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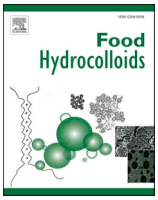

Encapsulation of salmon oil using complex coacervation: Probing the effect of gum acacia on interfacial tension, coacervation and oxidative stability

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ABSTRACT

The molecular characteristics of the food-grade polysaccharide gum acacia may vary depending on source, which could in turn significantly affect its behaviour as thickener, emulsifier and as a wall material in microencapsulation. In this study, five acacia gums (GA) from different sources were screened with respect to molecular weight distribution, interfacial tension, microencapsulation of salmon oil by complex coacervation and resulting oxidative stability of the oil. Bovine serum albumin (BSA) was used in combination with GA (BSA:GA = 1:1 w:w) for the coacervation. Interfacial tension was investigated for all GA alone and in combination with BSA at pH 5.5, pH 4.2 and pH 7, corresponding to the pH of emulsification, coacervation and neutral/reference conditions, respectively. Three of the five GA tested (GA from Sigma and the food grade GA Encapcia and Instant Gum BA from Nexira) resulted in stable complex coacervate microcapsules, with mean coacervate yields of the resulting microcapsules ranging from 34% to 76% depending on GA source, and a ~100% microencapsulation yield. The food grade GA Encapcia and Instant gum BA were found to provide significantly better protection against oxidation than the Sigma GA, both as a function of the microencapsulation process and after storage for 12 months. The differences in performance of the GA are discussed in terms of molecular weight, GA variety and impurities.

1. Introduction

Marine lipids are known as a source of bioactive polyunsaturated omega-3 fatty acids (n-3 PUFA), namely eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and for their promotion of human health, activity against different diseases and exhibition of cardioprotective, anti-platelet and anti-inflammatory effects among other (Yang et al., 2016). The Norwegian Directorate of Health and the European Food Safety Authority (EFSA) recommend a daily intake of 250 mg EPA/DHA, which can be covered by 2–3 fish meals a week. Despite the well-documented effects of polyunsaturated omega-3 fatty acids in fish oils on human health, a significant fraction of the population does not consume the 2–3 fish meals per week required to reap these health benefits, at least in part due to unwanted smell and taste of fish and fish products. Marine lipids are known to be highly susceptible to oxidation and the oxidation compounds are easily formed, leading to undesirable taste and odour (Larssen et al., 2018).

Microencapsulation of omega-3 and use of these microcapsules for fortification of food products could be an efficient way to increase the consumer intake of omega-3. Microencapsulation has the potential to improve stability and protect the oxidative status of the fish oil, limit the development of off-flavours, prolong shelf life and allow for controlled or delayed release into the surrounding physicochemical environment (Comunian & Favaro-Trindade, 2016; Eratte et al., 2015; Liu et al., 2014; Maki et al., 2003). While spray drying remains the most common method for microencapsulation of fish oil, complex coacervation is a promising alternative which has been reported to offer higher loading, better hydrothermal resistance and the possibility of controlled release (Leclercq et al., 2009; Xiao et al., 2014). Complex coacervation is a phase separation phenomenon which occurs upon mixing of oppositely but weakly charged polyelectrolytes. Complex coacervate microcapsules are core-shell structures, which allows for excellent barrier properties and thus protection of hydrophobic core materials from water in the external phase (Glomm et al., 2021). Depending on parameters such as

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Reynolds number during capsule formation and hardening and cooling rate, the resulting microcapsules can either be mononuclear, polynuclear or aggregated ("grape") coacervates (Lemetter et al., 2009). While any set of oppositely charged polyelectrolytes can be used, a combination of a protein and a polysaccharide – particularly gum acacia - is most commonly reported (see e.g. the review by de Kruif (de Kruif et al., 2004) and references therein). Gum acacia is a food grade ingredient (E 414 EC) commonly used as emulsifier, stabilizer and coating agent in food and non-food applications (Lopez-Torrez et al., 2015). Gum acacia is a complex mixture of glycoproteins and polysaccharides defined as "a dried exudate obtained from the stem and branches of Acacia *senegal* (L.) Willdenow or Acacia *seyal* (family Leguminosae)" (Joint FAO/WHO Expert Committee on Food Additives & Food and Agriculture Organization of the United Nations, 1999). Its composition can be described as a continuum of molecular species distinguishable by their protein to sugar ratio, molecular weights and charge density (Aphibanthammakit et al., 2018; Renard et al., 2006). Beyond differences related to Acacia specie, it has been reported that the molecular characteristics of gum acacia can vary depending on external factors such as tree location and age, weather conditions and way of tapping, as well as post-harvesting processes including storage conditions, maturation time, filtration heat treatments and more (Al-Assaf et al., 2009, 2012; Anderson & Farquhar, 1979; Assaf et al., 2005; Lopez-Torrez et al., 2015). Variations in the arabinogalactan protein fraction have also been shown to affect complex coacervate formation with gelatin via differences in the surface charge characteristics (Rousi et al., 2019).

Despite the wealth of reports on the use of gum acacia in complex coacervation and considering how external factors can affect the polysaccharide properties, there are significant knowledge gaps regarding differences in coacervation for different gum acacia sources. This is especially important in evaluating the robustness of an encapsulation process and elucidating any differences related to the quality and availability of the wall materials used. Here, we have encapsulated salmon oil via complex coacervation using a combination of bovine serum albumin (BSA) and a small library of acacia gums (GA) from different sources. This combination of wall materials was selected based on a recent study from our group, wherein BSA:GA 1:1 (w:w) was found to form stable coacervates for the microencapsulation and subsequent spray drying of peppermint oil (Glomm et al., 2021). The microcapsule walls were covalently crosslinked via the protein using glutaraldehyde. In the present study, the effect of gum acacia source on coacervation, coacervate yield and oxidative stability of the encapsulated salmon oil over time was investigated, as well as the ability of combinations of BSA and the different gum acacia samples to lower interfacial tension. This was done to elucidate differences in the film-forming properties of the acacia gums.

2. Materials and methods

2.1. Materials

Bovine serum albumin (BSA), gum acacia (GA, mixture of *Acacia Seyal* and *Acacia Senegal*), methanol, chloroform, iron thiocyanate, sodium hydroxide and glutaraldehyde were purchased from Sigma Aldrich Chemicals (Millipore Sigma, Merck KGa, Munich, Germany). The food grade acacia gums Encapcia (1:1 *Acacia Seyal: Acacia Senegal*), Encapcia Protect (*Acacia Seyal*), Eficacia (*Acacia Senegal*) and Instant Gum BA (*Acacia Seyal*) were obtained from Nexira. All the food grade acacia gums were reported to have an ash content of 3–4% and a protein content of 1–3%. Salmon oil was obtained as a gift from SINTEF Ocean and used as received. Reagents for peroxide value (PV) analysis; 37% hydrochloric acid, Iron (II) sulfate heptahydrate, ammonium thiocyanate and iron(III) chloride hexahydrate, were obtained from Merck (Millipore Sigma, Merck KGa, Munich, Germany), 30% hydrogen peroxide and 97% ethanol were obtained from VWR international. Medium-chain triglyceride (MCT) oil (WITARIX® MCT 60/40) for interfacial tension (IFT) analysis was obtained from IOI Oleo GmbH (Hamburg, Germany). All reagents were used as received without further purification.

2.2. Characterization of the wall materials

Zeta potential measurements for the wall materials BSA and the five gum acacias were measured via electrophoretic light scattering (ELS) using a Malvern Zetasizer Nano ZS (Malvern Panalytical Ltd., Malvern, UK).

The dynamic interfacial tension (IFT) of BSA and gum acacia samples at the oil–water interface was determined using an automated drop tensiometer OCA25 (DataPhysics Instruments GmbH, Filderstadt, Germany) at 25 °C. For the oil–water IFT measurement, a small drop of the protein/polysaccharide solution (0.1 wt% in distilled water, pH 5.5 or 7) was generated using the automated syringe into a glass cuvette filled with MCT oil. Every 10 s, the image of the pendant drop was recorded over 30 min. The drop shape was analysed using the Young-Laplace equation (equation (1)) as described in Yesiltas et al. (Yesiltas et al., 2019).

$$\Delta P = \gamma\left(\frac{1}{R_1}+\frac{1}{R_2}\right) \quad (1)$$

Here, ΔP (mN/m^2) is the pressure difference across the interface, γ (mN/m) is the interfacial tension and R_1 and R_2 (m) are the principal radii of curvature of the pendant drop. Changes in the IFT (mN/m) were plotted as a function of time (minutes). All measurements were performed in duplicate.

Molecular weight determination of the GA samples was done via gel permeation chromatography (GPC) using an Agilent 1260 Infinity II with a GPC RI detector (Agilent Technologies 1260 GPC/SEC MDS RI Detector). The instrument was equipped with two PL aquagel-OH columns from Agilent Technologies for the stationary phase (1x guard column, 8 μm, 5 μm; 1 × 300 × 7.5 mm, 8 μm, 6000–10 000 000 Da; 1 × 300 × 7.5 mm, 8 μm, 1000–500 000 Da) and a guard column (PL aquagel-OH, 8 μm). The analysis was run using $NaNO_3$ 0.15M/NaH_2PO_4 0.07M, NaN_3 adjusted to pH7 as the mobile phase. The columns were calibrated against PEG-PEO standards. Samples were dissolved in the mobile phase at 2 mg/ml.

2.3. Microencapsulation of salmon oil by complex coacervation

Microencapsulation of salmon oil by complex coacervation was done using a modification of the procedure reported by Glomm et al. (Glomm et al., 2021).

2.3.1. Polymer dissolution

4 wt% solutions of BSA and GA (either GA, Encapcia, Encapcia Protect, Eficacia or Instant Gum BA) in deionized (DI) water were prepared separately and allowed to hydrate at 40 °C under gentle stirring for 1h. Following hydration and dissolution, the two polymer solutions were mixed (BSA:GA = 1:1 w:w) and the pH was adjusted to 5.5 to avoid premature formation of coacervate nodules.

2.3.2. Emulsification

Salmon oil was added to the polymer solution, and the reaction mixture was emulsified using an Ultraturrax (T25 digital, IKA, Staufen, Germany) at 5000 RPM for 2 min, and subsequently transferred to a 100 mL reactor (cylindrical reaction vessel with thermostatic jacket, Lenz Laborglas GmbH). The reaction mixture was kept at 40 °C under stirring (400 RPM) for 30 min prior to the next process step. The amount of salmon oil and thus the oil to wall (o:w) ratio was kept constant at 2.5:1 with respect to the total polymer concentration.

2.3.3. Coacervation

Coacervation was initiated by adjusting pH to 4.2 – slightly below the isoelectric point of BSA - with continued stirring (400 RPM) at 40 °C. The reaction mixture was then gradually cooled to 5 °C at a rate of ~0.5 °C/min to facilitate hardening of the shell layer.

2.3.4. Crosslinking

All the coacervate microcapsules were chemically crosslinked via the protein using glutaraldehyde. After adjusting the pH of the reaction mixture to 9 using NaOH, a 50% aqueous glutaraldehyde solution was added (6.25 wt% glutaraldehyde with respect to protein). Following 2 h reaction at 5 °C with stirring (400 RPM), the reaction mixture was allowed to gradually reach room temperature over 12 h. Capsules were then washed with DI water.

2.3.5. Storage

Capsules were kept at 4 °C under a nitrogen blanket to minimize oxygen prior to further characterization. Capsules were held for long term oxidative stability studies in the same condition, and small analytical sample removed from the bulk after redispersion of particles to give a homogenous suspension by repeated inversion.

2.4. Characterization of the microcapsules

Optical microscopy images were collected using an Olympus BX43 equipped with an Olympus XM-10 digital camera (Olympus Corporation, Tokyo, Japan). Capsule size was determined using a Beckman Coulter LS230 laser diffraction Particle Size Analyzer (Beckman Coulter Inc., Brea, CA, USA) with water as the mobile phase.

The coacervate yield was determined using a modified version of the method described by Jun-Xia et al. (Jun-xia et al., 2011). Briefly, mixed polymer solutions as described in 2.3.1 were adjusted to pH = 4.2 with 1 M HCl to start the coacervation. After the solution was allowed to react at 40 °C with magnetic stirring for 30 min, the reaction mixture was centrifuged at 1073 RCF (relative centrifugal force corresponding to 4000 RPM) for 15 min, and the coacervates were harvested, dried and weighed. The coacervate yield was then determined according to equation (2). Note that in the method used, coacervate yield was determined without an oil phase present.

$$Coacervate\ yield\ (\%) = \left(\frac{Dry\ weight\ of\ coacervates}{Total\ weight\ of\ BSA\ and\ GA\ added}\right) x\ 100\% \quad (2)$$

The microencapsulation efficiency (MEY) was calculated as the percentage of salmon oil entrapped in capsules relative to the total amount added according to equation (3).

$$MEY\ (\%) = \left(\frac{Microencapsulated\ salmon\ oil}{Salmon\ oil\ in\ emulsion}\right) x\ 100\% \quad (3)$$

Primary oxidation products as represented by peroxide value (PV) were determined according to a modified Bligh and Dyer (Bligh & Dyer, 1959) method as follows: The salmon oil was extracted by placing 4 mL of microcapsule suspension in a DT-20 blender fitted to a ULTRA--TURRAX®-Tube drive, and adding 12 mL of methanol:chloroform 2:1 (v:v). After destruction of the capsules in the blender, the resulting mixture was transferred to a 50 mL centrifuge tube. The blender was washed with 2 mL DI water and 2 mL chloroform, and the washing solution was added to the centrifuge tube. After centrifugation (10 min at 3000 RPM) to aid phase separation, the aqueous and oil phases were separated by carefully removing the bottom (aqueous) phase with a pipette. The amount of oil extracted was subsequently weighed. 3 mL of the extracted oil in chloroform was then transferred into a preweighed glass evaporator tube, and the chloroform was evaporated under N_2 followed by 1h in vacuum oven at 25 °C. The glass tube was subsequently weighed and stored at −20 °C for further PV analysis. PV values for the extracts were subsequently determined by colorimetric detection of iron thiocyanate using a plate reader (BioTek Synergy H1 hybrid multi-mode microplate reader, BioTek, Winooski, Vermont, USA) at 500 nm. Measurements were undertaken in triplicate.

2.5. Statistical analysis

All measurements were performed in triplicate (analytical replicates) unless otherwise noted. Microsoft Excel was used for calculation of average and standard deviation, as well as for estimation of coacervate yield and microencapsulation efficiency. One-way ANOVA was performed via Excel using $\alpha = 0.05$. Curve fitting was done using GraphPad Prism version 9.1.0.

3. Results and discussion

3.1. Molecular weights, charge profiles and coacervate yield of wall materials used

Molecular weights for the acacia gums studied here as determined by GPC are shown in Table 1. All five acacia gums showed a trimodal molecular weight distribution $M_1 > M_2 > M_3$, with corresponding relative populations $\alpha_1 > \alpha_2 > \alpha_3$. Overall, the molecular weight distributions of the acacia gums studied here were similar and highly overlapping, with two potentially notable exceptions in the trends shown by the respective peak average molecular weights (M_p): Encapcia Protect has the lowest relative population of the high molecular weight species M1, whereas Eficacia has the lowest value for M1 found among the GA used here.

The pH-dependent zeta potentials of the wall materials studied here are shown in Fig. 1. For BSA, we determined the isoelectric point of BSA to be 4.8, which is in agreement with literature (pI_{BSA} = 4.6–4.8) (Glomm et al., 2007). All the acacia gums studied were negatively charged in the pH range studied (pH 2–7). With the possible exception of Eficacia, which appeared to be slightly more negatively charged above pH 2, the charge profiles of the acacia gums were very similar. At the coacervation pH (pH 4.2), the zeta potential profiles of BSA and the acacia gums are distributed on either side of neutral, indicating that the oppositely charged wall materials could form approximately neutral coacervate nodules which can self-assemble on the oil-water droplet interface. The formation of BSA:GA complexes is evident from the coacervate yields shown in Fig. 2. Average coacervate yields between BSA and the different acacia gums range from 34% (Instant Gum BA) to 76% (Eficacia). A one-way ANOVA revealed that there was a statistically significant difference in coacervate yields between the GA used here (F

Table 1
Overview of molecular weight distribution for the acacia gums studied here represented by peak (M_p), number (M_n) and weight (M_w) average molecular weight, as well as polydispersity (PD = M_w/M_n) and relative population (%) for each GA.

Gum acacia	Relative population (%)	M_p (Da)	M_n (Da)	M_w (Da)	PD
Sigma	67	72 630	41 839	82 817	1.979
	27	9828	1588	3474	2.188
	6	206	196	218	1.112
Encapcia	73	65 971	33 389	75 997	2.276
	20	6076	1199	2187	1.824
	7	222	190	211	1.111
Encapcia Protect	59	71 246	46 068	93 121	2.021
	34	12 379	1641	4078	2.485
	7	183	200	221	1.105
Eficacia	67	54 428	34 368	89 340	2.600
	27	3347	1273	2638	2.072
	6	191	178	195	1.096
Instant Gum BA	64	75 478	47 310	86 379	1.826
	29	12 143	1835	4167	2.271
	7	210	208	236	1.135

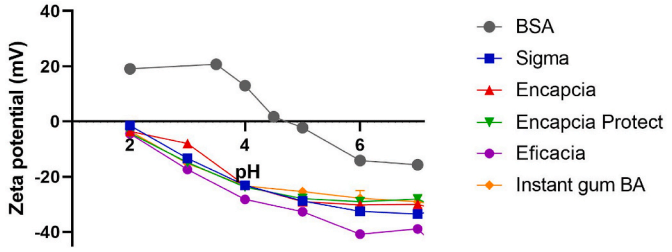

Fig. 1. Zeta potential as a function of pH for the wall materials used.

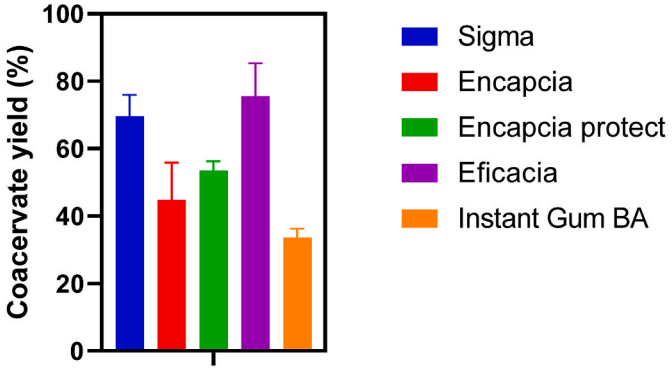

Fig. 2. Coacervate yield for 1:1 (w:w) ratios of BSA and the different gum acacias used at pH = 4.2.

$= 16.31 > F_{crit} = 3.48$; $p = 0.0002 < \alpha = 0.05$). Interestingly, the results indicate that there are three tiers of coacervate yields within the set of acacia gums used here: Eficacia, Sigma > Encapcia Protect, Encapcia > Instant Gum BA. Thus, from the zeta potential and coacervate yield measurements, Eficacia and Sigma should be more efficient at forming coacervate nodules at the oil-water interface than the other three acacia gums studied here. However, it should be mentioned that this method for determining coacervate yield does not take into account the effect of an oil phase on the formation of BSA:GA complexes.

3.2. Complex coacervation of BSA and GAs

The complex coacervation process described herein resulted in stable, well-dispersed spherical mononuclear microcapsules with similar sizes as the parent emulsions for three of the five BSA:GA systems – BSA:Sigma, BSA:Encapcia and BSA:Instant Gum BA. When using Encapcia Protect and Eficacia, no stable microcapsules were formed, and macroscopic phase separation was observed after the coacervation step (pH 4.2). Size distributions and morphology of the successful oil-containing microcapsule systems are shown in Fig. 3 and Table 2, respectively. All acacia gums used formed stable parent emulsions, likely due to both protein and polysaccharide being present during emulsification, with no signs of instability observed until after coacervation for Encapcia Protect and Eficacia.

While the microcapsule size as estimated by the differential volume profiles are overlapping for the successful coacervates, the corresponding Sauter mean diameters (Table 2) indicate an increase in diameter from Sigma to the two food grade acacia gums. The trend in Sauter mean diameter is also in agreement with the optical microscopy images of the capsules shown in Fig. 3, with larger microcapsules observed for BSA:Encapcia and BSA:Instant Gum BA. The Sauter mean diameter, also known as the surface-volume mean diameter, is a measure of the mean

Table 2
Overview of capsule size and microencapsulation yields for successful coacervate systems.

Gum acacia	Differential Volume Diameter (μm)	Sauter Mean Diameter (μm)	Dry weight (%)	MEY (%)
Sigma	23 ± 08	4	6.9	≥100
Encapcia	30 ± 11	6	7.3	≥100
Instant Gum BA	27 ± 10	11	5.2	≥100

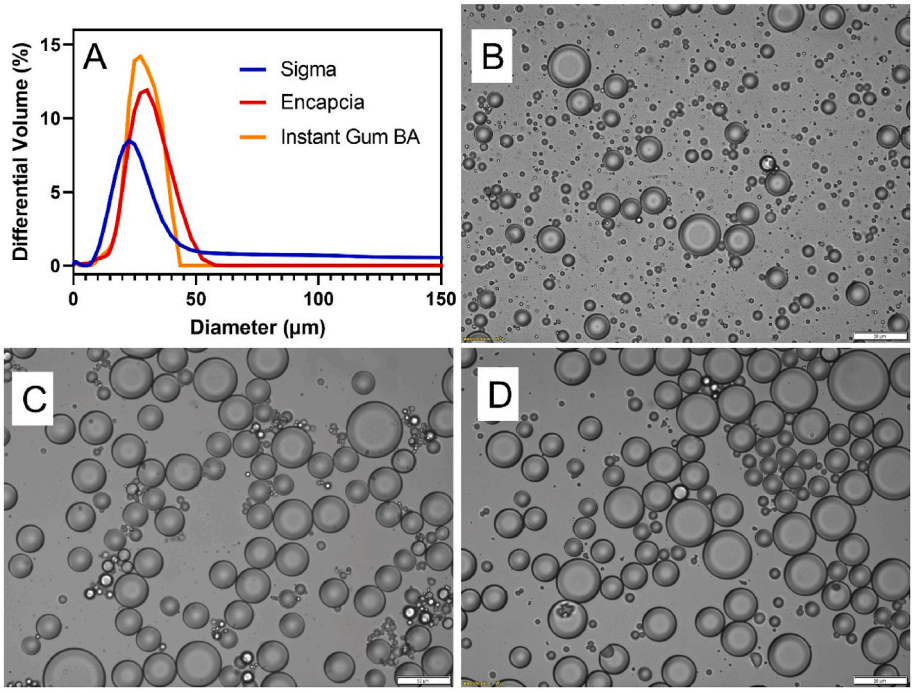

Fig. 3. Size distribution (A) and optical microscopy images of successful capsules of BSA:GA combinations: BSA:Sigma (B), BSA:Encapcia (C) and BSA:Instant Gum BA (D). The scale bar corresponds to 50 μm.

diameter by taking into account the volume-to-surface area ratio (Kowalczuk & Drzymala, 2016). Thus, for overlapping differential volume profiles, an increase in Sauter mean diameter indicates a lower total surface area, and thus either a more narrow size distribution, or a reduction of small particles such as free BSA:GA complexes.

As pH-mediated charge density matching of the anionic polyelectrolyte and the protein is a critical parameter in complex coacervation, the coacervation pH was adjusted based on the zeta potential profiles (Fig. 1). Specifically, as the zeta potential profile of BSA drops sharply above pH 4, a set of coacervation experiments was performed at pH = 3.8. This was done both in an attempt to improve charge density matching as well as to minimize experimental uncertainties in pH-adjustment in this region. However, adjusting pH did not yield stable microcapsules for Encapcia Protect and Eficacia (data not shown), and thus the coacervation process described here could not be successfully applied to these two polysaccharides.

The calculated MEY for the 3 successful capsule types was found to be $\geq$ 100%, indicating quantitative yield of the encapsulated material. No significant differences could be observed between the acacia gums used, despite correction for the degree of coacervation for each wall material pair. Interestingly, no apparent trend could be observed between successful microcapsule formation and coacervate yields for the acacia gums used here (Fig. 2).

3.3. Interfacial tension measurements of wall materials used

In order to elucidate differences in the film-forming properties of the gum acacias, interfacial tension (IFT) profiles of the five polysaccharides as well as of BSA were collected. The IFT profiles of the wall materials at neutral pH, and at pH values corresponding to the critical process steps of emulsification (pH 5.5) and coacervation (pH 4.2) are shown in Fig. 4. As expected, BSA significantly decreases IFT at all three pH values, with lower IFT observed at equilibrium for pH 5.5 and pH 4.2 compared to at pH 7.0. This can be attributed to higher interfacial activity and better packing of the protein close to its isoelectric point at pH 4.8 (Glomm et al., 2007). For the food grade acacia gums studied, the overall trend is

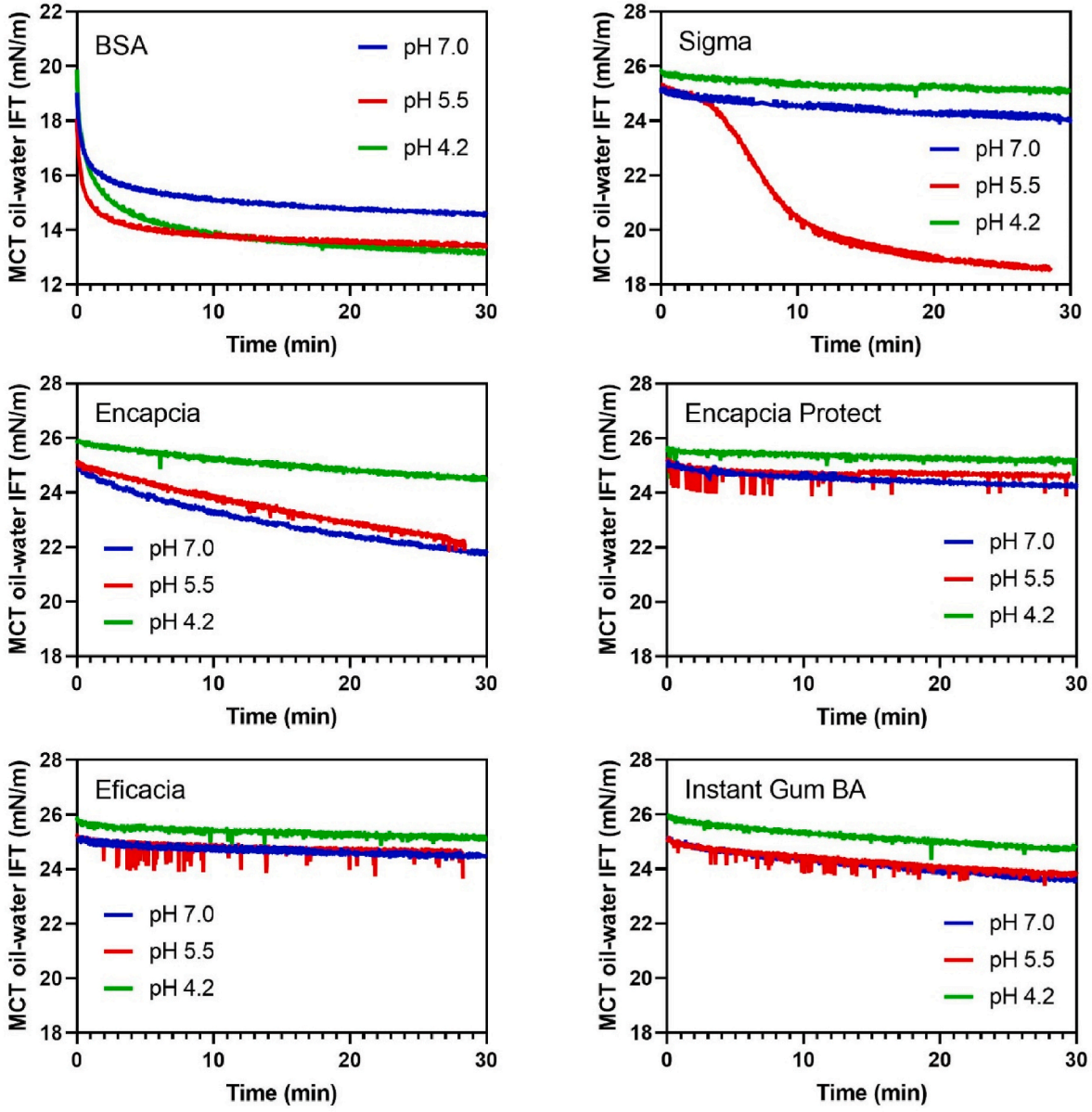

Fig. 4. Interfacial tension (IFT) profiles for the wall materials used in this study taken at pH 7.0 (blue line), pH 5.5 (red line) and pH 4.2 (green line). Medium-chain triglyceride (MCT) oil was used. Note the different scale for BSA.

that the polysaccharides decrease IFT to varying extents at pH 7.0 and pH 5.5 – albeit significantly less compared to BSA, but do not show any significant interfacial activity at pH 4.2. Thus, all the food grade acacia gums are more interfacially active at the emulsification pH (pH 5.5) than at the coacervation pH (pH 4.2). Interestingly, the Encapcia and to a lesser extent Instant Gum BA appear to lower the IFT more at pH 5.5 than the two food grade gum acacias that did not yield successful coacervate microcapsules. For the Sigma GA, the IFT is significantly lower at pH 5.5 than for the other two pH values, indicating that this gum acacia is highly interfacially active during emulsification. At the emulsification pH, the interfacial activity of the acacia gums studied can be ranked as Sigma $\gg$ Encapcia $>$ Instant Gum BA $\geq$ Encapcia Protect $=$ Eficacia. The higher interfacial activity of the Sigma does not appear to be linked to differences in molecular weight from the measured values listed in Table 1.

In order to assess the effect of interfacial activity on the mixed protein-polysaccharide film, IFT was measured at pH 5.5 and 4.2, comparing the benchmark system (BSA:Sigma 1:1) to the two acacia gums which did not yield successful coacervation (Encapcia Protect and Eficacia). From the interfacial tension measurements of the mixed BSA-gum acacia systems shown in Fig. 5, similar trends are observed for all the combinations tested. At pH 5.5, the IFT profile of the mixed BSA:gum acacia systems fall between those of the pure components, indicating that both protein and polysaccharide are present at the oil-water interface during emulsification. While the IFT profile of the BSA:Sigma system appears to be comprised of a higher fraction of the gum acacia than for Encapcia Protect and Eficacia, this observation does not by itself allow for any conclusions regarding differences in film composition for the emulsified system. However, it should be noted that Encapcia Protect and Eficacia differ from the remaining acacia gums with respect to molecular weight distribution (Table 1). Specifically, Encapcia Protect has the lowest relative population of the highest molecular weight, whereas Eficacia has the lowest peak average molecular weight. At the coacervation pH (pH 4.2), all the BSA:gum acacia systems show a lower IFT compared to the reference materials, indicating protein-polysaccharide complex formation. No significant differences could be

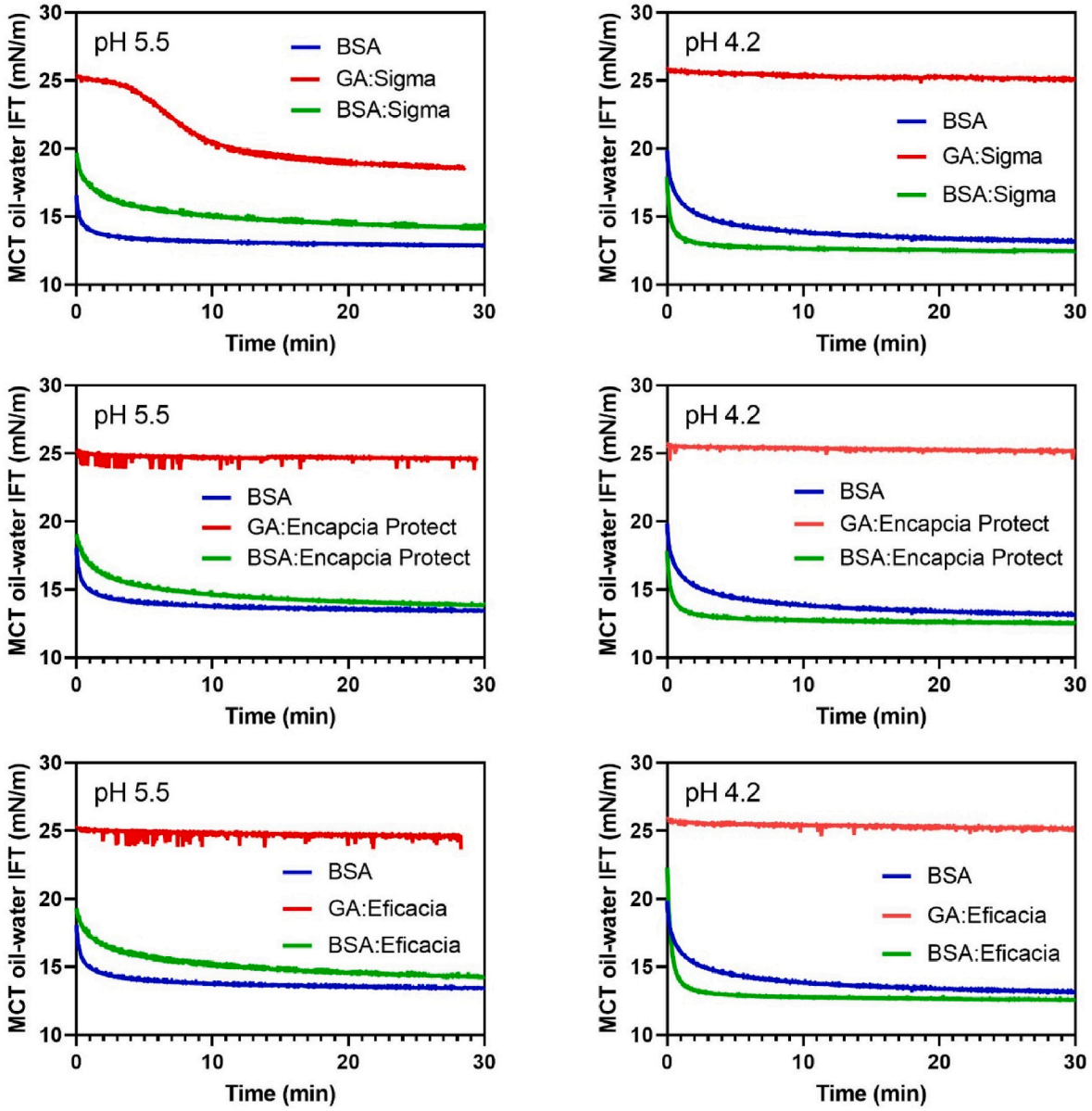

Fig. 5. Interfacial tension (IFT) profiles for 1:1 (w:w) combinations of BSA and acacia gums (GA) Sigma, Encapcia protect and Eficacia collected at pH values corresponding to the emulsification (pH = 5.5) and coacervation (pH = 4.2) steps. IFT profiles of BSA and the polysaccharide are included for comparison. Medium-chain triglyceride (MCT) oil was used.

observed between the BSA:gum acacia systems studied. This is in agreement with the coacervate yields shown above (Fig. 2) – all the BSA: gum acacia combinations studied here were found to result in formation of protein-polysaccharide complexes at pH 4.2. As the crosslinking strategy relies on access to the protein component of the microcapsule walls, we opted for using the well-documented low-molecular weight crosslinker glutaraldehyde (Habeeb & Hiramoto, 1968) which would have access to the protein irrespective of its relative orientation in the film.

Another possible explanation for the observed difference between the Sigma and the food grade acacia gums could be related to their adsorption behaviour at the oil-water interface. Specifically, the gum acacia could adsorb at the oil-water interface as single molecules or as colloidal particles, resulting in Pickering emulsions for the latter case. Differences in gum acacia interfacial properties could also lead to the formation of BSA-gum acacia particles as opposed to coacervate nodules. Formation of protein-gum acacia particles and subsequently of Pickering emulsions was recently reported for the combination of ovotransferrin and gum acacia (3:1 w:w) (Wei & Huang, 2020). The physical state of the polysaccharide at the oil-water interface, *i.e.;* whether it is present as single molecules or as particles, is likely to have a significant impact on the coacervation and crosslinking steps. Here, crosslinking is done via the formation of a Schiff base between the aldehyde and the amino groups on the protein (Habeeb & Hiramoto, 1968), and so entanglement with the polysaccharide is important to create a dense shell and to avoid inducing porosity which will destabilize the capsule walls. Differences in coacervation might also be directly attributed to differences between the acacia gums. The polysaccharides used in this study include both varieties of gum acacia – *Acacia Senegal* (Eficacia) and *Acacia seyal* (Encapcia Protect and Instant Gum BA) – as well as blends (Encapcia, Sigma). Additionally, Encapcia Protect contains phenols which should impart antioxidant properties. While both gum acacia varieties share a common hyperbranched structure and adopt an ellipsoid-like conformation in solution, they have been shown to vary in terms of average molecular weight, radius of gyration and intramolecular charge distribution (Lopez-Torrez et al., 2015). Specifically, *Acacia seyal* was found to have a slightly higher average molecular weight (820 kDa vs 680 kDa) yet have a lower radius of gyration (17 nm vs 31 nm) compared to *Acacia senegal*. It should be noted that the average molecular weights reported by Lopez-Torrez are significantly higher than for the commercial samples reported here (see Table 1). The more compact structure of *Acacia seyal* was at least partially attributed to a lower concentration of charged sugars and thus less intramolecular electrostatic repulsion, as well as self-assembly due to a higher content of long arabinose side chains (Lopez-Torrez et al., 2015). The reported difference in charged sugars is in agreement with the zeta potential measurements above, where the highest charge density was found for the pure *Acacia Senegal* variety (Eficacia). However, as both pure *Acacia seyal* (Encapcia Protect) and *Acacia Senegal* (Eficacia) are represented among the systems which did not yield stable microcapsules, successful coacervation could not be directly linked to the Acacia variety used. As described in the introduction, the molecular characteristics of gum acacia depend not only on specie, but also on external factors including age, harvesting method and processing conditions. For example, Rousi et al. (Rousi et al., 2019) investigated the effect of arabinogalactan protein fraction content for two Acacia Senegal samples on complex coacervate formation with gelatin, showing that the arabinogalactan content affected both the charge profile and the characteristics of the resulting microcapsules. Moreover, the gum acacias used likely are different with respect to type and concentration of impurities. Thus, it is not unexpected that different commercially available gum acacia might have different film-forming properties.

3.4. Oxidative stability of microencapsulated salmon oil

The time evolution of the oxidative stability of complex coacervate microcapsules relative to the salmon oil for the BSA:gum acacia systems studied here is shown in Fig. 6 below.

It should be mentioned that the salmon oil used in this study had a high PV value before encapsulation, and was not of food grade quality. In turn, this resulted in higher overall PV values than if a food grade salmon oil had been used. Future studies will be done using higher quality food grade salmon oil. From Fig. 6, the gum acacia used affects the oxidative stability of the oil, both during the encapsulation and as a function of storage time. A one-way ANOVA confirms that there is a statistically significant difference in oxidation between the GA used (e.g. after 12 months, $p = 2.32 \times 10^{-7} \ll \alpha = 0.05$; $F = 484.29 \gg F_{crit} = 5.14$). Immediately after encapsulation, no oxidation of was observed relative to the salmon oil when using Encapcia or Instant Gum BA, whereas significant oxidation was observed for the BSA:Sigma combination (Fig. 6A). For the stored samples, the BSA:Sigma system reveals much higher PV than the two other acacia gums, with an initial increase in oxidation which reaches a plateau after 10 months. The food grade gum acacias appear to follow the same overall trend over time, with a maximum, albeit significantly lower PV than for BSA:Sigma, observed after 10 months storage. Between the two food grade acacia gums, the BSA:Instant Gum BA system appears to attenuate oxidation more than BSA:Encapcia. Thus, the ability of the wall material combinations studied here to protect against oxidation of the salmon oil can be ranked in increasing order as BSA:Sigma ≪ BSA:Encapcia < BSA:Instant Gum BA. While the underlying reason for the observed differences remain to be elucidated, it is interesting to note that there appears to be differences related to the origin of the gum acacia used. Specifically, the best-

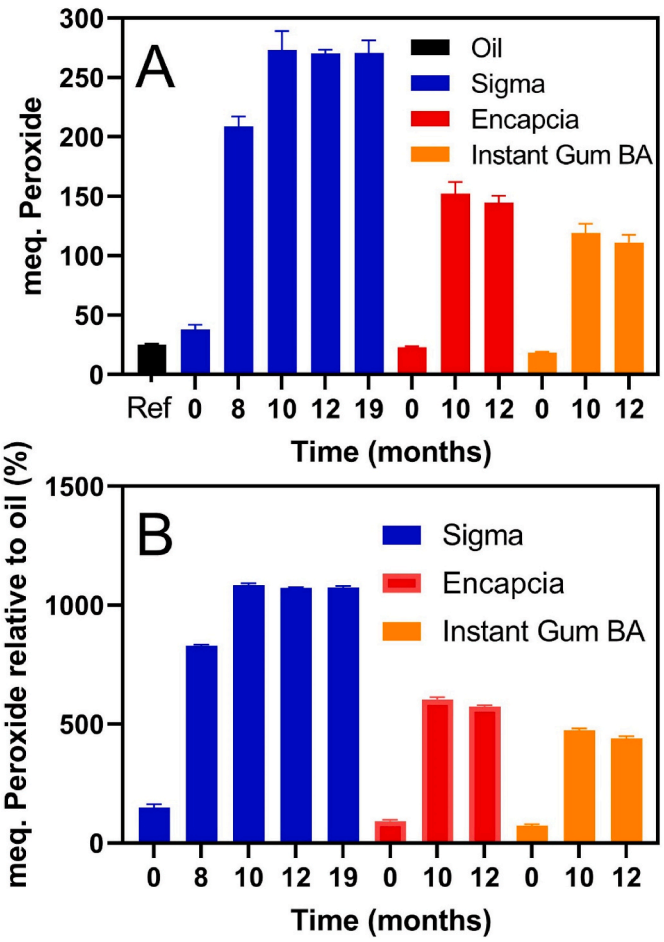

Fig. 6. Time evolution of the oxidative stability of microcapsules relative to the salmon oil. A: PV values for all systems, and B: oxidative stability relative to the salmon oil. Ref. Marks the oxidative stability of the salmon oil used.

performing gum acacia originates from *Acacia seyal* (Instant Gum BA), whereas Sigma and Encapcia are blends. Moreover, the susceptibility to oxidation appears to be positively correlated with coacervate yield, perhaps indicating that a higher coacervate yield results in a more porous coacervate shell.

4. Conclusion

The different GA tested show clear differences in terms of film-forming ability, formation of stable complex coacervate microcapsules, resulting coacervate yield and degree of protection against oxidation of salmon oil. Of the three GA which formed stable coacervate microcapsules, the two food-grade GA Instant Gum BA and Encapcia outperformed the GA from Sigma in terms of oxidative protection. The observed differences could be due to a combination of differences in molecular weight distribution, GA origin (*Acacia seyal* vs *Acacia senegal*), whether a single origin or a blend was used, as well as harvesting conditions and potential impurities. Considering the number of publications on microencapsulation using GA as one of the wall materials, knowledge of the differences directly related to the GA source is highly relevant both for design of stable microcapsules as well as for reproduction of earlier published results.

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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.

Data availability

Data will be made available on request.

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