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Original research

Gelation of gellan-stabilized oil-in-water emulsions using different gelling agents: fabrication and characterization

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ABSTRACT

Gellan gum was exploited to modulate the stability of sesame oil-in-water emulsion and fabricated stable droplets with mean size of~9 μm till 30 days, then gelified by $CaCl_2$ solution with final concentration of 55 mM 55 mM or 1% GDL. Enrichment with $CaCl_2$ increased the number of compact structures in gels in conjunction with decreasing WHC values and textural features modification. Gellan bundles as visualized by microscopic images conferred a remarkable influence on gel samples; enrichment with GDL or control gels increased the WHC value and formed less coarser networks. Based on Fourier transform infrared (FTIR) spectroscopy it was suggested that was no major changes in the functional groups of different gel samples, also according to the results of XRD analysis all the emulsion gels had an amorphous nature. The results indicated close relationships between physicochemical properties and microstructures of gellan-stabilized emulsion gels and would be of vital importance for extending the present knowledge about the preparation and properties of emulsion gels from gellan gum.

Keywords: Gellan gum; Emulsion; Emulsion gel; Functional properties; Textural attributes

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1. Introduction

Hydrogels are three-dimensional network structures consisting of cross-connected macromolecules that retain significant amounts of water or biological fluid (McClements., 2015). They are considered as convenient delivery systems for encapsulation, protection, and subsequent release of diverse water-soluble bioactive compounds (Komaiko & McClements., 2015). Both proteins and polysaccharides have been used to form hydrogels intended for drug delivery.

In addition to hydrophilic compounds, trapping hydrophobic compounds within polysaccharide hydrogels may improve their bioaccessibility. For instance, carotenoid-enriched lipid droplets were more bioaccessible when incorporated into starch-based hydrogels. The increased bioaccessibility was ascribed to inhibition of droplets flocculation in the stomach and small intestine by the polysaccharide gel (Mun et al., 2015).

Carbohydrates represent a large class of biomolecules with versatile technological functionalities and biological attributes. Gellan is a linear anionic exopolysaccharide with a tetrasaccharide repeating unit consisting of two β-D-glucose, one β-D-glucuronate, and α-L-rhamnose residues (Morris et al., 2012). Gellan molecules exist as random coils at high temperatures; however, during cooling, it undergoes transition to double helix configuration, which can result in the formation of an integer lattice through the Van der Waals forces and hydrogen bonds (Yamamoto & Cunha, 2007). It is possible to reinforce the texture of the resulting thermo-reversible hydrogel by adding mono and divalent cations (Morris et al., 2012). Gellan also gels at acidic condition; for example, the mixture of gellan and sodium caseinate was gelified by glucono-δ-lactone (GDL) and resulting hydrogels were used for probiotics encapsulation (Iurciuc et al., 2016).

Emulsions are kinetically stabilized systems of two or more immiscible liquids. Spontaneous emulsification is an inexpensive and low-energy technique that has been employed for the fabrication of emulsions at ambient condition (Chang & McClements, 2014; Anton & Vandamme, 2009). It is achieved by several methods including those that require solvents such as acetone, ethanol and tetrahydrofuran (Komaiko & McClements., 2015). Titration of an organic phase containing a hydrophilic surfactant into an aqueous phase introduced

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an alternative spontaneous emulsification method that does not require solvents (Anton & Vandamme, 2009). Recently, it was found that gellan gum allows spontaneous formation of a long-term stable emulsion of fish oil at a substantially low content of surfactant. It was concluded that the co-adsorption of gellan to the interface along with surfactant contributed to the emulsification (Moayedzadeh et al, 2018).

Oil-in-water emulsions can be transformed into a type of biphasic gel by gelation of the continuous aqueous phase. Transforming emulsion into gel increases its stability because problems such as phase separation are resolved. In addition, emulsion gels may provide better release of drugs as compared to other topical drug delivery systems (Alexander et al, 2013). Indeed, emulsion gels are considered as an effective delivery vehicle especially for controlled release of hydrophobic bioactive components (Yang & Tang, 2013). Tang et al compared the properties of emulsion gels induced by GDL, calcium chloride ($CaCl_2$), and microbial transglutaminase from the emulsions stabilized with unheated and heated soy protein isolate (Tang et al, 2011). Liang et al. also successfully developed a β-lactoglobulin-stabilized emulsion gel using Ca^{2+} ions as a carrier for α-tocopherol (Liang et al, 2010).

There was no report in the literature, to the best of authors' knowledge that transforms a spontaneously formed emulsion to emulsion gel using GDL. The objective of this study was therefore to gel the gellan-stabilized sesame oil-in-water emulsions formed via the spontaneous emulsification method using GDL and $CaCl_2$ and characterize the resulting emulsion and emulsion gel using different techniques. Sesame oil was employed for emulsification with respect to its outstanding biological properties such as anti-cancer and antioxidant activities furthermore it contains a wide range of polyunsaturated fatty acids and enriched in zinc (Budowski, & Markley, 1951).

2. Material and Methods

2.1. Materials

High acyl (HA) gellan gum was purchased from Kelcogel (Kelcogel, USA). Hydrochloric acid was obtained from Scharlau (Spain). Calcium chloride, GDL, sodium azide, sodium chloride, chloroform, tween 20 (polysorbate 20) and span 80 (sorbitan monooleate) were purchased from Merck (Darmstadt, Germany). Extra virgin sesame oil was obtained from a local market in Tehran, Iran. Distilled water was used throughout the study.

2.2. Preparation of sesame oil-in-water emulsion

The organic phase consists of the hydrophobic surfactant, span 80 mixed with sesame oil at different surfactant-to-oil ratios (0.1, 0.2, 0.5, and 1.0). The aqueous phase consisted of gellan and different concentrations of tween 20 (Bouchemal et al, 2004). Gellan powder was dissolved in phosphate buffer (10 mM, pH 7.3) at a concentration of 1.0 mg/g; sodium azide (50 ppm) was added as an antimicrobial agent. Then, the solution was heat-treated at 80 °C for 90 min at a constant stirring speed (500 rpm), cooled to 25 °C and supplemented with tween 20. The organic phase was titrated into the aqueous phase under stirring at 500 rpm for 10 min at 25 °C, initially appeared transparent but became opaque with time. The final emulsion contained 10 wt% sesame oil, 0, 1, or 5 wt% tween, 1, 2, 5, or 10 wt% span and 80 or 88 wt% gellan solution. The emulsions were stirred for 30 min at 500 rpm and then they were transferred to clear plastic containers (25 mm height and 15 mm inner diameter) and stored at 25 °C for 30 days to assess their stability.

2.3. Determination of emulsion stability

Various emulsion samples with different weight percentages of span, tween, and gellan were visually monitored during storage and any deviation from monophasic to biphasic system was considered as instability. Accordingly, three samples (Table 1) with at least one day stability were selected for further analysis. The samples were imaged by an optical microscope (BX51, OLYMPUS, Tokyo, Japan) equipped with a camera (Olympus DP25) at 40× objective magnification. For this purpose, emulsions were diluted with buffer solution (pH 7.3) and a drop of diluted samples was deposited on a glass slide and covered gently with a cover slip ensuring no air bubbles. At least 40 images were obtained for each sample at 25 ºC and the droplets average diameter was determined by measuring their size using Image-Pro Plus software (version 6, Media Cybernetic, Inc. Maryland, Montgomery, USA).

An electrical conductivity method was used to assess emulsion stability (Latreille, & Paquin, 1990). For this purpose, emulsion samples were centrifuged at 1330 × g for 30 min at 25°C for accelerating aging. The conductivity difference between lower aqueous phase and original emulsion was measured (HANNA instruments Inc., HI 8633, Rhode Island, USA) at 25°C as an index of stability.

2.4. Zeta-potential (ζ-potential) measurement

Zeta-potential analysis was performed to evaluate the surface charge of droplets by a zeta-sizer (SZ-100, HORIBA, Japan) at 25 °C. Three freshly prepared sesame oil-in-water emulsions with pH 6.8 ± 0.1 were analyzed at scattering angle of 90° and temperature of 25 °C upon application of a polystyrene zeta cell. Samples were diluted ten-fold with buffer solutions (same pH as sample) before analysis, to prevent multiple scattering effects.

2.5. Gelation of emulsions

Emulsion sample containing 10 wt% sesame oil, 1 wt% span 80, 1 wt% tween 20 and 88 wt% gellan solution was evidently stable for a minimum of 30 days. Therefore, it was selected to form emulsion gel. However, to form a self-standing gel a higher concentration of gellan (3.0 mg/g rather than 1.0 mg/g) was required. The emulsion was supplemented with either 3.7 M $CaCl_2$ stock solution to a final $CaCl_2$ concentration of 55 mM or 0.01 g GDL/mL. Samples were then incubated at 4 ºC for 45 h to form a self-standing emulsion gel. A gel-like sample with no added $CaCl_2$ or GDL was considered as control sample.

2.6. Fourier transform infrared (FTIR) spectroscopy of emulsion gels

The FTIR spectra of freeze-dried gel samples were acquired with a Perkin Elmer FT-IR spectrometer (Perkin Elmer Co., MA, USA) from 450 to 4000 cm^{-1} wavenumber.

2.7. X-ray diffraction (XRD)

The XRD analysis was carried out using an EQUINOX 3000 diffractometer (Inel, Artenay, France) equipped with a Cu Kα radiation source. For this purpose, freeze-dried emulsion gel samples were cut into pieces of 1cm ×1cm and were scanned in the 2θ range of 5–30º at an operating voltage of 40 kV and a filament current of 30 mA.

Table 1. Some properties of gellan-stabilized sesame oil-in-water emulsions. * Phase separation. ζ-potential was measured at first day. F_1= 5% tween, 80% gellan, 10% oil, and 5% span. F_2= 0% tween, 88% gellan, 10% oil, and 2% span. F_3= 1% tween, 88% gellan, 10% oil, and 1% span. Lower case letters indicate significant difference (p<0.05) for a given sample over time; upper case letters indicate significant difference between samples at identical time.

	Mean droplet diameter (µm)								
Sample	**Day 1**	**Day 5**	**Day 10**	**Day 15**	**Day 20**	**Day 25**	**Day 30**	**Zeta-potential[1] (mV)**	**Δ Conductivity (mS cm^{-1})**
F_1	7.13±0.04^{C}	-*	-	-	-	-	-	-2.7±0.02^{c}	1.20±0.03^{a}
F_2	7.58±0.05^{bB}	7.64 ±0.01^{b}	7.91±0.08^{a}	-*	-	-	-	-3.2±0.01^{b}	0.52±0.00^{b}
F_3	9.16±0.02^{dA}	9.21±0.06^{cd}	9.31±0.04^{c}	9.31±0.02^{c}	9.48±0.06^{b}	9.53±0.03^{b}	9.78±0.02^{a}	-7.7±0.01^{a}	0.29±0.01^{c}

2.8. Textural analysis of emulsion gels

Penetration test was performed using a texture analyzer apparatus (M350-10CT, Testometric, Lancashire, UK) to determine the strength of the gels. Cylindrical gel samples with 50 mm height and 15 mm diameter were penetrated to a depth of 20 mm by a cylindrical stainless-steel probe (5 mm diameter) at the constant speed of 0.55 mm s^{-1}. The maximum penetration force (N), defined as the force required for rupturing the gel, was expressed as gel strength.

2.9. Water holding capacity (WHC) and syneresis of emulsion gels

WHC of emulsion gel samples was determined according to the method described by Mao et al. with a slight modification. Samples were centrifuged at 10000 ×g for 15 min and WHC was calculated by the following equation (Mao et al, 2001):

$$\text{WHC}\ (\%) = \frac{W_g - W_{dw}}{W_g} \times 100 \quad (1)$$

where W_g and W_{dw} are gel weight (g) before centrifugation and drained water weight (g), respectively. For syneresis measuring, the gels were stored for 96 h at 4 ºC and the expelled water was quantified. The percentage of syneresis was calculated according to the following equation:

$$\text{Syneresis}\ (\%) = \frac{\text{Total weight of separated liquid (g)}}{\text{Total weight of filled hydrogel (g)}} \times 100 \quad (2)$$

2.10. Microstructure of emulsion gels

The microstructure of different gel samples was analyzed using a scanning electron microscope (SEM, Vega, Tescan, USA). Samples were freeze-dried, cut into small pieces, and submerged in chloroform for several times to remove the sesame oil and then placed under hood for evaporating chloroform. The samples were then gently broken at liquid nitrogen and fixed on a metal stub using copper tape followed by coating with a thin layer of gold before capturing the images.

2.11. Swelling experiment

The swelling capacity of emulsion gels after freeze-drying was measured following the method that described by Maltais et al. Accordingly, cylindrical gel specimens (about 0.5 g) were immersed in an enzyme-free simulated gastric fluid containing sodium chloride, hydrochloric acid, and distilled water with the final pH value of 1.2 for up to 2 h at 37 ºC. The specimens were removed from the solution periodically, blotted dry, and re-weighed accurately (Maltais et al, 2009). The following equation was used to calculate the swelling percentage:

$$\text{Swelling}\ (\%) = \frac{W_t - W_0}{W_0} \times 100 \quad (3)$$

where W_t is the gel weight (g) at time t and W_0 is the initial weight (g) of gel.

2.12. Statistical analysis

The data were analyzed by one-way ANOVA with the help of SPSS software version 16 (IBM software, NY, USA). Significant differences between the mean values were examined by using Duncan test and the level of significance used was $p < 0.05$. Each experiment was carried out in triplicate and the data are reported as means ± standard deviations.

3. Results and Discussion

3.1. Emulsion stability

It was necessary to include gellan at the continuous aqueous phase of emulsion; otherwise, no stable emulsion has been formed. The effect of gellan on emulsion formation and stability has been ascribed to the co-adsorption of gellan onto oil-water interface as well as increase the viscosity of continuous phase (Moayedzadeh et al, 2018). Three emulsion samples containing gellan in aqueous phase and with identical oil content (10%) were stable for at least 1 day. Fig. 1 illustrates the optical microscopy images of gellan containing sesame oil-in-water emulsion droplets immediately after spontaneous formation and Table 1 reports the droplet size of the samples over storage. The weight fraction of the hydrophilic and lipophilic surfactants and gellan solution (1 mg/mL) had a significant influence on emulsion stability and droplet size. A higher surfactant fraction (5 wt% tween and 5 wt% span) which was concomitant with a lower proportion of gellan (80 wt%) brought about a smaller droplet size immediately after preparation compared to a lower surfactant fraction (2 wt%). This is attributed to a less interfacial tension at the greater surfactant proportion. Davidov-Pardo and McClements observed that the mean droplet diameter of nanoemulsion formed by spontaneous emulsification decreased with increasing of surfactant concentration (Davidov-Pardo & McClements, 2015).

This phenomenon could be explained by the reduction in the interfacial tension caused by the increase of surfactant on the oil–water interface and/or due to the increase in the amount of surfactant diffusing from the oil phase to the aqueous phase, resulting in smaller droplets formed at the boundary. However, the emulsion with the higher proportion of surfactants (10% surfactant and 80% gellan) underwent creaming only after 1 day. Whereas those containing a lower content of surfactants (2%) but a higher proportion of gellan solution (88%) were stable for at least 10 days. It is argued that a sufficiently high viscosity in the continuous phase was required to prevent droplets coalescence and phase separation. Increasing the viscosity of the continuous phase using thickening or gelling agents like gellan gum can extend emulsion stability (Vilela & da Cunha,

2016). Creaming stability depends on the capacity of the continuous phase to immobilize the oil drops (Lorenzo et al, 2013).

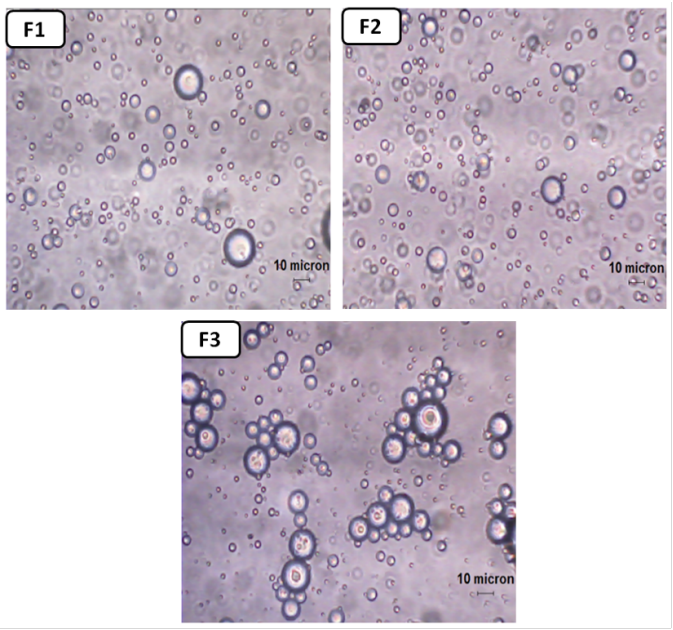

Fig. 1. Optical microscopy images of sesame oil-in-water emulsions immediately after preparation (F_1= 5% tween, 80% gellan, 10% oil, and 5% span, F_2= 0% tween, 88% gellan, 10% oil, and 2% span, and F_3= 1% tween, 88% gellan, 10% oil, and 1% span).

Ratio of the hydrophilic and lipophilic surfactants had a considerable effect on droplet size and emulsion stability. Adding hydrophilic surfactant, tween, into gellan solution (1.0 mg/mL) at the expense of the lipophilic surfactant, span, caused formation of larger oil droplets. Packing parameter of surfactant which is associated with its molecular geometry that determines the optimum curvature of a monolayer that a particular kind of surfactant forms, influences interfacial tension (Israelachvili, 2011; McClements, 2012). Span 80 and tween 20 have differences in CH chain length and of the presence of a double bond in the lipophilic group, tween 20 (C12:0) has saturated chain and span 80 (C18:1) has an unsaturated one (Guttoff et al, 2015). Wang et al. observed that the presence of a double bond in surfactant mixture tail favored spontaneous formation of smaller methyl oleate and ethyl oleate oil droplets and the effect has been attributed to less compatibility of the interface and would give rise to a looser film, therefore, generating emulsions with small droplet sizes (Wang et al, 2009). Guttoff et al. reported unsaturated tails in surfactants could affect the packing parameter, likewise in our study span 80 would expect to have a higher packing parameter due to a larger cross-sectional area of the tail group than tween 20 that have saturated linear chains (Guttoff et al, 2015).

Nevertheless, including tween into the aqueous phase prohibited oil droplets coalescence and yielded an emulsion stable for 30 days (Table 1). A better function was reported for certain mixture of surfactant in comparison with the pure surfactants (Huibers & Shah, 2007). Han et al. showed that the combination of four types of additives including ionic surfactant (sodium deoxycholate), non-ionic surfactant (Poloxamer 188 and Tween 80), and lecithin to obtain favorably stable nanostructured lipid carriers drug delivery system, which could stabilize the system for more than 1 year without phase separation at 4 ºC (Han et al, 2008). Al-Shannaq et al. studied the emulsion stability using mixed surfactants (sodium dodecyl sulfate and polyvinyl alcohol) and compared it with a system which was stabilized using a single surfactant (PVA) was used. Contrary to a system in which one surfactant was used, implementation of mixed surfactants induced long-term emulsion stability and drops had a mono- dispersed distribution (Al-Shannaq et al, 2015). Optical microscopy imaging showed that tween incorporation into the aqueous phase of emulsion (concomitant with decreasing span content in oil phase) resulted in oil droplets flocculation immediately after their formation (Fig. 1). It is hypothesized that tween competed with gellan to co-adsorb onto oil-water interface from the aqueous side, happening simultaneously by span 80 from the oil side. Spontaneous emulsification methods are not possible solely with proteins or polysaccharides because the rapid movement of the low-molecular weight surfactants into the aqueous phase is necessary to generate a large turbulent force at the oil–water interface as well as to form a significant increase in the oil–water interfacial area leading to the spontaneous formation of oil droplets surrounded through a budding process by aqueous phase (McClements, 2011). Low-molecular weight surfactants adsorb to droplet surface more rapidly than amphiphilic biopolymers (Qian & McClements, 2011), which is ascribed to the higher diffusion rate of small surfactants compared to large biopolymers. Low-mass surfactants are very mobile at the interface, and they can particularly reduce the interfacial tension. As a result, they rapidly coat the freshly created oil-water interface during emulsification. Biocompatible, biodegradable, and/or nontoxic emulsion-based formulations have great potential for incorporation in the food products (Kralova & Sjöblom, 2009). Pichot et al. proposed a mechanism for the stabilization process of O/W ‘‘food-grade” emulsions exhibited by the mixed emulsifier systems (Tween 60 or Sodium caseinate) and colloidal particles (silica particles). Initially, the low molecular weight surfactants stabilize the interfaces, which were formed during the emulsification process, lowering the interfacial tension and promoting the break-up of droplets (Pichot et al, 2007). In addition, surfactant adsorption reduces the occurrence of coalescence events and allows the less mobile particles to access the interface before the droplet size is significantly changed.

The inferiority of gellan to tween at interfacial adsorption probably reduced the anchoring sites of gellan into oil phase through its acetyl moiety, resulting in gellan accumulation in the immediate vicinity of oil drops in the continuous phase, which in turn promoted bridging flocculation of the drops. The flocculated drops were nonetheless stable to coalescence and the resulting emulsion remained stable for a period of 1 month.

Gellan conferred a negatively signed ζ-potential to oil droplets in spite of using non-ionic low-molecular weight surfactant in preparation of emulsions (Table 1). As expected, a higher gellan fraction resulted in a higher ζ-potential value. However, the ratio of hydrophilic surfactant to lipophilic surfactant had also a prominent influence on the magnitude of droplets charge. Including tween (1%) into emulsion, at the expense of span (reducing from 2% to 1%) caused a remarkable increase in ζ-potential, which supports our assumption that gellan was accumulated in the near vicinity of oil droplets as a consequence of preferential adsorption of tween from the aqueous side onto interface. It is noteworthy that the higher ζ-potential value of the sample with tween did not prevent oil droplets flocculation (Fig. 1). Indeed, ζ-potential values over |30| mV are required to achieve a long-term electrostatic stabilization and droplets flocculate progressively at ζ-potentials values between |5| and |15| mV (Marianecci et al 2013; Poletto et al, 2011).

3.2. Conductivity measurements

The differences in electric conductivity (Δ conductivity) of the lower aqueous phase obtained through the centrifugation and the original emulsion samples after one day are reported in Table 1. It is well known that Δ conductivity increases as a function of time due to creaming of fat globules (Latreille, & Paquin, 1990). Therefore, higher Δ conductivity indicates higher emulsion instability (Wang et al, 2011), which could be due to creaming of fat globules. These globules are nonconductors with respect to the water-gellan mixture which, on the other hand, is a good conductor therefore emulsion F_1 with higher organic phase ratio than others (10% oil and 5% span) is more unstable. The conductivity measurement test was fast and well correlated with the stability of emulsions.

3.3. Emulsion gels

In this research, we intended to transform produced sesame oil-in-water emulsions to gels. The existence of carboxyl group in glucuronic acid of gellan gum provides a special way for calcium ions and GDL promoted cross-linking by decreasing the electrostatic repulsion among the co-charged chains or being the less fraction of carboxyl groups dissociates; namely, the gellan chains become less anionic polyelectrolyte by adding GDL leading to the formation of junction zones and an interconnected three-dimensional gel network (Guo et al, 2009; Horinaka et al, 2004; Yamamoto & Cunha, 2007).

3.4. FTIR analysis

The FTIR spectra of control, Ca^{+2}- and GDL-induced cold-set emulsion gel samples are depicted in Fig. 2. The spectrum of all samples showed a similar pattern, suggesting that there were no major changes in the functional groups of control sample due to interaction between different gelling agents and gellan. It could be due to the presence of the gelling agents in small concentrations. The molecules are held together by a combination of weak intermolecular forces like hydrogen bonds, electrostatic, and Van der Waals forces as well as hydrophobic interactions. The FTIR spectra of the samples showed peaks at wavenumbers of 3009-3012 cm^{-1} which is concerned with OH group stretching vibrations (Sudhamani et al, 2003). This peak is generally associated with the presence of intra- and inter-molecular hydrogen bonding between the water containing gellan and the surfactant mixtures. The absorbance peak at 1160-1165 cm^{-1} corresponded to the glycosidic linkage present in control, GDL-, and $CaCl_2$-induced gels (Murillo-Martínez & Tecante, 2014). The characteristic peaks at the wavenumber range of 2927-2932 cm^{-1} were due to the C–H stretching vibration of the CH_2 groups of alkanes, present in the surfactants and sesame oil. The peaks at 2855 cm^{-1} (C–H stretching of the alkanes), 1748 cm^{-1} (-CO stretch of the carbonyl groups), and peaks at wavenumber of 1465 cm^{-1} (C–H bending vibrations of the alkanes) were due to the presence of both surfactants and sesame oil in the emulsion gels and the peak at 722 cm^{-1} may be associated with the CH rocking relating to the alkanes present in surfactants (Singh et al, 2016). The OH stretching vibration of saccharide units at 3009 cm^{-1} is shifted to 3012 cm^{-1} in the presence of GDL and $CaCl_2$. No significant shifting of the characteristic bands of gellan observed, it can be concluded that no complexation and effective surrounding of functional groups of gellan by GDL and divalent cations present in resultant emulsion gels was occurred. The addition of $CaCl_2$ to the emulsion caused a slight shift of the peak of C-H vibrations to lower number (from 2932 cm^{-1} to 2927 cm^{-1}).

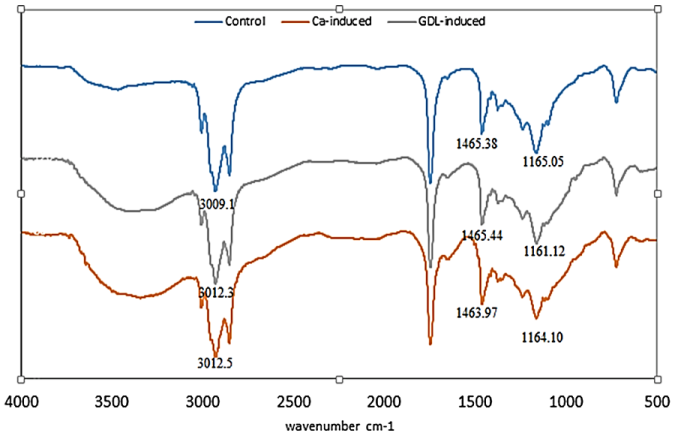

Fig. 2. FT-IR spectra of emulsion gel samples made of gellan-stabilized emulsions using different gelling agents.

3.5. XRD analysis

The structural organization of the emulsion gel samples was examined by XRD (Fig. 3). All the samples showed a broad hump at 2θ of~19.86º, which was attributed to the amorphous nature of the gel-based samples (Ahuja et al, 2013). The amorphous nature of the emulsion gel is due to the absence of the regular arrangements of the crystallite domains in a close range and/or sharp geometric structures. Results showed that the peaks intensities were followed in the order of control> GDL>$CaCl_2$, indicating that the control sample crystal-like ordered structure was destroyed apparently by adding of GDL and $CaCl_2$. The stronger interaction between carboxyl groups and calcium ions produced more amorphous structure than GDL-induced ones (Wang et al, 2016). This result was in good agreement with those from textural analysis.

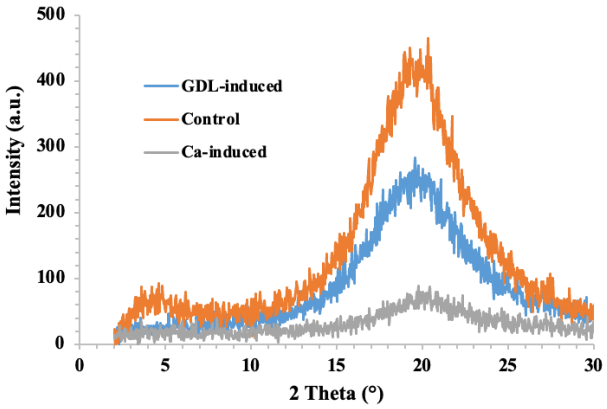

Fig. 3. XRD patterns of emulsion gel samples made of gellan-stabilized emulsions using different gelling agents.

3.6. Functional and textural analysis

The differences in microstructure were basically consistent with the differences in WHC and mechanical properties of the gels. Gel samples were investigated for their microstructural features by scanning electron microscopy. Fig. 4 illustrates exemplar SEM images of different freeze-dried gel samples. The matrix of the $CaCl_2$- induced gels was particulate and showed a more densely linked structure compared to the GDL-induced counterpart. In agreement with our results, Guo et al. also reported a particulate microstructure for

psyllium polysaccharide gel prepared with calcium ions (Guo et al, 2009). It is hypothesized that the establishment of numerous stronger and ionic bridges among junction zones decreased the flexibility of gellan chains leading to the formation of denser microstructures, resulting in a lower WHC value and enhanced strength of the gel. The GDL-induced gels had a more elongated structure with clear defects in their interior which would be responsible for their diminished strength (Phillips & Williams, 2009). The control gels had a more uniform structure than GDL-induced one (Fig. 4) which efficiently holds free water existing among the strands. It can be due to the higher count of junction zone interactions established by cooling compared to sample which was enriched with GDL as a gelling agent (Horinaka et al, 2004). Gellan bundles are clearly visible in the microstructural images of control gel sample (Fig. 4C) but these bundles changed for interacting with Ca^{2+} ions and GDL. The sample gelled through adding of GDL and control gel had 90.89 and 97.50% WHC and no syneresis (Table 2). It is concluded from the tabulated results that calcium-mediated crosslinking influenced the WHC and syneresis properties of the gel samples to a greater extent than other samples. The WHC and firmness of the GDL-induced gels were also lower than the control gels. The less WHC is related to the reduction of charged groups of the gellan in the system that might contribute to the water retention of the emulsion gels. In comparison with control sample, destruction of the gellan bundles by adding GDL formed the less coarse structure. Longer storage of samples for 96 h at 4 ºC and more, did not affect the syneresis and WHC levels (results not reported).

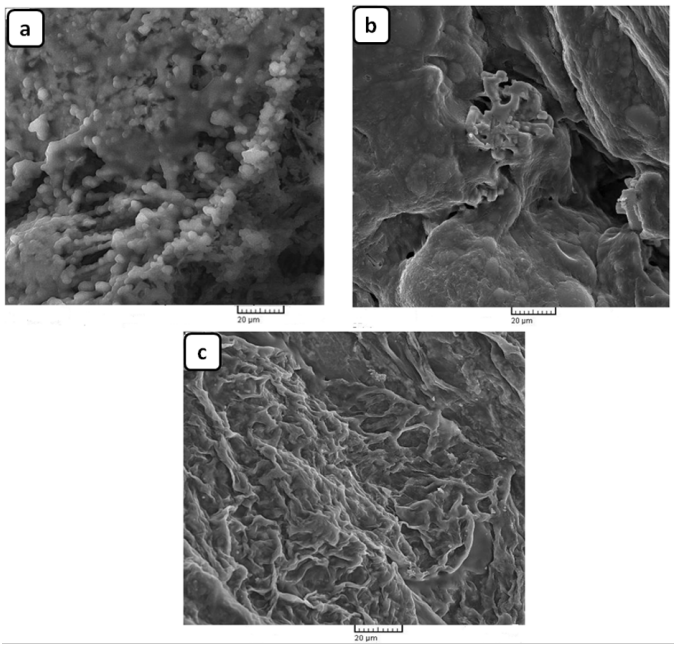

Fig. 4. SEM micrographs of (a) Ca^{+2}-induced, (b) GDL-induced, and (c) control emulsion gel samples made of gellan-stabilized emulsion.

Table 2. Firmness, water holding capacity, and syneresis of emulsion gel samples prepared by different gelling agents.

Gel samples	Firmness (N)	WHC (%)	Syneresis (%)
Ca^{+2}-induced	0.47 ± 0.01^{a}	74.98 ± 0.98^{c}	43.58 ± 0.55^{a}
GDL-induced	0.21 ± 0.01^{c}	90.89 ± 0.76^{b}	0.00 ± 0.00^{b}
Control	0.27 ± 0.02^{b}	97.50 ± 0.89^{a}	0.00 ± 0.00^{b}

Means within the same column with different superscripts differ significantly ($p < 0.05$).

3.7. Swelling experiment

Swelling behavior is important evaluation for a drug/nutraceutical delivery carrier. Table 3 indicates the swelling extent of freeze-dried cold-set emulsion gel samples over time of 2h. Samples immersed in simulated gastric media without digestive enzymes absorb water because of the osmotic pressure difference between the gel matrix and surrounding solution (Kalshetti et al, 2012). The swelling percentage of the $CaCl_2$ induced gel was lower than the other samples throughout the whole period. The control sample also showed a higher swelling capability than GDL enriched samples at pH 1.2. A correlative relation exists between the water imbibition capability and WHC of gel samples. The results indicated that the $CaCl_2$ crosslinking decreased synergistically the swelling ratio of emulsion gel. The particulate structure of the gel induced by $CaCl_2$ crosslinking barricaded the entrance of water into the matrix of emulsion gel. In addition, consumption of the COO^- groups of gellan resulting from crosslinking reaction also is expected to decrease the electrostatic repulsion within the gel network at acidic condition (pH 1.2) which in turn would decrease the inward water flux.

Table 3. The swelling ratio (%) of freeze-dried emulsion gels at acidic solution with pH value of 1.2.

Sample	Time (min)			
	15	45	90	120
Ca^{+2}-induced	34.17 ± 2.05^{bC}	41.59 ± 2.12^{bB}	47.17 ± 1.62^{cA}	50.75 ± 2.09^{cA}
GDL-induced	53.39 ± 0.70^{aB}	72.35 ± 1.80^{aA}	73.22 ± 1.68^{bA}	73.66 ± 1.06^{bA}
Control	57.95 ± 1.87^{aD}	74.63 ± 1.38^{aC}	86.27 ± 2.49^{aB}	93.51 ± 2.00^{aA}

Superscript lower-case letters indicate significant differences among different samples at a given time and superscript upper-case letters indicate significant differences at different times for one sample ($p < 0.05$).

4. Conclusion

Spontaneous emulsification technique was successfully employed to fabricate a gellan-stabilized sesame oil-in-water emulsion which was stable over 30 days. Resulting emulsion was transformed into emulsion gels using $CaCl_2$ and GDL. In Ca^{2+}-induced gel, cations promoted covalent crosslinking and increased the firmness of gel which was ascribed to formation of tighter gellan aggregates in gel microstructure. This network could expel water in emulsion gels, thereby decreased WHC of the gel. GDL was also implemented for cold-set gelation of gellan-stabilized sesame oil-in-water emulsions. GDL-induced and control gel samples had softer structure than $CaCl_2$-induced gel and immobilized more water, thereby decreasing syneresis and water expulsion from the gel. The lower firmness of these gels was attributed to the finely stranded gel microstructure. Ca^{2+}-induced gel had lower swelling extent than others attributing to its particulate structure. Generally, the results indicated that gellan-stabilized sesame emulsion produced by spontaneous emulsification could be transformed into emulsion gels with different structural and functional properties depending on type of the gelling agent. The resulting gels can be used in the formulation of edible gels as well as nutraceutical delivery systems. However, more detailed studies are required to assess their *in vitro* gastric digestibility and release behavior.

Conflict of interest

The authors declare that there is no conflict of interest.

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