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Quality control of gum arabic – Molecular basis and a new screening method

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ABSTRACT

Gum arabic (GA) is commonly used as a stabilizer in aroma oil and beverage emulsions due to its amphiphilic character. However, different GA batches exhibit varying levels of functionality, which so far cannot be related to a molecular parameter. Therefore, the aim of our study was to investigate the relationship between the industrial quality of eight selected GA samples and the (macro)molecular structure and functionality of their glycoprotein fractions in detail, to establish a new quality parameter, and to develop a simplified analytical method for a direct quality assessment. Each GA sample was separated into the three major glycoprotein fractions, which were analyzed for their structural and functional properties. GA quality strongly correlated with the amount of arabinogalactan protein (AGP) and its molecular expansion, expressed via the gyration radius. Based on these results, a new quality parameter was defined as the product of AGP content and the volume of AGP molecules. Furthermore, the spatial expansion of AGP showed a strong positive correlation with the rhamnose and glucuronic acid content, identifying both as a marker for the degree of branching and molecular expansion. For the development of a simplified analytical method, the HIC separation was successfully scaled down, enabling a rapid quantitative isolation of AGP and the subsequent determination of the quality parameter with HPSEC-RI/UV/MALLS. By applying the method to 40 GA samples, it was demonstrated that it allows for a fast and reproducible analysis of GA quality. Thus, the developed analytical approach can complement or replace time-consuming emulsion stability tests.

1. Introduction

Gum arabic (GA) is an exudate primarily harvested from *Acacia senegal*, a species native to the gum belt in Africa. GA is composed of different glycoproteins and owing to its unique physicochemical properties, e.g. its amphiphilic character, it finds widespread application in diverse fields. For example, the gum is used as an adhesive or binding agent, but its application as food additive, e.g. as emulsifier and stabilizer for aroma oils and flavors in beverages, is most important. The European Commission Regulation No 231/2012 on specifications for food additives defines various purity criteria for GA (E414), but none of these parameters provide information on the functionality of the gum (European Commission, 2012). The same applies to the parameters investigated by Karamalla et al. (1998) which included pH, viscosity, uronic acid content of around 1500 authentic and commercial GA samples. The characteristics analyzed in this study also proved to be insufficient for determining GA quality. In a previous study, we investigated molecular characteristics such as monosaccharide composition, protein content, and molecular weight in comparison to the long-term emulsion stability of 20 different gum arabic batches, however, it was not possible to establish a link between the characteristics of the native sample and the quality (Kersten et al., 2025). So far, some studies suggested that the amount, size, and shape of protein-rich molecules in GA correlated with improved interfacial interaction and emulsifying properties (Al-Assaf et al., 2003, 2009; Castellani et al., 2010). However, this knowledge has not yet been transferred to the analysis of a structural parameter for quality control and has only been executed on a small

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Abbreviations

AG	Arabinogalactan
AGP	Arabinogalactan protein
GA	Gum arabic
GP	Glycoprotein
HIC	Hydrophobic interaction chromatography
HPSEC-RI/UV/MALLS	High performance size exclusion chromatography with refractive index, UV/Vis and multi-angle laser light scattering detection
SPE	Solid-phase extraction

number of samples. Nevertheless, these results imply that a subfraction of the gum may be decisive for the quality as an emulsifier.

GA is a mixture of heterogenous glycoproteins, which differ in their polysaccharide-protein-ratio. Using hydrophobic interaction chromatography (HIC), these glycoproteins can be separated into three fractions: arabinogalactan (AG, ~90 wt%, 1 % protein, M_W ~2.79 × 10^5 g/mol), arabinogalactan-protein (AGP, ~10 wt%, 10 % protein, M_W ~1.45 × 10^6 g/mol), and glycoprotein (GP, ~1 wt%, 25–65 % protein, M_W ~2.5 × 10^5 g/mol) (Osman et al., 1994; Randall et al., 1989; Renard et al., 2006). The differences in the structural composition between AG, AGP, and GP result in varying degrees of amphiphilicity and thus different interactions with oil/water interfaces. Investigations of the interfacial behavior revealed that mainly protein-rich GA molecules with higher molecular weight adsorb at interfaces and are responsible for the stability of the emulsion (Atgié et al., 2019; Han et al., 2019; Li et al., 2025; Rhazi et al., 2020; Wang et al., 2021). In contrast, protease degraded gum was not able to produce stable emulsions. This makes the proportion, shape, and size of AGP particularly interesting as a quality criterion, because this fraction has a high molecular mass, a high protein content, and represents a significant amount of the total gum (Osman et al., 1994; Randall et al., 1988; Renard et al., 2006). The spatial structure of AGP is often approximated as a wattle blossom-type arrangement (Duvallet et al., 1989; Fincher et al., 1983; Mahendran et al., 2008). Small-angle neutron scattering and small-angle X-ray scattering experiments have provided a more refined structural characterization, revealing an AGP architecture described as a thin, ellipsoid, and open disk-like structure (Isobe et al., 2020; Sanchez et al., 2008).

Although different studies have already indicated that AGP molecules are relevant for the stabilization of oil droplets, a correlation of its (macro-)molecular structure and the industrial quality has not been established. In addition, there is no simple and fast method for the quantification and characterization of AGP from GA. The most commonly used approach for emulsifier quality assessment in the industry involves analyzing and comparing the emulsion droplet size distribution before and after accelerated aging of the emulsion as described by Al-Assaf et al. (2003). In this test, an emulsion, prepared with one GA batch, is heated in a water bath at 60 °C for 7 days to simulate long-term storage by inducing heat-stress alterations. However, this method is labor-intensive, time consuming, and thus also cost-intensive. Therefore, the aim of this study was to identify a structural parameter of the GA glycoprotein fractions which correlates with industrial quality, and to establish a straightforward analytical method for a simple, rapid, and reproducible screening of industrial quality.

2. Materials and methods

2.1. Materials

The eight GA samples used in this study were provided by Willy Benecke GmbH (Hamburg, Germany) and ADM Wild Europe GmbH & Co. KG (Eppelheim, Germany). The additional 40 GA samples for the verification of the screening method were provided by Willy Benecke GmbH. If not stated otherwise, all GA samples were obtained from *A. senegal*. The specification and classification of the quality according to industrial standard was carried out by the suppliers, using the acceleration test and the change in particle size as described by Al-Assaf et al. (2003). The gum batches were classified in five qualities, ranging from class 5 – average emulsifier up to class 1 – very good emulsifier. All chemicals used were "p.a." grade or better, and were, if not stated otherwise, purchased from Carl Roth (Karlsruhe, Germany), Merck (Darmstadt, Germany), Thermo Fisher Scientific (Waltham, MA, USA) and VWR (Darmstadt, Germany).

2.2. Hydrophobic interaction chromatography

Eight previously characterized GA samples with varying qualities were selected for HIC separation. Every GA sample was dissolved in 4.2 M NaCl (c = 70 mg/mL, V = 50 mL) and subsequently fractioned with HIC as previously described by Randall et al. (1989). For this purpose, a glass column (2 cm × 50 cm, Latek, Germany) was filled with Phenyl-Sepharose CL-4B (bed height 40 cm, Cytiva Europe GmbH, Germany) and operated with a HPLC pump (P4.1 S, Knauer, Germany) at 0.8 mL/min 4.2 M NaCl (A), 2 M NaCl (B) and ultrapure water (C) were used as eluents with the following gradient: 0–360 min 100 % A, 360–730 min 100 % B, 730–950 min 100 % C, re-equilibration after each run with eluent A for 150 min. The fractions were monitored by using an UV detector at 214 nm (UVD 2.1 S, Knauer) and collected with a 16-port valve (VU 4.1, Knauer). The fractions were extensively dialyzed against ultrapure water and then freeze dried. The yield of the individual fractions was determined gravimetrically.

2.3. Accelerated aging of GA and AGP solutions

The stability of native GA solutions and AGP solutions derived from the eight GA samples with varying industrial quality was monitored before and after heating similar to the accelerated aging test described by Al-Assaf et al. (2003). 20 wt% GA solutions and 2 wt% AGP solutions were prepared in ultrapure water in 1.5 mL Eppendorf tubes and incubated at 60 °C and 600 RPM for 7 days (neoLabLine neoMix, NeoLab, Germany). Aliquots were taken before and after incubation, freeze-dried, and re-dissolved in 0.2 M NaCl. These solutions were subsequently analyzed with HPSEC-RI/UV/MALLS as described in section 2.4.

2.4. Molecular size distribution with HPSEC-RI/UV/MALLS

High-performance size exclusion chromatography with refractive index, UV/Vis and multi-angle laser light scattering detection (HPSEC-RI/UV/MALLS) was conducted on an Agilent HPLC system (1100 Series, Agilent Technologies, Santa Clara, CA) as described by Kersten et al. (2025). The system was equipped with a TSKgel G5000 PWXL column (30 cm × 7.8 mm i. d., 10 µm particle size, 100 nm pore size, Tosoh Bioscience GmbH, Germany). The separation was carried out at 40 °C and a flow rate of 0.4 mL/min using 0.2 M NaCl as eluent solution. The RI detector was operated at 35 °C. Freeze dried samples were dissolved in eluent (c = 2 g/L). Weight average molecular mass M_W, number average molecular weight M_N, and radius of gyration R_G were calculated by using WinGPC software (PSS polymer solutions, Germany).

2.5. Monosaccharide composition analysis

Methanolysis was executed as described by Deruiter et al. (1992) with the modifications described by Wefers and Bunzel (2015). Briefly, 0.1 mg of each AGP was hydrolyzed with HCl in methanol (0.5 mL, 1.25 M) at 80 °C for 16 h. The acid was evaporated and the residue was hydrolyzed with trifluoroacetic acid (TFA, 0.5 mL, 2 M) at 121 °C for 60 min. After evaporation and co-evaporation with ethanol, the analytes

were dissolved in ultrapure water and diluted. The monosaccharide and uronic acid composition was analyzed by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) on an ICS-3000 system (ThermoFisher Scientific, Germany) equipped with a CarboPac PA-20 column (150 × 3 mm, 6.5 μm particle size, ThermoFisher Scientific) at 30 °C. Gradient and separation conditions were used as described by Kersten et al. (2025).

2.6. Amino acid analysis and protein calculation

Amino acid hydrolysis was executed according to Anderson et al. (1985) with minor modifications. Briefly, 1.25 mg AGP was hydrolyzed with HCl (6 M, 500 μL) at 110 °C for 24 h. To prevent amino acid oxidation, the headspace of the samples was flushed with argon prior to hydrolysis. After evaporation of the acid and co-evaporation with methanol, the amino acids were dissolved in ultrapure water, filtered (0.22 μm, 13 mm, cellulose acetate), diluted, and used for HPAEC-PAD analysis.

The hydrolysates were analyzed by HPAEC-PAD on a Dionex ICS-6000 (ThermoFisher Scientific) equipped with an AminoPac PA10 column (2 × 250 mm, ThermoFisher scientific) at 30 °C. Separation of 10 μL diluted sample was carried out at a flow rate of 0.2 mL/min. Gradient conditions were adapted from Jandik et al. (2004). The detection waveform was adapted from Rohrer and Avdalovic (2021).

The individual amino acids were qualified by comparing their retention time with an amino acid standard solution (Sigma-Aldrich). Quantification was carried out via external calibration. The nitrogen content was calculated as a sum parameter of the amino acids. The protein content was calculated by applying a conversion factor of 6.60 as implemented by Anderson (1986).

2.7. Preparation of emulsions and measurement of droplet size distribution

Aroma oil-in-water emulsions were formulated by dispersing 10 wt% weighted orange oil (disperse phase) in a 1 wt% AGP solution (continuous phase). The dispersed phase consisted of a mixture of Essential and Brazilian orange oils combined at a ratio of 1:5. In order to achieve a density equivalent to that of water, a glycerol ester was incorporated into the oil mixture at a 1:1 ratio. In this study, only the weighted orange oil was utilized, and it will be referred to as "orange oil" henceforth. The dispersion process was carried out using a high-speed mixer (Ultraturrax T-25 digital, IKA® Werke GmbH & Co. KG, Staufen, Germany) operating at 10,000 rpm for 30 s. Subsequently, the emulsion premixes were further dispersed for an additional 2 min at the same speed. Fine emulsions were obtained by homogenizing the coarse emulsions using a high-pressure homogenizer (Small Volume ShearJet HL60, Dyhydromatics LLC, Kämpfelbach, Germany) at 12 kpsi (~800 bar) for two passes. Each emulsion was prepared in triplicate.

The droplet size distribution of the prepared aroma oil emulsions was measured in accordance with the method described by Kersten et al. (2025).

2.8. Solid phase extraction for AGP isolation

Solid phase extraction (SPE) was carried out based on the HIC separation described above. For this purpose, a cartridge (15 cm × 2.5 cm i.d.) was packed with 25 mL Phenyl-Sepharose CL-4B (Cytiva) with a bed height of at least 4.5 cm and mounted on a cartridge holder through which negative pressure can be exerted. The column was preconditioned with 50 mL of 4.2 M NaCl and then loaded with 5 mL of a filtered GA-solution in 4.2 M NaCl (c = 25 g/L). The AG fraction was subsequently removed by washing the column with 50 mL of 4.2 M NaCl. Next, 50 mL of ultrapure water were used for rinsing, whereas the first 10 mL of eluate were discarded and the following 35 mL of eluate were collected in a graduated falcon tube.

The collected eluate can be directly analyzed by HPSEC-RI/UV/MALLS, as described in section 2.4. Quantification of AGP was carried out via external calibration with AGP solutions prepared in 0.2 M NaCl (c = 0.15; 0.25; 0.5; 0.75; 1.0 and 1.25 g/L).

3. Results and discussion

3.1. Hydrophobic interaction chromatography and gravimetric quantification

Eight GA samples with varying industrial qualities were separated into their glycoprotein fractions AG, AGP, and GP by using HIC. Both the fractionation and the gravimetric quantification of each fraction were performed in six replicates per sample. The quality levels and corresponding gravimetric proportions are summarized in Table 1. The eight samples showed clear differences in the portions of the three HIC fractions. The average contents (86.5 % AG, 11.8 % AGP and 1.7 % GP) were consistent with previously described amounts (Osman et al., 1994; Randall et al., 1989). Furthermore, the average deviations for the main fractions were low, with variances of 1.5 % for AG and 8.8 % for AGP. The portions of the protein-rich fractions (AGP and GP) were of particular interest due to their relevance for the emulsion-stabilizing properties (Aphibanthammakit et al., 2020; Atgié et al., 2019; Han et al., 2019; Padala et al., 2009; Randall et al., 1988). However, our results suggest that the GP content alone does not allow any reliable conclusions on the functionality of the respective sample. The contents determined in the eight GA samples were relatively similar across all samples, had a high average relative standard deviation of 27.1 %, and were generally too low to be considered as the sole criterion for emulsion stability. In contrast, the AGP contents provided an interesting differentiation: samples ranked in the highest quality 1 (GA1; GA4; GA6 and GA8) are enriched in AGP (>12.0 %) and depleted in AG, whereas samples of lower quality displayed a reduced AGP content (<12 %). However, samples GA2 and GA3 represent exceptions to this trend: Despite its comparably high AGP content, GA2 was classified as an average emulsifier (quality 5), while GA3, with one of the lowest AGP amounts, still showed good emulsion stabilization capacity (quality 3). These results demonstrate that better emulsion stability does not correlate directly with higher AGP levels. Therefore, the ability of a GA batch to stabilize emulsions does not solely depend on the proportion of protein-rich fractions alone. It appears likely that the structural properties of AGP play an important role on GA interfacial behavior, as it has been suggested previously (Davantès et al., 2021).

3.2. Determination of macromolecular properties before and after accelerated aging

In addition to the portion of protein-rich moieties, selected studies indicated that the molecular mass of AGP may impact the interfacial behavior of GA (Al-Assaf et al., 2003, 2009). To gain insights into the macromolecular properties of the AGP fractions, they were analyzed with HPSEC-RI/UV/MALLS. Furthermore, the native GA samples and their corresponding AGP fractions were subjected to an accelerated aging process, which is also used for the industrial quality determination. After the treatment, the samples were again analyzed by HPSEC-RI/UV/MALLS to gain information on the changes of the molecular characteristics due to extended periods of heating. The elution profiles of a representative native GA sample and its AGP fraction before and after accelerated aging are shown in Fig. 1, the elution profiles of all eight native GA samples and their AGP fractions before and after heating are presented in Figs. S1–S8. The treatment resulted in some changes in the elution profiles of the AGP and GA samples. For the AGP samples, the results suggested that early-eluting, high-molecular-weight molecules are slightly degraded, which is indicated by a slight decrease in both RI and UV signal intensity within the volume range of 5–6.2 mL and a minor reduction in the maximum peak intensity. In addition, a clear

Table 1
Industrial qualities (from class 5 = average emulsion stability up to class 1 = very good emulsion stability) and gravimetrically determined contents of the HIC fractions of eight GA samples.

	Quality	1				3	5		
	No.	GA1	GA4	GA6	GA8	GA3	GA2	GA5	GA7
HIC fraction/%	AG	83.0 ± 2.1	86.5 ± 0.6	83.4 ± 1.5	82.5 ± 2.2	90.0 ± 0.5	86.8 ± 1.0	89.8 ± 1.3	90.3 ± 0.9
	AGP	14.9 ± 1.6	12.0 ± 0.5	14.7 ± 1.1	16.3 ± 2.1	8.1 ± 0.4	11.6 ± 0.8	8.7 ± 1.1	7.9 ± 0.8
	GP	2.1 ± 0.5	1.5 ± 0.2	2.0 ± 0.8	1.2 ± 0.3	1.9 ± 0.5	1.7 ± 0.5	1.5 ± 0.2	1.8 ± 0.7

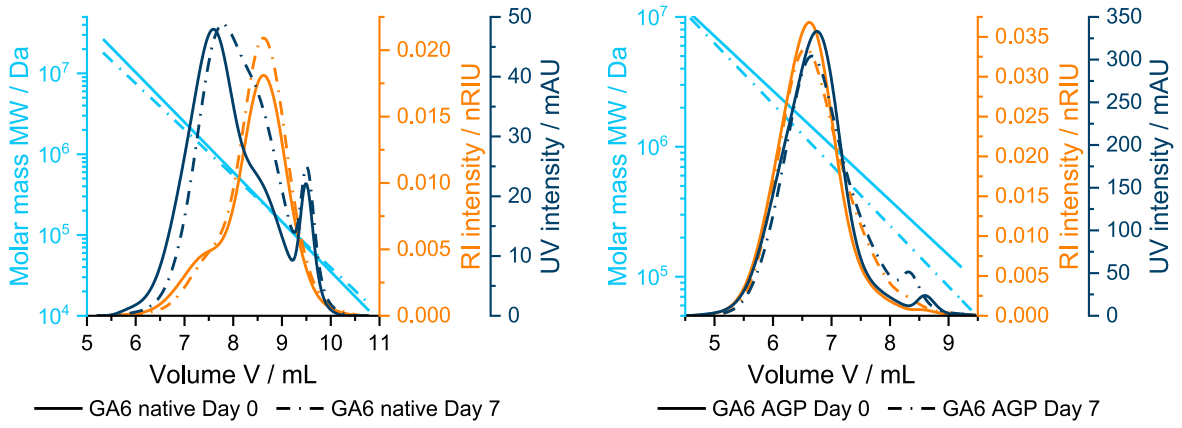

Fig. 1. HPSEC-RI/UV/MALLS elution profiles of the native GA6 sample before and after accelerated aging as well as GA6 AGP before and after accelerated aging.

increase in signal intensity in both the UV and RI detector can be observed between 7.3 and 8 mL which indicated the formation of low molecular weight degradation products. These results suggest that AGP, and particularly large AGP molecules, are sensitive to degradation upon heat exposure. The elution profiles of the native GA samples confirmed that especially high molecular weight molecules lose signal intensity in the elution range of 6–7.3 mL. In contrast, a pronounced increase in signal intensity was observed between 8 and 9 mL, corresponding to low-molecular weight degradation products. The protein content of the eluting molecules can be estimated by their UV absorption intensity at 214 nm. For AGP, a decrease in UV intensity is observed after accelerated aging. This implies that extended heat exposure over seven days likely leads to degradation of the protein portion within AGP. As a result, the protein-to-carbohydrate ratio shifts, effectively converting AGP into AG-like molecules accompanied by a reduction of the overall molecular weight.

The macromolecular characteristics of the eight AGPs before and after accelerated aging are shown in Table 2. Before accelerated aging, the AGP molecules of all eight GA samples have comparably high molecular masses (average M_W = 1445 kDa) with relatively low polydispersities (average M_W/M_N = 1.4). Notably, samples of good quality contained high molecular weight AGP >1400 kDa, whereas samples of lower quality contained low-mass molecules <1300 kDa. Once again, sample GA3 appears as an outlier, because it has a good functionality (quality 3), although its AGP showed a comparably low molecular mass.

Regarding the radius of gyration (R_G), which represents the average distance of a molecule's scattering sites from the center of mass, an interesting observation can be made. Although the differences in R_G between all eight samples were only minor, sample GA3 exhibits a comparably high radius before accelerated aging, demonstrating the presence of large AGP molecules. Apparently, the low portion of AGP in GA3 and its comparably low molecular weight is compensated by the significant spatial expansion of the corresponding molecules. The opposite can be observed for sample GA2, where the AGP content is high, but the molecules exhibit a clearly lower R_G. The macromolecular properties of the AGPs after accelerated aging demonstrate that the thermal exposure leads to structural alterations. In nearly all samples, a decrease in both molecular weight and radius of gyration of AGP was observed, accompanied by an increase in polydispersity. This confirms the substantial reduction in molecular size observed in the chromatograms (Figs. S1–S8) which was primarily attributed to the degradation of the protein moiety. Notably, samples of very good quality retained higher molecular weights (>1100 kDa) compared to those of lower quality which exhibited significantly reduced values (<950 kDa). Therefore, the degradation of high molecular weight AGP and/or the content of these components after heat treatment seems to play a role in the quality assessment by accelerated aging.

Table 2
Industrial qualities (from class 5 = average emulsion stability up to class 1 = very good emulsion stability) of eight selected GA samples and macromolecular characteristics of their AGP fractions determined with HPSEC-RI/UV/MALLS before and after accelerated aging.

	Quality	1				3	5		
	No.	GA1	GA4	GA6	GA8	GA3	GA2	GA5	GA7
AGP before accelerated aging	M_W/kDa	1513	1486	1606	2290	1151	1150	1271	1096
	M_W/M_N	1.3	1.4	1.4	1.3	1.4	1.5	1.5	1.5
	R_G/nm	27.4	26.2	27.6	38.4	27.4	19.1	25.6	21.2
AGP after accelerated aging	M_W/kDa	1144	1262	1166	2387	825	884	944	796
	M_W/M_N	1.6	1.6	1.6	1.5	1.6	1.7	1.8	1.8
	R_G/nm	17.0	17.8	20.0	35.0	15.6	12.1	17.8	16.4

3.3. Comparison and correlation of structural, macromolecular, and functional characteristics of the AGP fractions

To obtain information on the relation of different characteristics of the AGP fractions from the eight selected GA samples, they were additionally analyzed for their monosaccharide and uronic acid composition, as well as their protein content (results shown in Table S1). Furthermore, emulsions were prepared with each AGP and analyzed for their droplet size distributions of which the characteristic droplet size $x_{90,3}$ is reported (Table S1). The structural and functional properties of AGP were then correlated with each other using the Pearson correlation test. This test, which requires a normally distributed dataset, was applied to evaluate potential linear relationships, as well as the strength and direction of the linear correlations between functional and structural parameters. Fig. 2 shows the Pearson correlations of the different characteristics. For several parameter combinations, the correlation coefficient (R) fell within the range of −0.2 to 0.2, suggesting no significant linear relationship. However, certain parameters exhibited more pronounced correlations, which will be discussed in detail below.

As described in section 3.1. and 3.2., correlations can be observed between a) the industrial quality and the gravimetric portion of AGP, b) between the industrial quality and the spatial expansion (expressed via radius of gyration R_G) and c) between the spatial expansion and the molecular weight M_W. Those relationships are also reflected in the Pearson correlation matrix as all three parameters show a strong anti-correlation with the industrial quality ($R_{\%AGP} = -0.79$, $R_{Mw} = -0.71$; $R_{RG} = -0.67$). This again indicated that a high AGP content and a high molecular mass or radius of gyration of the AGP molecules is important for the industrial quality. Interestingly, no other structural or functional feature suggested such a pronounced linear correlation with the quality. However, several correlations between the various characteristics were observed. In order to structure these observations, one has to keep in mind, that R_G is directly proportional to M_W *(power law: $R_G \propto M_W^n$)*. Molecules possessing larger M_W will therefore also exhibit larger R_G values. This is also reflected in the Pearson correlations which gave an R of 0.91 demonstrating a linear relationship between R_G and M_W. Therefore, in the following, only the relationships between M_W and other structural parameters are discussed, since the same correlations also apply to R_G. Very strong correlations can be observed between the molecular weight and the relative monosaccharide composition: Arabinose (Ara) and galactose (Gal) show a (very) strong negative linear correlation with M_W ($R_{Ara} = -0.93$, $R_{Gal} = -0.68$), whereas rhamnose (Rha) and glucuronic acid (GlcA) exhibit very strong, positive linear correlations ($R_{Rha} = 0.92$, $R_{GlcA} = 0.87$). This leads to the conclusion that a higher portion of Gal or Ara and a lower portion of Rha and GlcA is associated with smaller AGP molecules. This interpretation aligns well with the literature: analyses of side chain structures after enzymatic digestion of the galactan backbone in GA have shown that Rha and GlcA dimers are found at terminal positions in the side chains of the galactan backbone (Cartmell et al., 2018; Sasaki et al., 2022). Additionally, methylation analysis of GA identified Rha residues predominantly in terminal positions of the polysaccharides (Akiyama et al., 1984; Lopez-Torrez et al., 2015; Nie et al., 2013). Therefore, the relative Rha content in AGP can be interpreted as a marker of the degree of branching and, consequently, of molecular expansion. This is confirmed by the results we have already published for native GA (Kersten et al., 2025). Interestingly, the portion of Ara in AGP was correlated with a lower molecular weight, even though Ara also appears in galactan side chains. But Ara may form only short oligosaccharide side chains, branching the galactan. Those occur frequently but, due to their short chain length, do not significantly contribute to the overall spatial expansion (Nie et al., 2013; Sasaki et al., 2021). Furthermore, Ara content correlated strongly negative with Rha ($R = -0.99$) and GlcA content ($R = -0.96$). Those results indicate that the ratio of Ara/Rha (or Ara/GlcA) significantly influences the spatial expansion of AGP molecules.

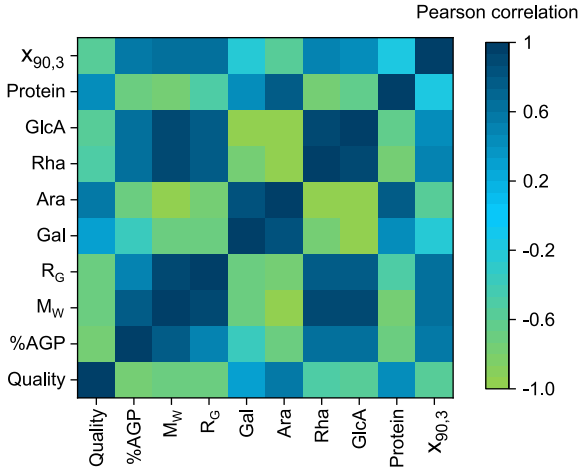

Fig. 2. Pearson correlation matrix of structural, macromolecular, and functional properties of AGP acquired from 8 GA samples with varying industrial qualities.

In addition to the correlations between spatial expansion and the monosaccharide composition, a strong negative linear correlation between M_W and the protein content in AGP could be observed ($R = -0.75$). This relationship indicates that an increasing protein content in AGP is associated with a decrease in AGP molecular weight. However, this correlation is less pronounced than the correlations between M_W and Rha or GlcA. These findings suggest that the parameters are interrelated as a shift in carbohydrate-to-protein-ratio is linked to an increase or decrease in molecular spatial expansion.

The characteristic droplet size $x_{90,3}$ of the emulsions also showed a positive linear correlation with the spatial expansion of AGP molecules, as indicated by molecular weight ($R = 0.63$). An increase in molecular weight or radius of gyration was associated with larger droplets in AGP stabilized emulsions. Interestingly, this trend contrasts with observations made for native GA, where $x_{90,3}$ correlated negatively with molecular weight, although the relationship was less pronounced (Kersten et al., 2025). Since the correlation in AGP between the M_W and $x_{90,3}$ is stronger than the one in native GA, it can be assumed that AGP is generally most relevant for functionality of GA. These findings agree with previous observations (Al-Assaf et al., 2009; Aphibanthammakit et al., 2020; Atgié et al., 2019; Castellani et al., 2010; Han et al., 2019; Li et al., 2025). The reduction in droplet size with lower M_W indicates that AGP is generally characterized by rather slow adsorption kinetics in comparison to the time given for interfacial stabilization within the homogenization process. Molecules with smaller spatial expansion typically exhibit faster adsorption kinetics. This can result in smaller emulsion droplet sizes provided that the interfacial stabilization is sufficient once the molecules have adsorbed (Schmidt et al., 2017). Regarding the emulsion stabilizing properties, one can also notice that there is no linear correlation between $x_{90,3}$ and the protein content of AGP ($R = -0.18$). This demonstrates that the protein content is of less significance to emulsion stabilization than other structural features such as branching. This is in line with previous results of our group that showed that the emulsifying properties of sugar beet pectin depended more on the Ara portion than on the protein content (Bindereif et al., 2021).

3.4. Establishment of a new quality parameter

The obtained results suggest that both the AGP content and the spatial expansion of the AGP molecules contribute to the long-term emulsion-stabilizing properties. To transfer this concept into a measurable and calculable quality parameter, the AGP content of the eight samples was multiplied by the estimated volume of the respective AGP

molecules. For an exact volume estimation, knowledge of the molecular shape is necessary. In the literature, the AGP molecules are described as thin, ellipsoidal discs (Isobe et al., 2020; Sanchez et al., 2008). An exact volume calculation would require additional data on the extension of the ellipsoid in all axial directions, which cannot be solely obtained from MALLS measurements. Therefore, AGP volume was estimated as a sphere and the quality parameter was calculated for each of the eight samples using the following equations:

$$\text{Quality parameter} = (\text{AGP}\% \times V_{AGP}) \ / \ 10,000 \quad \text{(equation 1)}$$

$$V_{AGP} = \left(\frac{4}{3} \times \pi \times R_G^3 \right) \quad \text{(equation 2)}$$

Equation (1) uses the gravimetrically determined AGP content after freeze-drying in percent, multiplied with the AGP molecules volume, which is based on the radius of gyration determined at the peak maximum (equation (2)). The result of the calculation is divided by a factor of 10,000 to enable a more intuitive and comparable representation of the quality parameter which should be considered as an empirical value rather than a mechanistic descriptor. The results of the calculation for the eight GA samples are shown in Fig. 3, and they clearly allow for a differentiation of the different industrial qualities by using the experimentally determined and calculated product as a quality parameter. Samples classified as quality level 1 exhibit high values (>90) compared to those of average quality (<60). Furthermore, GA3, assigned to quality level 3, is positioned between the other quality levels. Notably, the values for the quality parameter within a given quality category showed considerable variability. For quality level 1, values ranging from 90 to 385 were observed, while for category 5, the values ranged from 30 to 60. Although the ranges within each quality category are relatively broad, the categories are still clearly distinguishable from one another. These findings demonstrate that neither AGP content nor R_G provided a reliable indication of functional performance and that only the combined product of both characteristics allows for a meaningful correlation of molecular parameters and the industrial quality of GA. Notably, the simple multiplication of AGP content with R_G or M_W did not allow for a differentiation of the quality levels.

3.5. Development of a screening method for rapid quality assessment

Based on the findings in the previous sections, it can be concluded that the product of AGP content and AGP volume can be used to describe the industrial quality standard. However, since preparative HIC separation is both labor- and time-intensive, a quick and easy screening method was developed. For this, the HIC separation was scaled down and implemented as an SPE for the rapid isolation of AGP. The aim of this procedure was to separate AG from AGP, as these two fractions partially co-elute during the SEC separation and prevent the direct analysis of AGP by HPSEC-RI/UV/MALLS. Therefore, a cartridge (15 cm × 2.5 cm i. d.) was packed with 25 mL Phenyl-Sepharose CL-4B, the same material which is used for preparative HIC. The column was preconditioned with 4.2 M NaCl, to ensure maximum retention capacity, and then loaded with 5 mL of a filtered GA solution in 4.2 M NaCl. After elution of the AG fraction with 4.2 M NaCl, the AGP fraction was eluted by rinsing the column with 50 mL of ultrapure water. The first 10 mL of eluate were discarded as these mark the dead volume of the SPE column. Thus, this fraction does not contain any AGP, but high amounts of salt, which negatively impact the signal-to-noise ratio of the subsequent MALLS detection. The following 35 mL of eluate were collected in a graduated falcon tube. The collected eluate contains mainly AGP and negligible amounts of GP. Therefore, it can be directly analyzed by HPSEC-RI/MALLS without further dilution. The AGP yield was quantified via refractive index detection using an external calibration with AGP from GA1. The calibration was carried out by using the AGP fraction from only one sample, as the structural characterization revealed similar compositions and HPSEC-RI/UV/MALLS elution profiles for all eight analyzed AGPs (see also Figs. S1–S8). The characteristics of the calibration are shown in Fig. 4A. The AGP concentration and the signal intensity of the RI detector indicate a linear relationship. The data provided a very low degree of scatter and an excellent linear fit, as reflected by an R^2 value of 0.9997. The sample solutions obtained from the SPE cleanup fell within the lower to mid-range of the calibration curve (between 0.25 and 0.75 mg/mL).

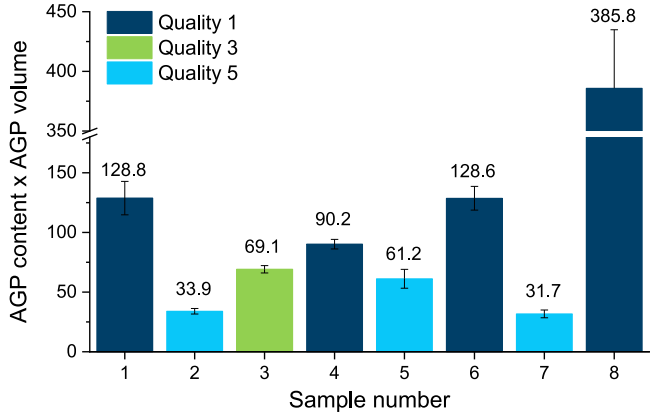

Fig. 3. Results of the quality assessment based on AGP content multiplied with the molecular volume of AGP molecules. The two parameters were determined gravimetrically and by using HPSEC-RI/UV/MALLS. The industrial qualities determined by the suppliers (quality 1 – very good up to quality 5 – average) are marked in different colors.

To evaluate the accuracy and repeatability of the scaled-down method, the eight previously fractioned GA samples were purified twice by using the adapted SPE protocol for AGP isolation and analyzed by using the developed HPSEC-RI/UV/MALLS method. The AGP concentration in the SPE eluate can be directly determined by placing the peak area into the linear regression equation. Taking the volume of the SPE eluate into account, the AGP content of the originally used GA can be calculated. For better comparability with the results from preparative HIC, it proved useful to normalize the AGP content to the dry matter of the GA sample. For spray-dried GA samples GA1, GA2, GA3, GA6 and GA7, a mean dry matter content of 90.7 % was used, and 86.4 % for kibbled gum samples GA4, GA5 and GA8 (mean values from Kersten et al. (2025)). A comparison of the AGP contents from preparative HIC and the two independent SPE separations is shown in Fig. 4B. On average, the AGP contents obtained from both SPE purifications showed very good recovery of the values determined by preparative HIC. The first SPE separation series yielded a mean recovery of 103.1 %, while the second series showed a mean recovery of 106.0 %. As also shown in Fig. 4B, some deviations can be observed in the results of the two independent SPE separations. In the first separation series, this includes the AGP contents of samples GA5 and GA6, and in the second series the AGP content of sample GA7, each deviating by approximately ±25 % from the expected value from preparative HIC. Since the outliers differ between the two SPE separation series, a random error can be assumed. This type of error can be minimized through careful sample weighing, precise execution of the SPE purification, and accurate integration of peak areas. Nonetheless, the robustness and reproducibility were tested via a third SPE series by determining the measurement uncertainty caused by SPE clean-up. For this, GA6 was again fractionated three times by using SPE. The following AGP contents were obtained: 12.24 %, 14.10 % and 13.88 %. The clean-up triplicate yielded a mean recovery of 91.5 % of the expected value from preparative HIC separation. On average, 13.41 % AGP was detected in the sample, with a deviation of ±1.02 %. This corresponds to a relative standard deviation of 7.6 %, which is acceptable for the chosen sample clean-up method. These results demonstrate a sufficient reproducibility and robustness of the SPE

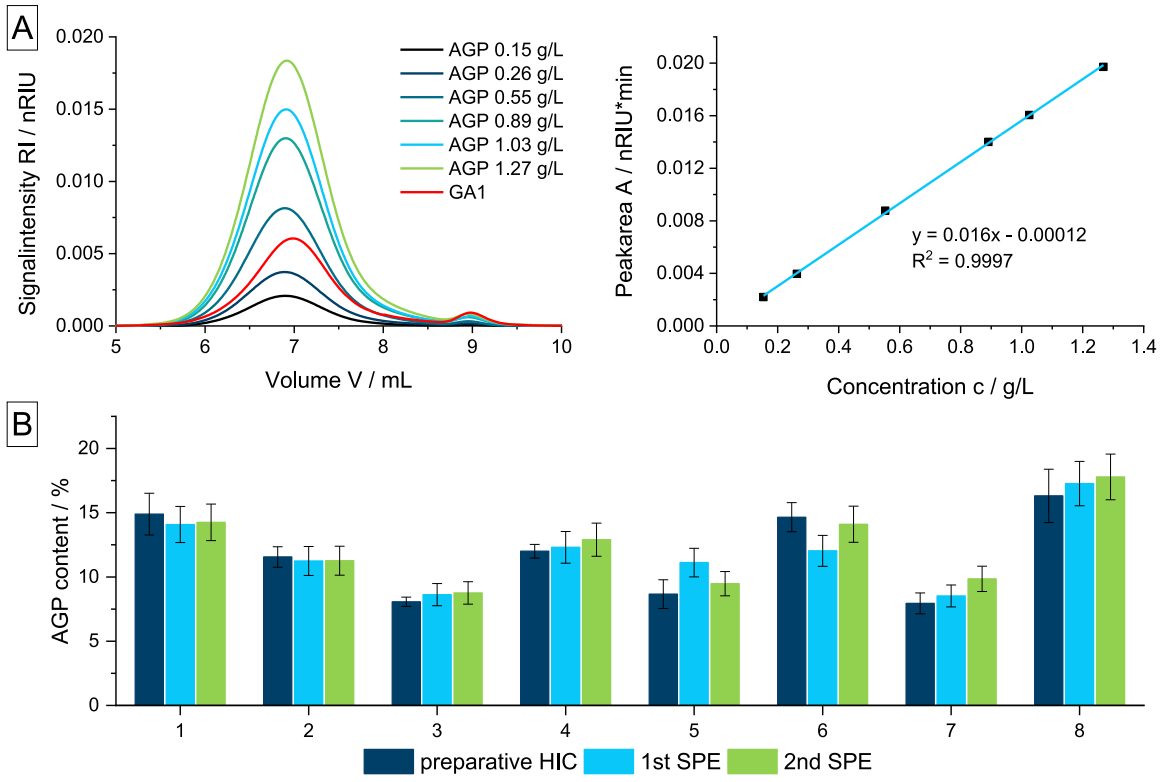

Fig. 4. A: Stacked HPSEC-RI chromatograms and calibration curve of the external calibration for AGP; B: comparison of the AGP contents of eight GA samples which were determined by preparative HIC and both SPE clean-up series.

clean-up. Considering the measurement uncertainty associated with the SPE clean-up and the chromatographic separation and detection technique, an overall measurement uncertainty of ±10 % can be assumed. The purification of AGP via SPE followed by direct quantification using HPSEC-RI/UV/MALLS by using an external calibration is therefore considered a quick, reliable, and reproducible screening method for determining AGP content and spatial expansion in native GA.

3.6. *Verification of the method for quality assessment*

To evaluate the suitability of the screening method as a tool for quality estimation of GA, a total of 39 *Acacia senegal* gums of known quality and one *Acacia seyal* gum were subjected to SPE purification and analyzed by HPSEC-RI/UV/MALLS. As described in the previous section, the newly introduced quality parameter was calculated by multiplying the AGP content of each sample with its spatial expansion (calculated from R_G). The calculated quality parameters as well as the industry quality classifications of the samples are shown in Fig. 5. The complete results (AGP content, R_G, and the calculated quality parameters) are shown in Table S2. The results clearly demonstrate that the calculated quality parameter correlates very well with the industrial quality and thus emulsion stability. Samples of very good quality exhibited higher AGP contents and larger radii of gyration compared to those of lower quality. Several samples classified as quality level 1 stand out significantly from the rest, with calculated values exceeding 150. However, the remaining samples are more closely clustered, with values ranging from

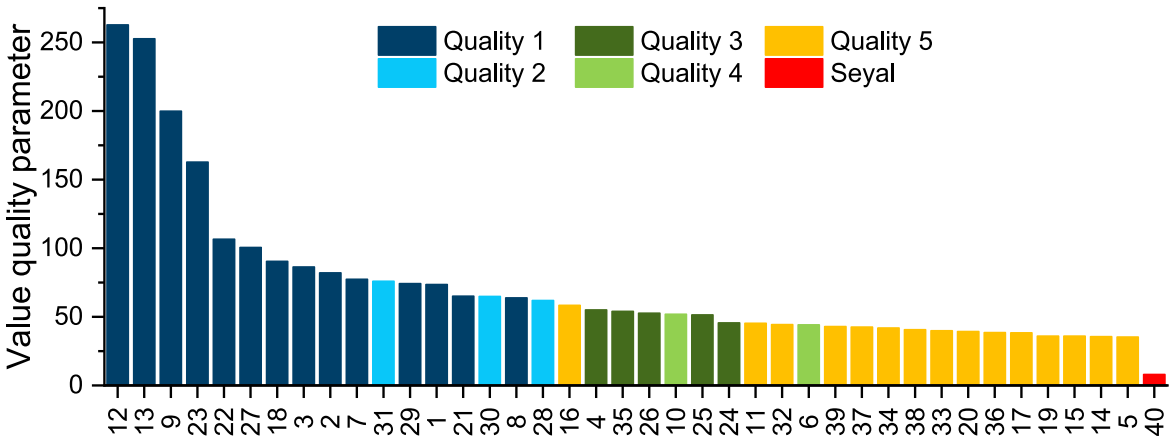

Fig. 5. Quality parameters (calculated from AGP content and spatial expansion of the AGP molecules) of 39 A. senegal gums and one A. seyal gum with varying industrial qualities (marked in different colors).

35 to 100. Some overlap between the quality levels could be observed, particularly for level 2, which partially appeared among samples classified as level 1. Similarly, quality level 4 cannot be clearly distinguished from levels 3 and 5. The overlap between these samples may be caused by slight variations in the determined AGP contents or R_G values, and performing multiple replicates could potentially improve the separation between the samples. Furthermore, variations in the assessment of industrial quality may contribute to the partially overlapping values. However, a distinction between five quality levels, as specified by the supplier based on the accelerated aging test, does not appear feasible with the developed experimental setup. It should also be noted that the industrial quality classification is subjective and may vary from one manufacturer to another. Nevertheless, the proposed screening method allowed for a similar but broader quality differentiation of only four quality levels, which translate into long-term emulsion stability: very good quality >60, good quality 45–60, moderate quality 35–45 and poor quality <35. One outlier is evident in the developed quality classification: GA16. Although this sample demonstrated average emulsion stability, it showed a relatively high AGP content, and particularly a large radius of gyration (Table S2). Thus, in this case, the experimentally determined quality parameter does not match the industrial quality even by taking the measurement uncertainty of 10 % into account. The outlier behavior of this sample may be attributed to the single measurement and potential inhomogeneity of the sample material, as only ~125 mg of the sample was used for SPE. Also, GA16 was an unprocessed, kibbled GA sample and therefore prone to a heterogeneous distribution of its glycoproteins throughout the batches nodules. For the application of the screening method in routine analysis, replicate measurements would be advisable for larger batch quantities and heterogeneous samples such as kibbled GA.

In addition to the 39 *A. senegal* gums, one *A. seyal* gum was analyzed. It was notable that the *A. seyal* gum exhibited a very low value for the calculated parameter (8.04), clearly distinguishing it from the *A. senegal* samples. Furthermore, the *A. seyal* sample can be classified as a sample with very poor quality. This suggests that it is not suitable for the production of long-term stable emulsions, which was also specified in the literature (Flindt et al., 2005; Gashua et al., 2016).

Altogether, the analysis of this large set of samples confirmed that the developed screening method is well suited for a rapid determination of the industrial quality of GA. Nevertheless, it is advisable to consider this method as complementary to existing approaches for assessing GA emulsifying behavior, as it cannot replace an analysis of the interfacial properties of GA in a specific formulation.

4. Conclusion

Although a lot is known about the interfacial behavior of gum arabic, a standardized parameter for predicting emulsion stability based on structural properties has not been established so far. With our results, we were able to demonstrate that a combination of the AGP content and the spatial expansion of the molecules (calculated from the radius of gyration) correlated with the functionality of a gum arabic batch in industrial applications. Furthermore, our results indicated a spatial enlargement with an increase in polymer branches containing rhamnose and glucuronic acid. Accelerated aging experiments revealed that extended periods of heat exposure degraded the protein core of AGP molecules, decreasing their molecular weight and spatial expansion. Moreover, HIC-based SPE purification and a subsequent direct analysis of isolated AGP with HPSEC-RI/UV/MALLS allowed for the development of a rapid and reliable screening method for the prediction of quality. Through the analysis of a set of 40 gum arabic samples with varying qualities, we were able to confirm that the method is suitable to differentiate four distinct quality levels. Therefore, the newly established quality parameter as well as the developed screening method are well suited for use in industrial practice complementary to existing quality analysis protocols.

CRediT authorship contribution statement

Frederike Kersten: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Désirée Martin:** Writing – original draft, Methodology, Investigation, Formal analysis. **Ulrike S. van der Schaaf:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization. **Daniel Wefers:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization.

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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.2026.112454.

Data availability

Data will be made available on request.

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