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Original article

Emulsifying properties of Ghanaian grewia gum

Fidelis Mawunyo Kpodo,[1] Jacob Kwaku Agbenorhevi,[2] Katerina Alba[3] & Vassilis Kontogiorgos[3]*

1 Department of Nutrition and Dietetics, University of Health and Allied Sciences, Ho, Ghana
2 Department of Food Science and Technology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana
3 Department of Biological Sciences, University of Huddersfield, Queensgate HD1 3DH, UK

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Abstract Grewia gums were extracted with phosphate (PB) and sodium metabisulphite buffers (SMB) and their emulsification properties in acidic oil-in-water emulsions on ageing were studied by means of droplet size distribution, ζ-potential measurements, interfacial composition analysis and viscometry. PB extracts showed smaller droplet sizes (~10 μm) than SMB isolates (>35 μm) and greater long-term stability. PB-stabilised emulsions also demonstrated the least polysaccharide (~0.6 mg m^{-2}) and protein (~0.2 mg m^{-2}) interfacial coverage compared with SMB counterparts (~1.5 mg m^{-2} for polysaccharide and ~ 1 mg m^{-2} for protein). ζ-Potential measurements revealed negative interfacial charge for all emulsions confirming the presence of polysaccharide-laden interfaces. Droplet size distribution also varied among emulsions during ageing indicating a complex relationship between interfacial composition and stability. The present work shows that different emulsifying properties may be obtained depending on the extraction technique employed that could be exploited in preparation of emulsions for flavour or bioactive-delivery applications.

Keywords Emulsion, *Grewia mollis*, polysaccharide, viscosity.

Introduction

Plant polysaccharides are one of the most abundant biological materials that are widely employed as functional ingredients to improve the physical properties of drug, food and non-food formulations (Amid & Mirhosseini, 2012, Vasile *et al*., 2016, Porto & Cristianini, 2018, Salarbashi & Tafaghodi, 2018, Crispín-Isidro *et al*., 2019, George *et al*., 2018, Ma *et al*., 2017). One of the functional properties that is very frequently exploited is their capacity to stabilise oil-in-water emulsions (Porto & Cristianini, 2018; Dickinson, 2018), as some polysaccharides may play an essential role as surface active agents that facilitate emulsion stability by lowering the interfacial tension between immiscible phases (Grein *et al*., 2013). The surface activity of polysaccharides that contributes to emulsion stability is largely attributable to the presence of hydrophobic groups for molecular attachment at the oil interface and to chain branching to sterically stabilise oil droplets (Kpodo *et al*., 2018, Dickinson, 2018, Alba & Kontogiorgos, 2017, Kontogiorgos, 2019).

Grewia gum is extracted from the inner stem bark of *Grewia mollis*, a tropical shrub widely distributed in tropical and sub-tropical Africa that belongs to the *Malvaceae* family (Kpodo *et al*., 2019, Nep *et al*., 2016). Grewia gum has been employed as hydrophilic matrix to control drug release because of its ability to hydrate and swell upon interaction with fluids (Nep *et al*., 2015). Grewia gum has also been explored as a binder and suspending agent in the formulation of pharmaceutical oral drugs (Nep & Conway, 2011, Nep *et al*., 2015). Although its use in food formulations has not been extensively evaluated, the pulverised inner bark from the stems of the shrub is already used as thickeners in ethnic food preparation. Grewia gum consists of polysaccharides that are acidic with substantial amounts of rhamnose, arabinose and protein (Kpodo *et al*., 2019) suggesting the presence of branches that may act as efficient steric barriers against coalescence. Studies have shown that polysaccharides of the same plant species with different extraction protocols exhibit variations in sugar composition, protein content, molecular weight and structure (Kpodo *et al*., 2018, Amid & Mirhosseini, 2012, Ghori *et al*., 2017, Samavati, 2013). In our previous studies, grewia obtained from Ghana was used to isolate polysaccharides with similar chemical composition but different macromolecular characteristics resulting in

*Correspondent: Email: v.kontogiorgos@hud.ac.uk

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distinct flow properties (Kpodo *et al*., 2019). Although the functional properties of polysaccharides from other members of the *Malvaceae* family such as *Abelmoschus esculentus* have been extensively evaluated (Kpodo *et al*., 2018, Alba *et al*., 2013, Alba *et al*., 2015, Alba *et al*., 2016), the emulsifying properties of those obtained from *Grewia mollis* remain unexplored. Furthermore, extracts isolated from *Grewia mollis* demonstrated significant diversity in macromolecular characteristics, which may uniquely influence its functionality as an emulsifier.

The present work builds on our previous investigations that have been targeting emulsifiers that may operate at acidic pH, as it is of substantial importance for two main reasons. Soft-drink industry continuously explores novel flavour-encapsulation materials in an attempt to optimise flavour-oil emulsification stability and flavour release. Since the majority of beverages are acidic with pH values frequently lower than 4.0, macromolecular emulsifiers that operate at low pH environments would be particularly desirable, as they may lead to different oral behaviour and flavour release (Hu *et al*., 2019). Additionally, the need to create advanced formulations that bypass gastric environment, delay lipid digestion and deliver bioactives in the gastrointestinal tract at the site of interest has boosted the research on the fundamental properties of polysaccharides at interfaces (Araiza-Calahorra *et al*., 2018, Gasa-Falcon *et al*., 2017, Hou *et al*., 2014, Ahmed *et al*., 2012). To that end, polysaccharide-based delivery systems may resist both proteolytic enzymes and the acidic gastric environment that limit the functionality of protein and surfactant-based formulations. Nevertheless, polysaccharides need to be tailored to enhance their adsorption strength and overall functionality at the interface (Kontogiorgos, 2019).

Hence, in the present study, the hypothesis that grewia polysaccharides of varied molecular architecture may proffer tailored functionality, as surface active agents in the fabrication of oil-in-water emulsions at acidic pH, was formulated. The objective, therefore, was to investigate the emulsifying properties of grewia gums isolated with different extraction buffers and elucidate how the different macromolecular structures may influence the formation and stability of oil-in-water emulsions in acidic environments.

Material and methods

Materials

The dried *Grewia mollis* inner stem bark was purchased from the local market in the Northern Region, Ghana. D-galactose (Gal), buffer salts, sodium azide, ethanol (96% w/w) and sodium metabisulphite were purchased from Sigma-Aldrich (Poole, UK). Deionized water was used throughout the extraction experiments. All reagents used were of analytical grade.

Extraction of grewia gum

The dried Ghanaian *Grewia mollis* inner stem bark was milled into a particle size of 450 μm (ZM 1000, Retsch, UK) and then subjected to extraction using sodium metabisulphite solution (SMB, 5 mM, pH 4.5) or phosphate buffer (PB, 100 mM, pH 6; Kpodo *et al*., 2019). The first extraction step yielded crude samples (SMBC, PBC) and exhaustive dialysis against deionized water for three days produced purified samples (SMBP, PBP). Key molecular characteristics of the isolated materials that are relevant to this work are reproduced in Table 1.

Emulsion preparation

Preliminary experiments on the optimum concentration of grewia extracts for emulsification were carried out with increments of 0.5% between 0.5% and 3% w/v. Reduction of droplet sizes levelled off at concentration of 1.5% w/v in the entire emulsion. As a result, the capacity of the four extracts to act as emulsifiers was investigated by means of emulsifying sunflower oil into an aqueous medium buffered at pH 2.0 containing 1.67% w/v of extract. 10 mL of oil was added to 90 mL of continuous phase so as to yield emulsions with $\varphi = 0.1$ of a nominal extract concentration in the entire emulsion volume of 1.5% w/v. Sodium azide (0.02% w/v) was added as preservative to prevent microbial growth. Emulsion preparation at 25 °C followed a two-step procedure, commencing with a primary homogenisation to obtain pre-emulsions with a high-speed homogeniser for 2 min (IKA T18 basic, Ultra-Turrax, Staufen, Germany) and followed by a secondary dispersion using ultrasonication (UP 100H Hielscher Ultrasonics, Teltow, Germany) with an MS7 tip at 30 kHz for 40 s with pulsed ultrasound (30% per second) at 100% amplitude.

Table 1 Chemical composition of grewia gums extracted in different buffers. Data reproduced with permission from Kpodo *et al.* (2019)

Sample	Protein (% w/v)	Total carbohydrates (% w/v)	Total uronic acids (mol%)	M_w ($\times 10^6$ g mol^{-1})
SMBC	16.5	56.5	45.7	2.8
SMBP	14.5	65.3	43.5	1.7
PBC	15.5	53.4	34.8	0.92
PBP	11.1	66.9	42.7	0.75

Determination of droplet size distribution

Droplet size distribution curves and average droplet sizes were measured at set time intervals (0, 5 and 15 days) using a Malvern Mastersizer 2000 (Malvern Instruments Ltd., Worcestershire, UK) laser diffraction particle size analyzer using the small volume sample dispersion unit Hydro 2000SM (Malvern Ltd., UK). Refractive index of sunflower oil and dispersion medium (HCl/KCl buffer, pH 2) were set to 1.435 and 1.333, respectively. Consequently, droplet sizes of the emulsions were described using the volume-weighted mean diameter ($d_{4,3}$).

Interfacial composition analysis and viscosity measurements

The interfacial composition of emulsions was characterised in terms of adsorbed protein and polysaccharide at the oil-water interface. Emulsions were centrifuged at 3000 ***g*** for 5 min (Centrifuge 5702, Eppendorf, Hamburg, Germany) in order to separate the dispersed phase (oil droplets) from the continuous phase (serum), and the serums were collected using a syringe. Concentrations of protein and polysaccharide (expressed as total carbohydrate) were measured in polysaccharide solutions (i.e. aqueous phase before emulsification) and in serums according to Bradford method (1976) using bovine serum albumin as calibration curve standard and phenol-sulphuric acid assay (DuBois *et al.*, 1956) using D-galactose as standard, respectively. Interfacial protein and polysaccharide concentrations (Γ, mg m^{-2}) were calculated as follows:

$$\Gamma = \frac{\text{mg of adsorbed protein or pectin}}{\text{SSA} \times \text{mL of oil in emulsion}}$$

where SSA is the specific surface area (SSA, m^2 mL^{-1}) obtained from the analysis output of the instrument (Malvern Instruments Ltd., Worcestershire, UK). The span of a volume-based size distribution that is defined as Span = $(d_{90}–d_{10})/d_{50}$ was also obtained by the analysis report of the instrument (Malvern Instruments Ltd., Worcestershire, UK) and is an indication of the width of distribution. ζ-Potential measurements of grewia-stabilised emulsions were performed using a ZetaSizer Nano Series ZEN2600 (Malvern Instruments, Malvern, UK) at 25 °C. Emulsions were diluted 1000 times in buffer to avoid multiple scattering effects. All measurements were performed in triplicate immediately after emulsion preparation (day 0) and after 5 and 15 days of storage. Viscosity curves of emulsions were obtained using a Bohlin (Gemini 200HR, Malvern, UK) nano-rotational rheometer equipped with cone-and-plate geometry (40 mm diameter, cone angle 4°). Steady shear measurements (0.01–1000 s^{-1} at 25 °C) were performed on fresh emulsions, and during storage (5 and 15 days).

Statistical analysis

Data obtained were analysed using Statgraphics (Graphics Software System, STCC, Inc. USA). Comparisons between the different treatments were done using analysis of variance (ANOVA) and differences between means were determined with LSD at 5% level of significance ($P < 0.05$).

Results and discussion

Droplet size distribution analysis

The average droplet size of emulsions stabilised by PBP, PBC, SMBP and SMBC samples were investigated at set time intervals for a period of fifteen days. Table 2 shows the results of $d_{4,3}$ values and span of the grewia-stabilised emulsions. PB-stabilised emulsions generally recorded smaller droplets compared with their SMB-stabilised counterparts indicating that extraction conditions influence polysaccharide functionality at the oil-water interface (Fig. 1). Polysaccharides extracted from different botanical sources exhibit diverse interfacial functionality yielding emulsions with a wide range of droplet sizes depending on the specific structure of the chains, functionalisation or protein content. For example, the average droplet size was comparable to that reported for emulsions stabilised with Angum gum or okra pectin (Jafari *et al.*, 2013, Kpodo *et al.*, 2018) but higher than those stabilised with basil seed gum (Osano *et al.*, 2014). The average droplet diameter generally increased during ageing with PB-stabilised emulsions manifesting slower coarsening kinetics than SMB-stabilised emulsions throughout storage. The smaller droplet sizes observed for emulsions stabilised with PB extracts show that extraction method modifies the macromolecular characteristics and influences the surface activity of grewia gum. This may be linked to the smaller molecular weight of PB samples (Table 1) that rapidly adsorb and stabilise the newly created interface during emulsification. Furthermore, $d_{4,3}$ values reported for PB samples remained fairly stable, particularly for PBC samples, throughout the fifteen days of storage whereas average droplet sizes of SMB samples increased (Table 2). It should be also noted that, substantial coarsening of PBP-stabilised samples was observed only after fifteen days of storage.

Considering the particle size distributions of the emulsions investigated, it was noticed that emulsions stabilised with sodium metabisulphite extracts demonstrated bimodal distributions characterised by higher modes after the fifth day of ageing (Fig. 1). However, a second mode was also developed for PB emulsions after

Table 2 Effect of grewia polysaccharides and storage time on average droplet diameters, ζ-potential and span of emulsions

Sample	Time (days)	$d_{4,3}$ (μm)	Span	ζ-Potential (mV)
PBP	0	12.7 ± 4.9	1.8 ± 0.0	−3.2 ± 0.1
	5	11.8 ± 8.2	2.5 ± 0.1	−2.9 ± 0.0
	15	74.9 ± 6.9	75.8 ± 7.7	−3.2 ± 0.5
PBC	0	8.4 ± 0.0	1.9 ± 0.0	−3.2 ± 0.0
	5	5.6 ± 0.2	4.8 ± 0.5	−2.9 ± 0.0
	15	8.7 ± 0.6	4.8 ± 1.2	−3.3 ± 0.1
SMBP	0	61.3 ± 2.1	1.8 ± 0.0	−3.6 ± 0.3
	5	55.6 ± 6.2	12.2 ± 2.5	−3.7 ± 0.0
	15	80.2 ± 7.1	15.2 ± 3.5	−1.0 ± 0.2
SMBC	0	35.0 ± 2.5	1.6 ± 0.0	−3.4 ± 0.2
	5	65.2 ± 3.9	12.3 ± 2.9	−2.8 ± 0.3
	15	148.7 ± 2.0	13.9 ± 0.2	−3.7 ± 0.1

Values indicate the mean ± SD of triplicates. PBP-phosphate buffer pure extract, PBC-phosphate buffer crude extract, SMBP-sodium metabisulphite pure extract, SMBC-sodium metabisulphite crude extract.

fifteen days of storage. The emulsion that contained PBC samples exhibited the highest stability, as the span of the distributions remained relatively constant during ageing without the development of higher modes. Enlargement of droplet size was more prominent in emulsions stabilised by sodium metabisulphite extracts. The emulsification capacity of the different extracts based on examination of droplet size distribution curve development over time generally followed the order of PBC > PBP > SMBP > SMBC. It should be, however, noted that PBP samples after fifteen days were completely destabilised, as evidenced not only by the high $d_{4,3}$ values but also by the span of the distributions. Generally, span values increased during storage for samples emulsified with PBP, SMBC and SMBP that showed considerable destabilisation of these emulsions during ageing. However, the lower span values that were recorded at the end of storage period for PBC emulsions (Table 2) indicate negligible droplet coarsening, as emulsion stability is higher when the disproportion between droplet sizes is lower (McClements, 2005). These observations for PBC emulsions suggest retarded droplet flocculation and coalescence, a behaviour that is frequently observed in polysaccharide-stabilised dispersions (Crispín-Isidro *et al*., 2019). On first inspection, these results indicate that proteinaceous fragments in grewia extracts play important role in the emulsification efficacy, as all samples contained relatively high protein between 10 and 16% w/v (Table 1). However, a closer look at the data reveals a more complex relationship between protein content and stability. For instance, despite that PBC and SMBC samples have comparable protein content they exhibit substantially different emulsification capacity. Similarly, PBP with lower protein than SMBP exhibits smaller $d_{4,3}$ values and uniform particle size distribution at the early stages of ageing although both samples destabilise at the end of the storage period. This is in accordance with the findings in other polysaccharide-stabilised emulsions where conformation of polysaccharides and adsorption strength at the interface seem to be the main mechanism behind their functionality at the oil-water interface (Kontogiorgos, 2019).

Molecular weight of grewia polysaccharides seems to play a critical role in emulsion stability when each sample is viewed separately. As regards phosphate buffer extracts, when molecular weight was increased from 0.75×10^6 g mol^{-1} (PBP) to 0.92×10^6 g mol^{-1} (PBC) emulsion stability improved. In contrast, a more complex behaviour appears with the SMB-stabilised emulsions, as increase of molecular weight from 1.7×10^6 g mol^{-1} (SMBP) to 2.8×10^6 g mol^{-1} (SMBC) does not correspond to improved long-term stability. Long-term stability of emulsions is usually determined by how well-adsorbed biopolymers conform around oil droplets (Cuevas-Bernardino *et al*., 2016). Other studies have also reported that increasing molecular weight enhanced emulsion stability by forming thicker polysaccharide layers, which protected the coated oil droplets and reduced coalescence and aggregation (Liu *et al*., 2019, Funami *et al*., 2011, Jung & Wicker, 2012). However, adsorption strength, interfacial rheology and conformational rearrangements may also influence the behaviour of polysaccharides at the interface (Kontogiorgos, 2019). Consequently, molecular weight cannot alone account for emulsion stability, as for example, SMBC samples with substantially higher Mw than the rest of samples do not perform particularly well during long-term storage. In polysaccharide extracts with substantial protein concentration, examination of the interface composition may disclose further details into their stabilisation mechanisms. Frequently, hydrophobic protein moieties act as anchoring points for polysaccharides although the relative contribution of each biopolymer to the overall stability may vary depending on the system (Kontogiorgos, 2019). Consequently, in the next part of the present investigation we examined the interfacial load of fresh grewia-stabilised emulsions).

Interfacial composition and flow behaviour

The interfacial load with the grewia extracts was investigated by determining the amount of polysaccharides and proteins adsorbed at the droplet interface (Table 3). The interfacial concentration of the polysaccharides (0.7–1.6%) was higher than that of proteins (0.2–1.0%) for all the different grewia extracts studied. Grewia gum extracted with the phosphate buffer demonstrated the least surface coverage in terms of polysaccharide and protein content at the interface (Γ) whereas sodium metabisulphite extracts had the

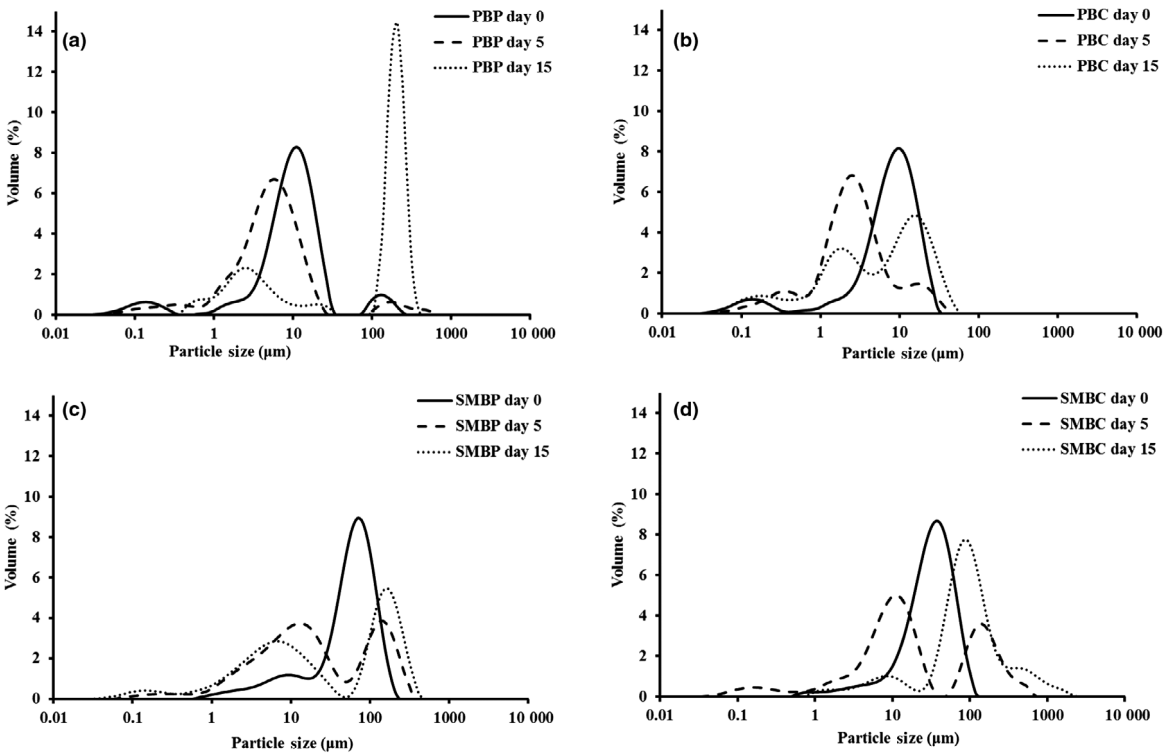

Figure 1 Droplet size distribution curves of emulsions ($\varphi = 0.1$, pH 2.0) prepared with different grewia polysaccharides: (a) PBP-phosphate buffer pure extracts, (b) PBC-phosphate buffer crude extracts, (c) SMBP-sodium metabisulphite pure extracts and (d) SMBC-sodium metabisulphite crude extracts.

highest polysaccharide and protein coverage. Interfacial adsorption of about 1 mg mL^{-1} corresponds to a monolayer 2D-interface coverage whereas greater values indicate adsorption of secondary biopolymer layers. In PB samples, mixed interfaces were formed with polysaccharides being the predominant biopolymer. The sum of Γ_{ps} and Γ_{pr} was ~1 mg mL^{-1} indicating single layer formation. On the contrary, SMB samples appear to form thicker interfaces with multiple layers with Γ_{ps} and Γ_{pr} exceeding 2 mg mL^{-1}. Thick interfacial layers are frequently observed in polysaccharide-stabilised emulsions where the interfacial concentration regularly exceeds the threshold value of 1 mg mL^{-1} (Alba *et al*., 2016, Akhtar *et al*., 2002, Siew & Williams, 2008).

The lower interfacial coverage of PB samples reinforces the observations of the previous section and puts the emphasis on the arrangement and adsorption strength of the polysaccharides at the interface rather than on the interfacial thickness. Intuitively, thick interfacial layers should provide better steric stabilisation, however, this is insufficient for long-term emulsion stability when the anchoring strength is weak and polysaccharides desorb from the interface. In the present work, such desorption is evidenced in the most unstable samples (SMBC) by the increase of viscosity after fifteen days of storage (Fig. 2). Fresh and aged emulsions exhibited shear thinning flow irrespectively of grewia sample (Fig. 2) a common behaviour reflecting the influence of the polysaccharide on the viscosity of the aqueous phase. Sample ageing did not considerably influence viscosity for most emulsions studied, except for SMBC-stabilised emulsions. In addition, the

Table 3 Polysaccharide and protein interfacial loads of fresh emulsions stabilised with grewia polysaccharides

Sample	Adsorbed polysaccharide (%)	Γ_{ps} (mg m^{-2})	Adsorbed protein (%)	Γ_{pr} (mg m^{-2})
PBP	86.4 ± 0.1^{c}	0.7 ± 0.1^{a}	42.5 ± 0.1^{a}	0.2 ± 0.1^{a}
PBC	72.9 ± 0.1^{b}	0.5 ± 0.1^{a}	47.8 ± 6.7^{a}	0.3 ± 0.1^{a}
SMBP	55.8 ± 0.1^{a}	1.5 ± 0.1^{b}	60.6 ± 1.2^{b}	1.0 ± 0.1^{c}
SMBC	78.5 ± 6.4^{bc}	1.6 ± 0.2^{b}	64.9 ± 5.4^{b}	0.8 ± 0.1^{b}

Values indicate the mean ± SD of triplicates. PBP-phosphate buffer pure extract, PBC-phosphate buffer crude extract, SMBP-sodium metabisulphite pure extract, SMBC-sodium metabisulphite crude extract. Means sharing the same letters in a column are non-significant ($P > 0.05$).

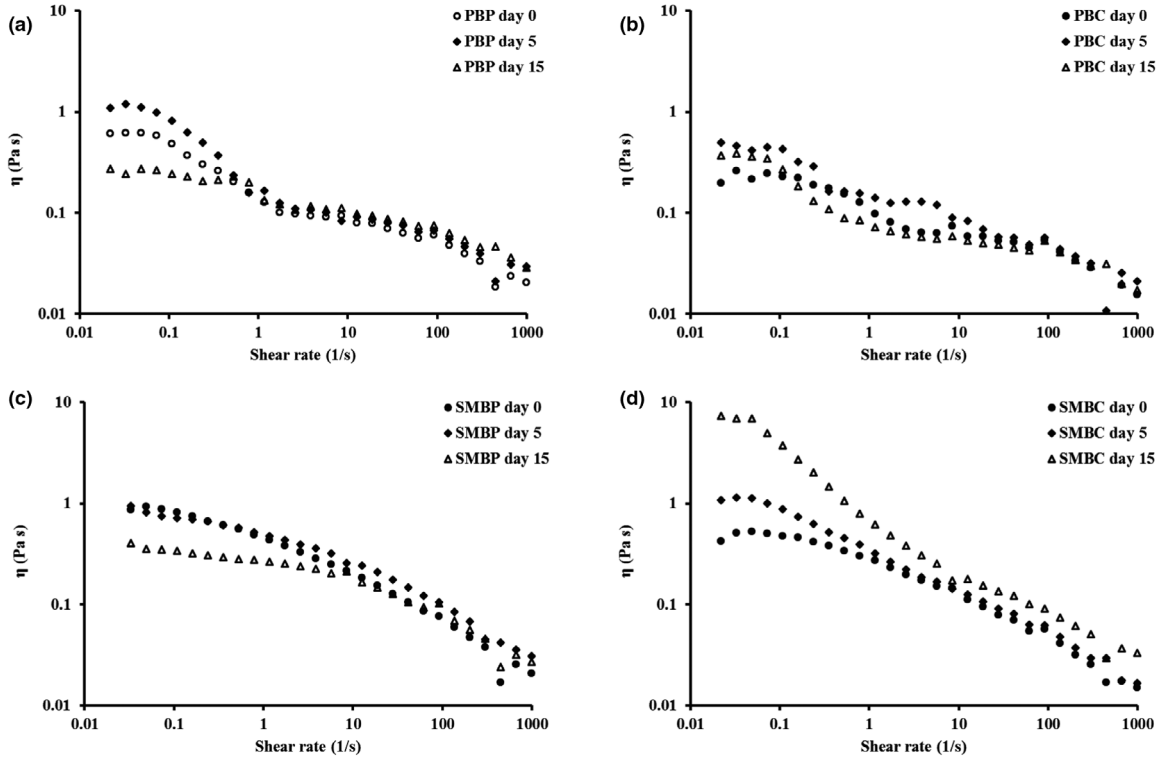

Figure 2 Viscosity curves during ageing of different grewia-stabilised emulsions: (a) PBP-phosphate buffer pure extracts, (b) PBC-phosphate buffer crude extracts, (c) SMBP-sodium metabisulphite pure extracts and (d) SMBC-sodium metabisulphite crude extracts.

higher zero shear viscosity of SMB samples do not correlate with emulsion stability, as SMB samples destabilise rapidly compared with those fabricated with PB extracts. It is clear that higher molecular weight with the concomitant increase in the viscosity of the continuous phase is not in harmony with the stability of emulsions during storage.

ζ-Potential values are indicative of the contribution of electrostatic forces towards emulsion stability. Usually, strong electrostatic interactions occur between positively charged proteins and negatively charged polysaccharides, which improve the surface activity of biopolymers (Gao *et al*., 2019). The ζ-potential values showed that the interfacial charge of all emulsions was negative (Table 3) and remained relatively constant after fifteen days of storage. The negative ζ-potential confirms the presence of polysaccharide-laden interfaces since proteins would normally possess a positive charge at pH 2. However, the charge is low and electrostatic stabilisation is not expected to contribute substantially to emulsion stability. Lack of electrostatic stabilisation is common in emulsions prepared at low pH, as protonation of carboxyl groups of uronic acids at pH values below their dissociation constant results in the predominance of steric stabilisation (Alba *et al*., 2016, Kpodo *et al*., 2018).

Conclusions

The emulsification capacity of grewia gum at acidic pH obtained with different extraction buffers was assessed with a set of complementary analytical techniques. Grewia gum extracted with phosphate buffer exhibited appreciable emulsifying capacity, as evidenced by the low average droplet size values of fresh emulsions. Additionally, phosphate buffer extracts demonstrated better emulsification capacity and long-term emulsion stability than those extracted with sodium metabisulphite. Conformational properties and adsorption strength of the polysaccharides at the oil-water interface seemed to control destabilisation, as no link was observed between molecular weight, interfacial load, protein content and long-term emulsion stability. The present findings show that different extraction protocols tailor polysaccharide structures resulting in materials with substantially different functional properties that may be exploited as surface active agents in oil-in-water emulsions with potential uses in flavour or bioactive delivery dispersed systems.

Data availability

Research data are not shared.

Ethical guidelines

Ethics approval was not required for this research.

Conflict of interest

None.

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