

# Effect of different polysaccharides on swelling of composite whey protein hydrogels: A low field (LF) NMR relaxometry study

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## Abstract

Hydrogels are usually prepared from hydrophilic polymers and when different types of polymers are blended or emulsified with an oil phase the resulting gel is usually known as a composite gel. The objective of this study was to analyze the water uptake characteristics of whey protein-polysaccharide containing composite hydrogels. Composite and composite emulsion gels were formulated in this study using whey protein, xanthan (XN), pectin (PC), alginate (AL), and sunflower oil. Water absorption of hydrogels was studied using Nuclear Magnetic Resonance (NMR) relaxometry through transverse relaxation time ( $T_2$ ) measurements, and Non-Negative-Least-Square (NNLS) analysis. Swelling ratios (SR) of different gels were determined. Swelling of the gels was also evaluated mathematically and water uptake of hydrogels was explained by the power law model and diffusion rate constants based on power law model were determined. Results showed that NMR relaxometry could be used to differentiate water uptake mechanisms of composite hydrogels.

## Practical applications

Biodegradable hydrogels provide controlled delivery of nutrients, bioactive agents such as antioxidants and antimicrobial agents at a desired site and time and at a specific rate. Incorporating bioactive agents to these gels also protects sensitive nutrients and may increase the shelf life of some potential food products. The swelling characteristics of hydrogels provide information about the possible release behavior of the hydrogels. When a hydrogel is loaded with an agent and placed in an aqueous medium, usually the release rate of the agent from the gel and the swelling ratio (SR) of the gel due to solvent uptake from the surrounding medium is proportional to each other. In this study, effects of different polysaccharides on the SR of composite whey protein hydrogels were determined by NMR relaxometry. NMR enabled fast and easy monitoring for hydrogel swelling. Furthermore, NMR provided more detailed information on swelling mechanism than conventional methods proving potential for further applications.

## KEYWORDS

composite hydrogel, emulsion gel, NMR relaxometry, swelling, whey protein isolate

## 1 | INTRODUCTION

Hydrogels are three-dimensional micro or nanocapsule structures used in food systems prepared by food grade polymers capable of providing gelation and emulsifying properties for designing delivery matrices (Argin, Kofinas, & Lo, 2014; Heelan & Corrigan, 1998; Hennink & van

Nostrum, 2012; Lee & Rosenberg, 1999). Biodegradable polymers such as whey proteins, xanthan (XN) gum, alginate (AL), and pectin (PC) are widely used for hydrogel formulations (Gunasekaran, Xiao, & Ould Eleya, 2006; Oztop & McCarthy, 2011; Vandenberg, Drolet, Scott, & De La Noue, 2001; Wichchukit, Oztop, Mccarthy, & Mccarthy, 2013). One of the main areas of using hydrogels is controlled delivery of

nutrients, bioactive compounds, and molecules in food systems at a desired site and time at a specific rate (Argin et al., 2014). The main mechanisms taking place in hydrogel formation during heat-induced gelling is crosslinking interactions between polymers that are mainly noncovalent including ionic interactions, hydrogen bonding, hydrophobic, and covalent interactions such as S-S bridges (Nicolai & Durand, 2007; Oztop, McCarthy, McCarthy, & Rosenberg, 2012). In biomaterial research, food technology and biotechnology, hydrogels that form physical and chemical crosslinking have an importance due to their ability to provide a control release mechanism for bioactive agents entrapped in the matrix (Argin et al., 2014; Hoffman, 2012).

When exposed to water, hydrogels usually swell due to the penetration of the hydrophilic solvent resulting from polymer chain relaxation. The extent of swelling relies on the degree and the balance between polymer-polymer, polymer-ion, and polymer-water interactions (Gunasekaran et al., 2006; Kong, Oztop, Singh, & McCarthy, 2013; Oztop & McCarthy, 2011; Oztop et al., 2012). Swelling ability and composition of the hydrogels as well as the surrounding aqueous medium in which release will occur, determine the release behavior of the hydrogel (Siepmann & Peppas, 2001). Release of encapsulated compounds can be explained by swelling controlled, diffusion controlled or both mechanisms (Lee and Rosenberg, 2000). When mass transfer of the encapsulated agent is faster than hydrogel swelling, swelling controlled release occurs. Diffusion controlled release, where swelling of particles has no effect on diffusion, can be described by Fick's law of diffusion (Lin & Metters, 2006). In our study, a mathematical model for swelling of hydrogels was also developed to further investigate the swelling kinetics of hydrogels formulated using various polymers.

Whey protein which is a common food protein is widely used for gel preparations and a valuable by product of the cheese industry (Ersus, Oztop, McCarthy, & Barrett, 2010). Whey protein and other biodegradable polymers are used for heat-induced gel making. During heat gelation of whey proteins a series of consecutive events take place. These include; heat-induced protein unfolding, -SH group activation of  $\beta$ -lactoglobulin and molecular aggregate formation leading to formation of a three-dimensional network (Aguilera, 1995). Protein composition, protein concentration, ionic environment, and the pH affect the structure, formation, and physicochemical properties of hydrogels prepared by whey protein (Gunasekaran et al., 2006; Ziegler, 1990).

AL is a hydrophilic colloidal carbohydrate polymer, composed of unbranched binary copolymers of 1-4-linked  $\beta$ -D-mannuronic and  $\alpha$ -L-guluronic acid residues of widely varying in composition and sequence (Rhim, 2004). Alginic acid includes carboxyl groups in each residue. So, AL has the ability to interact with polyvalent metal cations due to the presence of carboxyl groups to form strong gels or insoluble polymers (Rhim, 2004). AL is a biopolymer that has its unique colloidal properties which are thickening, stabilizing, suspending, film forming, gel producing, and emulsion stabilizing abilities (Lee & Mooney, 2012; Liling et al., 2016; Rhim, 2004).

PC is a structurally complex heteropolysaccharide containing three pectic polysaccharides that are galacturonic acid as the linear back-

bone, 1-4-linked  $\alpha$ -D-Galacturonic acid and Rhamnogalacturonan (Willats, Knox, & Mikkelsen, 2006). PC is primarily used as a gelling and stabilizing agent in the food industry, including jam, jelly, fruit juice, confectionary, and bakery filling productions (Argin et al., 2014). PC's carboxyl groups attract cations that are present in the medium resulting in water uptake to the structure. This creates an osmotic pressure on the gel which causes the gel to swell (Williams, Oztop, McCarthy, McCarthy, & Lo, 2011). Gel making and swelling properties of PC hydrogels are utilized in a wide range of food systems for delivery purposes (Belscak-Cvitanovic et al., 2015; Sriamornsak, Prakongpan, Puttipipatkachorn, & Kennedy, 1997).

XN is a natural heteropolysaccharide that contains a cellulosic backbone with side chains of two mannose and one glucuronic acid on each glucose residue (Argin et al., 2014; Garcia-Ochoa, Santos, Casas, & Gomez, 2000). XN gum is an important industrial biopolymer and used in a wide variety of foods for emulsion stabilization, temperature stability, compatibility with food ingredients, and its pseudoplastic rheological properties (Garcia-Ochoa et al., 2000). XN gum has a tendency for increasing solution viscosities thus, widely used as a thickening agent in aqueous solutions (Whitcomb, 1978). By increasing the viscosity of the solution, XN reduces water mobility and decreases permeability of such systems (Garcia-Ochoa et al., 2000). This behavior results in enhanced oil recovery in mediums containing oil as a dispersed phase implying that oil can be incorporated into the XN gel structure. XN hydrogels are also used for delivery matrices for their desirable gelling characteristics (Argin et al., 2014; Dumitriu & Chornet, 1997).

When polymer solutions are mixed with lipids, composite emulsion gels are obtained. Composite emulsion gels consist of at least two phases which are continuous matrix and a dispersed phase. The continuous network of the gel containing polymers interact with lipids and three-dimensional junction zones are formed so that two constituent phases of the composite are physically connected (Mor-Rosenberg, Shoemaker, & Rosenberg, 2004). Composite gels can change the swelling behavior. Each biodegradable polymer blended for gel making, could interact differently with the lipid dispersed in the gel network. Since release phenomenon depends on swelling ability of the gel and generally release is proportional to the swelling capability of the gel, composite emulsion gels can be used to control the rate of release of hydrogels.

NMR relaxometry is a noninvasive and nondestructive method that is used to obtain information about the mobile protons of a sample. Low field (LF)  $^1\text{H}$  NMR relaxometry is a practical way to analyze proton relaxation in food systems (Ersus et al., 2010; Oztop et al., 2012). Since NMR signal is composed of all protons in a sample, mobility and distribution of protons can give information about the internal structure of the sample.  $T_2$  which is also described as spin-spin relaxation time provides information about relaxation and mobility of hydrogen molecules. Short spin-spin relaxation times are attributed to the hydrogen nuclei in an immobile structure while long spin-spin relaxation times belong to hydrogen nuclei in mobile structures (Hashemi, Bradley, & Lisanti, 2010). Therefore, the swelling of hydrogels can be

investigated by LF  $^1\text{H}$  NMR relaxometry since gels uptake water during swelling and this results in changes of the  $T_2$  times of water immersed hydrogels.

$T_2$  relaxation spectrum analysis provides detailed information about the relaxation spectra of the samples so that the contribution of each proton pool to the relaxation time can be investigated (Ersus et al., 2010). Relaxation spectrum analysis makes it possible to understand the water distribution within the hydrogels and further investigate the polymer-water, polymer-polymer, and polymer-oil interactions.

The primary aim of our study was to investigate the swelling properties of different composite hydrogels and composite emulsion gels and explain the water uptake behavior of such systems using mainly LF  $^1\text{H}$  NMR relaxometry accompanied with a simple mathematical modeling approach. Relaxation spectrum analysis was also conducted to obtain detailed information on the swelling phenomenon of gel systems.

## 2 | MATERIALS AND METHODS

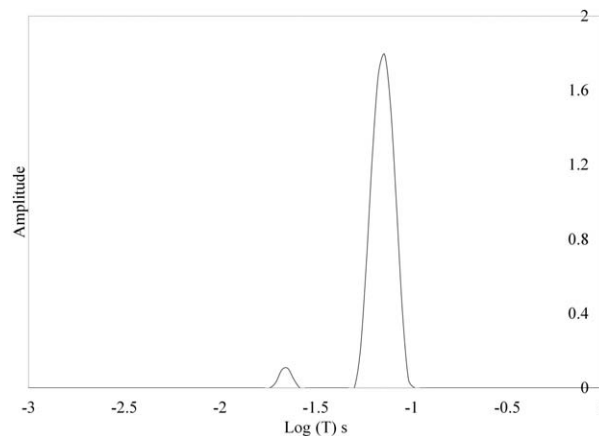
XN (Sigma-Aldrich Corp., Taufkirchen, Germany), PC (Sigma-Aldrich Corp., Taufkirchen, Germany), and AL (Fluka, Steinheim, Germany) were used as the polysaccharides. Whey protein isolate (WPI) with protein content of 88.5% determined by Kjeldahl method was used as the main gelling ingredient (Hardline Nutrition, Kavi Food Ltd. Co., Istanbul, Turkey).

### 2.1 | PREPARATION OF THE COMPOSITE GELS

First, 15% WPI (w/w), 0.5% polysaccharide (w/w), 0.2%  $\text{NaNO}_3$  (w/w), and 84.3% distilled water (w/w) were stirred. WPI, in the absence of polysaccharides, were used as control. Emulsion gels were also prepared by substituting 10% of water with sunflower oil in the final mixture. Polymer solutions were stirred at 15,000 rpm for 5 min using Ultra Turrax T-18 (IKA Corp., Staufen, Germany). Before mixing, all polymer solutions were centrifuged at 1,000 rpm for 2 min for degassing. Afterwards, the mixture was stirred overnight. Gelation was conducted in cylindrical shaped tubes (5 cm long, 1.5 cm diameter) immersed in  $90^\circ\text{C}$  water bath for 30 min (Wisd, Wertheim, Germany). Following gelation, tubes were placed into ice for 15 min. Swelling experiments were conducted at distilled water at a pH of 7.0 that was adjusted with 2.0 M NaOH. Gels were placed into plastic mesh baskets and weight of the hydrogels was recorded at predetermined times. Swelling data were fitted to power law model as seen in Equation 1 where  $m_t$  denoted the measured weight and  $m_\infty$  was the weight at equilibrium,  $k$  was the rate constant, and  $t$  was the time and  $n$  was the release exponent.

$$\frac{m_t}{m_\infty} = kt^n. \quad (1)$$

Furthermore, SR of hydrogels was calculated as in Equation 2:



**FIGURE 1** A representative  $T_2$  relaxation spectrum of the xanthan composite hydrogel

$$SR(\%) = \frac{m_t - m_0}{m_0}. \quad (2)$$

For NMR experiments, each hydrogel was placed on a 0.32 T NMR system at 0, 3, and 6 h (Spin Track SB4, Mary El, Russia) to measure  $T_2$  relaxation times. For  $T_2$  measurements, Carr-Purcell-Meiboom-Gill sequence was used with 1 ms echo time, 500 echoes 32 scans, and 3 s repetition time.

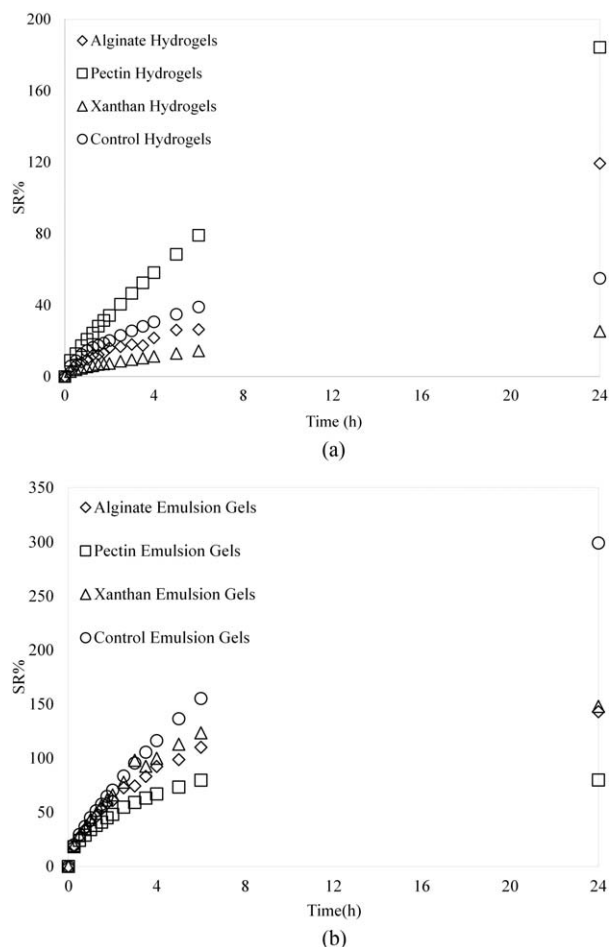
Statistical data analysis was performed using MINITAB 1.6 (Minitab Ltd. Co., Coventry, UK) with at least three replications for each experiment. Tukey test was used as the multicomparison test at 5% significant level when significant difference was detected between the treatments.

In order to perform relaxation spectrum analysis, Prospa 3.1 NNLS module (Magritek, CA) was used. Peak's number, time, and area were investigated with  $\alpha = 10^8$ ,  $\beta = -1$  as shown in Figure 1. NNLS module is based on Lawson and Hanson algorithm that depends regularization function that seeks to find a smooth spectrum of exponentials that satisfies the data in a chi-squared (Ersus et al., 2010).

## 3 | RESULTS AND DISCUSSION

### 3.1 | Swelling ratio characterization of hydrogels and composite emulsion gels

SR of all hydrogels increased due to increased water uptake during 24 h water immersing of gels. As shown in Figure 2A, PC hydrogels had the highest SR (184%) at the end of 24 h. AL hydrogels followed PC hydrogels with a SR of 119% whereas the XN hydrogels had the lowest SR with 25% which was even lower than the SR of control hydrogels prepared with just WPI. SR results showed that the PC hydrogels absorbed the highest amount of water from the surrounding medium with respect to other gel types indicating that the PC polymer provided a better gel matrix under provided gelling conditions since gelation was mainly associated with the solvent holding capacity of that matrix (Oztop et al., 2012). PC is a complex group of heteropolysaccharides comprising a branched structure in solutions and carries a negative charge at the pH 7 which is the immersion pH value used in



**FIGURE 2** (A) Swelling ratio of composite hydrogels. (B) Swelling ratio of composite emulsion gels

the experiments (Mohnen, 2008). During heat-induced gelation of a polymer blend, heat-induced interactions between the polymers lead to formation of molecular aggregates, interactions among these aggregates to form a network and finally absorption of solvent when placed in a solvent containing medium. For each polymer the extent, type and degree of these interactions show differences causing a variety of distinct gelling properties. PC, in this case, formed a suitable gel network with WPI under heating conditions probably due to the enhanced interactions between polymers. Inter and intramolecular disulfide bonds, crosslinking, weak interactions such as hydrophobic interactions and hydrogen bonding may have contributed to the distinct gelling properties of PC (Oztop et al., 2012). Moreover, when PC hydrogel was placed into the water having the pH of 7 that was prepared by addition of sodium hydroxide (NaOH), PC hydrogel showed an enhanced swelling capacity. One possible reason was the hydrophilic structure of the inner part of the PC gel enabling gel to interact strongly with water resulting in higher swelling ratios. Branched portions of PC such as L-Aceric acid, D-Apiose, L-Arabinose, L-Fucose, D-Galactose, L-Galactose, and L-Rhamnose have higher affinity to water than its rigid backbone due to conformation characteristics of PC after gelling (Mohnen, 2008). In addition, PC has negatively charged side

groups mainly carboxylic groups that can crosslink further with cations present in the solvent contributing to the better gel properties.

On the other hand, AL hydrogels showed intermediate SR compared to PC and XN hydrogels as in Figure 2A. One of the differences between the PC and AL polymers is their chemical structures. AL possesses a more linear structure with respect to branched PC polymer. Both of the polymers are negatively charged under the neutral pH but the magnitude of the charge may be different for each polymer. The zeta potential of AL solutions were found to be negative across the pH range of 2–7, changing from strongly negative (–68 mV) at the pH 7 to slightly negative (–10 mV) at the pH 2 (Zhang, Zhang, Zou, & McClements, 2016). So, AL is strongly negatively charged at the pH 7. At this pH, the negatively charged molecules in AL are believed to repel each other strongly causing a weakening of the crosslinks thus the gel structure itself. The physical observations of AL hydrogels also showed that the AL gels had a very porous matrix which could be attributed to the impaired crosslinking resulted in a poor gel structure. The linearity of the AL molecules may have contributed to the high extent of electrostatic repulsion in the gels (Wichchukit et al., 2013). Although PC was also negatively charged at the immersion pH, its more branched and complex structure may have reduced the effect of charge repulsion and provided an improved gel structure.

The lowest SR values were obtained by XN hydrogels as shown in Figure 2A. Low SR was attributed to the chemical structure and conformation of the XN molecules. XN is a big and highly branched molecule composed of a main chain of  $\beta$ -D-glucose units with trisaccharide side chains. XN is categorized as an anionic polysaccharide due to the presence of side chains containing acetic and pyruvic acids. The trisaccharide branches appear to be closely aligned with the polymer backbone. The molecules of XN interact strongly with each other and form helical or random coil structures in solutions (Santos, Casas, & Go, 2000; Tao et al., 2016). XN gels were reported to have small pores that were attributed to a high crosslink density (Tao et al., 2016). In our study, it is hypothesized that the ionizable groups of XN gum which are mostly carboxyl groups, crosslinked with  $\text{Na}^+$  ions coming from NaOH solution intensely since carboxyl groups attract the  $\text{Na}^+$  ions during diffusion of water into the gel complex. This incident may have led to a strong crosslinking of XN gels and resulted in a lower SR (Argin et al., 2014; Shiroodi, Rasco, & Lo, 2015).

The composite emulsion gels that were obtained by addition of 10% (w/w) sunflower oil showed different swelling characteristics. The incorporation of oil into the gel matrix almost did not alter the SR of AL gels as expected since AL had poor gelling capability at the pH of 7 due to high electrostatic repulsion at that pH which might have resulted in a decreased crosslink degree. As a result of the porous structure, AL gel network had a high permeability characteristic with lacking the necessary barrier effects and that could have prevented oil from incorporating into the gel structure efficiently (Belscak-Cvitanovic et al., 2015).

The most drastic effects of oil addition to polymers were observed in XN and PC composite emulsion gels as seen in Figure 2B. XN hydrogel initially showed a very low SR (25%) but XN emulsion had SR of

148% which was the highest SR among others. XN gum forms viscous solutions and gels in the absence of oil (Santos et al., 2000). This behavior is usually associated with the complex conformational structure of the XN (Tao et al., 2016). The oil addition enhanced the swelling ability of XN hydrogels drastically ( $p < 0.05$ ). Oil incorporation into the XN gel matrix could have reduced the initial high viscosity and made it possible for gel to absorb higher amount of water. The complex conformation of the gel network could have also been effected by the oil incorporation and the intense interactions between the molecules of XN could have been interrupted. While XN hydrogel swelling capacity increased by oil addition, PC gels showed a decreased SR implying less swelling capacity. One proposed reason was the change in PC molecule's interaction with water after oil incorporation into the gel matrix. Initially, PC was able to absorb and hold high amount of water due to its highly hydrophilic branches (Ventura & Bianco-Peled, 2015). Oil addition clearly changed the ability of gel network to interact with water and less water was able to be retained in the gel matrix. The high hydrophilic characteristic of the PC gel was impaired by the oil addition.

### 3.2 | NMR measurements, relaxation spectrum analysis, and $T_2$ vs. swelling ratio comparisons

NMR relaxometry allows identification of water distribution and molecular interactions of gums without destruction of the sample (Williams et al., 2011). NMR relaxometry was also employed to study gelation process of hydrocolloid blends and their interactions with water (Vittadini, Dickinson, & Chinachoti, 2002; Wang et al., 2002). Spin-spin relaxation ( $T_2$ ) times of the gels were found highly correlated with water uptake in Pearson correlations ( $r > 0.99$ ). The interactions between water and gel structures were also explained with  $T_2$  measurements (Williams et al., 2011).  $T_2$  measurements were conducted for a 6 h interval and all gels showed an increase in  $T_2$  values as observed in Figures 3A,B. This increasing trend was related to the water uptake of gels and indication of increase in the amount of water present in the gels. In order to interpret the relation between SR and  $T_2$  in more detail,  $T_2$  percent changes were also calculated for 0–6 h time interval of the experiment. Although  $T_2$  was mainly associated with the degree of swelling thus the water uptake of the gels, a high percent increase in the  $T_2$  was observed in XN gels (61.86%, Table 1) which was close to percent  $T_2$  change of PC gels (63.70%, Table 1), indicating that the interactions between the gel structure and water also played a significant role on the of  $T_2$  values besides water uptake of hydrogels. In spite of low water uptake of XN gels, high increase in  $T_2$  value suggested that water-polymer interactions predominated in XN hydrogels (Wichchukit et al., 2013). This phenomenon could be explained in XN gels by high viscosity and decreased permeability of the gel network (Santos et al., 2000).

PC gels showed the highest  $T_2$  increase at the end of 6 h of swelling (63.70%) as shown in Table 1. Since the SR of PC gels was considerably higher than the XN gels, 184% and 25%, respectively, the high  $T_2$  values of PC gels were attributed to the high amount of water uptake in these gels. Although interactions between PC gel network

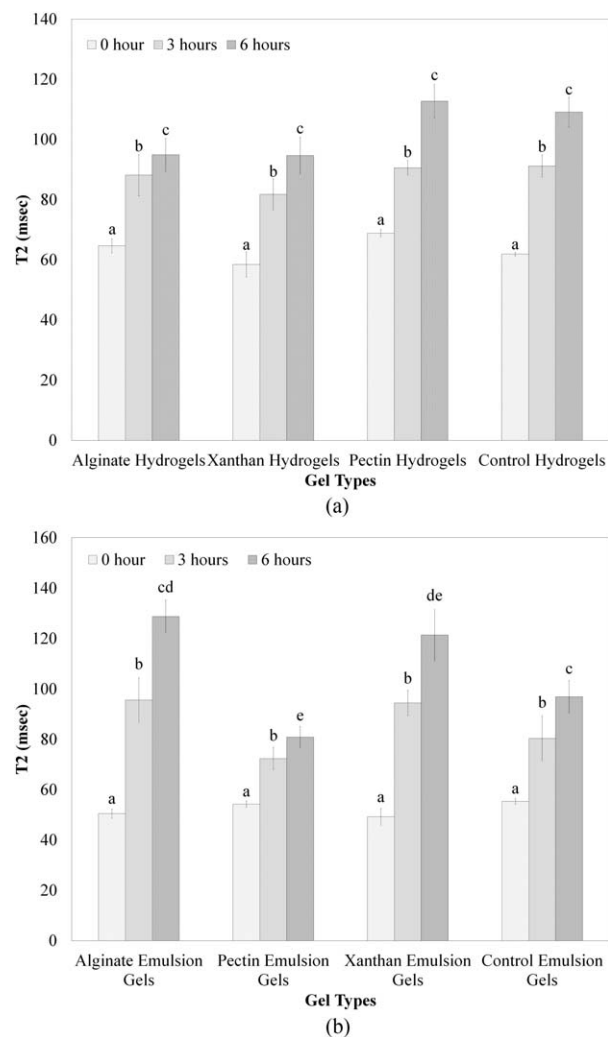


FIGURE 3 (A)  $T_2$  relaxation times of composite hydrogels. (B)  $T_2$  relaxation times of composite emulsion gels

and water were expected to decrease the  $T_2$  of the system, high amount of water present in the gel predominated over interactions and resulted in longer  $T_2$ 's.

As seen on Table 1, the  $T_2$  increase of AL was found to be 46.80% for 0–6 h time interval as lowest among the others. This was again mostly related to the amount of water absorbed as it was in the PC case. The poor gelling properties and porous structure of AL could not provide an efficient gel network to uptake and hold the water in desired amounts (Zhang et al., 2016).

The  $T_2$  changes of XN and PC composite emulsion gels were consistent with their SR results. The percent increase in  $T_2$  of XN emulsion hydrogels was higher compared to XN gels prepared without oil, 146.43% and 61.86%, respectively. SR of XN composite emulsion gels were also higher than XN hydrogels indicating that high  $T_2$  percent increase in XN emulsions originated from better water uptake capabilities of XN emulsions. Reasons behind this phenomenon were discussed in SR explanations of XN emulsions. PC composite emulsion gels exhibited lower  $T_2$  increase (49.05%) with respect to PC gels prepared without oil (63.70%) and this was the same trend that was

TABLE 1 Percent change in  $T_2$  relaxation times of composite hydrogels and emulsion gels

	Alginate emulsion gels	Pectin emulsion gels	Xanthan emulsion gels	Control emulsion gels	Alginate hydrogels	Pectin hydrogels	Xanthan hydrogels	Control hydrogels
0–6 h	155.05%	49.05%	146.43%	75.01%	46.80%	63.70%	61.86%	76.24%
0–3 h	89.31%	33.43%	91.70%	45.16%	36.28%	31.56%	39.81%	47.32%
3–6 h	34.72%	11.71%	28.55%	20.56%	7.72%	24.43%	15.76%	19.63%

observed in SR experiments of PC gels. Looking at the change in  $T_2$  results, it was validated that addition of oil to PC gels decreased PC's ability to form a proper gel network. Finally,  $T_2$  changes of AL composite emulsion gels showed a drastic increase from 46.80% to 155.05% after oil addition. SR of AL gels also exhibited an increase from 119% to 143% as shown in Figure 2A, but that was not as severe as the increase in  $T_2$  value. Apparently, oil did not incorporated into the AL gel system efficiently but at the same time oil could have implemented further damage in the gel matrix causing an increase in the amount of water in the system. The increase in  $T_2$  of AL emulsion gel could have stemmed from this incident.

When the SRs of hydrogels containing different polymer types and their emulsions were compared with their corresponding  $T_2$  values, a high correlation was observed ( $r > 0.99$ ). This indicated that NMR relaxometry can be used for characterization of polymers in terms of their gelling abilities based on their interactions with water and NMR relaxometry technique is an alternative and reliable method for hydrogel swelling experiments.

In order to analyze  $T_2$  changes in more detail, relaxation spectrum of hydrogels and composite emulsion gels were investigated by NNLS analysis. Table 2 summarizes the relative peak times and areas obtained through the relaxation spectra. Each peak represents a distinct proton

TABLE 2  $T_2$  relaxation spectrum analysis results of composite hydrogels and emulsion gels

Types of gels	Number of peaks	Peak's time (ms)			Peak's area		
		0 h	3 h	6 h	0 h	3 h	6 h
Alginate Hydrogels	First peak	17.333 ± 3.382 <sup>a</sup>	9.300 ± 3.021 <sup>a</sup>	14.366 ± 3.502 <sup>a</sup>	1.44 ± 1.26 <sup>a</sup>	1.43 ± 0.47 <sup>a</sup>	1.43 ± 0.19 <sup>a</sup>
	Second peak	68.666 ± 3.666 <sup>b</sup>	90.000 ± 0 <sup>b</sup>	90.000 ± 0 <sup>b</sup>	97.14 ± 1.35 <sup>b</sup>	95.96 ± 1.23 <sup>b</sup>	94.27 ± 0.46 <sup>b</sup>
	Third peak	*	390.000 ± 0 <sup>c</sup>	880.000 ± 0 <sup>c</sup>	*	6.29 ± 0 <sup>c</sup>	5.08 ± 0 <sup>c</sup>
Composite emulsion gels	First peak	8.933 ± 3.225 <sup>a</sup>	15.933 ± 6.598 <sup>a</sup>	65.333 ± 6.064 <sup>a</sup>	2.28 ± 0.17 <sup>a</sup>	3.89 ± 2.75 <sup>a</sup>	34.06 ± 7.43 <sup>a</sup>
	Second peak	44.666 ± 2.333 <sup>b</sup>	71.666 ± 20.480 <sup>b</sup>	200.000 ± 0 <sup>b</sup>	81.17 ± 3.37 <sup>b</sup>	45.14 ± 16.12 <sup>b</sup>	54.73 ± 8.46 <sup>a</sup>
	Third peak	*	1153.333 ± 1023.398 <sup>c</sup>	*	*	36.95 ± 11.22 <sup>b</sup>	*
Pectin Hydrogels	First peak	20.700 ± 5.000 <sup>a</sup>	21.550 ± 1.016 <sup>a</sup>	*	411 ± 1.64 <sup>a</sup>	6.25 ± 4.73 <sup>a</sup>	*
	Second peak	65.000 ± 0 <sup>b</sup>	80.333 ± 16.000 <sup>b</sup>	76.000 ± 0 <sup>a</sup>	90.23 ± 0.42 <sup>b</sup>	71.12 ± 18.95 <sup>b</sup>	59.96 ± 5.49 <sup>a</sup>
	Third peak	280.000 ± 0 <sup>c</sup>	660.000 ± 44.090 <sup>c</sup>	240.000 ± 23.000 <sup>b</sup>	7.46 ± 0.71 <sup>a</sup>	34.91 ± 2.56 <sup>b</sup>	39.84 ± 5.48 <sup>a</sup>
Composite emulsion gels	First peak	*	*	18.000 ± 0 <sup>a</sup>	*	*	0.02 ± 0 <sup>a</sup>
	Second peak	55.000 ± 0 <sup>a</sup>	72.333 ± 3.666 <sup>a</sup>	80.666 ± 4.666 <sup>b</sup>	92.85 ± 0.6 <sup>a</sup>	85.04 ± 1.51 <sup>a</sup>	81.79 ± 1.78 <sup>b</sup>
	Third peak	*	*	120.00 ± 0 <sup>c</sup>	*	*	15.39 ± 0 <sup>c</sup>
Xanthan Hydrogels	First peak	*	11.066 ± 4.975 <sup>a</sup>	26.800 ± 1.633 <sup>a</sup>	*	0.57 ± 0.07 <sup>a</sup>	1.66 ± 1.25 <sup>a</sup>
	Second peak	55.000 ± 0 <sup>a</sup>	76.000 ± 0 <sup>b</sup>	90.000 ± 0 <sup>b</sup>	0.49 <sup>a</sup>	96.36 ± 0.09 <sup>b</sup>	95.09 ± 1.34 <sup>b</sup>
	Third peak	*	3200.000 ± 0 <sup>c</sup>	750.000 ± 0 <sup>c</sup>	*	2.38 ± 0 <sup>a</sup>	5.53 ± 0 <sup>c</sup>
Composite emulsion gels	First peak	*	43.000 ± 1.193 <sup>a</sup>	34.833 ± 16.333 <sup>a</sup>	*	23.76 ± 13.67 <sup>a</sup>	15.97 ± 8.56 <sup>a</sup>
	Second peak	49.666 ± 2.666 <sup>a</sup>	116.666 ± 3.333 <sup>b</sup>	128.333 ± 32.188 <sup>b</sup>	84.96 ± 1.51 <sup>a</sup>	61.37 ± 15.07 <sup>b</sup>	47.89 ± 5.73 <sup>b</sup>
	Third peak	*	*	170.000 ± 0 <sup>b</sup>	*	3.46 ± 0 <sup>c</sup>	55.27 ± 0 <sup>c</sup>
Control Hydrogels	First peak	*	*	*	*	*	*
	Second peak	65.000 ± 0 <sup>a</sup>	90.000 ± 0 <sup>a</sup>	115.000 ± 4.082 <sup>a</sup>	96.70 ± 0.22 <sup>a</sup>	96.95 ± 0.60 <sup>a</sup>	97.34 ± 0.68 <sup>a</sup>
	Third peak	*	*	*	*	*	*
Composite emulsion gels	First peak	*	4.800 ± 0 <sup>a</sup>	*	*	0.34 ± 0 <sup>a</sup>	*
	Second peak	55.000 ± 0 <sup>a</sup>	90.000 ± 0 <sup>b</sup>	103.333 ± 6.666 <sup>a</sup>	93.83 ± 0.58 <sup>a</sup>	89.05 ± 0.18 <sup>b</sup>	83.12 ± 4.15 <sup>a</sup>
	Third peak	*	*	*	*	*	*

\* Peak not detected.

Lower case letters denote significance level at 5%.

population located in a sample. The first peak time and area values of all hydrogels and composite emulsion gels were lower than the other two peaks represented as in Table 2. Therefore, the first peak was attributed to the interactions between polymers since solid-solid interactions were reported to have low relaxation times (Hashemi et al., 2010). The third peak which had the highest relaxation time that was close to water's was attributed to the water entrapped in the gel matrix during solvent uptake. The third peak area of PC hydrogel showed an increase from 7.46% to 39.84% after six hours of immersion in water. PC hydrogel had the highest SR as mentioned before, thus the increase in the area of the third peak of PC hydrogel during swelling originated from water uptake into the gel matrix. A similar trend was observed for other hydrogels and also for the composite emulsion gels. The presence of the second peak, on the other hand, could be explained by the interactions between polymers and water, since relaxation times belonging to this peak stands in the middle of first and the third peak values. Furthermore, the control sample containing WPI as the sole polymer source showed one peak which was the second peak, indicating that polymer-water interactions predominated the system.

When relative peak areas of PC hydrogel were investigated it was observed that the second peak area decreased as immersion time increased. High water uptake of PC hydrogels caused a decrease in polymer-water interactions since excess water was not able to interact with the polymer. The increase in the area of the third peak representing the water entrapped in the gel matrix also justified this explanation. The increase in the relative third peak areas of AL and XN hydrogels were not as severe as the increase in the PC hydrogel. XN hydrogel had an area of 5.53% for the third peak at the end of six hours immersion which was consistent with the low SR of XN samples indicating that XN hydrogel matrix was not capable of entrapping as much water as PC hydrogel matrix.

The PC composite emulsion gel's SR was lower than PC hydrogel's SR and this was also validated by the relaxation spectrum analysis of PC composite gels. In PC composite gels, amount of water entrapped in the gel structure thus SR reduced since the third peak of PC composite gels nearly disappeared compared to PC hydrogel third peak areas. The reason could be the high surfactant ability of PC (Kaltsa, Paximada, Mandala, & Scholten, 2014). PC could have made possible for oil to incorporate into the gel structure more efficiently resulting in an enhanced barrier effect against water uptake. In contrast, areas of the third peak in XN composite emulsion gels increased compared to their XN hydrogel correspondents. XN composite gels also showed an increased SR with respect to XN hydrogels, which confirmed the changes in the relative areas. Clearly, oil addition decreased the viscosity of XN hydrogels (Garcia-Ochoa et al., 2000). Furthermore, oil incorporation into the XN gel matrix could have induced polymer chain

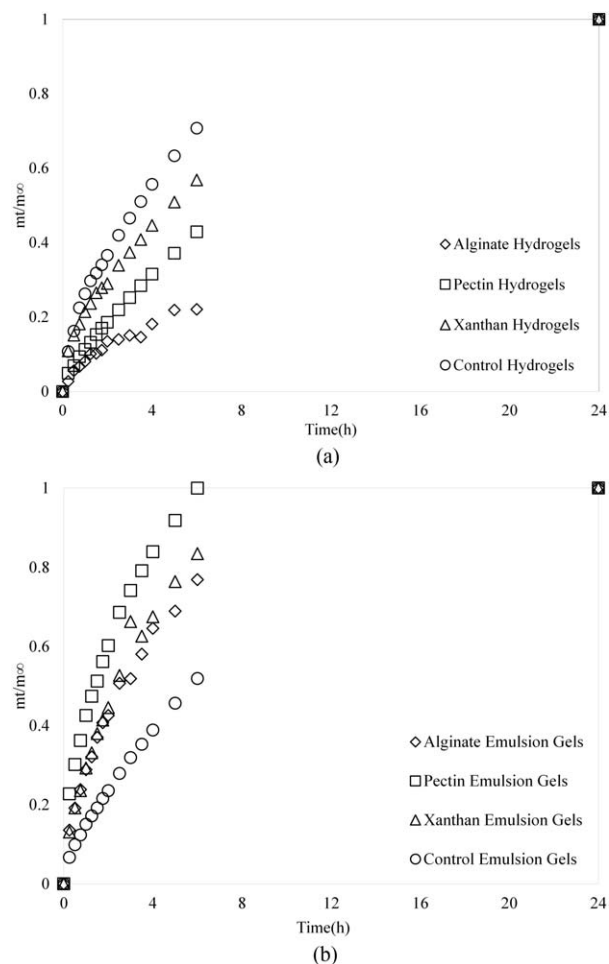


FIGURE 4 (A) Water uptake of composite hydrogels. (B) Water uptake of emulsion gels

relaxation of the polymer so that an enhanced water uptake was observed in XN composite gels.

### 3.3 | Mathematical modeling

A power law model for different transport mechanisms for solute release of water soluble drugs from various polymers were introduced previously (Peppas, 1985). In this study, power law model ( $M_t/M_\infty = Kt^n$ ) was used for kinetic characterization of swelling of composite hydrogels and emulsion gels at  $M_t/M_\infty < 0.6$  (Argin et al., 2014; Kikkinides et al., 1998; Wichchukit et al., 2013). When power law model was applied to water uptake data of composite hydrogels and emulsion gels (Figures 4A,B), it was observed that the data fitted well to the model ( $R^2 \geq 0.95$ ). Therefore, the swelling behavior of gels can be described by power law model as stated before. Table 3 shows the calculated diffusion rate constant ( $k$ )

TABLE 3 Power law model fitting results of water uptake by composite hydrogels and emulsion gels

	Alginate emulsion gels	Pectin emulsion gels	Xanthan emulsion gels	Control emulsion gels	Alginate hydrogels	Pectin hydrogels	Xanthan hydrogels	Control hydrogels
$k$	0.2890	0.4289	0.2955	0.1540	0.0801	0.1212	0.2071	0.2418
$n$	0.5581	0.4805	0.6061	0.6554	0.6507	0.7001	0.5326	0.5573

and release exponent ( $n$ ) values for each system. As seen on the results, apart from control gels having diffusion rate of  $0.2418 \text{ h}^{-1}$ , the highest and lowest diffusion rates belong to XN and AL gels,  $0.2071 \text{ h}^{-1}$  and  $0.0801 \text{ h}^{-1}$ , respectively. PC gel diffusion rate stands somewhere in the middle of these rates as  $0.1212 \text{ h}^{-1}$ . The diffusion rates gave information about how fast swelling occurred. Thus, XN apparently swelled more rapidly compared to the other polymer gels. Although the water uptake of XN gels occurred faster, because of the limited swelling ability of XN gel matrix, fast swelling did not contribute to the high SR of these gels indicating that the speed of swelling was not proportional to SR. Moreover, the lowest diffusion rate thus slower swelling of AL gels can be attributed to the poor gel network characteristics of the AL gels.

The diffusion rates of composite emulsion gels, somehow, were higher than their hydrogel correspondents except for control emulsion samples, as given in Table 3. Higher rates of water diffusion into the emulsion gels could be the result of the changes in the permeabilities of gel matrices due to oil incorporation into the gel network.

Table 3 also lists the release exponent data of gels and emulsions. All release exponents for all types of hydrogels and composite emulsion gels were larger than 0.45 ( $n > 0.45$ ) and smaller than 1.0 ( $n < 1.0$ ) which was consistent with power law estimation, revealing that the water uptake of gels pursued both diffusion controlled and swelling controlled mass transfer characteristics (Ritger & Peppas, 1987).

#### 4. | CONCLUSION

To evaluate and characterize the swelling behaviors of composite hydrogels and emulsion gels, SR and  $T_2$  measurements were conducted. Swelling characteristics of different food polymers were evaluated in terms of their chemical structures and their interactions with water. SR and  $T_2$  values showed a high correlation proving that NMR relaxometry is an alternative method for monitoring swelling experiments. PC hydrogels showed the highest SR (184%) and thus the highest increase in  $T_2$  value (63.70%) at the end of 6 h of swelling compared to AL and XN hydrogels. AL emulsion gels having a SR of 143% exhibited the highest  $T_2$  increase as 155.05% during the 6 h of swelling among the other emulsion gels and the reasons behind this phenomenon were discussed in terms of polymer-water interactions.  $T_2$  relaxation spectrum analysis results also confirmed the water uptake into the gel matrix since the third peak area of samples with high relaxation time values represented the proton population coming from the entrapped water in the gel matrix. The third peak area of PC hydrogel indicated an increase from 7.46% to 39.84% at the end of 6 h water immersion. A power law diffusion model was also estimated and the experimental water uptake data confirmed the model. PC hydrogels had the biggest release exponent ( $n$ ) value (0.7001) and all samples had  $n$  values in the range of both diffusion and swelling controlled mass transfer characteristics. This study provided detailed information on swelling mechanisms and characteristics of heat-induced composite hydrogels and emulsion gels prepared with WPI and various polysaccharides by NMR relaxometry.

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