Emulsifying activity of potato proteins in the presence of k-carrageenan at different pH conditions

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A B S T R A C T

Oil in Water (3:1) emulsions were prepared using potato proteins in the presence or absence of 0.2% k-carrageenan at different pH conditions (3.0, 7.0, and 4.8). These emulsions showed different droplet sizes, stability, appearance, and rheological properties. The best emulsion stability was achieved combining potato proteins and k-carrageenan at pH 3.0, where uniform and small oil droplets (30 μm) were observed. The rheological properties of the emulsions were also different. The highest viscosity and G' were shown by the emulsion prepared with the addition of k-carrageenan at pH 3.0, this being attributed to the onset of a gel-like viscoelastic structure in these conditions. SDS-PAGE indicated that the superior properties of the emulsion prepared with k-carrageenan at pH 3.0 can be attributed to an electrostatic interaction between the positively charged potato proteins and the anionic polysaccharide. This interaction allowed the formation of a strong molecular network able to stabilize the system.

Introduction

Emulsions are present in many foods, such as cream, milk, butter, margarine, mayonnaise, juices, soups, cakes, pastries, sauces, ice creams, and salad dressings. Food emulsions consist of three main phases with different chemical and physical characteristics: the dispersed droplets, the continuous phase surrounding the droplets and the interface between these two phases (Lam & Nickerson, 2013; McClements, 2005). In the latter region, surfactants are adsorbed avoiding the spontaneous coalescence of the dispersed phase. In addition to the so-called "small surfactant molecules", such as, for example, lecithin, this action can be exerted also by those polymers, in particular proteins, possessing distinct hydrophilic and hydrophobic clusters of monomers that allow the interaction with both the oil and the water phase. For example, several proteins from milk (Taherian et al., 2011), legumes (Zhou et al., 2022), or egg (Russell et al., 2021), show a strong tendency to adsorb at the interfaces, thus avoiding the coalescence of the dispersed emulsion droplets (Lam & Nickerson, 2013). On the other hand, in the case of oil in water (O/W) emulsions, polysaccharides generally contribute to emulsion stabilization by increasing the viscosity of the continuous phase acting as thickening or gelling agents (McClements, 2005). However, in certain conditions, proteins and

polysaccharides can interact each other giving rise to molecular networks able to modify the rheological behavior of the system and to enhance emulsion stability by maintaining the dispersed droplets physically separated (Dickinson, 2009). As this phenomenon is desired in many food preparations, it is important to study the environmental conditions leading to these molecular interactions.

Among the proteins with emulsifying activity, potato proteins have been shown to give promising results (Hussain et al., 2021). These proteins, which can be extracted from potato juice, an abundant by-product of the starch industry (Josefsson et al., 2020), can be divided into three classes. The first comprises the Patatin family ($\approx$ 40% of the total), which are 39–43 kDa glycoproteins with an isoelectric point (pI) ranging from 4.45 to 5.17. The second are the protease inhibitors ($\approx$ 50% of the total) showing lower MW (4–20 kDa) and pI from 5 to 9. Finally, the third group consists of various enzymes with different MW and pI (Bárta et al., 2012; Jørgensen et al., 2011).

In general, potato proteins show a high nutritional quality and no allergenicity (Hussain et al., 2021), but also relevant foaming and emulsifying properties, mainly attributed to the Patatin fraction (Lomolino et al., 2015; Schmidt et al., 2018). Thanks to properties, potato proteins are a promising ingredient for different food preparations including infant foods, food supplements, bakery products, and pet

foods (Hussain et al., 2021).

In industrial processes, potato proteins are commonly obtained by a combination of acid and heat treatment of the potato juice, resulting in irreversible protein denaturation and precipitation. In addition, acidification also affects the surface activity of these proteins and, consequently, their foaming and emulsifying properties (Lomolino et al., 2015; Schmidt et al., 2018). Attempts have been made to improve these properties by heat, enzymatic and chemical treatments (for a review see Hussain et al., 2021). Recently, the interaction between potato proteins and k-carrageenan was described (O'Sullivan et al., 2017). K-carrageenan is an anionic polysaccharide consisting of D-galactose chain bearing one sulfate group every two of units (Azevedo et al., 2013) commonly used as a thickening/gelling agent in several food applications (Bixler & Porse, 2011). The anionic character of k-carrageenan allows interactions with the potato proteins at acidic pH producing submicron and micron complexes leading to an improved emulsion stability (O'Sullivan et al., 2017). However, the precise effect of pH, which obviously can affect the extent of the electrostatic interaction, was not described. Therefore, the aim of this research is to study the effect of the pH conditions on the stability, appearance and rheological properties of O/W emulsions obtained with potato proteins in the presence of k-carrageenan.

Materials and methods

Potato protein recovery and quantification

Potatoes (*Solanum tuberosum* cv. Bintje), were washed, cut into large pieces and ground by a domestic juice extractor (Moulinex, Écully, France). Before extraction, sodium bisulfite (1 g/100 g fresh potato) and polyvinylpoly-pyrrolidone, (PVPP; Sigma-Aldrich, S. Loius, USA) (50 mg/100 g fresh potato) were used to prevent enzymatic browning and to remove phenolic compounds, respectively. The slurry was stirred for about 10 min and centrifuged (10 min, 5000 rpm, 10 °C) using a Universal 320 centrifuge (Andreas Hettich GmbH & Co, Tuttlingen, Germany). The supernatant was then centrifuged again before being paper filtered using Whatman 1 filters (Whatman, Maidstone, UK) and dialyzed (MWCO 3500 Da) with CelluSep membranes (Thermo Fisher, Waltham, USA) against deionized water at 4 °C. After dialysis, this protein solution was freeze-dried obtaining a powder whose nitrogen content was measured by the Kjeldahl method (N × 6.25).

Preparation of emulsions

Emulsions were prepared with 10 mg/mL of potato protein powder in McIlvaine buffer at pH 3.0, 4.8 and 7.0 (which are values below, equal, and above the Patatin isoelectric point, respectively) with a ionic strength of 0.5 M. Each solution was added with 3 volumes of corn oil and kept at 4 °C. The mixtures were then homogenized for 30 *sec* with Ultra-Turrax (IKA-Werke GmbH & Co., Staufen im Breisgau, Germany) and then sonicated at 20/22 KHz with a Sonoplus Ultrasonic Homogenizer HD200 (Bandelin electronic, Berlin, Germany). To avoid thermal denaturation of proteins, the oil/buffer mixtures were kept in ice during sonication, which was performed at intervals of 15 *sec* for 4 times.

Emulsions in presence of k-carrageenan (Sigma-Aldrich, S. Loius, USA) were prepared in the same way but dissolving 0.2 % of k-carrageenan in the McIlvaine buffer. Three cycles including alternated microwave heating and stirring steps (10 s each) were applied to achieve complete solubilization.

Image analysis

The different emulsions were observed by a Lumar V12 stereomicroscope (Zeiss, Oberkochen, Germany) at 80x magnification. One mL of emulsion was placed on a glass support and the images were acquired and processed by using the Axio Vision 4.8.2 software (Zeiss,

Oberkochen, Germany) that allowed to measure the droplet diameter. About 10 images were acquired for each sample while the diameters of 1,200 droplets were measured individually.

Droplet size was reported as the volume-weighted mean diameter (d_{43}), defined by the following equation (Jafari et al., 2007):

$$d_{43} = \frac{\sum_{i=1}^{k} n_i d_i^4}{\sum_{i=1}^{k} n_i d_i^3}$$

Where n_i is the number of particles with diameter d_i. Each emulsion/time of observation were carried out, six times. Each emulsion was studied at room temperature (22 °C ± 1 °C) by 4 observations during the eight days of storage (days 1, 2, 5 and 8).

CiELab analysis

Emulsions were analyzed by the CiELab system using a CR 410 colorimeter (Konica Minolta, Chiyoda, Japan). The analyzes were standardized by always taking the samples (10 mL) from the central part of the tube. The collected volume was placed in a Petri dish (60 mm diameter), and then analyzed. The evolution of appearance of the emulsions was then determined for eight days using the following formula which considers the L, a and b parameters of the tristimulus system by calculating the difference of the samples' color and the white reference standard (Minolta CR-A44):

$$\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$$

Emulsion creaming

Emulsion creaming was determined according to Ye and Singh (2006), with some modifications. Ten mL of freshly prepared emulsions were poured into a graduated cylinder (0.1 mL division) and stored at room temperature (22 °C ± 1 °C) for 24 h. The height of the watery phase formed at the bottom of the cylinder was measured at different times for 24 h. The rate of creaming (as %) was defined as:

$$Creaming(\%) = \frac{H_w}{H_t} \bullet 100$$

Where H_w is the height of the watery layer at the bottom and H_t the total height of the sample.

Furthermore, to better observe the emulsion behavior, the six samples were poured into glass bottles and stored at 4 °C for 8 days to minimize the risk of microbial spoilage. Pictures were taken at the beginning (1 h after the preparation) and after 8 days of storage.

Sodium dodecyl Sulphate-Polyacrylamide gel electrophoresis (SDS-PAGE)

The proteins of the emulsions were collected by the method described by Romero et al. (2011) with some modifications. Two mL of each sample were mixed with 2 mL of sucrose solution (500 mg/mL) prepared in the 3 McIlvaine buffers (pH 3.0, 4.8, and 7.0). Two mL of each of the resulting mixtures were carefully put at the bottom of a tube containing 10 mL of the corresponding buffer. After centrifugation (3000 rpm, 2 h at 5 °C), no pellets were observed, while 3 phases separated: a yellowish upper phase containing the oil; an intermediate white creamy phase containing the emulsion, and a transparent bottom watery phase. The 3 separated phases were carefully sampled and individually analyzed by SDS-PAGE.

One hundred μL of each of the three phases were added with 50 μL of a 1.33 M Tris, pH 7.4 buffer, containing 40% (v/v) glycerol and 8% (w/v) SDS. Nine μL of the reducing agent 2-mercaptoethanol were added and samples were heated at 95 °C for 2 min. SDS-PAGE with a total acrylamide concentration of 15% was carried out in a Mini Protean II cell (BIO-RAD, Milan, Italy) at a constant voltage of 100 V. Gels were stained by Coomassie brilliant blue and their images were acquired using the Gel Logic 112 apparatus (BIO-RAD, Milan, Italy).

Rheological characterization

A Searle, Coaxial Cylinder Viscometer, was used for the rheological measurements. The instrument (Haake Viscotester 550, Thermo Fisher Scientific, Waltham, USA) was equipped with MV-DIN and MV1 rotary cylinders (MV-DIN cylinder radius 19.36 mm/height and rotary beam MV1 20.04 mm). Ninety mL of each emulsion were put in the cylinder. The experiment was carried out at 20 °C and the shear flow test was carried out from 1 to 100 s^{-1}. The emulsion phases (six replicates) of each emulsion were tested at the first and at the eighth day of storage. Stress sweep test at a frequency of 1 Hz was carried out for all the emulsions studied at time 1 and after 8 days of storage to estimate the dynamic linear viscoelastic range (Calero et al., 2013).

Statistical analysis

Data were statistically processed by Excel 2016 (Microsoft Corporation, Redmond, USA), Origin 2018 Graphing and Analysis (OriginLab Corporation, Northampton, USA) and Statgraphics Centurion XVI (StatPoint Technologies Inc., Warrenton, USA). A descriptive statistical study, using bar charts and inferential analysis of variance (p < 0.05), was carried out.

Results

Potatoes were extracted at the laboratory scale in a way reproducing

that used in the potato starch industry. From 100 g of fresh potato about 40 mL of juice were obtained. The juice contained 79.3% of protein on dry weight, a value in line with that reported by others (Romero et al., 2011). This extract confirmed to possess emulsifying activity when added to a mixture of oil and water. Therefore, six emulsions were prepared by sonication at pH 3.0, 4.8 and 7.0 and in absence and presence of k-carrageenan. These emulsions were then characterized as follows.

Stereomicroscopic analysis

Since average size and size distribution of the dispersed droplets are main factors in determining the quality of an emulsion (McClements, 2004), these parameters were studied by processing the data derived from optical stereomicroscopy of the emulsions using images taken immediately after preparation and after 2, 5 and 8 days of standing at RT (not shown). In general, the droplets' diameters were in the range typical of "macroemulsions" (1–1000 μm) (Dickinson, 2012), with differences detected among the different samples. In particular, at pH 3.0, the presence of k-carrageenan caused a shift of the peak of the droplets' diameter distribution from around 130 μm to around 30 μm and a lower polydispersion (1 to 180 μm vs. 1 to > 300 μm) was also noted (Fig. 1A, left panel). The emulsions prepared at pH 4.8 showed a similar behavior, but in this case the diameter at the peak was significantly larger for both the samples (without and with k-carrageenan, ≈ 170 and ≈ 70 μm, respectively) (Fig. 1A, central panel). At pH 7.0, both samples showed
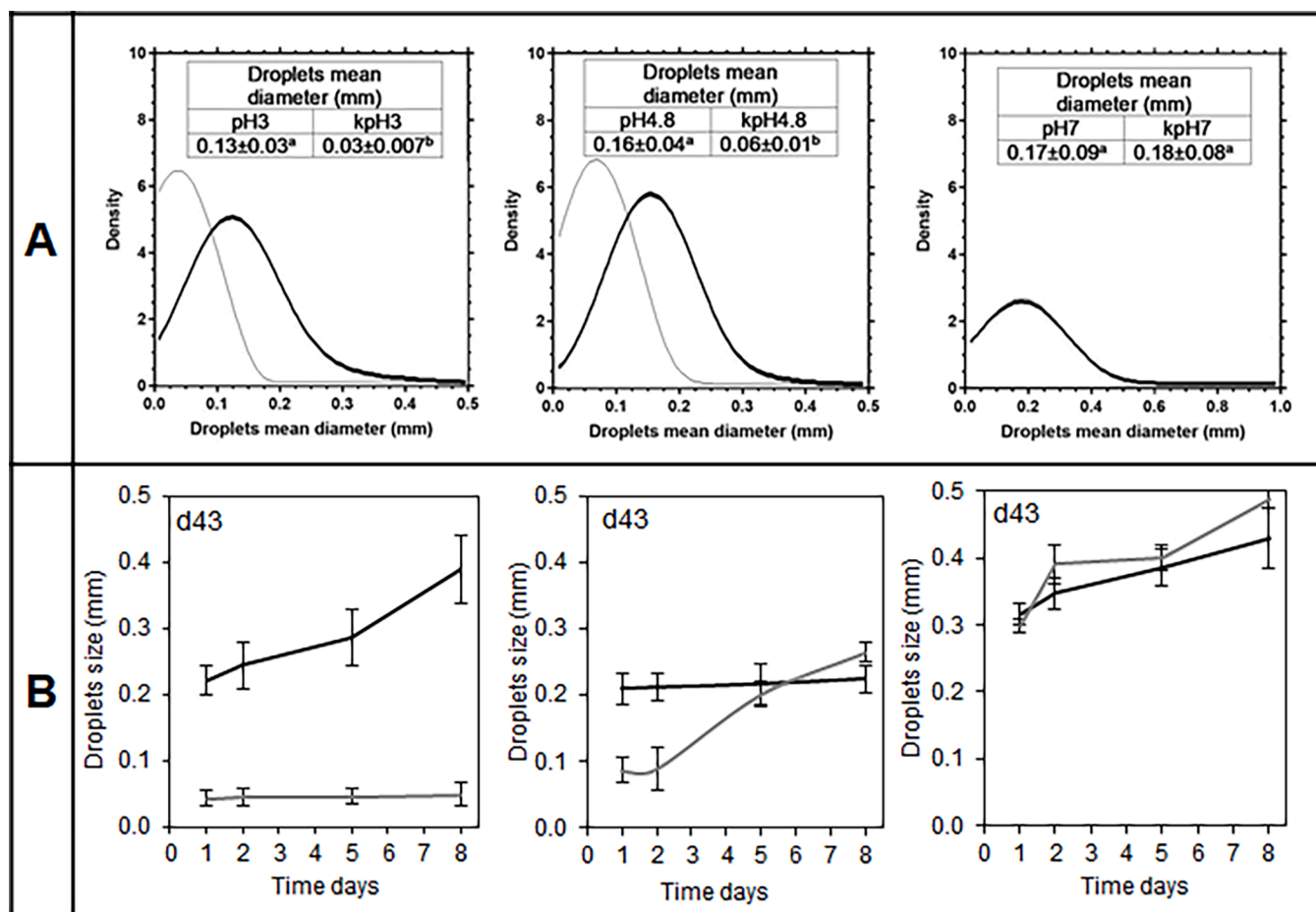

Fig. 1. A) Droplet's size distributions and mean droplets diameters (statistically processed by LSD 95% test) taken at the first day of storage of the potato proteins emulsions obtained with (kpH3, kpH4.8, kpH7) and without (pH3, pH4.8, pH7) k-carrageenan at pH 3.0 (kpH3 and pH3), 4.8 (kpH4.8 and pH4.8) and 7.0 (kpH7 and pH7). Curves related to the two samples at pH 7.0 appear as one as they overlap. B) d_{43} parameter evolution of the droplets in potato proteins emulsions with (k, grey line) and without (black line) k-carrageenan at pH 3.0, 4.8 and 7.0 from the first to the eighth day of storage. The diameters were obtained by stereomicroscopic analysis. Assays were performed in 6 replications.

droplets with the largest average sizes (≈ 180 μm) and, in this case, the presence of k-carrageenan had no significant effects on the mean diameter of the droplets (Fig. 1A, right panel).

With the aim of further investigate the differences in particle size distribution, the time evolution of the volume-weighted mean diameter (d_{43}, De Brouckere mean) was measured. At pH 3.0, the sample prepared with the proteins alone showed a d_{43} value ≈ 5 times higher and increasing instability compared to that containing k-carrageenan, whose d_{43} did not change during the 8 days of storage (Fig. 1B, left panel), indicating high stability of this latter emulsion. At pH 4.8, a different behavior was seen, because in this case the sample containing k-carrageenan shown an increase of the d_{43} value starting from day 2. In contrast, the sample with the protein alone showed to be stable, even if its d_{43} was higher at the initial times (Fig. 1B, central panel). At pH 7.0, both the emulsions showed increasing d_{43} values (Fig. 1B, right panel) and no effects of the presence of k-carrageenan were detected.

Emulsion creaming

Emulsion creaming was recorded over 24 h by measuring the height of the watery layer formed at the bottom of the emulsions maintained at room temperature (Fig. 2). This approach provides a measure of emulsion stability (Seta et al., 2013). Among the different samples, that prepared at pH 3.0 with k-carrageenan was the most stable as it did not show any creaming during 24 h of observation. Good stability was also shown by the 2 emulsions prepared at pH 4.8 with the one made with k-carrageenan displaying a lower creaming rate compared to that prepared with the protein alone. However, at this pH, the stabilizing effect of the polysaccharide was lower than at pH 3.0 (Fig. 2). At pH 7.0, the emulsions showed the highest creaming value, with a minimal effect due to the presence of k-carrageenan (Fig. 2). A visual assessment of the six emulsions at the beginning of the experiment and after 8 days (SF 1) confirmed these results.

Emulsion appearance

Appearance can be considered an important characteristic of an emulsion because it affects the perception of its quality by the consumer (McClements, 2002a, 2002b). The overall color change, measured as ΔE (Rubilar et al., 2015) took place in the early hours after the emulsion's preparation (Fig. 3). Even though R^2 was always lower than 0.9, trends curves could fit log functions showing a rapid and significant change in

Fig. 2. Creaming % of the potato proteins emulsions with and without k-carrageenan at pH 3.0, 4.8 and 7.0, in the first 24 h of observation. Samples labelling as in Fig. 1.

the overall color (until a ΔE value close to 8 in all cases except for the sample at pH 3.0 with k-carrageenan) followed by a plateau. At pH 4.8 and 7.0 curves overlapped, indicating no effects of k-carrageenan, whereas this polysaccharide showed to strongly inhibit the ΔE variation over time of the sample prepared at pH 3.0 (Fig. 3).

The emulsion brightness (L), which is largely due to light scattering phenomena related to the characteristics of the dispersed particles, including their size and concentration (McClements, 2002b), showed high values at time 0 in all cases (Fig. 3). However, a decrease in brightness was observed overtime for all the samples obtained in the absence of k-carrageenan, while its presence resulted in an improvement of the brightness stability in each case. Again, the strongest effect was seen for the sample at pH 3.0, whose original brightness was maintained until the end of the experiment (Fig. 3). This indicated that the characteristics of the particles of this latter emulsion did not vary in time confirming its stability.

Flow properties

The rheological behavior of the six emulsions was studied immediately after preparation and after 8 days of storage to characterize their flow properties and their time variations.

The shear rate dependence of viscosity fitted well the Cross model ($R^2 > 0.999$), which is extensively used to describe food emulsions (Joyner, 2019).

$$\eta_a = \eta_\infty + \frac{\eta_0 - \eta_\infty}{1 + (\dot\gamma/\dot\gamma_c)^m}$$

The viscosity profiles obtained by testing the 6 different emulsions are shown in SF 2. Those obtained with the potato proteins alone showed similar trends, characterized by a Newtonian region at low shear rates followed by a shear thinning behavior. In addition, the zero-shear rate viscosity did not change statistically for the emulsions prepared at pH 3.0 and pH 4.8 after eight days of storage (Table 1, SF 2). Instead, a slight, but significant increase of this parameter was observed at pH 7.0, probably due to an increase in the volume fraction deriving from the high creaming tendency of this latter emulsion (Shokrollahi, 2016) (Fig. 2).

The presence of k-carrageenan generally increased the viscosity of the emulsions at both day 1 and 8, except for the emulsion at pH 7.0 on day 8, where the viscosity did not show a significant change (Table 1). The strongest effect of k-carrageenan on viscosity was observed at pH 3.0 at both times (Table 1), suggesting the development of the strongest structure at this pH which was even much enhanced after 8 days. It is noteworthy that when the viscosity of k-carrageenan alone was measured in the same conditions and pH values, the highest viscosity was detected at pH 4.8 (not shown) and not at pH 3.0, indicating that the enhancement on the emulsion viscosity at pH 3.0 should not be due only to the k-carrageenan behavior in these conditions.

The presence of k-carrageenan also affected the critical shear rate values of the different emulsions (Table 1). In all cases, this parameter decreased when the polysaccharide was present, but this effect was particularly evident at pH 3.0, where it approached the 0-value indicating the onset of a strong gel-like structure (Calero et al., 2013; Ebagninin et al., 2009; Liao et al., 2020; Paximada et al., 2016; Winuprasith & Suphantharika, 2015). Also, the 8-day storage impacted the critical shear rate, but only for the samples containing k-carrageenan, where this parameter significantly decreased for the emulsions at pH 3.0 and 4.8, but not in that prepared at pH 7.0 (Table 1).

The shear stress sweep test at 1 Hz (Fig. 4) showed that the elastic component G' dominated on the viscous one G'' in all the samples, indicating the presence of a gel-like structure (Santos et al., 2015). Among the samples, that showing the highest G' and G'' values was that prepared with the addition of k-carrageenan at pH 3.0, which, again, proved to be the best conditions for the behaviour of the potato proteins.

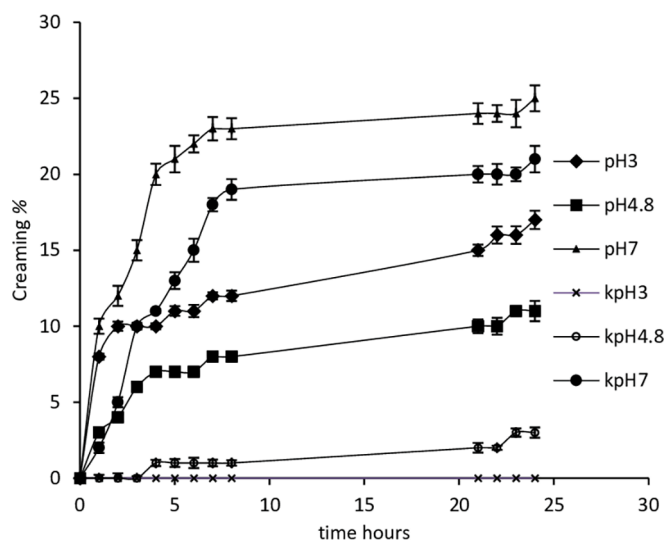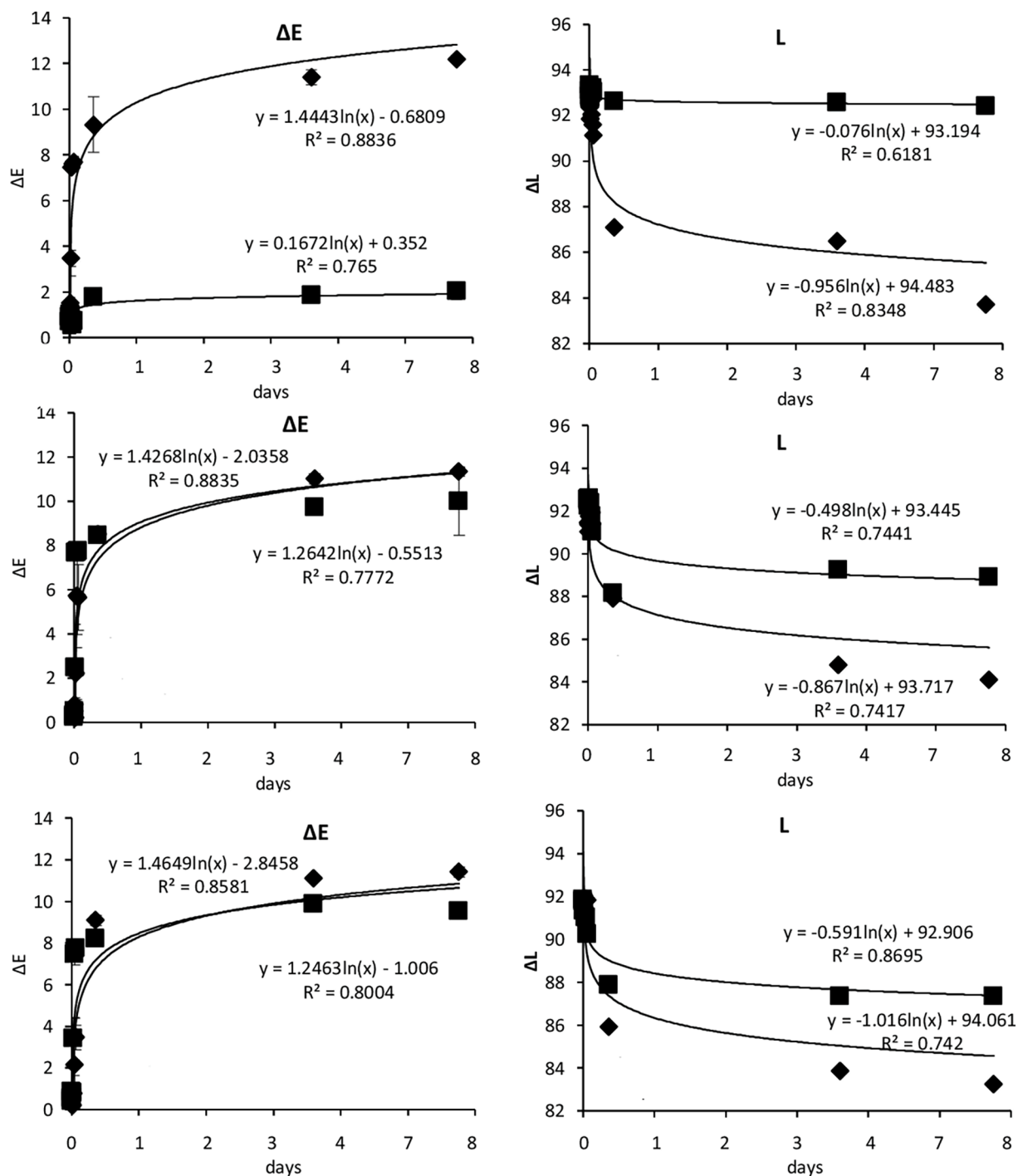Considering the time evolution, except the sample at pH 7.0, G' and

Fig. 3. ΔE and L evolution of the potato proteins emulsions with (■) and without (◆) k-carrageenan at pH 3.0, 4.8 and 7.0, over 8 days of storage. Each data is the mean of six replications. Samples labelling as in Fig. 1.

G'' decreased from day 1 to day 8 in the emulsions with the potato proteins alone, while, when k-carrageenan was present, the same parameters increased after 8 days of storage (Fig. 4), indicating a reinforcement of the gel structure.

SDS-PAGE

In order to detect the proteins present in the emulsions, the samples were centrifuged and the three separated phases (oily at the top, creamy in the middle, and watery at the bottom) were examined by SDS-PAGE in denaturing conditions. The upper oily phase did not show any band (not

shown), indicating the all the proteins were included in the other two phases, which in fact showed to contain different protein bands (Fig. 5).

The patterns of the creamy phase of the samples obtained without k-carrageenan were almost exclusively represented by a sole band whose apparent MW (42 kDa, Fig. 5A) corresponded to that of the Patatin protein fraction (Kim et al., 2008), although the sample prepared at pH 7.0 showed also a band with higher MW. This result indicates that in each case, Patatin is the protein involved in emulsion formation. A smaller amount of the same protein band was however present also in the watery phase of the samples obtained at pH 4.8 and 7.0, but not in that at pH 3.0, suggesting that in this latter case all the available Patatin

Table 1

Flow curves fitting parameters (η_0 and γ_c) for Cross model ($R^2 = 0.999$) of potato proteins emulsions with and without k-carrageenan. The analysis was carried out in six replications and the results were processed by Tuckey's test ($p \leq 0.05$). Samples labelling as in Fig. 1.

Emulsions	η_0 (Pa*s)		$\dot{\gamma}_c$	
	Day 1	Day 8	Day 1	Day 8
pH3	3.05 ± 0.47^a	4.31 ± 0.98^a	2.02 ± 0.29^a	1.73 ± 0.33^a
pH4.8	4.48 ± 0.37^a	4.13 ± 0.85^a	1.52 ± 0.05^b	2.03 ± 0.09^a
pH7	0.72 ± 0.12^b	2.00 ± 0.30^a	2.87 ± 0.31^a	2.68 ± 0.35^a
kpH3	18.35 ± 3.48^b	120.37 ± 18.34^a	0.0036 ± 0.0003^a	0.00018 ± 0.00002^b
kpH4.8	8.93 ± 1.18^b	21.65 ± 1.39^a	0.55 ± 0.06^a	0.11 ± 0.02^b
kpH7	2.05 ± 0.48^a	1.29 ± 0.37^a	1.61 ± 0.41^a	1.8 ± 0.29^a

was present in the emulsion droplets (Fig. 5A).

The other protein bands of lower MW which can be found in total potato protein extract (mainly corresponding to protease inhibitors, lane PTP) were instead all present in the watery phase at all the pH tested (Fig. 5A), indicating that they were not involved in the formation of the emulsion droplets.

This situation, however, changed for the emulsions prepared in the presence of k-carrageenan. In these samples, all the protein components of the potato extract seemed to move in the creamy phase, where they were more concentrated in comparison to the corresponding liquid phases. This phenomenon was particularly evident in the sample prepared at pH 3.0, where, in addition to Patatin, all the bands of MW in the

range 21–29 kDa (Fig. 5B) were detected only in the creamy phase.

Discussion

The interest of the food industry in the use of ingredients from natural sources is increasing, and this applies also to emulsifiers (McClements & Gumus, 2016). Among natural emulsifiers, potato proteins showed to be good candidates for their functionality, low cost, and high availability (Josefsson et al., 2020; Lomolino et al., 2015). However, to better exploit potato proteins, a better understanding of the conditions which modulate their behavior as emulsifiers is necessary.

Therefore, the effect of pH and of the presence of the anionic polysaccharide k-carrageenan on the emulsifying properties of the potato proteins was here studied. With this aim, a potato proteins extract was produced in a way resembling that used in the potato starch industry.

From the here reported results, it is confirmed that the pH has a main effect on potato protein functionality (Schmidt et al., 2018), either in the presence and in the absence of k-carrageenan, and this is clearly related to the charge acquired by the potato proteins at different pHs. In particular, at the most acidic pH (3.0) all the proteins present in the extract are below their IP, whereas at pH 4.8 the Patatin fraction, being around its IP (Bárta et al., 2012), would lose its charge, while the other main proteins, i.e. the protease inhibitors, should maintain their positive charge. In contrast, at neutrality (pH 7.0) all the potato proteins are negatively charged (O'Sullivan et al., 2017; Schmidt et al., 2018). This pH-dependent charge variations were previously shown by measuring the zeta potential of the whole potato protein isolate (Dachmann et al., 2020) and also that of separated protein fractions (Schmidt et al., 2018).

Patatin is considered the protein component with the highest emulsifying activity (Schmidt et al., 2018) and, in fact, this protein was the only found by SDS-PAGE analysis (Fig. 5) in the emulsions prepared with
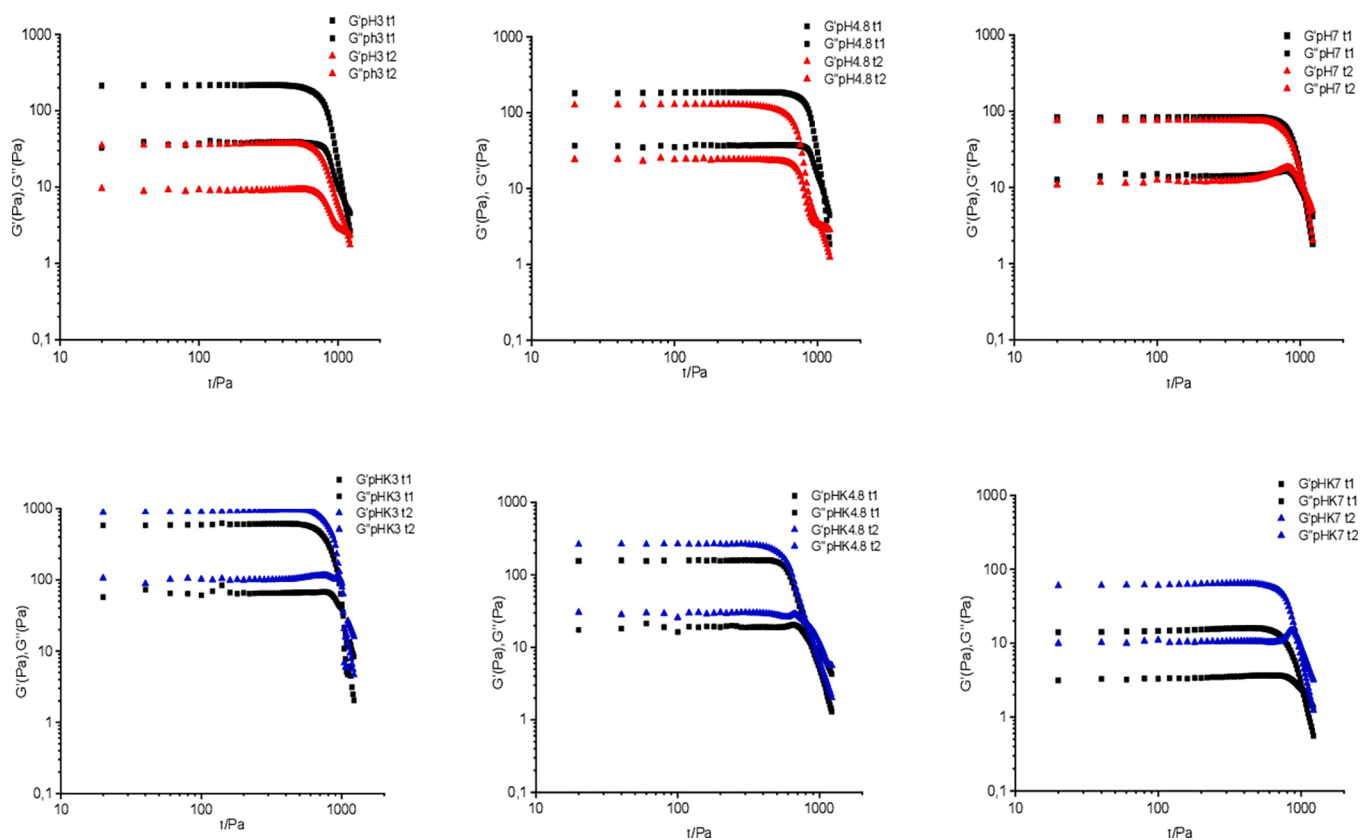
Fig. 4. Stress sweep tests under oscillatory shear of potato proteins emulsions with (up) and without k-carrageenan (down) at pH 3.0, 4.8 and 7.0 at the first (black) and the eighth (red/blue) day of storage. Frequency: 1 Hz. Samples labelling as in Fig. 1. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
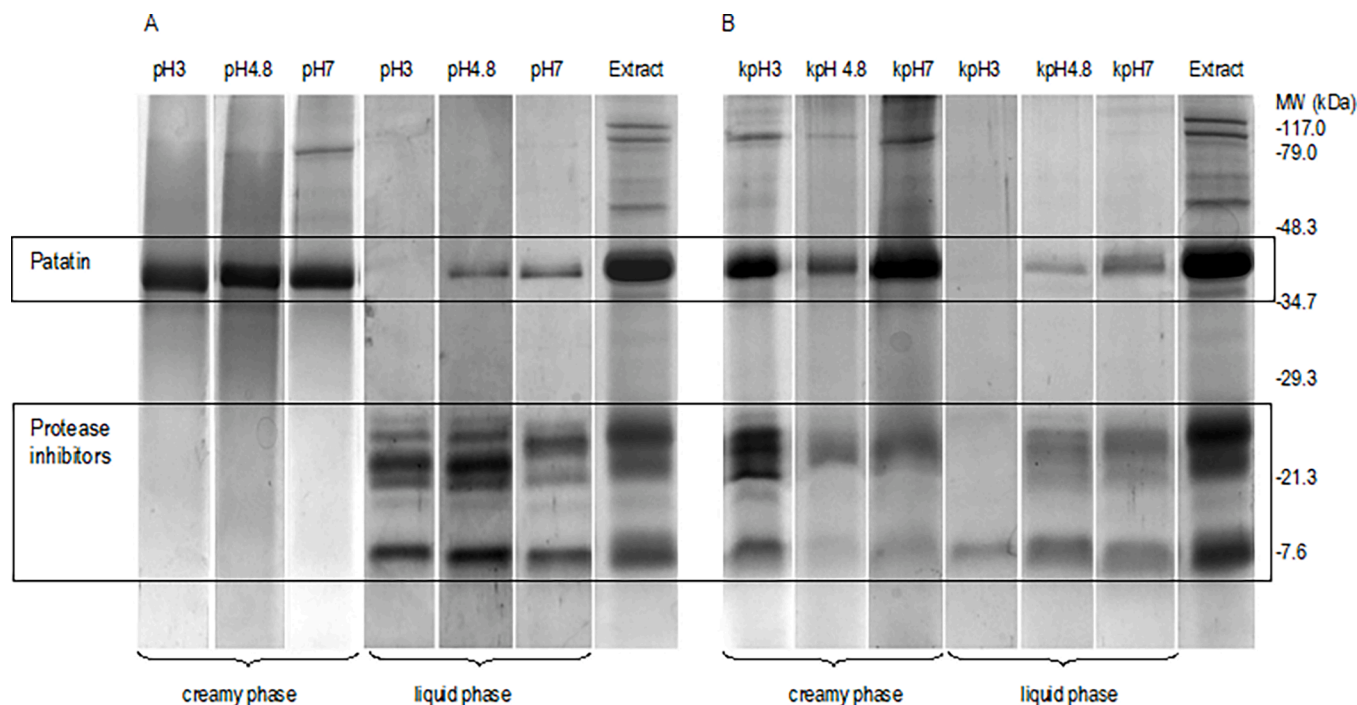
Fig. 5. SDS-PAGE, 15%. Electrophoretic patterns of creamy phase (potato proteins adsorbed on droplets) and liquid phase of emulsions with (panel B) and without (panel A) k-carrageenan, at pH 3.0, 4.8 and 7.0, in comparison with the whole potato protein extract. Patatin and Protease inhibitors bands are indicated in the boxes. Samples labelling as in Fig. 1.

the potato proteins alone. This occurred at each of the pH tested, indicating a high affinity of this protein for the oil/water interface. However, the complete adsorption of Patatin was achieved only at pH 3.0, indicating a certain improvement when the protein is positively charged. In contrast, the other proteins with lower MW were never found not to be associated with the emulsion by SDS-PAGE (Fig. 5), indicating that these components are always unable to participate to the emulsion formation, although previous studies indicated some emulsifying activity at acidic pH also for them (Schmidt et al., 2018).

By using the potato protein alone, the best results in terms of emulsion stability, measured creaming rate (Fig. 2) and maintenance of the original d_{43} (Fig. 1) were shown by the emulsion prepared at pH 4.8, indicating a major effect of Patatin at this pH. A low destabilization rate for emulsions prepared with Patatin in the pH range 4.0–5.0 was also reported by (Ralet & Guéguen, 2000) while, at the same pH, the surface activity of solubilized potato proteins started to reach its maximum value (Dachmann et al., 2020). At this pH, Patatin should be virtually unchanged, allowing the formation of protein aggregates due to the absence of electric repulsion (Dachmann et al., 2020; Schmidt et al., 2018), which instead should occur at pH 3.0 and 7.0. It is then likely that the Patatin aggregates formed at the IP are adsorbed at the water–oil interface thus forming a continuous layer around the oil droplets which avoids coalescence, as confirmed by the stability of the droplet size (d_{43} value), which was not seen in the samples prepared at pHs in which electric repulsion among Patatin molecules should occur (3.0 and 7.0) (Fig. 1B). These results are confirmed also by looking at the zero-shear viscosity, which for the sample at pH 4.8 was the highest and remained unchanged after 8 days (Table 1). This sample also showed the highest storage (G') and loss (G'') moduli after 8 days (Fig. 4), confirming the high quality of the emulsion obtained with Patatin at its pI.

The results above described strongly changed when the anionic polysaccharide k-carrageenan was included in the system. This polysaccharide carries sulfate groups with an apparent pKa of 1.2 (Vleugels et al., 2018), which means that they are negatively charged at all the pH here tested, making this hydrocolloid available for electrostatic interactions with other charged species.

When k-carrageenan was added, the SDS-PAGE pattern of the proteins extracted from the emulsions showed the presence, in addition to Patatin, also of other low MW proteins, absent in the emulsions prepared with the potato proteins alone (Fig. 5). This was particularly evident for the sample prepared at pH 3.0 where all the proteins were included in the emulsion, whereas almost none of them were found in the watery phase. At this pH an electrostatic interaction of the negatively charged k-carrageenan and positively charged proteins can be expected, leading to the formation of a mixed molecular complex which would be responsible for their presence in the emulsion phase. This phenomenon is much less evident in the other samples confirming a major effect of the charge acquired by the proteins at the different pHs in determining the extent of the interaction.

The presence of k-carrageenan also caused a shift to lower values of the particle size of the emulsions at pH 3.0 and 4.8, which was however maintained in the time only at pH 3.0 (Fig. 1B). This confirms the importance of the complexes deriving from electrostatic interactions between proteins and k-carrageenan for the formation of a stable interfacial layer (O'Sullivan et al., 2017), which results in increased stability of the system in terms of both d_{43} values (Fig. 1B), creaming rate (Fig. 2) and also appearance (Fig. 3).

Looking at the flow behavior of the different emulsions (Table 1), it can be noted that the zero-shear viscosity of that prepared with k-carrageenan at pH 3.0 was significantly the highest and strongly increased in time, indicating a strengthening of the structure of the system. In particular, considering the absence of a Newtonian region, the very low critical shear rate (Table 1) and the strong increase of the storage modulus (G', Fig. 4) of this sample, a shift to a gel-like viscoelastic structure can be envisaged (Calero et al., 2013; Ebagninin et al., 2009; Liao et al., 2020; Paximada et al., 2016; Winuprasith & Suphantharika, 2015), which is much less evident in the other samples.

Taken together, these outcomes could indicate the establishment of a strong k-carrageenan/potato proteins network produced by the electrostatic attractions taking place below the pI of the potato proteins (pH 4.8) which therefore involves not only the Patatin present at the interface but also the other potato proteins of lower MW. However, this latter

fraction could be involved in the system as an element that contributes to the formation of electrostatic bridges among k-carrageenan molecules in the continuous watery phase of the emulsion contributing in such a way to the formation of the network which explains its rheological behaviour. This suggested scheme is consistent with the complex coacervation phenomenon previously described in the presence of k-carrageenan (De Kruif et al., 2004). In the present case, the network formation can occur only at pH 3.0, thus explaining why the emulsions prepared at higher pHs behave worst. In particular, at pH 4.8, where however the oil/water interface is stabilized by the uncharged Patatin aggregates, the structure of the entire system is weaker due to the low attraction between the proteins and the polysaccharide localised in the continuous phase. Finally, at pH 7, where all the components are negatively charged, the network formation is inhibited by the repulsive forces that took place among them, giving rise to an emulsion with poor stability.

In summary, it can be hypothesized that the excellent properties of the emulsion prepared at acidic pH in the presence of k-carrageenan is be due to a complex mechanism involving, on one hand, the stabilisation of the oil/water interface due to the adsorption of Patatin-k-carrageen electrostatic complexes, and, on the other, to the presence of a dense gel-like protein polysaccharide network able to entrap and immobilize the dispersed oil droplets, thus avoiding their interaction.

Conclusions

This study confirms that potato proteins can be used as emulsifying agents in oil/water systems and that their behavior is affected by the pH of the system. Moreover, the effects of these proteins can be modulated by the addition of the anionic polysaccharide k-carrageenan, a widespread food hydrocolloid which allows to improve the structure of the emulsion by interacting with the potato proteins in acidic conditions. The characterization of the emulsion here studied allowed to determine the best conditions for the use of potato proteins in the presence of k-carrageenan and to hypothesize the possible mechanisms determining the emulsion properties in the different conditions.

In conclusion, the combined use of potato proteins and k-carrageenan, seems to be a promising approach to produce stable food emulsion, even if the pH of the food must be considered, because it can strongly affect emulsion stability, appearance, and rheological properties. Finally, it is noteworthy to consider that both the macromolecules here studied are natural products that could substitute synthetic emulsifiers and that the use of potato proteins allows to exploit an abundant by-product thus adding value to the potato starch industry.

Data availability statement:

The data that support the findings of this study are available from the corresponding author upon reasonable request

Ethical approval:

Ethics approval was not required for this research

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2022.100232.

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