Research Paper

Influence of cations, pH and dispersed phases on pectin emulsification properties

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ARTICLE INFO

ABSTRACT

The cooperativity of six cations (Ca^{2+}, Mg^{2+}, Zn^{2+}, Al^{3+}, Cr^{3+} and Fe^{3+}), three pectins (sugar beet, high and low methyl esterified), three dispersed phases (medium chain triglycerides (MCT), orange oil and hexadecane), time (30 days) and pH (2.0 and 6.0) has been investigated in the formation and stability against coarsening of oil-in-water emulsions. Cations generally influenced emulsion stability in the following order (most stable) $Ca^{2+} > Mg^{2+} > Al^{3+} > Cr^{3+} > Zn^{2+} > Fe^{3+}$ (least stable). This order largely coincided with that of the strength of pectin-cation interactions showing that the higher the affinity of cation for pectin the less stable the emulsion. More stable emulsions were formed with sugar beet pectin, which was also unresponsive to the presence of cations, followed by high- and then low-methyl esterified samples. At pH 2.0 all pectins showed their best emulsification performance whereas shifting pH to 6.0 severely impaired emulsification capacity and longer term stability against droplet growth. Smaller droplets were created with hexadecane under all conditions studied followed by MCT and orange oil in agreement with their aqueous solubilities. The present results advance our understanding of the stabilisation of emulsions using pectin and allow us to tailor their functionality for applications in food, pharmaceutical and biomedical industries.

1. Introduction

Pectins are composed primarily of α-(1→4) linked α-D-galacturonic acid (GalA) residues and they are defined as acidic hetero-polysaccharides. Pectic polysaccharides have two main domains all of which contain GalA residues. These two domains are the homogalacturonan region (HG), which is made up of α-(1→4) linked α-D-GalA residues, and the rhamnogalacturonan I (RG-I) region with a backbone made up of repeating units of α-(1→4) linked α-D-GalA and α-(1→2) linked α-L-rhamnose (Rha). The α-(1→2) linked α-L-Rha monomers are covalently connected to neutral sugar side chains formed mostly of arabinose, galactose and xylose (Ropartz and Ralet, 2020). Commercial pectins are classified into two main types depending on the degree of methyl esterification (DM) and are obtained most frequently (85%) from citrus products (Fidalgo et al., 2016). They are classified either as low methylesterified pectins (LM) which have less than 50% of methyl ester groups and high methylesterified pectins (HM) which have over 50% of methyl ester groups. The methyl ester is found at the C-6 of the galacturonic acids of HG segments, as their counterparts in RG-I segments are not esterified. Sugar beet pectin (SBP) which is extracted from sugar beet pulp is known to have better emulsification capacity compared to HM or LM pectins and this has been attributed to the presence of acetyl groups and ferulic acids (Bai et al., 2017; Karnik and Wicker, 2018; Leroux et al., 2003).

The chemical and macromolecular characteristics of pectins influence their functionality as emulsifiers. Recent critical assessment of the literature has revealed that pectins are suitable emulsifiers for oil-in-water emulsions when they contain protein in the range of ~3% with degree of acetylation of ~10% and are enriched in RG-I and ferulic acids (Alba and Kontogiorgos, 2017). Furthermore, once at the interface, pH-dependant conformational rearrangements and the degree of methylation influence coarsening of pectin-stabilised emulsions. At pH < ~3.5 pectins attain compact conformations that are relatively unaffected by the degree of methylation and steric stabilisation, is generally efficient. At pH > ~3.5 chains of low methoxylated pectins attain extended conformations with space occupancy being less efficient leading to facile desorption (Alba and Kontogiorgos, 2020). In addition, at pH > 3.5, gradual increase in the degree of methylation leads to compact

conformations due to lower charge density and ineffective steric hindrance because of the presence of methyl groups (Alba et al., 2018). Consequently, pectins have been found to be efficient emulsifiers predominantly in acidic environments (Alba et al., 2016; Verkempinck et al., 2018). Recently, the interactions between multivalent cations and pectins have started attracting renewed research interest, as it has been found that they have a strong impact on pectin functionality (Huynh, Lerbret, Neiers, Chambin and Assifaoui, 2016a; Maire du Poset et al., 2019). For example, inclusion of divalent and trivalent cations in pectin films at pH 2 modifies their glass transition temperature by up to 25 °C (Kalathaki et al., 2019) a finding that constitutes an advance in tuning the properties of pectin-based biomaterials. Nevertheless, the influence of cationic species on emulsification properties of pectin is largely unknown and the present work attempts to bridge this gap in our understanding. Consequently, a hypothesis was formed that inclusion of cations would influence emulsification performance of pectins. The objectives of the present work focused on providing an in-depth understanding of emulsion formation and stabilisation using a multivariate experimental design of a large number of environmental conditions including different cations, pectins, pH, oils and ageing (i.e., time).

2. Materials and methods

2.1. Materials

Unstandardised low-methoxyl (LM, GENU type ML-12 CG-Z), high-methoxyl (HM, GENU type B rapid set-Z) and sugar beet pectin (SBP, GENU BETA) were purchased from CPKelco (Lille Skensved, Denmark). Chloride salts used in emulsion preparation ($CaCl_2$, $MgCl_2$, $ZnCl_2$, $AlCl_3$, $CrCl_3$ and $FeCl_3$) were purchased from Sigma-Aldrich (Gillingham, UK). Medium chain triglyceride oil (MCT) was obtained from IOI Oleochemical (Witten, Germany), whereas orange oil and hexadecane were obtained from Sigma-Aldrich (Gillingham, UK). De-ionised water was used in all experiments. Molecular characteristics of pectins used in the present work are presented in Table S2.

2.2. Emulsion preparation

Pectins (SBP, HM, or LM) were dispersed at 1% w/v in deionised water and left stirring overnight at room temperature. All final emulsions containing salts ($CaCl_2$, $MgCl_2$, $ZnCl_2$, $AlCl_3$ and $CrCl_3$) except $FeCl_3$ were prepared with the same ionic strength (10 mM) with the addition of 0.001% w/v NaN_3, as a preservative. Ionic strength was calculated using:

$$I = \frac{1}{2} \sum_{i=1}^{n} c_i z_i^2$$

where c_i is the concentration (mol/L) of ion i, and z_i is the charge number of the ions. Emulsions containing $FeCl_3$ were adjusted to 1 mM, as in preliminary experiments all emulsions containing iron at 10 mM gelled. 10 mM HCl or NaOH solutions were used to adjust pH for both pectin and electrolyte solutions to either 2.0 or 6.0. Stock emulsions with $\phi = 0.12$ of either MCT, orange oil or hexadecane were prepared into two stages. In the first stage, pre-emulsions were prepared using a high-speed homogeniser (IKA T18, Ultra-Turrax, Staufen, Germany) at 25000 rpm for 2 min. In the second stage, ultrasonic treatment using an ultrasound processor at room temperature (Model UP 100H, Hielscher Ultrasonics, Teltow, Germany) equipped with 7 mm diameter MS7 tip was applied to the coarse emulsion (10 mL) for 40 s with a cycle of 0.3 and amplitude of 100% (100 W, 30 kHz).

Stock emulsions were then diluted dropwise under stirring with the appropriate amount of electrolyte solution to yield final emulsions with pectin concentration of 0.7% w/v in the entire emulsion volume and volume fraction of final emulsion of $\phi = 0.1$. Dilution with deionised water adjusted to either pH 2.0 or 6.0 yielded "control" emulsions (i.e., no additional salt). All emulsions were prepared and stored at room temperature.

2.3. Droplet size distribution analysis and ζ-potential measurements

Droplet size distribution measurements were made directly after emulsion preparation (fresh, "time zero") and then after 1, 5, 10, 20 and 30 days in order to investigate the long term stability of the dispersions. The measurements were carried out using a Malvern Mastersizer 2000 (Malvern Instruments Ltd., Worcestershire, UK) laser diffraction droplet size analyser with a small volume sample dispersion unit Hydro 2000SM. The refractive indices of MCT oil, orange oil and hexadecane were 1.445, 1.473 and 1.434, respectively, whereas 1.333 was used for both aqueous phases (pH 2.0 and 6.0). Droplet growth rate constants were assessed using non-linear regression with GraphPad Prism v.7 (GraphPad software, San Diego, USA). Electrokinetic measurements were performed using a Zetasizer Nano Series ZEN2600 (Malvern Instruments, Malvern, UK) at 25 °C. Emulsions were diluted 1:1000 before measurements to prevent multiple scattering effects with pH-adjusted deionised water. pH of water was adjusted to pH 2.0 or pH 6.0 with a few drops of 10 mM HCl prior to diluting the emulsions. Emulsions formed at pH 2.0 were diluted with water at pH 2.0 whereas those formed at pH 6.0 were diluted with water at pH 6.0. All measurements were performed at least in duplicate. As more than one thousand measurements were taken during this work, only the most significant findings and general trends are presented and discussed in detail.

3. Results

3.1. Emulsions at pH 2.0

In this work, emulsions were stabilised in the presence of six cationic species, three divalent (Ca^{2+}, Mg^{2+} and Zn^{2+}) and three trivalent (Al^{3+}, Cr^{3+} and Fe^{3+}), and changes in droplet size distribution curves and average droplet sizes were monitored for a period of thirty days. Irrespectively of the oil phase or pectin that was used, droplet size distributions were bimodal with a second peak frequently developing at higher modes during storage, particularly for orange oil, showing that flocculation and/or coalescence are the major destabilisation mechanisms. At pH 2.0 fresh SBP-stabilised emulsions (Fig. 1a) had smaller droplet sizes ($d_{4.3}$–2 μm) than those fabricated with HM or LM ($d_{4.3}$–5 μm or ~10 μm, respectively) under all conditions studied (Fig. 1b and b, inset). It has been also observed that trivalent cations may improve the long-term stability of HM-stabilised orange or MCT oil emulsions (Fig. 1b, inset). This is in agreement with previously published work where HM-pectin has been found to stabilise emulsions having as dispersed phases a range of terpenoids even at the sub-micron droplet range (Guerra-Rosas et al., 2016). In contrast, LM emulsions exhibited poor stability with trivalent cations.

Stability-plots provide a visual overview of emulsion stability under all different environments at pH 2.0 (Fig. 2). SBP-stabilised samples are not shown, as they were able to form emulsions under all experimental conditions at pH 2.0. LM pectin, forms stable emulsions with hexadecane and MCT but it was not an efficient emulsifier for orange oil (Fig. 2a). This is in stark contrast with HM-stabilised emulsions (Fig. 2b) that were able to emulsify orange oil in the presence of all salts, a clear indication that degree of methylesterification is critical for emulsification of low viscosity oils. HM-stabilised emulsions performed particularly well with all oils and cations apart from those formed with Cr^{3+} in hexadecane. Electrokinetic potential measurements of all emulsions irrespectively of the formulation yielded a value of ~ −6 mV that remained constant throughout storage. The low ζ-potential is a clear manifestation of the presence of pectin at the interface as protein-laden interfaces normally carry positive charge at pH 2.0. In addition, it has been recently confirmed that in emulsions stabilised by SBP-protein complexes, protein adsorbs at the droplet interface controlling its interfacial viscoelasticity whereas pectin chains are oriented towards the aqueous phase
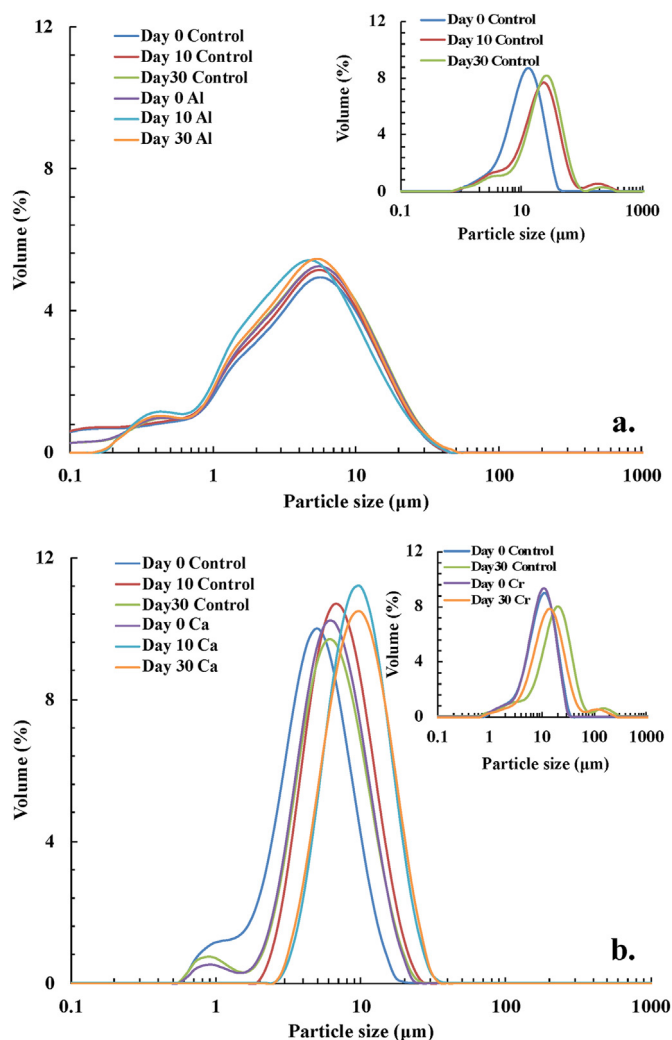
Fig. 1. Typical droplet size distribution curves of emulsions ($\phi = 0.1$, pH 2.0) prepared with different pectins and oil type. a) MCT emulsions stabilised with SBP and Al^{3+}. Inset shows control orange oil emulsions stabilised with HM. b) Hexadecane emulsions stabilised with LM and Ca^{2+}. Inset shows example of improvement of stability of HM-stabilised orange oil emulsions with trivalent cations. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

controlling the electrical characteristics of the droplets (Artiga-Artigas et al., 2020). This relationship seems to persist in emulsion systems where pectin is not complexed with protein but is present as contaminant (Alba et al., 2016). The low negative ζ-potential also indicates that steric stabilisation should be the prevalent mechanism instead of electrostatic interactions behind long-term emulsion stability.

3.2. Emulsions at pH 6.0

Generally, emulsions at pH 6.0 were less stable than those prepared at pH 2.0 for all samples studied, particularly for HM and LM pectin, in agreement with recent findings for emulsions stabilised with carrot pectin having different ultrastructures (Neckebroeck et al., 2020). SBP-stabilised emulsions exhibited superior stabilisation capacity at pH 6.0. Droplet size distributions of SBP emulsions remained stable over the study period (Fig. 3a) and results were largely comparable to those obtained at pH 2.0. LM pectin generally formed more stable emulsions with hexadecane and MCT, although the exact state of affairs depended on the type of ion present (Fig. 3b). For example, Al^{3+} and Zn^{2+} did not show good stability behaviour with any of dispersed phases used, as they have

mostly gelled or did not form a homogeneous dispersion (Fig. 2c). HM showed poor emulsification behaviour under most conditions at pH 6.0 in contrast to its exceptional behaviour at pH 2.0. HM-stabilised emulsions gelled in the presence of $ZnCl_2$, $CrCl_3$ and $AlCl_3$ for all oils and all pectin types. However, HM-stabilised emulsions showed good overall stability with Ca^{2+} and Mg^{2+} (Fig. 2d). This is in agreement with recent findings where the presence of Ca^{2+} in emulsions formed with HM pectin enhances stability possibly through a microgel-formation induced mechanism (Fan et al., 2020). Surface charge for emulsions at pH 6.0 was ~ -35 mV and remained constant throughout storage indicating that electrostatic stabilisation should contribute to droplet interactions in contrast to those formed at pH 2.0.

3.3. Kinetic analysis of droplet growth

Quantification of the influence of environmental conditions (i.e., type of cation, oil, pectin and pH) on the evolution of droplet size with time may be followed using an empirical equation that describes pseudo-first order exponential growth kinetics:

$$d_{4,3} = d_o + \left(d_{eq} - d_o\right)\left(1 - e^{-kt}\right) \tag{1}$$

where $d_{4,3}$ is the volume weighted mean diameter (μm), d_o is the $d_{4,3}$ at time zero, d_{eq} is the $d_{4,3}$ after thirty days of storage, k is the rate constant (min^{-1}) and t is time (min). Data fitting was carried out for emulsions formed with a divalent (Ca^{2+}) and a trivalent (Cr^{3+}) cation. These cations were selected as they were found to be the best performing among those with relevance to food systems. Equation (1) was found to describe sufficiently well droplet coarsening, as all samples followed exponential growth kinetics (Fig. 4a, Table S1). Rate constants of samples at pH 6.0 showed fast destabilisation at the early stages of ageing followed by a quasi-equilibrium plateau after five days of storage (Fig. 4a). In contrast, samples at pH 2.0 did not show appreciable droplet growth and tended to reach quasi-equilibrium after one day of storage (Fig. 4a). Collecting and plotting rate constants k for all conditions studied may give an in-depth appreciation of the emulsification capacity of pectins (Fig. 4b and c). It immediately transpires that a two-fold and in some cases an order of magnitude increase occurs in growth rates at pH 6.0 (Table S1, Fig. 4c). SBP prevents droplet growth more efficiently than the other pectins whereas faster destabilisation has been observed using hexadecane at both pH values. Calcium exhibited a stabilising effect at pH 2.0, albeit marginal. In addition to the rate constants, d_{eq} is also important characteristic of emulsion stability, as it represents the size at which droplet growth stabilises. Observation of d_{eq} values (Table S1 and Figure S1) showed that coalescence in SBP emulsions is particularly slow as they are not far from those of fresh emulsions (only ~ 2 μm higher after 30 days of storage). This is followed by HM samples that perform better at acidic conditions. In addition, orange oil seems to be the least stable dispersed phase at both pH values studied, as it results in the highest d_{eq} values (Figure S1) suggesting that it may be challenging to emulsify flavour oils using pectin.

To better visualise trends in droplet sizes over time, principal component analysis (PCA) was used to minimise the data in this multi-dimensional data set. For clarity and consistency with kinetic data only droplet sizes of emulsions stabilised with the control (no cations), a divalent (Ca^{2+}) and a trivalent (Cr^{3+}) cation were selected (54 samples). The aim of a PCA is to obtain a small number of principal components (PC), which explain variations in the data and between samples. This then generates an orthogonal transformation of the droplet size data, which is easier to visualise (Denman and Morris, 2015; Kpodo et al., 2017; Lo et al., 2007). Analysis of the data (Mintab version 18.1, Minitab Inc. Pennsylvania, USA) emphasized differences between the pectins (Fig. 5). In the plot, PC1 and PC2 described 91.4 and 4.4% of the variation between the different samples. As can be seen clearly in Fig. 5, those samples which increased in droplet size to the greatest extend over time have higher values for PC1 and these are all either LM and to a lesser
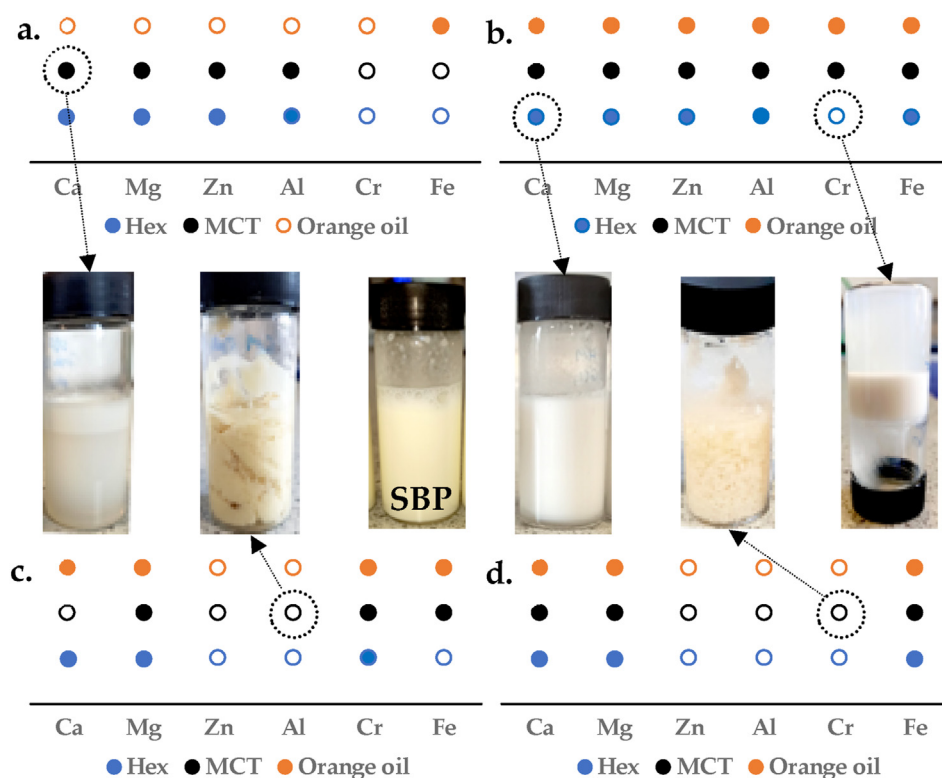
Fig. 2. Stability plots of pectin-stabilised emulsions using different dispersed phases, pectin, and cations. a) LM at pH 2.0, b) HM at pH 2.0, c) LM at pH 6.0 and d) HM at pH 6.0. Sugar beet pectin stabilises emulsions under all conditions studied (not shown). Photos in the middle show examples of the macroscopic appearance of emulsions. The third photo from the left (without arrow) is SBP-stabilised emulsion at pH 2.0 with Ca^{2+} and orange oil. Open symbols represent emulsions that do not form or destabilise rapidly after emulsification (<5 days). Closed symbols represent emulsions that are formed and are stable throughout storage period (30 days). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

extent HM pectins. Moreover, all but two of these samples where PC1 > 1.2 were prepared at pH 6.0, the other two unstable samples were LM pectin samples prepared at pH 2.0 which were presumably destabilised by Cr^{3+} cations. This is consistent with emulsions at pH 6.0 being less stable than those prepared at pH 2.0 for HM and LM pectin and that SBP emulsions remained largely comparable to those obtained at pH 2.0 and that Cr^{3+} cations destabilise emulsions to a greater extent to Ca^{2+} cations. Furthermore, this confirms the stability order (most stable) SBP > HM > LM (least stable).

4. Discussion

Association between multivalent cations and pectins leads to formation of three dimensional networks with their functional properties (e.g., elastic modulus) being dependant on the chemical nature and the amount of cations that are present (i.e., ionic strength). In the current study, we carried out all experiments at the same ionic strength (10 mM) with the exception of Fe^{3+} (1 mM). The strong affinity of Fe^{3+} for pectin leading to gelation of the continuous phase did not allow formation of emulsions at 10 mM and the experiments had to be terminated and the ionic strength was adjusted to 1 mM. Rapid gelation during emulsification has been avoided by our emulsion fabrication strategy (see section 2.2), as cross-linking commences at the interface and in continuous phase only after droplets have been formed. Under iso-ionic strength conditions, ionic interactions are independent of electric field screening provided by the presence of electrolytes and is solely based on cation characteristics (e.g., valence shell or coordination number). This approach allows derivation of fundamental knowledge on the influence of the chemical nature of cations on pectin emulsification. Polyelectrolytes, such as pectins, undergo conformational changes in response to chances in pH and presence of charged species (Dobrynin and Rubinstein, 2005). These conformational rearrangements have the capacity to change the functionality of the macromolecule. In pectin, the effect of negative charge may be muted by the number and distribution of methyl esters along the polygalacturonate chain yielding pectins with distinct cation-induced cross-linking properties (Irani et al., 2017). The distribution of

carboxyl groups also needs to be considered in the case of pectins with very similar degree of methylesterification (Daas et al., 1999; Daas et al., 2001; Williams et al., 2003).

In the present work we have found that at both pH values the presence of cations influences emulsion stability in the following order (most stable) $Ca^{2+} > Mg^{2+} > Al^{3+} > Cr^{3+} > Zn^{2+} > Fe^{3+}$ (least stable). Generally, interaction of cations with pectins proceeds through their association with the carboxylate groups, a process that strongly depends on pH. At pH above the dissociation constant of galacturonic acid (pH > ~3.5) carboxylate groups are negatively charged and they interact readily with cationic species. In contrast, at pH < ~3.5 interactions are not facilitated although they exist. This is demonstrated by the negative ζ-potential values at both pH values (~−5 and −35 mV) indicating that interactions may occur at both environments albeit to a different strength and extent. Differences in emulsification performance cannot be solely explained by the effect of pH. Interactions of pectins with metals depend on the affinity of the cation for water and on the geometry of their hydration shells (Huynh, Lerbret, Neiers, Chambin and Assifaoui, 2016b). In addition, crosslinking starts at a molar ratio that is specific to each cation. This was demonstrated with Fe^{3+} where a tenfold dilution was required (1 mM vs. 10 mM) to achieve homogeneous emulsions. Interaction dynamics of Mg^{2+} with galacturonic acids residing on pectin chains are different to those of Ca^{2+} and Zn^{2+}. In the case of Mg^{2+}, counterion condensation occurring around pectin chains (Huynh et al., 2016b) reduces the charge density between neighbouring galacturonic acid charges (Dobrynin et al., 2005). This translates to inability of pectin chains to cross-link, as Mg^{2+} cannot release water from its first hydration shell although it may lead to thicker steric barrier that protects from coalescence. In contrast, Ca^{2+} and Zn^{2+} are able to cross-link pectin chains with different strengths, as Zn^{2+} forms more stable cross links than Ca^{2+}, but at different concentrations (Celus et al., 2018). Furthermore, Zn^{2+} interacts with both carboxylate and hydroxyl groups of galacturonic units whereas Ca^{2+} only with carboxylate groups (Assifaoui et al., 2015). Transition metals (Zn^{2+}, Fe^{3+}, and Cr^{3+}) form strong coordination-covalent bonds, whereas alkaline earth metals (Ca^{2+} and Mg^{2+}) form ionic bonds with galacturonic acids (Agulhon et al., 2012)
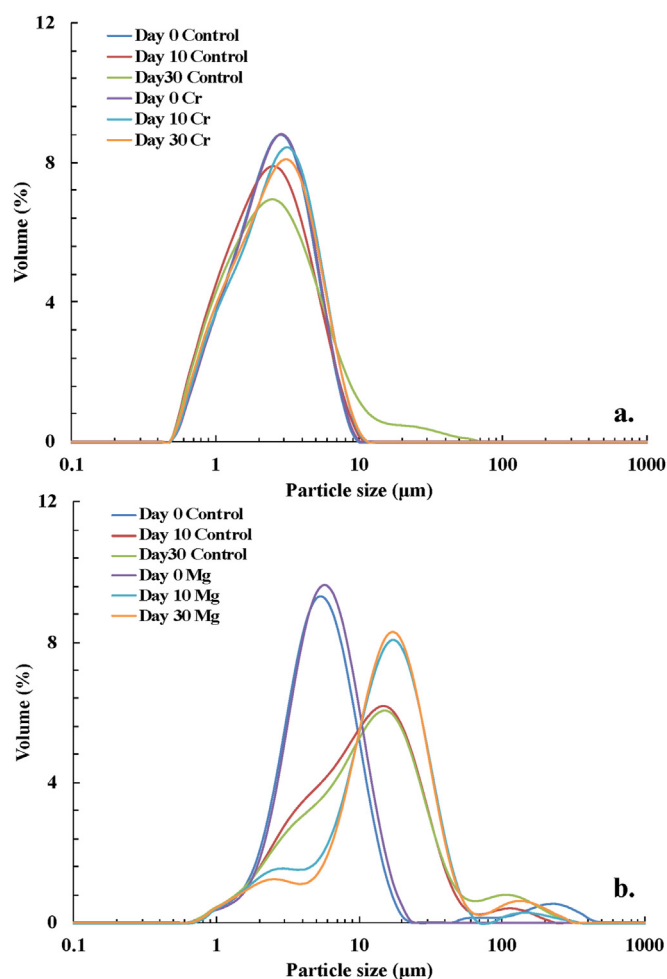
Fig. 3. Typical droplet size distribution curves of emulsions ($\phi = 0.1$, pH 6.0) prepared with: a) Hexadecane, SBP and Cr^{3+}, and b) orange oil, LM and Mg^{2+}. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Fig. 4. Kinetics of droplet growth over time. a) Typical non-linear regression analysis curves of emulsions. Curves shown are for LM-stabilised emulsions, b) rate constants of all emulsions at pH 2.0, and c) rate constants of emulsions of all emulsions at pH 6.0. Ctrl: control sample, Ca: samples with Ca^{2+}, and Cr samples with Cr^{3+}.

which helps to explain the substantial differences in emulsion stability between Ca^{2+}, Mg^{2+} and Zn^{2+} despite the fact that they have the same oxidation state.

The influence of trivalent cations on pectin properties has not been explored in the literature as thoroughly as those of divalent cations. Generally, the type of trivalent cation seems to influence pectin chain association by differences in their specificity of binding yielding junction zones of different strength (McKenna et al., 2010; Surabhi et al., 2017). Trivalent chromium has generally higher affinity for pectin than divalent cations (Berestova et al., 2020). Additionally, highly electronegative Fe^{3+} binds more strongly to pectin in comparison to the less electronegative Al^{3+} (Günter et al., 2019). These metals have been found to perform sub-optimally compared to Ca^{2+} and Mg^{2+} (it should be reminded that Fe^{3+} was used at 1 mM). Strong cross-linking restricts sufficient conformational rearrangements of pectin at the interface, as they favour pectin-pectin associations instead of pectin unfolding at the interface. Such interplay creates exposed patches in the droplet interface that are prone to bridging flocculation or coalescence. This has also been exemplified by influence of pH where ionisation of carboxyl group at pH 6.0 accelerates cross-linking and thus gelation kinetics. At this juncture, we should mention that another plausible path to data interpretation could be through a Hofmeister series approach. The central tenet of Hofmeister series is that water molecules rearrange in the hydration shell around ions disrupting the continuity of hydrogen bonding and form structures that are different than that of bulk water (Marcus, 2009). The
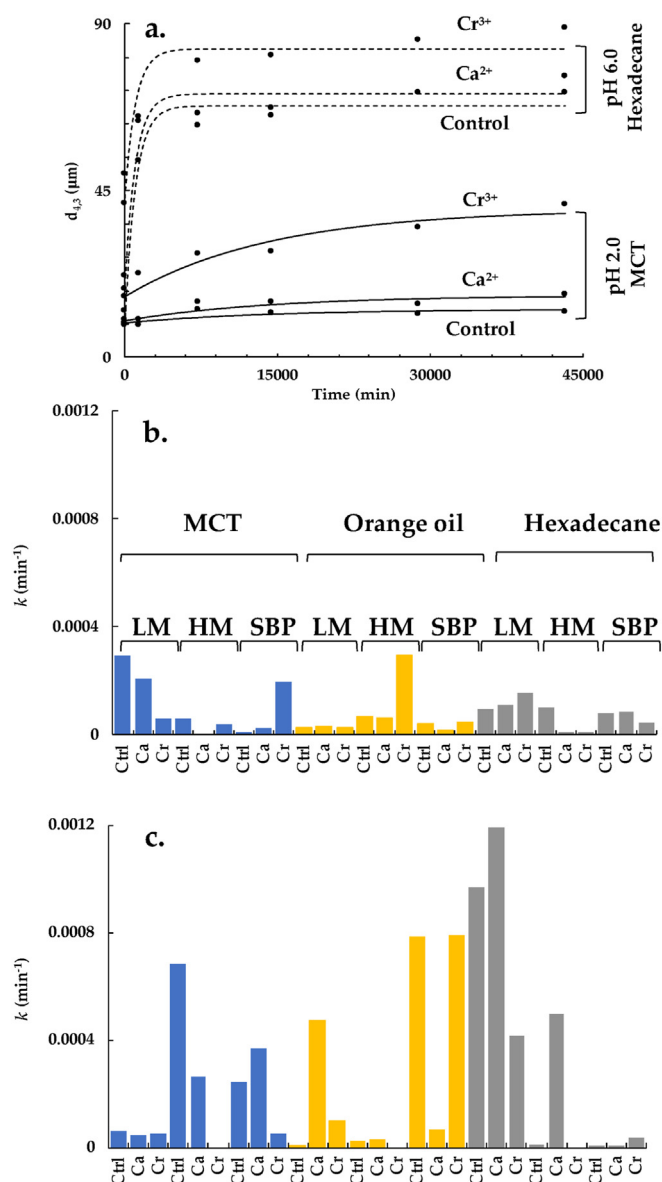
ability of ions to "break or make" water structure leads to their different capacity to precipitate proteins. Similarly, altering water structure may be the underlying fundamental cause leading to changes in pectin-water interactions that influence its conformation and emulsification characteristics. Although it is rather difficult to advance further this argument based on the data of the present work this approach offers an alternative route to data interpretation that worth to be further explored in future studies. In conclusion, the results of the present investigation show that the higher the affinity of cation for pectin the less stable the emulsion.

Pectins with substantially different molecular characteristics have been also used to probe their emulsification potential (Table S2). Stability of emulsions generally followed the order (most stable) SBP > HM > LM (least stable). Weight average molecular weight (M_w) ranged between 105 and 250×10^3 g mol^{-1} which is within the range for optimal emulsification of pectins (Alba et al., 2017). Recently, smaller citrus pectin chains with M_w in the region of 250×10^3 g mol^{-1} have been found to be excellent emulsifiers due to better interfacial coverage
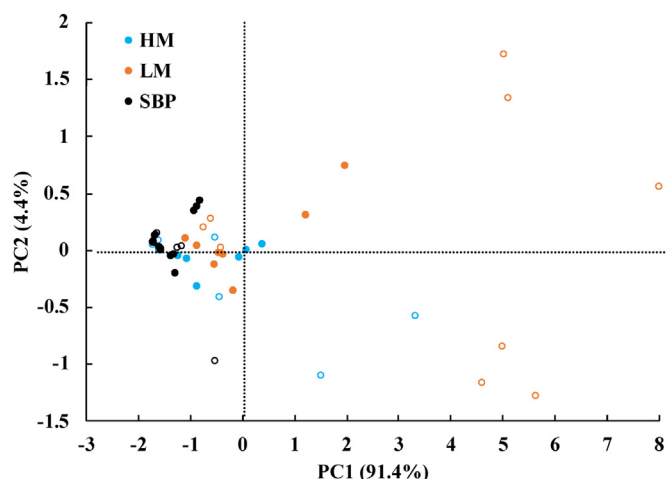
Fig. 5. Principal component analysis (PCA) plot for all emulsions made with LM, HM and SBP pectins. Closed markers are emulsions at pH 2.0 and open marker at pH 6.0.

resulting in thick steric layers (Wang et al., 2021b). Radius of gyration at elution conditions (i.e., pH ~6.6) was particularly close for all samples (Table S2) signifying that chain dimensions were similar for emulsions prepared at pH 6.0 although they would be somewhat different when pH was reduced to 2.0. Emulsification properties of pectins have been linked to their space-occupancy characteristics that depend on pH, ionic strength and degree of esterification (methyl and acetyl) with dense structures resulting in good long term emulsion stability (Alba et al., 2020; Wang, Qiu, Chen and Yan, 2021a; Zhao et al., 2020). With high-DM pectin this is normally achieved at low pH, as compactness of chains increases under these conditions (Alba et al., 2018). As a result, it is not surprising that HM-stabilised emulsions performed particularly well at low pH. The presence of acetylation in SBP samples (~15%) enhances emulsification, a finding that is generally well-documented in the literature (Chen et al., 2018; Karnik et al., 2018). Remarkably, SBP-stabilised emulsions were essentially unresponsive to the presence of cations an observation that opens new avenues for the use of this biopolymer in the stabilisation of water-in-oil emulsions that need to operate in complex cation-loaded environments.

The dispersed phases that have been used allow us to pinpoint pectin performance in a broad range of industrially relevant environments. The chemical nature of the dispersed phases are substantially different (Table S3) and are expected to play central role not only at the emulsification stage but also during ageing. The oil phase influences droplet size of emulsions and generally the higher the solubility the faster the coarsening kinetics of emulsions during and after emulsification (Chivero et al., 2014; Weiss et al., 1999; Wooster et al., 2008). At pH 2.0, regardless of the other experimental factors (e.g., pectin type, cation, storage time, etc.), droplet size followed the order (smaller) hexadecane < MCT < orange oil (larger) that coincides with their aqueous solubilities (Table S3). At pH 6.0 where the emulsions are not stable the order was (smaller) hexadecane < orange oil < MCT (larger) indicating that oil viscosity during emulsification may come into play (Table S3) (e.g., restriction of efficient oil droplet break up).

4. Conclusions

In the present work the hypothesis that inclusion of cations influences emulsification performance of pectins due to formation of interfacial cross-links was tested. Indeed, it was found that at both pH 2.0 and 6.0 the presence of cations generally influences emulsion stability in the following order (most stable) $Ca^{2+} > Mg^{2+} > Al^{3+} > Cr^{3+} > Zn^{2+} > Fe^{3+}$ (least stable). This order largely coincides with that of the strength of pectin-cation interactions showing that the higher the affinity of cation

for pectin the less stable the emulsion. Pectins generally influence stability in the order (more stable) SBP > HM > LM (least stable) with higher solution space occupancy leading to greater stability. At pH 2.0 where pectin shows its best emulsification performance dispersed phases followed the order (smaller $d_{4,3}$) hexadecane < MCT < orange oil (larger $d_{4,3}$) which coincides with their aqueous solubilities.

The present work showed that cation-induced interfacial cross-linking is a strategy to control emulsification behaviour of biological polyelectrolytes with interfacial activity. In addition, conformational changes of polymer chains are critical for long term stability and appear to be more important that the strength of interfacial anchoring. The guiding principles of these findings may be applied to other biological or synthetic polymer systems that form ionotropic gels. Further studies should elucidate the role of ionic strength and oil volume fraction to fine tune stability for specific applications. The parameters studied in this investigation seem at times to be inextricably intertwined. However, present results lend impetus to the understanding of the designing principles of oil-in-water emulsions using pectin under a broad range of industrially relevant conditions and provide scientific guidance for the growing application of pectins in the food, pharmaceutical and biomedical industries.

CRediT authorship contribution statement

Şerife Ürüncüoğlu: Investigation, Data curation. **Katerina Alba:** Investigation, Data curation, Formal analysis, Writing – original draft, Writing – review & editing, Visualization. **Gordon A. Morris:** Supervision, Formal analysis, Writing – review & editing. **Vassilis Kontogiorgos:** Conceptualization, Methodology, Formal analysis, Writing – original draft, Writing – review & editing, Supervision, Project administration.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crfs.2021.05.008.

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