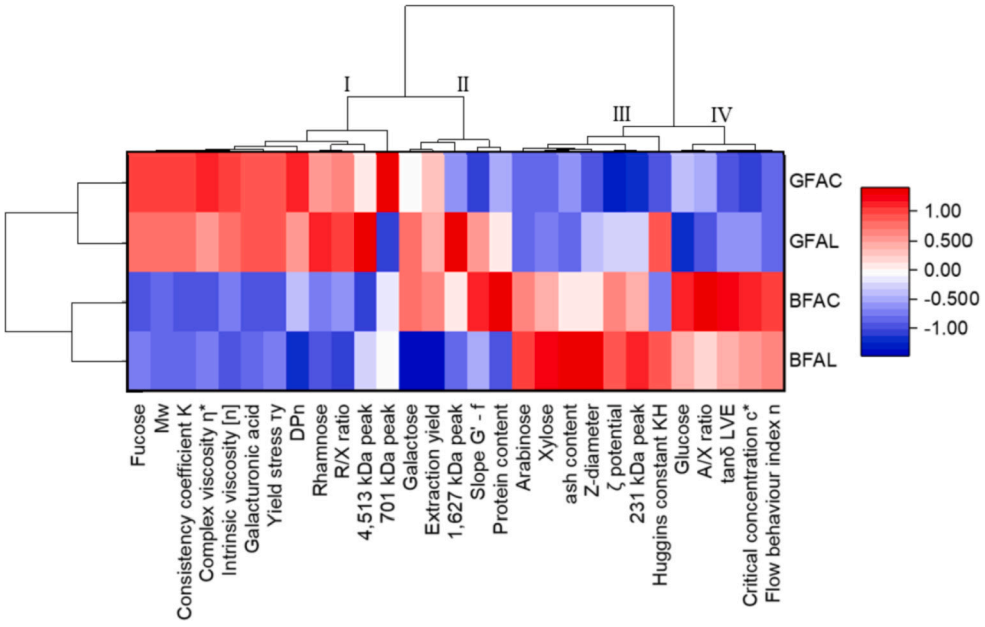

Fig. 9. Heat-map of flaxseed gum extracts as a function of their main physico-chemical, molecular, conformational and rheological properties. Flaxseed gum extracts are clustered by rows and properties by columns.

population comprising cluster II, a mixed effect was observed compared to the extraction conditions and flaxseed phenotype. Therefore, it appears that gum extraction yield, residual protein content and the partition of the AX-RG-I polymer composites in the total polysaccharidic fraction are concomitantly influenced by both the flaxseed phenotype and the pH of the aqueous extraction medium.

4. Conclusions

An alternative approach was studied for the extraction of water-soluble gum from flaxseed husk at pH conditions close to the isoelectric point of flaxseed proteins. Gum extraction at pH ≈ pI did not enable the significant deproteinisation of the gum extracts, most probably due to the intermolecular association of the residual proteins with the polysaccharides. The flaxseed phenotype (golden vs brown) was the most influential parameter of the osidic composition and structure conformational properties of the gum extracts. Four major polysaccharide fractions attributed to arabinoxylans (4513 kDa), rhamnogalacturonan-I (231 kDa) and two AX-RG-I composites (1627 and 701 kDa) were identified. Despite the compositional complexity of the gum extracts, it was observed that the acidic-extracted gums were characterised by lower amounts of AX and RG-I polysaccharide fractions compared to their alkaline counterparts. That explained the better solvation affinity and lower surface charge density of the acidic flaxseed gum extracts. Nevertheless, the polymer – polymer interchain associative phenomena (in both water and lyotropic media) were influenced only by the flaxseed phenotype. The steady state flow behaviour and viscoelasticity of the obtained flaxseed gum solutions (1–5% wt) was cross-dependent on the flaxseed phenotype and pH of the extraction media. All gum extracts exhibited good thermal and mild-acidic pH stability indicating their technological tangibility for food product applications.

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CRediT authorship contribution statement

Thierry Hellebois: Conceptualisation, Investigation, Formal analysis, Writing Original Draft: Writing-Review-Editing; **Jennyfer Fortuin:** Investigation; Formal analysis; **Xuan Xu:** Investigation, Formal analysis, Writing-Review-Editing; **Alexander Shaplov:** Investigation, Writing-Review-Editing; **Claire Gaiani:** Writing-Review-Editing, Supervision (TH); **Christos Soukoulis:** Conceptualisation, Writing-Review-Editing, Supervision (TH and JF), Project administration, Funding acquisition.

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