

Interaction of novel hydrocolloids from indigenous New Zealand ferns with κ -carrageenan and low-acyl gellan gum

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ABSTRACT

Growing demand for sustainable hydrocolloids has spurred the exploration of polysaccharides from New Zealand's diverse fern flora. This study investigates interactions between polysaccharides extracted from *Sphaeropteris medullaris* (mamaku gum, MG) and *Parablechnum novae-zelandiae* (kiokio gum, KG), with κ -carrageenan (κ C) and low-acyl gellan (LAG), selected from a preliminary screening of commercial hydrocolloids. MG and KG are non-gelling anionic glucuronomannans exhibiting unusual shear-thickening behaviour, but their interactions with other polysaccharides remain unexplored.

Mixtures were characterised using rheology, TPA, FTIR, cryo-SEM, syneresis, water-holding capacity (WHC) and freeze-thaw stability across different blend ratios, temperatures, ionic strengths, and pH values. Crude and purified forms of MG and KG impacted differently in these mixtures; κ C and LAG gels containing crude fern gums were significantly firmer, with functionality dominated by endogenous ions. Conversely, κ C and LAG gels containing purified fern gums were softer, heterogeneous and displayed micro-phase separation, which was exacerbated at pH 3 and pH 9. These findings are consistent with a segregative interaction resulting in a phase-separated gel network.

From a formulation perspective, purified fern gums were able to modulate gel microstructure and functionality independently of ionic effects, offering advantages for ion-sensitive formulations. The LAG-purified MG blend also maintained its WHC after one freeze-thaw cycle, highlighting potential for frozen products. Overall, the distinct behaviour of crude and purified fern gums offers an adaptable approach to texture modification in mixed polysaccharide systems.

1. Introduction

In recent years, there has been increasing interest in water-soluble mucilaginous polysaccharides extracted from ferns due to their potential as sustainable alternative natural sources of functional additives in both food and non-food applications (Pei et al., 2022; Zeng & Lai, 2019; Zhao et al., 2019). New Zealand can potentially play a pioneering role in advancing this research area since it is home to approximately 200 species of ferns, of which an estimated 40 % are endemic to the country (Department of Conservation, n.d.). Furthermore, Māori, the Indigenous people have a long history of utilising ferns such as *Sphaeropteris medullaris* (black tree fern/mamaku) and *Parablechnum novae-zelandiae* (palm leaf fern/kiokio) as food and medicine. The white pith of mamaku fronds is known to be slimy and has been used as poultice or to relieve diarrhoea. The vastly abundant fronds of kiokio have also been used as a food wrapper during cooking and the young, curled fiddleheads also consumed (Crowe, 2009). Translating Māori knowledge into contemporary polysaccharide research provides a culturally and historically grounded rationale for examining whether the physicochemical functionality of these indigenous resources can be developed into

value-added applications. In particular, understanding their interactions with established commercial polysaccharides may provide a pathway towards incorporating locally sourced fern polysaccharides into formulated food systems while simultaneously expanding the functional repertoire of these underexplored novel hydrocolloids.

Intensive research has been conducted on mamaku polysaccharide for almost two decades, accumulating comprehensive knowledge on its rheological, physicochemical and structural properties, the effects of harvesting age, chemical (ions, pH and urea), processing (temperature, shear and drying) and commercial upscaling conditions, as well as its impact on gastrointestinal function and colon fermentation (Bisht, 2023; Bisht, Goh, & Matia-Merino, 2023; Bisht, Goh, Sims, et al., 2023; Carnachan et al., 2019; Goh et al., 2007, 2011; Jaishankar et al., 2015; Matia-Merino et al., 2012; Tresidder, 2018; Wee, 2015; Wee et al., 2014, 2015a, 2015b, 2017). More recently, extensive research has also been conducted on kiokio polysaccharide. Similarly, knowledge on its rheological, physicochemical and structural properties, as well as the effects of processing and chemical environments have been acquired (Peh, Goh, et al., 2026; Peh, Matia-Merino, et al., 2026). However, the interactions of mamaku and kiokio polysaccharides with commercial

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polysaccharides remain largely unexplored.

Mamaku polysaccharide is a non-gelling, shear-thickening gum with a high molecular weight ($\sim 3 \times 10^6$ Da) and semiflexible random coil conformation (Goh et al., 2007, 2011). It is an anionic heteropolysaccharide, with a glucuronomannan backbone consisting of methylesterified 4-GlcpA and 2-Manp (Wee et al., 2014). Additionally, it is sensitive to the removal of ions from the crude gum via dialysis, which results in the loss of shear-thickening, since this property is conferred by transient intermolecular hydrogen bonding promoted by the presence of cations (Jaishankar et al., 2015; Wee et al., 2015a). However, re-addition of 0.05 M NaCl restores shear-thickening in the dialysed gum; furthermore, both cation concentration and valency have significant effects on this recovery (Wee et al., 2015a). Nonetheless, in the crude form, it demonstrates good tolerance to high NaCl concentrations (up to 1 M) and pH (especially between pH 3 to 9) (Matia-Merino et al., 2012). Its shear-thickening property is however reduced above 75 °C and lost at 95 °C, due to thermal depolymerisation (Bisht, Goh, Sims, et al., 2023). Kiokio polysaccharide shares several key characteristics with mamaku gum—it is likewise a non-gelling, shear-thickening, high-molecular weight ($\sim 2.5 \times 10^6$ Da) and random coiled anionic heteropolysaccharide with a glucuronomannan backbone (Peh, Goh, et al., 2026)—but differs in important aspects. In contrast to mamaku gum, dialysis of kiokio gum leads to an irreversible loss of shear-thickening which cannot be restored with the re-introduction of ions. Its crude gum is also highly sensitive to ionic strength increases, and deviations away from its native pH of 5, which results in great reductions in viscosity and shear-thickening (Peh, Goh, & Matia-Merino, 2026). As with mamaku, crude kiokio gum is susceptible to high temperatures (>55 °C) due to thermal depolymerisation, which reduces its viscosity and diminishes shear-thickening behaviour (Peh, Matia-Merino, et al., 2026).

In the food industry, non-starch polysaccharide (NSP) mixtures are used at varying concentrations to achieve desired organoleptic and physicochemical properties while maintaining cost-effectiveness. In mixed polysaccharide systems, polymers may behave independently (segregative) or interact synergistically (associative), depending on their thermodynamic compatibility. Segregative interactions result in phase separated or entrapped gel networks, whereas associative interactions produce interpenetrating or coupled gel networks (Morris et al., 2012; Sikora & Kowalski, 2007; Williams et al., 1994). Identifying the dominant interaction mode is therefore essential for predicting and controlling mixture functionality. These interactions are influenced by factors such as cations, polysaccharide charge, and chain conformation (Williams, 2009), which together determine the microstructure, macroscopic properties, and functional performance of food systems (De Kruif & Tuinier, 2001; Gao et al., 2017; Gentile, 2020; Goh et al., 2020).

Yet despite the fundamental knowledge available on mixed polysaccharide behaviour, the interactions of fern-derived polysaccharides with commercial hydrocolloids remain largely unexplored. The present study therefore aimed to conduct a systematic characterisation of the functional properties of mixed polysaccharide systems comprising fern-derived polysaccharides (mamaku and kiokio) and two commercially available hydrocolloids (κ -carrageenan and low-acyl gellan), selected following preliminary screening, across different blend ratios, ionic strengths, pH conditions and storage regimes, with the goal of elucidating the nature of their interactions. It was hypothesised that the crude and purified forms of each fern gum would interact differently with the commercial gums—the crude forms acting predominantly through their endogenous ions, and the purified forms through the polysaccharide chains themselves. Comparing the two forms was therefore used as a means of separating ionic from polymeric contributions to the mixed-system behaviour.

κ -carrageenan is a sulphated galactan extracted from the cell walls of red algae (Rhodophyta), composed of a linear backbone of alternating residues of 3-linked β -D-galactose and 4-linked α -D-galactose (in the form of 3,6-anhydro-D-galactose). It is most stable at pH 9 but

depolymerises below pH 4 and its chemical susceptibility is affected by counterions and co-solutes which influences the polysaccharide conformation. Synergistic interactions between κ -carrageenan and locust bean gum, tara gum, xanthan and konjac glucomannan have been reported (Tuvikene, 2021). Low-acyl gellan gum is a fully deacetylated extracellular polysaccharide secreted by *Sphingomonas elodea*. It is an anionic linear tetrasaccharide with repeating units of $\rightarrow 3$ - β -D-Glcp-(1 $\rightarrow$ 4)- β -D-GlcpA-(1 $\rightarrow$ 4)- β -D-Glcp-(1 $\rightarrow$ 4)- α -L-Rhap-(1 $\rightarrow$ (Sworn & Stouby, 2021). Both κ -carrageenan and low-acyl gellan gums are classified as gelling gums and typically form firm, brittle and thermoreversible gels. They produce strong and stable three-dimensional structures by forming junction zones through hydrogen bonding, cation-mediated cross-linking or hydrophobic interactions. Gelation can be induced mainly through heating, cooling, acidification, and enzymatic treatments amongst other processes. Under heating, these gums solubilise and exist in a disordered random coil state. During cooling, a coil to helix transformation occurs. These helices then aggregate to form a structure and is promoted by cations that reduce the electrostatic repulsion between chains. Aggregation is promoted by K^+ and Ca^{2+} for κ -carrageenan and low-acyl gellan gum, respectively (Sworn & Stouby, 2021; Tuvikene, 2021). While several studies have suggested synergistic interactions between unconventional non-starch polysaccharides like yellow mustard gum and senna tora gum with κ -carrageenan (Fang et al., 2024; Wang et al., 2022), this study is the first to introduce a naturally occurring, non-gelling, novel fern polysaccharide with distinctive shear-responsive behaviour into κ -carrageenan and low-acyl gellan gel networks. In contrast to the fern-derived gums, both κ -carrageenan and low-acyl gellan are gelling polysaccharides, making their mixtures with the non-gelling fern gums a particularly interesting system for investigating how a non-gelling polysaccharide component, influences the structure and functionality of an established gel network.

Understanding the interactions between mamaku and kiokio polysaccharides with these widely utilised commercial polysaccharides is essential for establishing whether these novel fern-derived polysaccharides can be deployed to modulate the physicochemical properties and functionality of food and non-food products.

2. Materials and methods

2.1. Extraction of fern gums

The fronds of *Parablechnum novae-zelandiae* (kiokio) were harvested from the Manawātū-Whanganui region in New Zealand. The warm water extraction process to obtain water-soluble polysaccharides (crude gum) from the fronds is as described in Peh, Matia-Merino, and Goh (2026). The crude gum was freeze-dried before further purification or analysis. Frozen crude gum from *Sphaeropteris medullaris* (mamaku) at 4.9 % (wt/wt) total solids (TS), was extracted as reported earlier (Tresidder, 2018) and used in this study.

Prior to purification or analysis, the freeze-dried crude kiokio gum powder was hydrated in Milli-Q water for 15 h at 20 °C (200 rpm) to achieve the required TS (% wt/wt), while the frozen crude mamaku gum was defrosted overnight at 20 °C. The samples were then centrifuged at 3100 g for 20 min at 20 °C to remove any residual insoluble particles.

2.2. Purification of fern gums and quantification of total polysaccharide content

Two purification approaches were compared alongside the crude gums – dialysis alone, and ethanol (EtOH) precipitation followed by dialysis. The crude extracts (2 % TS wt/wt) were dialysed (dialysis tubing molecular weight cut-off: 12,000–14,000 Da) against Milli-Q water with multiple changes of water within 72 h at 4 °C. The dialysed samples were then concentrated using a rotary evaporator (23 mbars, 40 °C) until the desired TS was achieved. The fern gum obtained through this process was named the “dialysed fern gum”. The dialysate was also

retained and concentrated for further analysis.

For the ETOH precipitated and dialysed samples, the crude extracts (3 % TS wt/wt) were subjected to a single-step ETOH precipitation (80 % v/v) to obtain purified polysaccharide precipitates. The precipitates were then redissolved in Milli-Q water. According to the procedure mentioned above, the samples were then dialysed and concentrated in a rotary evaporator until the desired TS was achieved. The samples obtained through this purification process were termed “purified fern gums”.

The total carbohydrate (TC) content of crude, dialysed and purified fern gums was quantified using the phenol-sulfuric acid method modified from Dubois et al. (1956). Briefly, 400 µL of each sample was pipetted into each test tube and 200 µL of phenol solution (5 % wt/wt) was added. The tubes were mixed on a vortex mixer before 2 mL of concentrated sulfuric acid were added rapidly by jetting it directly onto the surface of the mixture at ~45° angle. The mixtures (230 µL) were then transferred onto 96-well microplates and read at 490 nm. Sugar mix standards containing the main constituent sugars of mamaku and kiokio gums at their respective proportions as previously reported (Peh, Goh, et al., 2026), were used to construct the calibration curves. Briefly, the sugar mix prepared for mamaku gum contains glucuronic acid (42 %), galactose (25 %), mannose (14 %), xylose (12 %) and glucose (7 %). The sugar mix representing kiokio gum consists of arabinose (25 %), galactose (24 %), xylose (16 %), glucuronic acid (16 %), mannose (9 %) and glucose (10 %). While the TC of purified fern gums was assumed to be representative of the polysaccharide content (PC), the TC of the crude fern gums was contributed mainly by the free sugars. Hence, to determine the PC in the crude fern gums, a further calculation was performed using Equation 1.

$$PC \text{ in crude fern gums (\%)} = \frac{\text{Weight of freeze-dried purified gum (g)}}{\text{Initial weight of crude gum powder used in purification (g)}} \times TC \text{ of purified gum (\%)} \quad \text{Equation 1}$$

2.3. Proximate analysis and mineral content determination

The proximate analyses of the fern gums were conducted by an accredited Nutrition Laboratory, Massey University, Palmerston North, New Zealand. Dry matter (AOAC 925.10/930.15), ash (AOAC 942.05), crude protein (AOAC 968.06), crude fat (AOAC 2003.06), starch (AOAC 996.11, Megazyme kit), total free sugars (phenol-sulfuric method) and crude fibre (AOAC 962.09/978.10) were quantified. Mineral content was determined using an inductively coupled plasma-optical emission spectrometer (ICP-OES).

2.4. Screening process to select commercial gums for interaction studies

Six non-gelling and ten gelling commercial gums were screened in this preliminary investigation. The non-gelling gums were xanthan gum, guar gum, locust bean gum, tara gum, gum arabic, and sodium carboxymethylcellulose. The gelling gums screened were high methoxyl (HM) pectin [67–71 % degree of esterification (DE)], low methoxyl (LM) pectin (35–42 % DE), low methoxyl amidated (LMA) pectin [31 % DE, 19 % degree of amidation (DA)], low acyl (LA) gellan gum, high acyl (HA) gellan gum, sodium alginate, κ-carrageenan, ι-carrageenan, and chitosan [both low (50–190 kDa) and medium molecular weight (M_w) grades].

Stock solutions of commercial and fern gums were hydrated overnight in Milli-Q water at an appropriate concentration. Chitosan was hydrated in 1 % acetic acid solution (pH 2.65) instead, and after solubilisation, it was readjusted to pH 5.5.

Once hydrated, commercial gum solutions were heated in a water bath at 60 °C for 15 min (or 95 °C for 30 min for hot-water soluble gums), before the fern gum solutions were added and the mixture held at 60 °C for a further 15 min. The gum solutions were mixed at various ratios, with commercial gum concentrations ranging from 0.01 to 0.50 % TS (wt/wt) and crude fern gum concentrations ranging 1.50–1.99 % TS (wt/wt), such that the total solids of each mixture was fixed at 2 % TS (wt/wt). Controls containing only the commercial gum or fern gum were included, and where necessary, anhydrous glucose (Glc) was added to make up for the TS content. The tubes were then cooled in an ice bath for 30 min and chilled (4 °C) overnight for gelation to occur. The tubes were equilibrated to room temperature (20 °C) before observation.

In order to detect gelation or synergistic increases in viscosity, the tubes were inverted and the flow behaviour of the mixtures upon inversion was recorded and classified as free-flowing, viscous or non-flowing (gelled). The solutions or gels were then removed from the tubes and placed on a Petri dish to assess whether the mixture exhibited viscous flow or gel-like behaviour. From this preliminary screening, only xanthan (out of the non-gelling gums) showed a synergistic increase in viscosity. However, this interaction will not be covered here, due to the already lengthy manuscript. Only two gelling gums [κ-carrageenan (κC) and LA gellan (LAG)] were selected to investigate their interactions with the fern gums, based on detectable gel formation. For further analysis, gel mouldability was an important criterion for performing tests such as TPA, syneresis and WHC. Thus, from the wide range of concentrations tested during the screening, 1 % TS (wt/wt) commercial gum was selected as the appropriate concentration for subsequent analysis, due to their superior gel formation, mouldability and suitability for downstream characterisation.

2.5. Preparation of commercial-fern gum mixtures and gels

Prior to mixing with the commercial gums, the freeze-dried crude kiokio gum powder was hydrated, and frozen crude mamaku gum was defrosted before centrifugation as described in Section 2.1. The frozen dialysed or purified gum samples were also defrosted overnight at 20 °C and diluted to the required TS (% wt/wt) before use.

The selected hot-soluble commercial gelling gums (κ-carrageenan and LA gellan) were hydrated overnight without stirring for swelling to occur and heated at 90 °C for 10 min to ensure complete dissolution. The fern gums were also pre-heated to 90 °C for 10 min to prevent instantaneous gelation upon mixing. Both gums were then mixed and heated at 90 °C for another 10 min. Thereafter, if pH adjustment was necessary, the mixtures were held above 70 °C during adjustment to prevent instantaneous gelation. The mixtures were then transferred into the concentric cylinder geometry (CC27) pre-heated to 90 °C in the rheometer. A layer of oil was applied to the surface to prevent evaporation during measurement. Aliquots of 4 g of the heated mixtures were transferred into cylindrical silicone moulds (2 cm in diameter and height). They were then cooled at room temperature before covering and chilling at 4 °C for 15 h for gel formation. Further analyses were conducted on the gelled samples, including texture profile analysis (TPA), syneresis, water-holding capacity, Fourier transform infrared spectroscopy (FTIR) and cryo-scanning electron microscopy (cryo-SEM). Prior to analysis, the gels were equilibrated at 20 °C for at least 1 h before unmoulding. For freeze-thaw stability analysis, the gels were chilled as described above, before transferring them to the freezer.

(−16 °C) and storing for 15 h. They were then allowed to thaw at 20 °C for at least 2 h before analysis.

The gums were mixed to make up a solution of a fixed concentration of 2 % TS (wt/wt) at two blend ratios [commercial gum (% TS):fern gum (% TS)] – 0.5:1.5 and 1.0:1.0. Controls containing only commercial gums at their respective concentrations were also included, with Glc added to make up the total solids content to 2 % TS (wt/wt).

2.6. Ion adjustment using various solvents

When studying the influence of ions on the interactions, three types of aqueous solutions of varying ionic composition were used for the commercial gum controls to isolate and identify the effects of endogenous ions present in the crude fern gums. Firstly, Milli-Q water was used to represent an ion-free environment. Secondly, to identify the effects of a single-ion system, potassium chloride (KCl) was dissolved in Milli-Q water to obtain a similar ionic strength and conductivity as the one found in the crude fern extracts at a given TS (% wt/wt). KCl was selected since potassium was found to be the most abundant mineral present in the crude fern gums (Peh, Goh, et al., 2026). Thirdly, to simulate the multi-ion environment of the crude fern gums, concentrated dialysate (prepared according to Section 2.2) was used. The conductivity of the dialysate was adjusted to match that of the crude fern gums at a given TS (% wt/wt).

2.7. pH adjustment

In order to investigate the influence of pH on the interactions, the mixtures were adjusted to pH 3 and pH 9 using HCl and NaOH solutions (0.1 M and 1 M as appropriate). Mixtures containing gelling gums were kept above 70 °C in a hot water bath during adjustment to prevent instantaneous gelation. Adjustments were performed using an Orion Dual Star pH/ISE meter fitted with an Orion Single Junction Epoxy Bodied pH/ATC Triode (Thermo Fisher Scientific, Massachusetts, United States).

2.8. Rheological analysis - temperature and frequency oscillatory sweeps

MCR 302 rheometer (Paar Physica, Anton-Paar, Graz, Austria) fitted with a concentric cylinder (CC27) geometry was used to measure the rheological profiles. Data were recorded using Rheoplus/32 software (Version 3.62). All rheological measurements were carried out in duplicates at 20 °C unless otherwise specified.

Time-temperature profiles were obtained for all mixtures using a constant frequency (1 Hz) and a constant strain of either 0.01 % or 0.1 %, selected depending on the linear viscoelastic region (LVER) determined from a preliminary amplitude sweep. Samples were first pre-sheared at 100 s^{−1} for 1 min to eliminate residual structure and establish a reproducible initial condition, and then held at 90 °C for 10 min. They were then cooled to 4 °C (cooling rate: 2 °C/min) and held at 4 °C for 30 min to allow gelation to occur. Subsequently, the temperature was increased to 20 °C (heating rate: 2 °C/min) for final viscoelastic characterisation at the measurement reference temperature. A frequency sweep was then carried out from 0.01 Hz to 20 Hz at a constant strain of either 0.01 % or 0.1 %, within the LVER as determined by an amplitude sweep from 0.1 % to 100 % strain.

2.9. Texture profile analysis (TPA)

A TA.XT Plus Texture Analyser (Stable Micro Systems Ltd., Surrey, UK) with a 5 kg load cell was used for textural characterisation. TPA was performed on the gels using a trigger force of 3 g, wait time between compressions of 10 s, test and post-test speeds of 2 mm/s, 51 mm compression platen (P/51 mm) and 70 % strain. This strain level was selected to simulate the compressive forces experienced during mastication, consistent with the original design intent of TPA. Given the

brittle nature of the gels and the high strain applied, fracturing during the first compression was anticipated, rendering springiness, cohesiveness and chewiness values invalid (Bourne, 2002; Johnson, 2015), and only hardness as well as fracturability values were reported. All gels were subjected to TPA and measured at least four times.

2.10. Syneresis measurements

Syneresis measurements were performed following the methods of Ako (2015) with modifications. Gels were unmoulded at 20 °C and blotted dry with a fluted filter paper (Whatman Grade 1 cellulose filters, 90 mm diameter, 11 µm pore size). The same filter paper was used to absorb any residual liquid remaining in the mould prior to blotting. The total liquid expelled from the gels was recorded. The syneresis ratio in percentage was calculated using Equation 2 as follows:

$$\text{Syneresis (\%)} = \frac{W_3}{W_1 + W_2} \times 100 \quad \text{Equation 2}$$

where W_1 is the initial weight of the gel, W_2 is the weight of the residual liquid in the mould and W_3 is the weight of the liquid absorbed by the filter paper when blotting the gel.

All measurements were performed in duplicates using an analytical balance (Mettler Toledo, ML204, Switzerland) with a resolution of ± 0.1 mg.

2.11. Water holding capacity (WHC) measurements

WHC measurements were performed on the freeze-thawed gels using the filter paper press method reported by Niknia and Kadkhodae (2017) but with some modifications with respect to the dimensions of the filter paper, weight used and compression duration. The gels were compressed between two fluted filter papers (Whatman Grade 1 cellulose filters, 90 mm diameter, 11 µm pore size), under a fixed weight of 40 g for 10 min at 20 °C. The WHC in percentage was calculated using Equation (3) as follows:

$$\text{WHC (\%)} = \frac{W_5}{W_4} \times 100 \quad \text{Equation 3}$$

where W_4 is the initial weight of gel and W_5 is the final weight of the dried gel after compression.

All measurements were performed in duplicates using an analytical balance (Mettler Toledo, ML204, Switzerland) with a resolution of ± 0.1 mg.

2.12. Fourier transform infrared spectroscopy (FTIR)

The spectra of freeze-dried fern gums and gels were recorded using an attenuated total reflectance–Fourier transform infrared spectrometer (Nicolet iS5 fitted with an iD7 ATR accessory, Thermo Fisher Scientific Inc., MA, USA), fitted with a diamond crystal and using 32 scans at a resolution of 4 cm^{−1}. Fresh background spectra were acquired periodically before each new sample was analysed. It was collected under the same experimental conditions but with no samples loaded on the crystal. The raw reflectance measurement data were converted to transmission spectra. The baselines of the spectra were then corrected, and the peaks were detected using Omnic 9.2 software (Thermo Fisher Scientific Inc., MA, USA).

2.13. Cryo-scanning electron microscopy (Cryo-SEM)

Gel samples were frozen at −180 °C using a Bal-Tec JFD 030 Jet freezer (Bal-Tec AG, Balzers, Liechtenstein) and loaded into the preparation area of a Gatan Alto 2500 cryo preparation chamber and cryo stage (Gatan Inc, Pleasanton, California, USA). The frozen sample was fractured to expose the internal microstructure, then etched at −90 °C

for 15 min to remove surface ice by sublimation, before the temperature was lowered to -120°C . The sample was then sputter coated with platinum (15 nm). Subsequently, the sample was loaded into the viewing chamber of the Jeol JSM 6500F FE-SEM (JEOL Ltd, Tokyo, Japan), where the temperature was maintained at -120°C . Images were obtained using a secondary electron imaging (SEI) detector with an accelerating voltage of 3 kV.

2.14. Statistical analysis

For each experiment, measurements were performed in duplicate (rheology, syneresis, WHC, FTIR) or quadruplicate (TPA). The values were averaged prior to statistical analysis. One-way analysis of variance (ANOVA) at 95 % confidence level was performed and significant differences among mean values were determined by Tukey post hoc test, using a statistical analysis software [IBM SPSS Statistics, Version 30.0.0.0 (172)]. Owing to the limited availability of the novel fern gums, replication was restricted to duplicate or quadruplicate measurements, and differences identified from the duplicate assays should therefore be regarded as indicative.

3. Results and discussion

3.1. Effect of the purification degree of fern gums on the interactions

Based on established knowledge in the literature, ion-mediated gel formation was the most likely explanation for the gelation of κC and LAG with the crude fern gums, as observed during screening (Section 2.4). However, it was hypothesised that the gel properties would differ between systems containing crude fern gums — with their endogenous ions and polysaccharides — and those containing aqueous ionic solutions alone. It was therefore necessary to test whether the observations were solely an ionic effect or whether the polysaccharides in the fern gums also contributed, necessitating purification of the crude fern gums as discussed in this section.

As listed in Table 1, the proximate composition of both crude mamaku and kiokio gum powders revealed high total carbohydrate contents (59.60–70.30 %), comprising largely of free sugars (17.00–45.60 %). They also contained high ash contents (25.80–31.90 %) due to the large amounts of endogenous minerals.

Amongst the minerals listed in Table 2, potassium was the most abundant mineral in both crude mamaku (9.59 % out of 12.64 % of total minerals) and kiokio (12.05 % out of 14.71 % of total minerals) gums. It was therefore selected as the mineral to be used to prepare aqueous ionic solutions that simulate the ionic environment of the crude fern gums (at 1 % TS wt/wt) – 0.03M KCl, 2.37 mS/cm KCl or dialysate (conductivity of crude mamaku gum), and 3.44 mS/cm KCl or dialysate (conductivity of crude kiokio gum). Additionally, potassium was also used as a benchmark to determine the effectiveness of the various degrees of purification conducted – dialysis only, ETOH precipitation only, as well as ETOH precipitation and dialysis. Potassium content of dialysed as

Table 1
Proximate composition of crude mamaku and kiokio gum powder.

	Crude mamaku gum	Crude kiokio gum
Moisture (% wt/wt wet basis)	16.60	7.90
Proximate composition (% wt/wt dry basis)		
Ash	25.80	31.90
Crude protein	1.90	8.40
Fat	2.00	0.30
Starch	1.60	2.50
Total free sugars	45.60	17.00
Crude fibre	0.50	1.00
Total carbohydrates ^a	70.30	59.40

^a Values calculated by difference: [Total carbohydrates (%) = 100 – (Moisture + Ash + Protein + Fat)].

Table 2

Mineral composition of crude mamaku and kiokio gum powder.

	Crude mamaku gum		Crude kiokio gum	
	mg/kg	% wt/wt dry basis	mg/kg	% wt/wt dry basis
Potassium	80,000	9.592	111,000	12.052
Sodium	20,000	2.398	8900	0.966
Calcium	3500	0.420	6400	0.695
Magnesium	870	0.104	8700	0.945
Aluminum	840	0.101	47	0.005
Iron	138	0.017	270	0.029
Manganese	47	0.006	162	0.018
Zinc	39	0.005	0.025	Negligible
Copper	10	0.001	32	0.003
Lead	0.19	Negligible	0.64	Negligible
Selenium	0.02	Negligible	0.02	Negligible
Mercury	0.01	Negligible	0.1	Negligible
Total	105,444.22	12.64	135,511.79	14.71

Table 3

Changes in potassium concentration in samples of different degrees of purification, based on a standardised sample concentration of 1 % TS (wt/wt).

	Potassium (mM)
Mamaku gum	
Crude	31
Dialysed	< 4
ETOH precipitated	9
ETOH precipitated and dialysed	< 4
Dialysate (at 2.37 mS/cm, representative conductivity of 1 % TS (wt/wt) crude mamaku gum)	13
Kiokio gum	
Crude	28
Dialysed	< 4
ETOH precipitated	12
ETOH precipitated and dialysed	< 4
Dialysate (at 3.44 mS/cm, representative conductivity of 1 % TS (wt/wt) crude kiokio gum)	20

well as ETOH precipitated and dialysed samples were similarly low (<4 mM), indicating that the majority was effectively removed from the crude sample (28–31 mM) (Table 3). It was also noted that ETOH precipitation only was not as effective since it retained a higher amount of potassium after the process (9–12 mM). These results also indicate that majority of potassium was removed in the dialysate. It should be noted however, that the dialysis process did not remove all the ions present as some ions may be bound to the fern-derived polysaccharides. Thus, the calculation of the molarity of KCl solution based on the potassium content in the crude fern gum powders may be slightly overestimated. Although potassium was the dominant ion in the crude fern gums, use of the dialysate enabled the investigation of the effects of the full profile of cation types and proportions (Table 2). While dialysates may also contain minor components such as polyphenols, their profiles and contribution to the rheological properties are unclear in this study and are beyond the scope of the current investigation. Regardless, the importance and ability of endogenous ions to modulate network strength and influence chain associations, has been previously highlighted as the dominating effect in crude mamaku gum (Matia-Merino et al., 2012; Wee et al., 2015a). Thus, retaining this native ionic environment was essential to capture authentic structure–function behaviour.

With increasing levels of purity, it was also expected that the polysaccharide content of the fern gums would increase accordingly. The polysaccharide content (PC) of the fern gums after various purification processes was therefore measured in the form of total carbohydrate content (TC) using the phenol-sulfuric acid assay. The TC content of crude fern gums (Table 4) was likely underestimated by this assay since it was lower than the approximated TC content obtained by proximate

Table 4

Total carbohydrate (TC) content of samples at different degrees of purification and the equivalent polysaccharide content (PC) in 1 % TS (wt/wt) gum solutions used for standardising PC in subsequent analysis.

	TC (%)	PC (1 % TS)
Mamaku gum		
Crude	49.96 ± 2.34 ^b	0.10
Dialysed	64.33 ± 1.56 ^d	N. D.
ETOH precipitated and dialysed	70.98 ± 0.93 ^e	0.71
Kiokio gum		
Crude	27.70 ± 0.68 ^a	0.19
Dialysed	57.06 ± 1.09 ^c	N. D.
ETOH precipitated and dialysed	69.57 ± 1.29 ^e	0.70

Note. TC values obtained using the phenol-sulfuric acid assay method (Dubois et al., 1956). Average values are presented with standard deviations. Different superscript letters indicate significant differences at $\alpha \leq 0.05$ ($n = 6$). Values that were not determined were represented by "N. D.".

analysis (Table 1). This was likely due to interference from the presence of other compounds in the crude gum solutions. Nonetheless, the PC content was accurately determined (using Equation (1)) from the accurate TC content and yield of the ETOH precipitated and dialysed samples. As expected, the TC increased with increasing degree of purification and achieved a high TC of 70 % for ETOH precipitated and dialysed samples (Table 4). This indicated a high degree of material purity and high polysaccharide content, since majority of the other components would have been removed. Thus, the TC was assumed to be equivalent to PC for this sample. This degree of purity is comparable to that of many commercial gums, including the κ C used in this study. Other researchers have achieved purity levels between 19.7 % and 80 % by using similar purification processes applied to plant-sourced polysaccharides (Bai et al., 2020; Fan et al., 2020; Ghosh et al., 2019; Radzki et al., 2023; Romdhane et al., 2017; Ross et al., 2015). Thus, ETOH precipitated and dialysed samples (hereafter termed 'purified') were selected for subsequent analysis instead of the dialysed only or ETOH

precipitated only samples. Since both the purified gum samples and the commercial gum samples used in this study have a similar PC of 70 %, comparison on a TS basis as commonly practiced in the industry was justified.

Additionally, to determine if this purification process resulted in any alterations in the molecular composition and functional groups of these fern-derived polysaccharides, their chemical fingerprints were compared using FTIR (Fig. 1).

When analysed by FTIR (Fig. 1), crude versus purified mamaku (Fig. 1A) showed similar bands, with the exception of bands 11–15 (910–632 cm^{-1}), which were present only in crude mamaku. This region is commonly assigned to skeletal mode vibrations, or deformational modes of C–C–C, C–C–O, and O–C–O groups in polysaccharides and mono-, di- or tri-saccharides (Wiercigroch et al., 2017). Since bands in this region are often associated with ring torsion modes which is consistent with the assignment to simple sugars, these peaks may thus have been contributed by the free sugars in crude mamaku powder, which constitute a high percentage (45.60 %, Table 1). Purification of kiokio gum did not noticeably alter the FTIR spectra when compared to the crude (Fig. 1B). Generally, both purified mamaku and kiokio gums showed similar spectra (Fig. 1C), with peaks and bond assignments typical of polysaccharides (Cerna et al., 2003; Čopíková et al., 2001; Gómez-Ordóñez & Rupérez, 2011; Guo et al., 2015; Hong et al., 2021; Ke et al., 2023; Mikusheva et al., 2025; Sedaghat et al., 2019; Silva-Correia et al., 2011; Webber et al., 2012). Therefore, it could be concluded that the purification processes did not alter the molecular composition and functional groups of these fern-derived polysaccharides, but primarily removed the free sugars, minerals and possibly proteins in the crude gums.

The effects of purification on the viscoelastic and structural properties of these fern gums are illustrated in Figs. 2 and 3, respectively using LAG and mamaku as an example.

As observed in the rheological results (Fig. 2), 1 % LAG gels containing crude mamaku had much stronger viscoelastic properties as

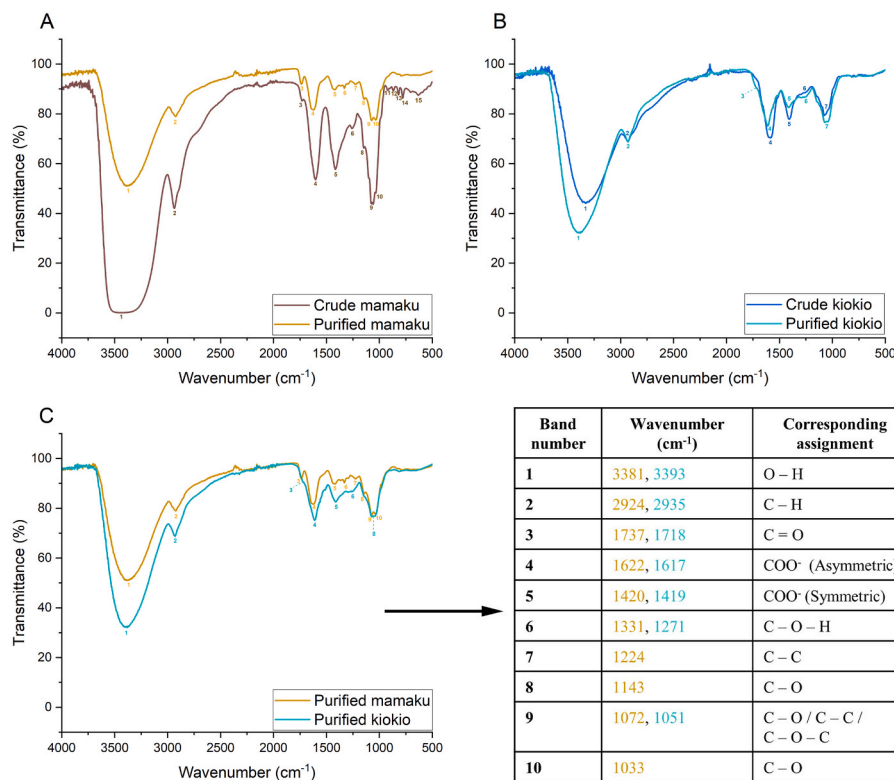


Fig. 1. FTIR spectra of (A) crude versus purified mamaku gum, (B) crude versus purified kiokio gum, and (c) purified mamaku versus kiokio gum with a table of functional group assignments to the respective wavenumber.

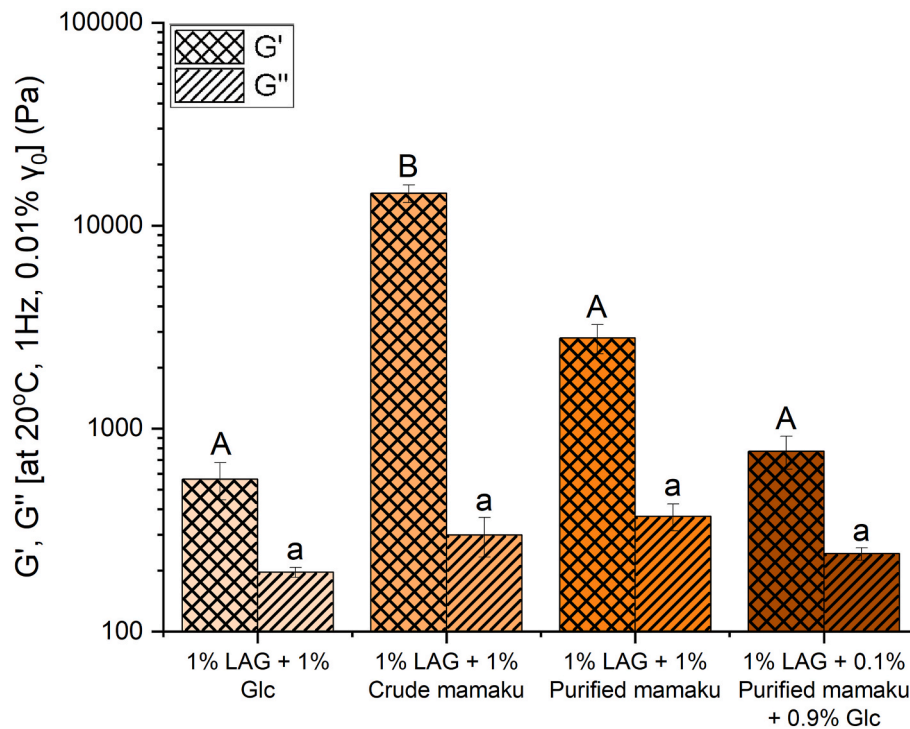
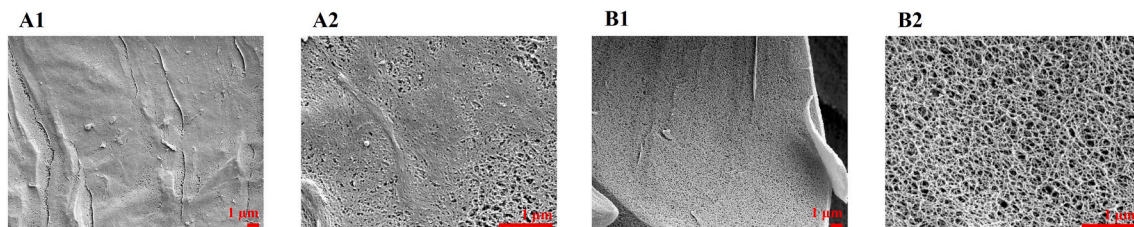


Fig. 2. Effect of mamaku gum purification on the viscoelastic properties (G' and G'') of mixed gels made with LAG.

1% LAG + 1% Crude mamaku



1% LAG + 1% Purified mamaku

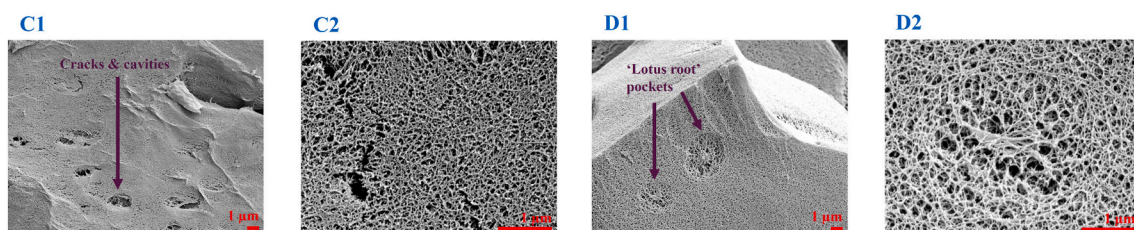


Fig. 3. Cryo-SEM images of 1 % LAG + 1 % crude mamaku gel (A1-B2) and 1 % LAG + 1 % purified mamaku gel (C1-D2). All concentrations represented as % TS (wt/wt). Note. Each letter corresponds to a different location explored in each sample. Images labelled as “1” and “2” were captured at 5000 $\times$ and 25000 $\times$ magnification, respectively.

compared to gels containing purified mamaku. This was likely due to the stronger network promoted by ions in the crude gum. When an equivalent amount of polysaccharide (0.1 % TS wt/wt) in the crude gum was added in the form of purified gum, no significant change resulted relative to the control gel containing LAG only, suggesting that gel strength was contributed predominantly by LAG, which was also true for the other commercial gums tested. Equally, when the purified fern gum was further increased (1 % LAG + 1 % purified mamaku) the overall gel strength (~ 2500 Pa) remained statistically similar to the control (1 % LAG + 1 % Glc, ~ 600 Pa) even though G' values were higher on average. This highlights the critical role of ions in the gelation of these gelling

gums, as already established in the literature. Thus, the important influence of ions was further investigated and is discussed in the following sections.

The rheological results were also corroborated by cryo-SEM images (Fig. 3). LAG gels containing crude mamaku displayed smoother surfaces (Fig. 3 A1-A2) with a more uniform pore network (Fig. 3 B1-B2), compared to the gels containing purified mamaku, which showed more cracks and cavities on the surfaces (Fig. 3 C1-C2), alongside signs of possible phase separation observed as ‘lotus root’ pockets (circular voids characteristic of lotus rhizome cross-sections) (Fig. 3 D1-D2). These cracks, cavities and pockets may induce greater gel heterogeneity and

disrupt gel connectivity, thereby weakening the gel strength relative to gels containing crude mamaku, consistent with the rheological results.

However, this is one example only and the effects observed for other mixtures are expanded upon in the subsequent discussions. For the rest of this study, crude mamaku gum (CM), purified mamaku gum (PM), crude kiokio gum (CK) and purified kiokio gum (PK) are compared in parallel to elucidate the effects of endogenous ions and polysaccharides in the fern gums on the physicochemical properties of the commercial gums.

3.2. Interaction with κ C and LAG

3.2.1. Influence of ions

3.2.1.1. Gel hardness and syneresis. As shown in Table 5, both κ C and LAG exhibited significantly greater gel hardness in the presence of ions, as expected. The use of a single ion type (KCl) or a mixture of ions (dialysate, CM or CK), did not significantly affect the gel hardness and syneresis. Although residual low-molecular-weight components such as free sugars and polyphenols were not independently quantified after purification (as this was beyond the scope of the present study) and may contribute to the observed gel behaviour, the ability of KCl and dialysate alone to reproduce the principal effects observed with crude fern gums indicates that endogenous ions were a major contributor to the enhanced gel strength. Notably, despite the higher polysaccharide content, gels made with purified fern gums were significantly softer than gels made from crude gums, suggesting that ions played a dominant role in strengthening the gel network. Furthermore, gels containing purified fern gums were not significantly harder than the controls (water only), except for LAG + PK. This suggests that PM and PK behave independently of κ C and LAG to form either a phase separated gel network or a network formed by a singular polysaccharide with another polysaccharide trapped within. Interestingly, LAG + PK was the only purified combination to exceed the control (water only) hardness, whereas PM combinations did not — a divergence that merits comment. Although both are purified glucuronomannan gums, kiokio undergoes an irreversible loss of shear-thickening upon dialysis that cannot be restored by ion re-addition (Peh et al., 2026), whereas mamaku recovers its shear-thickening under the same conditions (Wee et al., 2015a). This irreversibility suggests that ion removal induces a more fundamental structural reorganisation in kiokio, which may expose mannan backbone segments that, without the steric or electrostatic shielding provided by ions, are free to associate with LAG chains under the conditions tested here. Whether this reflects differences in substitution pattern, chain flexibility, or the degree of exposed mannan backbone between the two gums remains to be established through more detailed molecular characterisation. Nevertheless, the consistent tendency for PK to contribute more to network reinforcement than PM — observed here in TPA and subsequently in rheology — suggests a structural rather than purely concentration-driven origin. Additionally, PM and PK gels also had significantly lower syneresis compared to samples containing ions. They also conferred a darker brown colour to the gels, which may be an important consideration for future product applications. Overall, these results demonstrate that purified fern gums can decouple texture modification from ionic content, which is a major formulation advantage, especially in ion-sensitive formulations such as dairy-protein and plant-protein systems. However, the hardness value obtained solely from TPA may not holistically represent the mouthfeel and swallowing attributes that consumers may experience. Future work should therefore include sensory evaluations to provide deeper insights.

3.2.1.2. Gelation behaviour and network viscoelasticity. In-situ gelation was also conducted in the rheometer, and the time-temperature gelation profiles (Fig. 4) and final viscoelastic moduli obtained during a frequency sweep (Fig. 5) were recorded. It should be noted that rheological

Table 5

Hardness (obtained from TPA), syneresis and visual appearance of gels consisting of 1 % TS (wt/wt) of commercial gum combined with 1 % TS (wt/wt) Glc or fern gums in various aqueous ionic (KCl or dialysate) and non-ionic (H_2O) solutions.

Sample	Hardness (g) ⁺	Syneresis (%) [#]	Visual appearance
κC + (Glc or CM or PM)			
κ C + Glc (H_2O)	2772.37 $\pm$ 9.33 ^g	1.66 $\pm$ 0.17 ^{b-d}	
κ C + Glc (0.03 M KCl)	5100.06 $\pm$ 384.89 ^h	9.26 $\pm$ 0.06 ^f	
κ C + Glc (2.37 mS/cm KCl)	5802.32 $\pm$ 175.43 ⁱ	2.292 $\pm$ 0.586 ^d	
κ C + Glc (2.37 mS/cm dialysate)	5538.15 $\pm$ 312.54 ^{hi}	8.46 $\pm$ 0.83 ^f	
κ C + CM	3295.81 $\pm$ 133.11 ^{hi}	5.83 $\pm$ 0.40 ^e	
κ C + PM	1399.62 $\pm$ 176.09 ^{b-e}	0.79 $\pm$ 0.01 ^{a-c}	
κC + (Glc or CK or PK)			
κ C + Glc (3.44 mS/cm KCl)	5900.34 $\pm$ 200.67 ⁱ	8.60 $\pm$ 0.43 ^f	
κ C + Glc (3.44 mS/cm dialysate)	5701.47 $\pm$ 490.76 ⁱ	8.45 $\pm$ 0.02 ^f	
κ C + CK	5728.93 $\pm$ 114.14 ⁱ	4.82 $\pm$ 0.50 ^e	
κ C + PK	2293.30 $\pm$ 141.74 ^{fg}	1.75 $\pm$ 0.04 ^{cd}	
LAG + (Glc or CM or PM)			
LAG + Glc (H_2O)	369.80 $\pm$ 23.21 ^a	0.35 $\pm$ 0.14 ^a	
LAG + Glc (0.03 M KCl)	1146.79 $\pm$ 49.14 ^{bc}	0.43 $\pm$ 0.02 ^a	
LAG + Glc (2.37 mS/cm KCl)	1209.12 $\pm$ 282.38 ^{b-d}	0.467 $\pm$ 0.025 ^a	

(continued on next page)

Table 5 (continued)

Sample	Hardness (g) ⁺	Syneresis (%) [#]	Visual appearance
LAG + Glc (2.37 mS/cm dialysate)	1539.99 ± 521.40 ^c _e	0.52 ± 0.06 ^{ab}	
LAG + CM	1736.13 ± 110.22 ^d _f	0.80 ± 0.09 ^{a-c}	
LAG + PM	325.86 ± 31.61 ^a	0.58 ± 0.08 ^{a-c}	
<i>LAG + (Glc or CK or PK)</i>			
LAG + Glc (3.44 mS/cm KCl)	1122.79 ± 233.24 ^{bc}	0.37 ± 0.06 ^a	
LAG + Glc (3.44 mS/cm dialysate)	2640.08 ± 50.03 ^g	0.61 ± 0.21 ^{a-c}	
LAG + CK	1838.48 ± 56.28 ^{ef}	0.28 ± 0.03 ^a	
LAG + PK	872.34 ± 79.92 ^{ab}	0.25 ± 0.06 ^a	

Note. All samples deformed upon the first compression at 70 % strain; therefore, values obtained for springiness, cohesiveness and chewiness were invalid. Only hardness values were reported which were equivalent to the fracturability values for all samples. Average values are presented with standard deviations. + Different superscript letters indicate significant differences at $\alpha \leq 0.05$ ($n = 4$). # Different superscript letters indicate significant differences at $\alpha \leq 0.05$ ($n = 2$). Dashed lines link all letters encompassed within that group in alphabetical order (e.g. b-d represents b, c, and d).

comparisons were based on duplicate measurements ($n = 2$); while this limits definitive statistical conclusions, the observed trends were consistent across multiple independent techniques including TPA, cryo-SEM and FTIR, lending confidence to their interpretation. The time-temperature gelation profiles showed similar profiles regardless of the ionic environment. The general trend observed was that the sol-gel transition temperature was higher in the presence of ions. For controls (water only) and samples containing PM or PK, the gelation temperatures ranged from 25.5 °C to 31.5 °C, except for LAG + PK (44.5 °C) (Fig. 4D). For samples containing KCl or dialysate, the gelation temperatures ranged from 33.3 °C to 41.1 °C. Additionally, in LAG gels (Fig. 4C and D), the gelation temperatures were higher for dialysates as compared to KCl solution even at the same conductivity, possibly due to the presence of di- and tri-valent ions which promote the formation of helices and aggregation. Notably, while most of the curves were smooth, fluctuations in moduli values were observed in some κ C gels (Fig. 4A and B) when the samples were held at 4 °C. This suggested that there were likely competing events occurring concurrently, such as phase separation and gelation, thereby leading to gel heterogeneity and resulting in the observed fluctuations in moduli values measured during gelation. This has been observed by other researchers in these heterogeneous systems (Matia-Merino et al., 2004). Nonetheless, moduli values showed consistent growth throughout, and the final moduli values during the frequency sweep were as expected.

As shown in Fig. 5, for κ C gels, both PM and PK had higher G' values

than CM and CK gels, and especially significantly higher for PK (Fig. 5B). This may be due to the higher polysaccharide content in the purified gums (as established in Table 4). However, this explanation alone is insufficient as it was not true for LAG + PM, which had a lower G' than LAG + CM (Fig. 5C). These results therefore indicate that the difference was not entirely an effect of the total polysaccharide content, but rather an influence of the ion type as well — specifically because κ C gel networks may be weakened by other ions such as Na^+ , although gel promoting ions such as K^+ and Ca^{2+} were also present (Tuvikene, 2021). Therefore, when dialysates, CM and CK were used, the viscoelastic properties were slightly weaker than the control (water only), PM and PK gels. In contrast, LAG gels containing dialysate, CM, and CK, had slightly higher G' than KCl samples, likely because the divalent ions were more effective crosslinkers than monovalent ions in LAG gels (Sworn & Stouby, 2021).

For both κ C and LAG, when an equivalent amount of polysaccharide (0.1 % TS (wt/wt) PM and 0.19 % TS (wt/wt) PK) in the crude gum was added, no significant changes in the viscoelastic properties were detected as compared to the controls (water only). This suggests that gel strength was modulated predominantly by ions at low polysaccharide concentrations, with the polysaccharide fraction contributing only at higher concentrations (e.g. 1 % TS (wt/wt) PM or PK), particularly for LAG.

Because TPA and small-deformation oscillatory rheology probe fundamentally different deformation regimes (large, destructive strain versus small, non-destructive oscillation within the linear viscoelastic region), the two are not expected to agree in every case, and where they diverge the comparison is itself informative. For example, TPA results for κ C showed significantly greater gel hardness in the presence of ions, which was not detected by small deformation rheology. From the TPA results, PM and PK gels were significantly softer than CM and CK gels, which was also opposite to the rheology results except for LAG + PM. These differences likely stem from the fundamental nature of the tests — small deformation within the linear viscoelastic range (non-destructive oscillatory conditions in rheometer) versus large deformation (70 % strain in a destructive two-cycle compression test for TPA). Under small amplitude oscillation, the rheometer predominantly measures subtle differences in strength and connectivity of the undisturbed network while TPA measures macroscopic textural attributes of the gel during mastication. The weakly crosslinked network can exhibit limited elastic storage under small oscillatory deformations, resulting in low G' values. However, under the large compressive strains applied during TPA, additional resistance mechanisms can become dominant, including pore collapse, water redistribution, polymer chain densification, and frictional interactions between collapsed junction zones. These nonlinear structural rearrangements lead to a marked increase in resistance to deformation, manifesting as elevated hardness despite the comparatively weak small-strain viscoelastic response. Complementary techniques such as large amplitude oscillatory shear, microrheology and sensory testing would be needed to obtain a more comprehensive assessment of both mechanical and sensory-relevant properties, which ultimately better predicts consumer experience (Bourne, 2002; Jeddou & Bouallegue, 2026).

3.2.1.3. Microstructural evidence for phase separation. In order to understand how the microstructure of the gels may have influenced the gel hardness and viscoelastic properties, selected controls and PM and PK gels were visualised by cryo-SEM (Fig. 6). κ C and LAG controls showed smooth surfaces and a homogeneous microstructure (Fig. 6 A1-A2, D1-D2). κ C + PM gel (Fig. 6 B1-B2) contained many cracks, consistent with the lower gel hardness observed in TPA. κ C + PK gel (Fig. 6 C1-C2) contained many 'lotus root' pockets (circular voids resembling the air canals seen in lotus rhizome cross-sections), consistent with internal micro-phase separation, which is plausible since PK is non-gelling. It is possible that sizable inclusions of PK reside as spherical droplets inside a

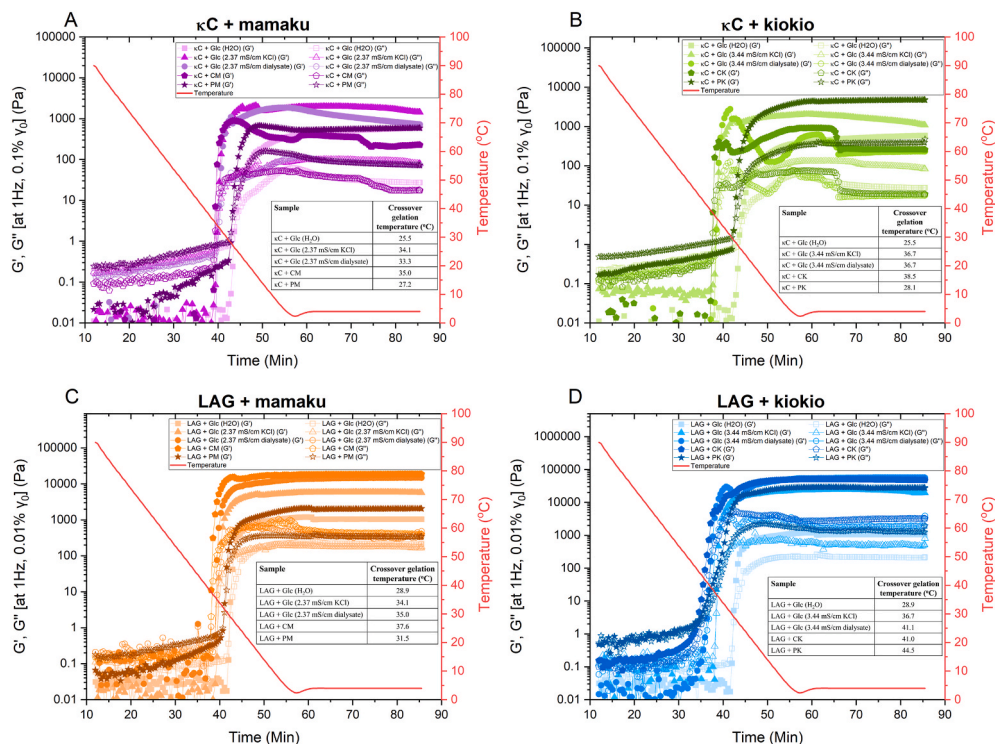


Fig. 4. Time-temperature gelation profiles and corresponding crossover gelation temperatures of selected gels consisting of 1 % TS (wt/wt) commercial gums combined with 1 % TS (wt/wt) Glc or fern gums in various aqueous ionic (KCl or dialysate) and non-ionic (H₂O) solutions – (A) κ C + (Glc or CM or PM), (B) κ C + (Glc or CK or PK), (C) LAG + (Glc or CM or PM) and (D) LAG + (Glc or CK or PK). All combinations were fixed at 2 % TS (wt/wt).

gelled matrix, analogous to a water-in-water emulsion formed from an initially unstable mixed polymer solution that is gelled thermally (Amici et al., 2000). Similar heterogeneous ‘lotus root’ pockets were also observed in LAG + PM (Fig. 6 E1-E2) and LAG + PK (Fig. 6 F1-F2) gels. LAG + PM gel also contained many cracks and cavities on its surface. These gels also had a larger pore size than LAG control.

These observed cracks, cavities, ‘lotus root’ pockets and apparently larger pore sizes provide qualitative microstructural support to indicate greater microstructural heterogeneity and disrupted connectivity in these gels, consistent with the lower gel hardness observed in TPA. The gel heterogeneity was also supported by the fluctuations in the G' and G'' values observed in the time-temperature profiles during gelation, suggesting that competing kinetic processes such as phase separation and gelation were occurring simultaneously. Once gelation set the network, phase separation would be halted within that time frame producing the observed ‘lotus root’ pocket morphology. These observations support the hypothesis that the commercial and purified fern gums behaved segregatively to form a phase-separated bicontinuous gel network, in which the purified fern gums conferred some network disruption and heterogeneity, although they remain intimately mixed with κ C and LAG without being confined to only one part of the total gel volume.

3.2.1.4. Molecular characterisation by FTIR. In order to substantiate this hypothesis, FTIR was employed on selected samples to examine for any changes in molecular compositions and functional groups, and to qualitatively identify patterns (Figs. 7–9 below).

In order to isolate the effects of ions on κ C and LAG only, controls (water only), KCl solutions and dialysate solutions at their respective conductivities were compared (Fig. 7). The different ionic types did not result in major shifts in κ C and LAG and all peaks corresponded to the expected assignments typical of the respective commercial gums (Cerna et al., 2003; Čopíková et al., 2001; Gómez-Ordóñez & Rupérez, 2011; Guo et al., 2015; Hong et al., 2021; Ke et al., 2023; Mikusheva et al., 2025; Sedaghat et al., 2019; Silva-Correia et al., 2011; Webber et al.,

2012). In κ C with dialysate gel (Fig. 7A), there was a slight shift in peak 3 to a lower wavenumber (1647 cm^{-1}) although it still remains within the same assignment range (COO^- , asymmetric), since the range for each functional group assignment reported in the literature is broad. A similar shift in the presence of divalent ions has been observed in cation-polygalacturonic acid mixtures and was attributed to a decreased bonding strength in the COO^- groups due to cation interaction (Bhattarai et al., 2026). However, this was not observed in LAG (Fig. 7B), suggesting that κ C and LAG differ in their sensitivity to divalent ion interactions.

When κ C and LAG were combined with PM or PK, the FTIR spectra of the gels generally showed an averaging effect for all combinations (i.e. the spectra of the gels are a simple weighted average of the gel components for all combinations with no new peaks formed) (Fig. 8). As seen in the detailed functional group assignments in Fig. 9, the mixed gels contained characteristics present in the individual commercial and purified fern gums they comprised. Although no new peaks were formed, there were slight shifts in wavenumbers of some peaks, suggesting that the local environment surrounding those functional groups had changed, yet the wavenumber for the shifted peak still remained within the same functional group regions as reported in the literature. For example, the slight shift in peak 4 (1623 cm^{-1}) of KC + PK (Fig. 9B) may indicate a change in the local chemical environment which might include changes in hydrogen bonding around the COO^- groups. In LAG + PK gel (Fig. 9D), peak 8 (1104 cm^{-1} , C – O, C – C, C – O – C) was absent, which was present in the LAG control gel. This was likely due to peak overlapping or masking within the highly crowded region. Nonetheless, for all samples, the overall averaging trend and absence of significant shifts or new peaks were consistent with phase-separated polysaccharide networks rather than strong molecular interaction, thus, supporting the hypothesis that the commercial and purified fern gums retained independent structures. A similar conclusion was reached for a phase separated system comprising a starch-gelatine mixture, which had a spectrum where bands for starch and gelatine were individually

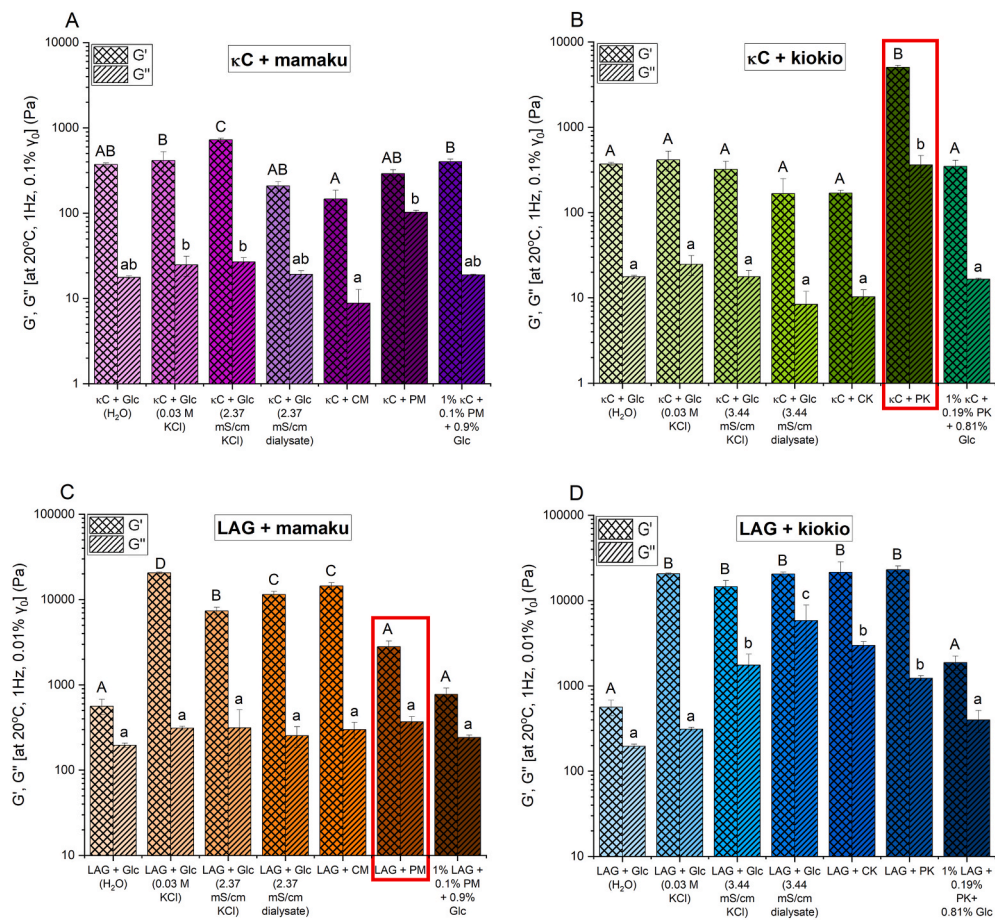


Fig. 5. Comparison of viscoelastic properties (G' and G'') of gels consisting of 1 % TS (wt/wt) commercial gums combined with 1 % TS (wt/wt) Glc or fern gums in aqueous ionic (KCl or dialysate) and non-ionic (H_2O) solutions – (A) $\kappa\text{C} + (\text{Glc or CM or PM})$, (B) $\kappa\text{C} + (\text{Glc or CK or PK})$, (C) LAG + (Glc or CM or PM) and (D) LAG + (Glc or CK or PK). All combinations were fixed at 2 % TS (wt/wt). Note. Average values are presented with standard deviations. Different superscript letters indicate significant differences at $\alpha \leq 0.05$ ($n = 2$).

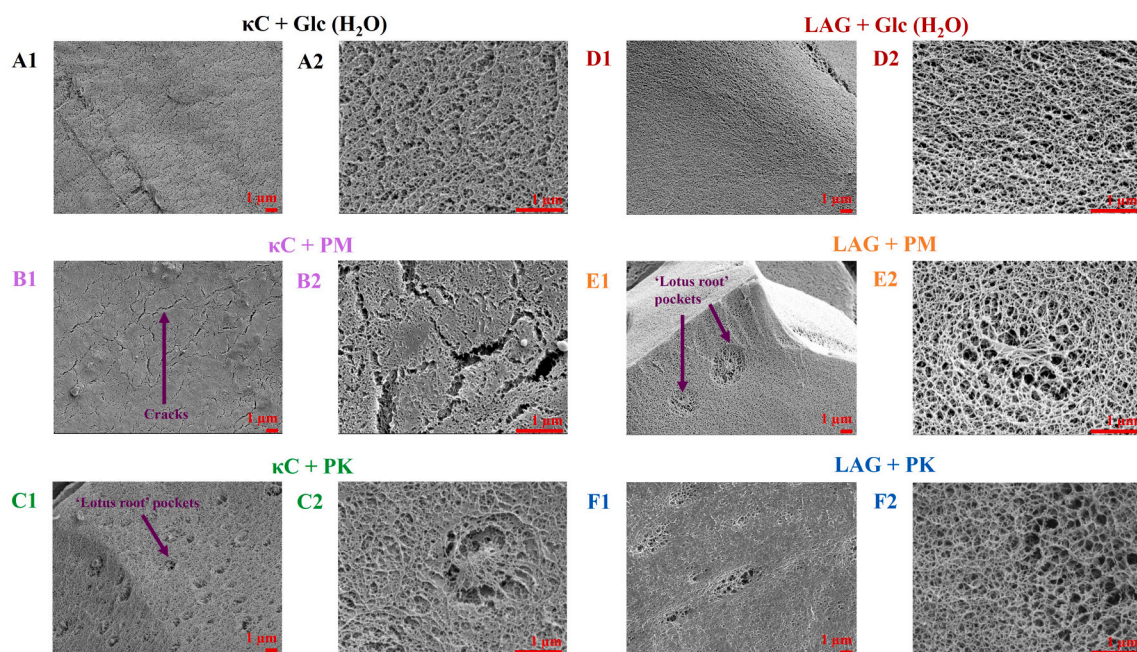


Fig. 6. Cryo-SEM images of gels consisting of 1 % TS (wt/wt) commercial gums combined with 1 % TS (wt/wt) Glc or fern gums – (A1-A2) $\kappa\text{C} + \text{Glc (H}_2\text{O)}$, (B1-B2) $\kappa\text{C} + \text{PM}$, (C1-C2) $\kappa\text{C} + \text{PK}$, (D1-D2) LAG + Glc (H_2O), (E1-E2) LAG + PM, and (F1-F2) LAG + PK. Note. Each letter corresponds to a different location explored in each sample. Images labelled as “1” and “2” were captured at 5000 $\times$ and 25000 $\times$ magnification, respectively.

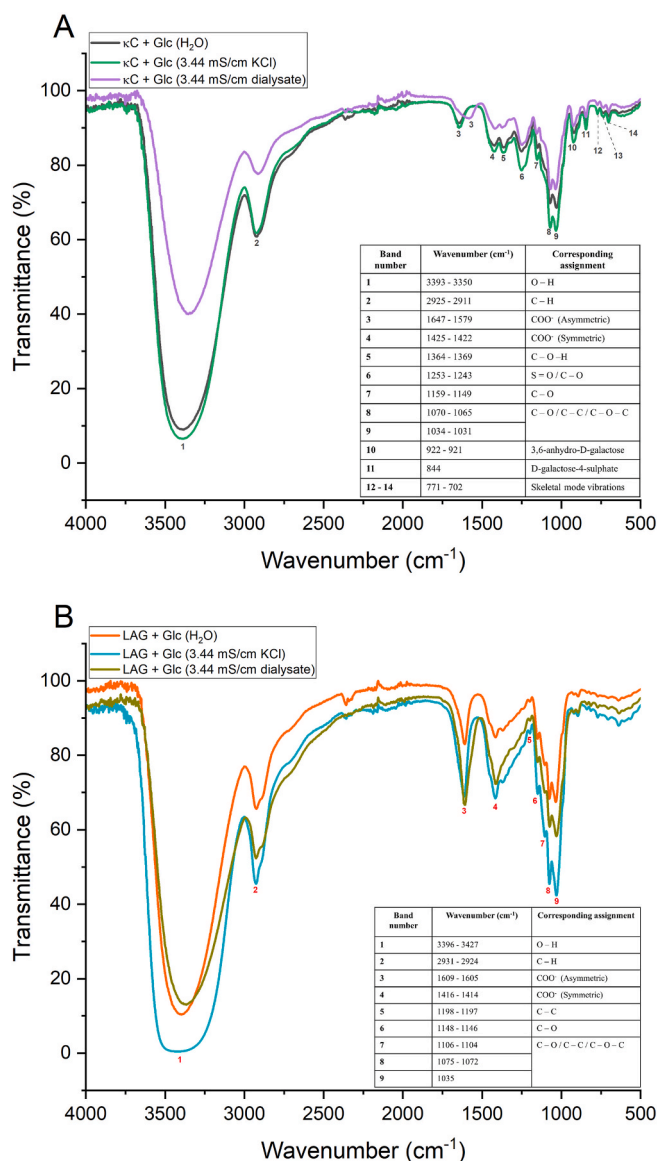


Fig. 7. FTIR spectra of gels consisting of 1 % TS (wt/wt) commercial gums combined with 1 % TS (wt/wt) Glc in either water, 3.44 mS/cm KCl or dialysate (using the conductivity of 1 % TS (wt/wt) kiokio gum as an example) – (A) κ C and (B) LAG. Tables indicating functional group assignments to the respective wavenumber were included.

identified with no new bands detected (Zhang et al., 2013).

In summary, the results of the analyses conducted indicate that when crude fern gums (CM and CK) were mixed with κ C and LAG, the endogenous ions, and not the low concentration of fern-derived polysaccharides present in these crude gums, contributed to any changes in the physicochemical properties of the mixed gels; these ions promoted the formation of an ordered gel network by forming cation-mediated junction zones, thus tightening the aggregation of κ C and LAG helices. This was evidenced by the observation that similar results could be achieved by aqueous ionic solutions containing no fern-derived polysaccharides (i.e. KCl solutions and dialysates). However, when purified fern gums (PM and PK) were used, the combined evidence suggests that κ C and LAG interacted segregatively with PM and PK when mixed, and formed a phase separated bicontinuous gel network with disrupted connectivity and greater gel heterogeneity due to the high concentrations of purified fern-derived polysaccharides. Similar phase separated gel networks have been reported for κ C–gellan and gellan–calcium alginate systems (Morris et al., 2012). The two types of hypothesised gel

networks that can be formed by κ C and LAG when mixed with either crude or purified fern gums are depicted in Fig. 10 below.

3.2.2. Influence of pH

This section investigates the influence of pH on the two types of gels that can be formed by κ C and LAG when mixed with either crude or purified fern gums: (i) ordered gel network with cation mediated junction zones (CM and CK gels) and (ii) phase separated bicontinuous gel network (PM and PK gels). In this study, pH 5 represented the native pH environment of gels containing crude or purified fern gums. In addition, pH 3 and pH 9 were selected for comparison. Extremely acidic or alkaline conditions (e.g. pH 1 and pH 12) were not included as it resulted in severe acid or alkaline depolymerisation, which inhibited gelation.

The hardness, syneresis and visual appearance of the gels formed after pH adjustment are presented in Table 6. As expected, all cation mediated gels containing CM and CK formed firm and self-sustaining gels suitable for TPA and syneresis measurements. All κ C gels (with CM and CK) at pH 9 were significantly harder than gels at pH 3. For κ C + CM, the gel at pH 9 was also significantly harder than at native pH 5. Additionally, syneresis of κ C gels at pH 3 was significantly reduced even in the presence of ions. However, as pH increased from pH 5 to pH 9, syneresis increased accordingly. These differences may be attributed to the strength and compactness of the network formed in κ C gels under different pH conditions. In contrast, for all combination of LAG gels with CM or CK, hardness significantly decreased as pH increased from pH 3 to pH 9. Expectedly, syneresis remained low regardless of the pH level. Generally, the brown colouration of these ion mediated gels containing CM and CK also became darker with increasing pH, as also previously reported for crude mamaku gum solution (Matia-Merino et al., 2012).

Notably, for PM and PK gels, gelation was incomplete for some combinations when pH was adjusted [κ C + (PM or PK) at pH 3 and LAG + PM at pH 9], resulting in the coexistence of viscous fluids and gelled pieces. For combinations that could gel completely, the gel formed were either too soft and mushy (LAG + PK at pH 9) or extremely hard and brittle [LAG + (PM or PK) at pH 3], making them challenging to unmould, fracturing under minimal handling. Furthermore, macroscopic phase separation was also noted in some combinations [(κ C or LAG) + PM at pH 9 and LAG + (PM or PK) at pH 3]. For example, at pH 9, both κ C and LAG gels containing PM showed clear gel parts containing κ C or LAG only, and brown gel parts containing PM that were mainly located towards the bottom of the gel. At pH 3, LAG + (PM or PK) gels also showed similar phase separated gel parts as visually observed. However, it could not be captured by the camera's resolution since it was not as obvious as at pH 9. These observations are consistent with the gel separating into a dispersed phase containing the purified fern gums which was surrounded by a continuous phase containing the commercial gums. Although this differs from the phase separated bicontinuous network observed at native pH, in which the two polysaccharides are intimately mixed and not confined to only a part of the total gel volume, the interaction is nonetheless classified as a segregative interaction (Morris et al., 2012). Alkaline conditions appeared to exacerbate and accelerate phase separation process more than at native pH 5, likely due to an increased electrostatic repulsion of the anionic polysaccharides. Due to these observations, gels containing PM and PK were therefore rendered unsuitable for TPA and syneresis measurements. Hence, rheological analysis was also conducted to supplement the findings.

In-situ gelation was conducted under small oscillatory shear and changes in pH did not affect the crossover gelation temperature of κ C + (CM or PM) gels, shown as a representative in Fig. 11. This was also observed for all other gel combinations, with the full data set shown in Fig. S1 (Supplementary). As observed previously in Fig. 4, sol-gel transition temperatures of gels containing crude fern gums were higher than purified fern gums attributed to the presence of ions in the crude gums. Fluctuations in moduli values were also observed in some samples when they were held at 4 °C. This confirmed that competing events were occurring concurrently (e.g. phase separation and gelation),

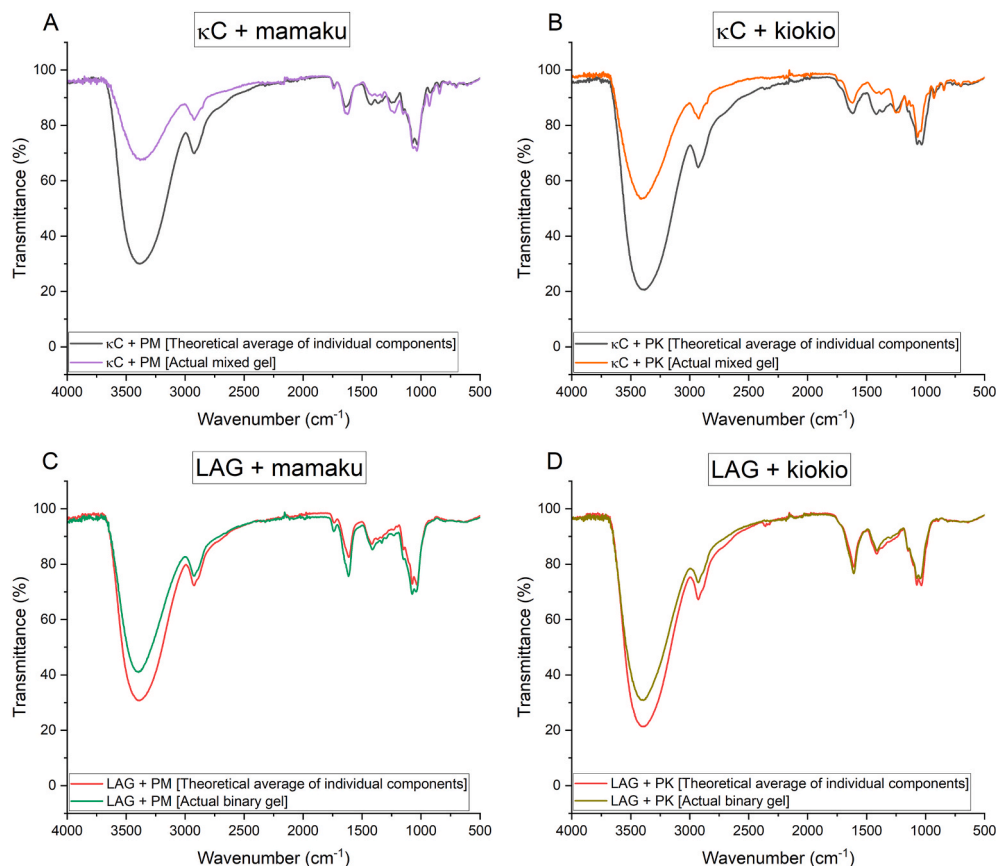


Fig. 8. FTIR spectra of gels consisting of 1 % TS (wt/wt) commercial gums combined with 1 % TS (wt/wt) purified fern gums – (A) κ C + PM, (B) κ C + PK, (C) LAG + PM and (D) LAG + PK. Note. The theoretical numerical average was calculated using the measured spectrum of each individual gums used in the actual mixed gel to produce a single spectrum, which was compared against the measured spectrum of the actual mixed gel.

thereby leading to gel heterogeneity, even at the various pH values.

The visual observations recorded in Table 6, also provide insights into the final G' and G'' values of the gels (Fig. 12). For example, the coexistence of viscous fluids and gelled pieces in κ C + (PM or PK) gels at pH 3 (Fig. 12A and B) is consistent with the low viscoelastic moduli values, inconsistencies and high standard deviations observed in the rheological results, given the heterogeneity of the system in contact with the surface of measurement. Nonetheless, several TPA results were consistent with the rheological findings. For example, LAG gels showed decreasing gel strength with increasing pH (Fig. 12C and D), except for CK gels, for which pH alterations did not significantly affect the viscoelasticity. A similar trend was observed for κ C + (CM or PM) gels (Fig. 12A). However, no clear trend was observed for CK and PK gels.

Generally, the observed changes in the physical properties of κ C and LAG gels when mixed with either crude or purified fern gums at various pH values can be attributed to alterations in the polysaccharide charge and physicochemical properties as a function of pH. At pH 3, κ C may undergo partial acid hydrolysis with chain cleavage primarily at the 1,3-glycosidic linkages, resulting in reduced molecular weight. Since susceptibility toward degradation increases in absence of counterions (Tuvikene, 2021), this may explain why κ C forms a self-sustaining gel with crude fern gums but not with purified fern gums at pH 3. Based on the known behaviour of κ C with pH, the resultant disrupted double-helix junction zones form a weak and less elastic network that is unable to sustain internal stresses that drive syneresis at pH 3 (Ako, 2015), consistent with the significantly lower syneresis and lower gel hardness than at pH 5 or pH 9 observed in Table 6 for κ C + CM or κ C + CK. At pH 5, acid hydrolysis of κ C is unlikely and the sulphate groups become increasingly deprotonated, generating greater electrostatic repulsion. This may result in localised contraction and network heterogeneity

when the formation of helix aggregation and junction zones was promoted by ions. At pH 9, alkaline depolymerisation was unlikely, but the sulphate groups were expected to be fully ionised with strong repulsion between chains. Thus, in the presence of ions at this pH, non-uniform aggregation could occur, forming a combination of dense junction zones and weak regions. A plausible interpretation is that contraction of the polysaccharide-rich network drives water into and out of more open, less crosslinked regions; the resulting structural heterogeneity would be consistent with the higher syneresis observed at this pH, although the present data do not permit a direct mechanistic conclusion. Nonetheless, κ C is known to be most stable at pH 9, consistent with the highest gel hardness at this pH (Tuvikene, 2021).

For LAG, the trend observed when mixing it with the crude fern gums (CM or CK) was opposite to that of κ C gels, as the gels strength decreasing progressively with increasing pH from 3 to 9. At pH 3, the carboxyl groups in LAG would mostly be protonated and with reduced electrostatic repulsion, the polysaccharide chains would allow closer chain packing, promoting strong double-helix aggregation and junction zones which result in a dense and compact network with higher hardness. With reduced charge competition, cations would also bridge chains more efficiently. Furthermore, LAG can also form acid gels without metal ions (Sworn & Stouby, 2021). As pH increases from pH 5 to pH 9, the carboxyl groups become increasingly deprotonated, generating increased electrostatic repulsion between chains. The higher density of charged carboxyl groups that must be screened may also lead to more diffuse ion binding and less effective crosslinking. These factors collectively result in weaker aggregation and a looser gel network with lower hardness, consistent with the results presented in Table 6.

Overall, acid and alkaline pH modulate the properties of κ C and LAG gels mixed with crude fern gums through the maintenance of an ordered

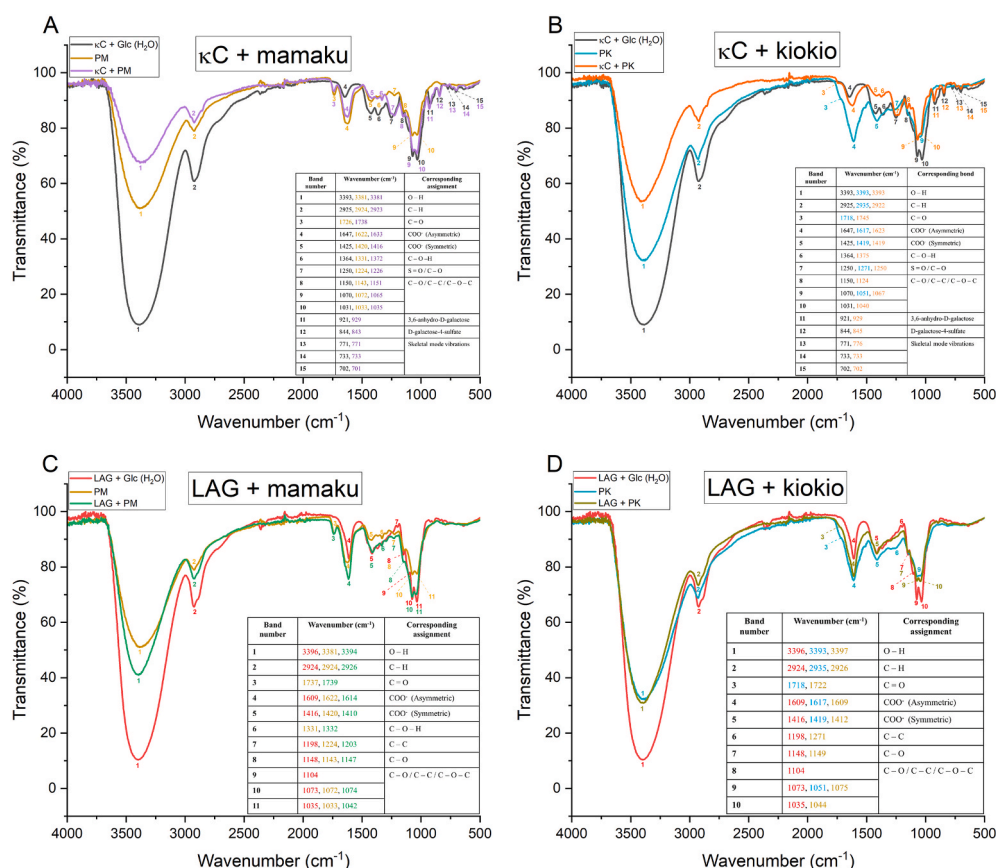


Fig. 9. FTIR spectra of gels consisting of 1 % TS (wt/wt) commercial gums combined with 1 % TS (wt/wt) purified fern gums – (A) κ C + PM, (B) κ C + PK, (C) LAG + PM and (D) LAG + PK. Controls containing only κ C, LAG, PM or PK were also shown for comparison purposes. Tables indicating functional group assignments to the respective wavenumber were included.

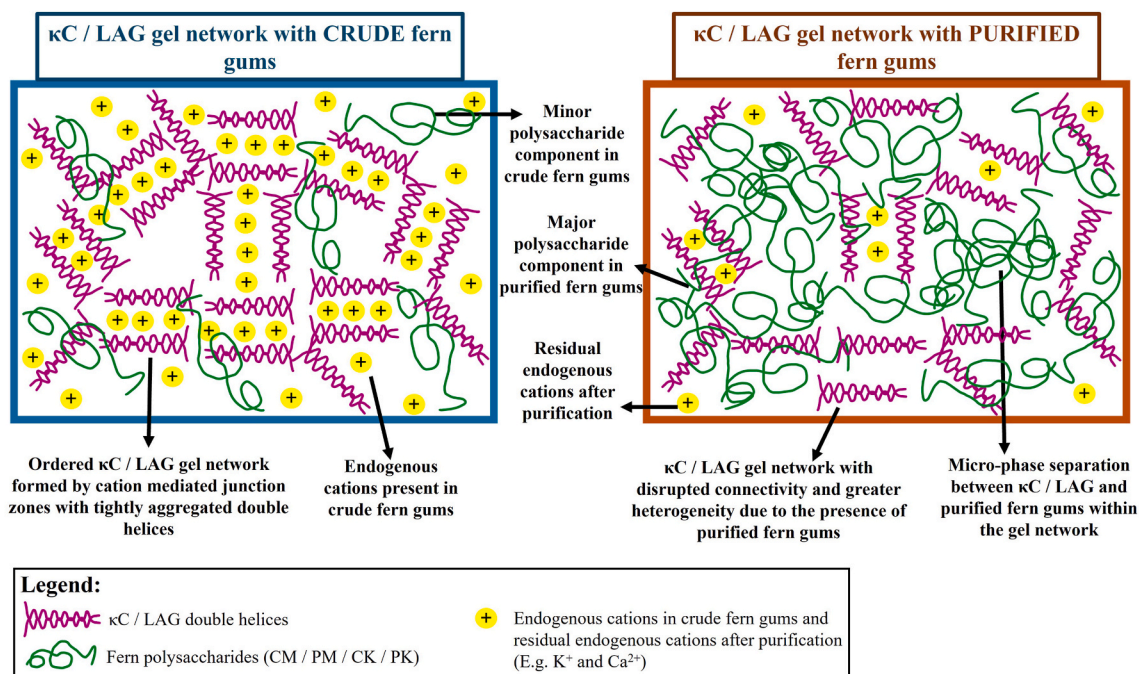


Fig. 10. Schematic illustrating the differences in gel network formed by κ C and LAG when mixed with either crude or purified fern gums.

gel network via cation mediated junction zones. The enhanced hardness of κ C gels containing crude fern gums at pH 9 may be advantageous in alkaline food systems such as alkaline noodles or traditional Chinese

alkaline dessert gels. However, when κ C and LAG were mixed with purified fern gums at pH 3 and pH 9, complete gelation was either hindered or a severely phase separated gel was formed. A summary on

Table 6

Hardness (obtained from TPA), syneresis and visual appearance of gels consisting of 1 % TS (wt/wt) of commercial gum combined with 1 % TS (wt/wt) fern gums at various pH.

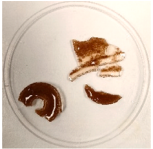
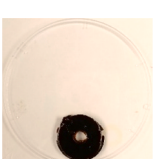
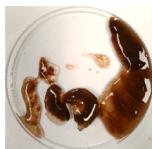
Sample	Hardness (g) ⁺	Syneresis (%) [#]	Visual appearance
κ C + (CM or PM)			
κ C + CM (pH 3)	2301.84 ± 54.66 ^g	0.81 ± 0.23 ^a	
κ C + PM (pH 3)	N.A.	N.A.	
κ C + CM (pH 5)	3295.81 ± 133.11 ^h	5.83 ± 0.40 ^d	
κ C + PM (pH 5)	1399.62 ± 176.09 ^{cd}	0.79 ± 0.01 ^a	
κ C + CM (pH 9)	4587.40 ± 101.76 ^j	8.20 ± 0.07 ^f	
κ C + PM (pH 9)	N.A.	N.A.	
κ C + (CK or PK)			
κ C + CK (pH 3)	195.75 ± 50.26 ^a	0.18 ± 0.04 ^a	
κ C + PK (pH 3)	N.A.	N.A.	
κ C + CK (pH 5)	5728.93 ± 114.14 ^k	4.82 ± 0.50 ^c	
κ C + PK (pH 5)	2293.30 ± 141.74 ^g	1.75 ± 0.04 ^b	
κ C + CK (pH 9)	4055.79 ± 401.13 ⁱ	7.08 ± 0.29 ^c	
κ C + PK (pH 9)	N.A.	N.A.	
LAG + (CM or PM)			
LAG + CM (pH 3)	1924.75 ± 44.95 ^{e-g}	0.83 ± 0.12 ^a	

Table 6 (continued)

Sample	Hardness (g) ⁺	Syneresis (%) [#]	Visual appearance
LAG + PM (pH 3)	N.A.	N.A.	
LAG + CM (pH 5)	1736.13 ± 110.22 ^{de}	0.80 ± 0.09 ^a	
LAG + PM (pH 5)	325.86 ± 31.61 ^a	0.58 ± 0.08 ^a	
LAG + CM (pH 9)	1085.13 ± 144.26 ^{bc}	0.59 ± 0.07 ^a	
LAG + PM (pH 9)	N.A.	N.A.	
LAG + (CK or PK)			
LAG + CK (pH 3)	2154.33 ± 206.30 ^{fg}	0.39 ± 0.13 ^a	
LAG + PK (pH 3)	N.A.	N.A.	
LAG + CK (pH 5)	1838.48 ± 56.28 ^{ef}	0.28 ± 0.03 ^a	
LAG + PK (pH 5)	872.34 ± 79.92 ^b	0.25 ± 0.06 ^a	
LAG + CK (pH 9)	1658.27 ± 169.79 ^{de}	0.08 ± 0.02 ^a	
LAG + PK (pH 9)	N.A.	N.A.	

Note. All samples deformed upon the first compression at 70 % strain; therefore, values obtained for springiness, cohesiveness and chewiness were invalid. Only hardness values were reported which were equivalent to the fracturability values for all samples. Average values are presented with standard deviations. + Different superscript letters indicate significant differences at $\alpha \leq 0.05$ (n = 4). # Different superscript letters indicate significant differences at $\alpha \leq 0.05$ (n = 2). Dashed lines link all letters encompassed within that group in alphabetical order (e.g. e-g represents e, f, and g).

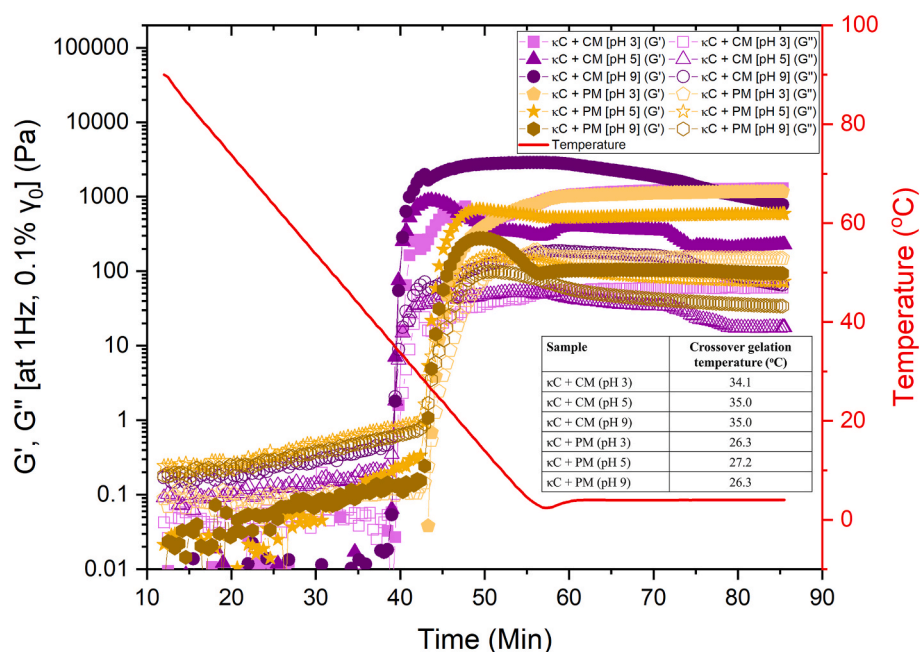


Fig. 11. Time-temperature gelation profiles and corresponding crossover gelation temperatures of gels at various pH, using the example of 1 % TS (wt/wt) κC combined with 1 % TS (wt/wt) CM or PM. The full data set can be found in Fig. S1 (Supplementary).

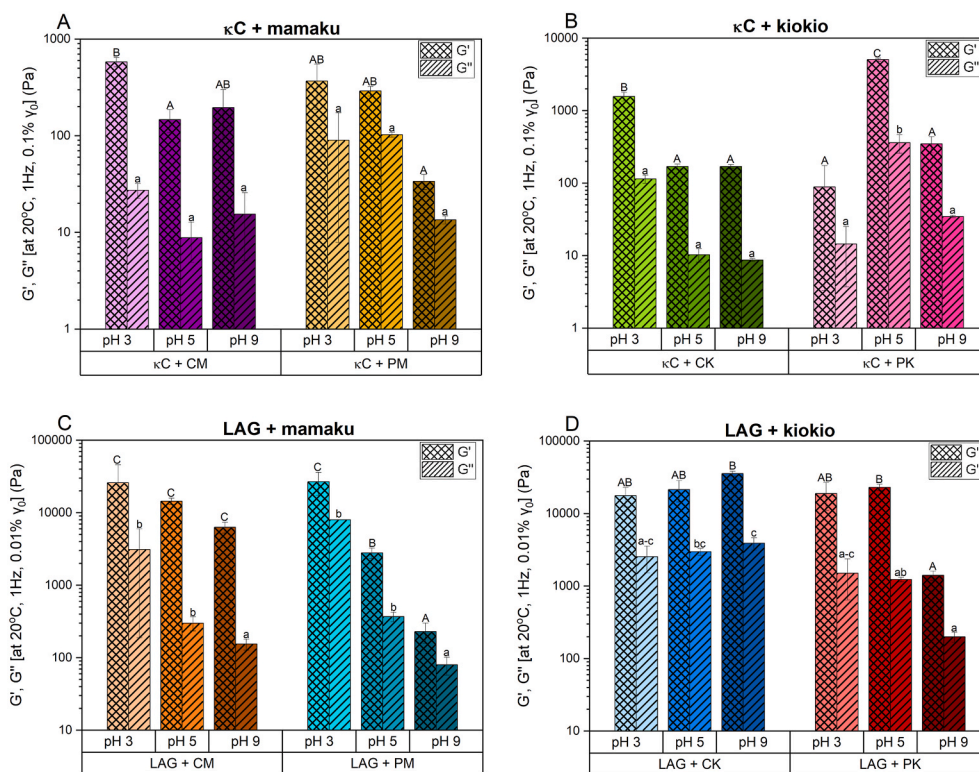


Fig. 12. Comparison of viscoelastic properties (G' and G'') of gels consisting of 1 % TS (wt/wt) commercial gums combined with 1 % TS (wt/wt) fern gums at various pH – (A) κC + (CM or PM), (B) κC + (CK or PK), (C) LAG + (CM or PM) and (D) LAG + (CK or PK). All combinations were fixed at 2 % TS (wt/wt). Note. Average values are presented with standard deviations. Different superscript letters indicate significant differences at $\alpha \leq 0.05$ ($n = 2$), and dashed lines link all letters encompassed within that group in alphabetical order (e.g. a-c represents a, b, and c).

the influence of pH on the various types of gel networks is presented Fig. 13.

3.2.3. Freeze-thaw stability

Having established that both ionic content and polysaccharide

concentration significantly influence the network structure of κC and LAG gels containing fern-derived gums, the freeze-thaw stability of these systems was evaluated — a property of direct relevance to food applications involving frozen storage or thermal cycling. A potential application of these gels is in frozen desserts or ready-to-heat meals.

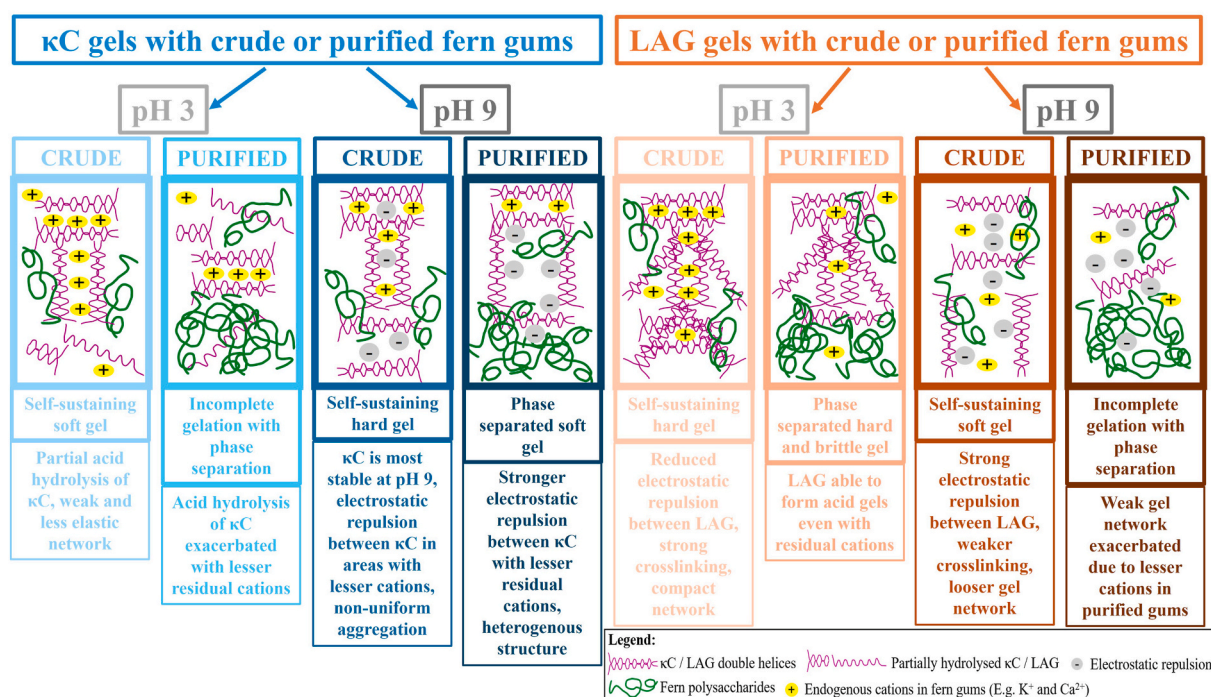


Fig. 13. Schematic illustrating the different gel networks formed by κC and LAG when mixed with either crude or purified fern gums at pH 3 and pH 9.

Moreover, freeze-thaw cycling is a common industrial process. It was therefore of interest to evaluate the freeze-thaw performance (through a preliminary single cycle assessment) of κC and LAG gels containing

either crude or purified fern gums by measuring the WHC. WHC was selected as the primary measure of freeze-thaw stability as it quantifies the capacity of the gel network to retain water following ice crystal

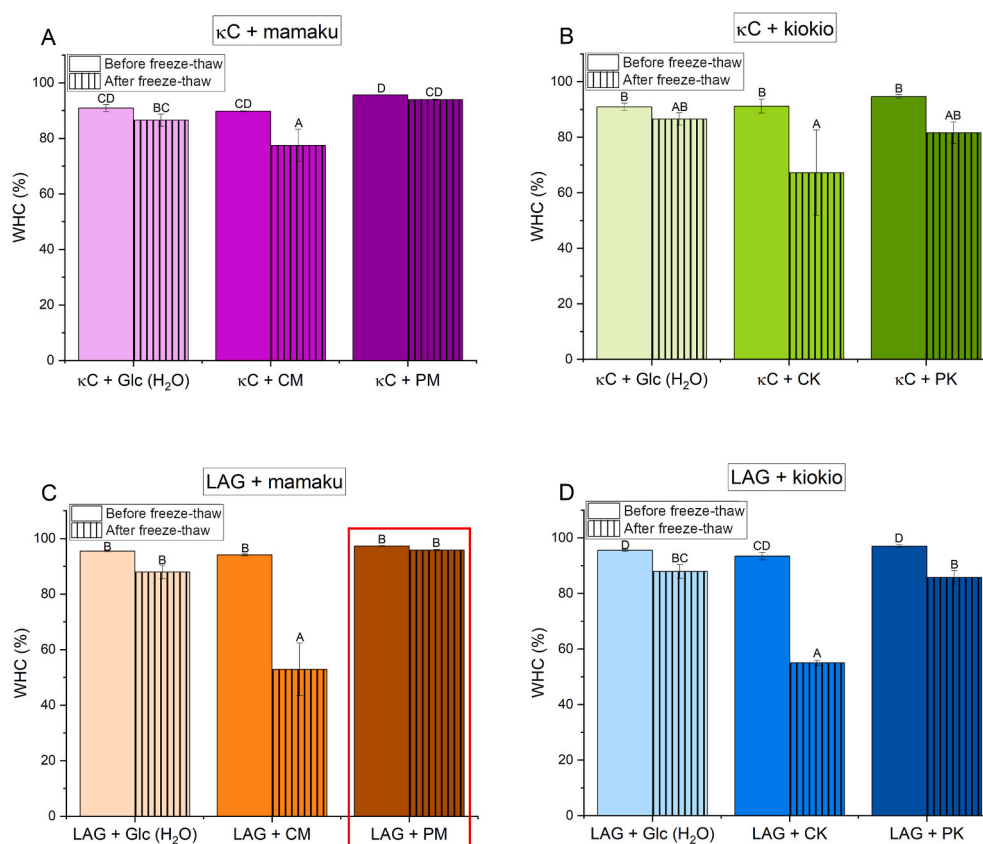


Fig. 14. Comparison of WHC (%) of gels consisting of 1 % TS (wt/wt) commercial gums combined with 1 % TS (wt/wt) Glc or fern gums – (A) κC + (Glc or CM or PM), (B) κC + (Glc or CK or PK), (C) LAG + (Glc or CM or PM) and (D) LAG + (Glc or CK or PK). All combinations were fixed at 2 % TS (wt/wt). Note. Average values are presented with standard deviations. Different superscript letters indicate significant differences at $\alpha \leq 0.05$ (n = 2).

formation and subsequent melting, which is directly relevant to product quality and texture in frozen applications.

As shown in Fig. 14, WHC of all gels before a freeze-thaw cycle, was not significantly different. Overall, κ C gels showed greater freeze thaw stability than LAG gels although both are known to have poor freeze-thaw stability (Mao et al., 2001; Tuvikene, 2021). Generally, after one cycle of freeze-thaw, the WHC of gels did not decrease substantially in the absence of ions, since the controls and PM and PK gels retained significantly higher WHC than CM and CK gels. LAG + PM (Fig. 14C) demonstrated the greatest freeze-thaw stability, with no significant differences observed before and after cycling. This suggests that purified fern gums may aid in preserving the physicochemical properties of products during freeze-thaw processes. It has been reported that the freeze-thaw stability (also quantified via WHC) of κ C improved when mixed with senna tora gum, due to increased intra- and inter-molecular hydrogen bonding between the two polysaccharides, resulting in the tightening of the lamellar structure of κ C and formation of a more compact, ordered network (Fang et al., 2024).

Since LAG + PM gel showed almost no change in WHC after freeze-thaw, cryo-SEM images of this gel were compared before and after freeze-thaw cycling (Fig. 15). Before freeze-thaw (Fig. 15 A1-A2, B1-B2), the gel was characterised by the presence of small cracks, cavities and pockets of micro-phase separation as described in Section 3.2.1.3. Nonetheless, majority of the gel consisted of relatively uniform small pores. After freeze-thaw cycling, while some areas of uniform small pores remained (Fig. 15 C1-C2), areas with much larger pores (Fig. 15 D1-D2), and wider cracks (Fig. 15 E1-E2) emerged. These structural changes were expected as free water expelled during thawing, leaving behind a more porous network. Interestingly, despite evidence of large ice crystals causing bigger pore sizes and greater heterogeneity in the gel microstructure, the WHC was not affected. However, such microstructures may nonetheless affect gel texture — an aspect not assessed in the present study but warranting future investigation given its direct impact on consumer acceptability of freeze-thawed products.

4. Conclusions

The novel non-gelling fern-derived polysaccharides MG and KG can

be mixed with κ C and LAG to modulate the physicochemical properties and functionality of these commercial gums. MG and KG were evaluated both in the crude and purified forms, and various permutations of purification processes involving ETOH precipitation and/or dialysis were explored. The results show that MG and KG could be utilised in both the crude and purified forms to produce different structures and properties in these mixtures.

When the fern gums were mixed with κ C and LAG, two types of gels could be formed: (i) ordered gel network with cation mediated junction zones (with crude fern gums) and (ii) phase separated gel network (with purified fern gums). In the first type of ordered gel network, the endogenous ions in the crude fern gums were responsible for promoting junction zone formations and tight helix aggregations that resulted in firm self-sustaining gels even at pH 3 and pH 9. In the second type of phase separated gel network, purified fern gums conferred greater gel heterogeneity and disrupted gel connectivity, resulting in softer gels. This segregative interaction behaviour was exacerbated at pH 3 and pH 9 and resulted in distinct phase separated gels likely containing purified fern gums dispersed within a continuous network of κ C or LAG. Since the proposed segregative interactions and phase-separated networks were primarily inferred from TPA, rheology, cryo-SEM and FTIR, further assessment using confocal microscopy would provide a strong confirmation. Additionally, the absence of ions in the purified fern gums also resulted in incomplete gelation in some combinations at various pH values. The selected pH conditions demonstrate that acidic, native and alkaline environments can substantially alter gel formation, network strength and phase separation; however, further investigation across a continuous pH range would be required to establish more detailed pH-structure-function relationships. These results demonstrate that purified fern-derived polysaccharides can alter gel microstructure and functionality without relying on ionic interactions, making them beneficial for formulations sensitive to ions or where a paste-like consistency may be required.

Furthermore, LAG + PM also demonstrated good WHC after one freeze-thaw cycle. This was a preliminary indicator of its potential in frozen food applications although further assessment of repeated freeze-thaw cycling, and textural attributes should be conducted. Sensory evaluation would also aid in strengthening potential formulations for

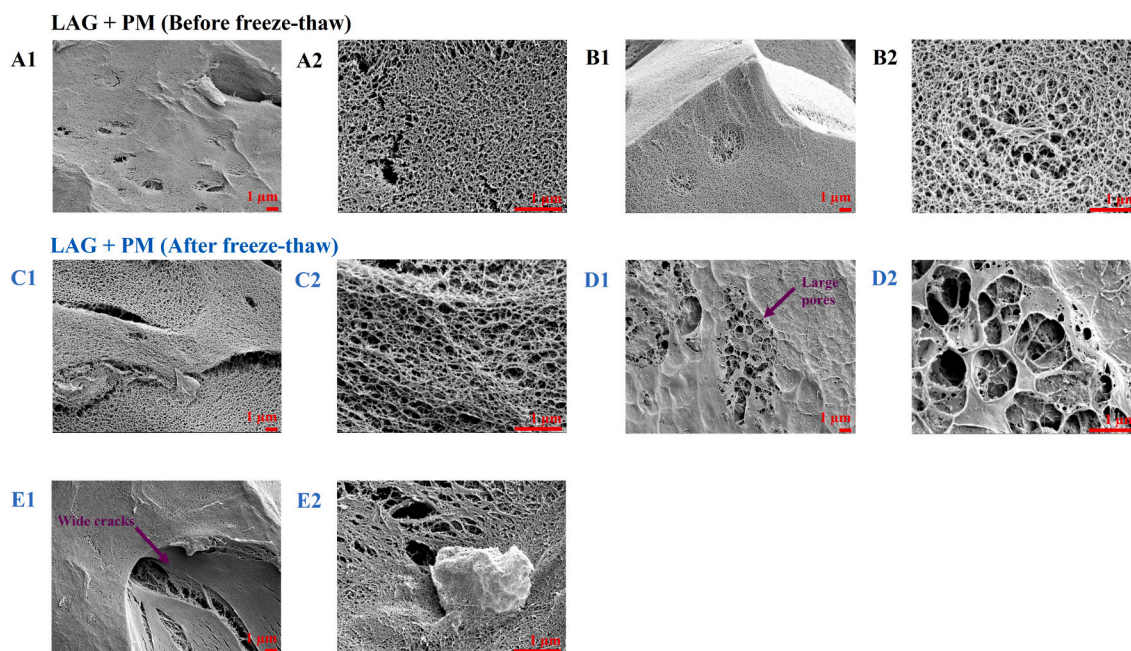


Fig. 15. Cryo-SEM images of LAG + PM gels (combined at 1 % TS wt/wt of each gum) before and after one freeze-thaw cycle – (A1-B2) LAG + PM (Before freeze-thaw), and (C1-E2) LAG + PM (After freeze-thaw). Note. Each letter corresponds to a different location explored in each sample. Images labelled as “1” and “2” were captured at 5000 $\times$ and 25000 $\times$ magnification, respectively.

future applications, since the TPA and small-amplitude oscillatory rheology employed in this study probed different deformation regimes and therefore produced some divergent trends, with neither technique fully capturing sensory properties.

Nonetheless, it can be concluded, that these novel fern-derived polysaccharides show potential for tailoring texture and gel structure in mixed polysaccharide systems, including ion-sensitive protein-based formulations. However, further work is needed before commercialisation, particularly regarding sustainable supply, purification costs, and the impact of the natural brown colour on product appearance and consumer acceptance. This study therefore provides mechanistic evidence of functionality rather than demonstrating commercial readiness.

CRedit authorship contribution statement

Felicia Zhi Wen Peh: Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Conceptualization. **Xu Yong Han:** Software, Methodology, Investigation, Formal analysis. **Kelvin K.T. Goh:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization. **Mark R. Waterland:** Writing – review & editing, Methodology. **Lara Matia-Merino:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Data curation, 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.2026.113294>.

Data availability

Data will be made available on request.

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