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Preparation and characterization of sodium caseinate/xanthan gum complexes in acidic conditions and their use for the stabilization of oil-in-water emulsions

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Abstract

In this work, complexes formed by sodium caseinate (NaCn) and xanthan gum (XG) were tested as novel natural stabilizers. They were prepared at pH 4, using a constant concentration of XG (0.4%) and different NaCn concentrations. Then, they were characterized in terms of turbidity, surface tension (ST), and rheology. The acquired results indicated that the ST of NaCn solution and NaCn/XG dispersion decreases with NaCn concentration. In addition, the ST of NaCn/XG is higher than that of NaCn solution indicating interactions between NaCn and XG. In addition, the turbidity of the dispersions increases while ST decreases with NaCn concentration. Fourier transform spectroscopy (FTIR) spectra confirmed the formation of a complex between NaCn and XG through electrostatic interactions. Structural analyses demonstrated that the morphologies of the complex particles are different compared to the pure biopolymer ones. Furthermore, the oil-in-water (O/W) emulsions based on NaCn/XG complexes formed into acid conditions were studied. The obtained results showed that the stability, the average diameter of the droplets, and the rheological behavior of the formulated emulsions depend on the NaCn:XG ratio. Emulsion formulated with 0.5:1, 1:1, 1.5:1, 2:1 NaCn:XG ratio presented good creaming stability during 1 month of storage at 25°C. On the other hand, samples formulated with a 1:1 ratio of NaCn/XG exhibited the best stability. Their average diameter (29.026 μm) remained constant during the storage. Emulsions formulated with higher ration PR:PS (2.5:1, 3:1) were unstable and have larger droplets. Also, all formulations showed a shear-thinning and elastic behavior.

KEYWORDS
complexation, emulsions, rheology, sodium caseinate, stability, xanthan gum

INTRODUCTION

Due to their easy formulation and protection of active agents, emulsions are utilized in many applications, such as pharmaceutical, cosmetic, and food (Kiss et al., 2011; Kowalska et al., 2015; Tan & McClements, 2021). Their stabilization usually requires the use of synthetic emulsifiers. However, these ingredients are increasingly raising long-term environmental and health concerns, mainly in the food and pharmaceutical fields (Bouyer et al., 2012; Cox et al., 2021). The requirements of biodegradability and respect for the environment have led to the search for natural compounds. To this end, biopolymers, which are abundant and inexpensive, have been widely used in different applications (Bouyer et al., 2012; Dai et al., 2018). These products compared to synthetics have a major advantage related to their polyfunctionality; they are often integrated for their gelling, texturing, and for their surface properties (Evans et al., 2013). Proteins (PR) and polysaccharides (PS) are among the most

Abbreviations: CI, creaming index; G', storage modulus; G'', loss modulus; NaCn, sodium caseinate; O/W, oil-in-water; PR, protein; PS, polysaccharide; ST, surface tension; XG, Xanthan gum.

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used biopolymers for the replacement of synthetic surfactants (Silva et al., 2015). The stabilization of emulsions by PR alone or in combination with PS has been the objective of several previous works (Perugini et al., 2018; Seddari et al., 2022).

The complexation between PR and PS has also been studied because of the benefits that can bring in the stabilization of dispersed systems when carried out under appropriate conditions (HadjSadok et al., 2010; Jones et al., 2011; Warnakulasuriya & Nickerson, 2018). It has been observed that most often, the complexation between PR and PS macromolecules results from a non-covalent association, mainly due to attractive electrostatic interactions (Albano et al., 2019; Dai et al., 2022).

Sodium caseinate (NaCn) which is the major protein in milk, can be used as a natural emulsifier for the formulation of oil-in-water (O/W) emulsions owing to its amphiphilic properties (Tavasoli et al., 2022). However, emulsions stabilized by NaCn are unstable at pH near to its isoelectric point (pI ∼4.6) due to the reduction of electrostatic repulsion between oil droplets (Perugini et al., 2018). There are many pharmaceutical forms especially topical ones that are prepared at acidic pH (Angelova-Fischer et al., 2018; Blaak et al., 2017; Kilic et al., 2019). Acid food emulsions based on the whey protein isolate/xanthan gum complex have also been successfully formulated (Matsuyama et al., 2021). They are manufactured and distributed at a pH close to 4. Therefore, it would be interesting to study the possibility of formulating stable emulsions with NaCn under acidic conditions.

Complexation of PR with some anionic PS through electrostatic interactions can significantly improve the stability of emulsions (Guzey & McClements, 2007). In the case of NaCn, adding a PS such as xanthan gum (XG) can strengthen the protein network formed at the oil interface (Seddari & Moulai-Mostefa, 2015). In pharmaceutical, XG is employed as a stabilizing agent in oral and topical formulations (Singhvi et al., 2019), for the production of extended-release matrix tablets (Fan et al., 2008), or for its mucoadhesive properties (Laffleur et al., 2016). Several works were devoted to the evaluation of the properties of NaCn/XG dispersions at acidic pH (Gravelle et al., 2021; Hidalgo et al., 2016; Liu et al., 2012). However, the complexation between NaCn and XG to form stable emulsions under acidic conditions has not yet been studied to our knowledge.

The main objective of the present study was to examine the possibility of formulating stable acid O/W emulsions using NaCn/XG complexes prepared at pH 4. The stability of emulsions can be enhanced by using the electrostatic interactions between PR and PS. For this, two approaches were proposed; one of them is called the "layer by layer" training method. This approach consists in improving the stability of the emulsion by adsorbing the PS at the droplet interface of the emulsion by electrostatic interaction between the two biopolymers (Evans et al., 2013). This technique was employed in many investigations on the use of interactions between NaCn and XG to improve emulsion stability. The second method consists at first in preparing PR/PS complexes, then using them for emulsification (Dickinson, 2017). For this method, the emulsion stabilization mechanism has not been thoroughly clarified.

In this work, NaCn/XG complexes were prepared in advance at pH 4 and then used for the formulation of O/W emulsions using the second approach. The physicochemical properties of the different complexes prepared were determined by measurements of turbidity and surface tension. Their rheological behavior in solution was also studied. Structural characterization of the complexes was performed by scanning electron microscopy (SEM) and Fourier transform spectroscopy (FTIR). The stability of the emulsions formulated with the NaCn/XG complexes was monitored for 1 month of storage at 25°C. In parallel, microscopic and particle size analyses were carried out on the various formulated systems. The study of the rheological behavior of the different formulations was also achieved.

Overall, we demonstrate that the correlation of rheological and physiochemical properties with microstructures can provide valuable insight for the stabilization of acidic food emulsions with relatively low concentrations of NaCn and XG and by an industrially feasible process.

MATERIALS AND METHODS

Materials

NaCn, obtained from bovine milk, was produced by Sigma Life Science (New Zealand). Food grade XG was purchased from Rhodia (Algeria). Sodium azide, used as a preservative, was provided from Sigma-Aldrich (Buchs, Switzerland), and hydrochloric acid (HCl) was supplied from Fujifilm Wako Pure Chemical Co. Ltd (Osaka, Japan). The organic phase (olive oil) was purchased from a local supermarket.

Preparation of NaCn/XG complexes

Different NaCn solutions (0.2%, 0.4%, 0.6%, 0.8%, 1%, and 1.2%) were prepared by dispersing the powder in distilled water containing sodium azide (0.01%) to prevent any microbial development. The protein suspension was stirred using a magnetic stirrer. The obtained solutions were stored for 24 h at room temperature to supplement their hydration. In parallel, a 0.4% XG solution was solubilized in distilled water containing sodium azide (0.01%) at room temperature

using a magnetic stirrer for 2 h to complete their dissolution. The concentration (0.4% XG) was chosen from a previous study and some results reported in the literature (Rahmati et al., 2018; Seddari & Moulai-Mostefa, 2015). In addition, a large number of experiments were realized using different concentrations of XG which enabled us to choose this optimal concentration (less than 0.4% of XG, the emulsions prepared with the NaCn/XG complex were unstable and more than 0.4%, all emulsions were stable).

NaCn/XG dispersions were prepared by the method used by Matsuyama et al. (2021) with some modifications. A volume of 50 mL of NaCn was mixed with 50 mL of XG by adding HCl (0.1 M) in the same time. The agitation was carried out using a homogenizer (Ultra-turrax T-25, IKA, Germany) at 9000 rpm for 3 min. The final pH of the dispersion was 4.

Preparation of emulsions

O/W emulsions were prepared by adding 20% of the oil phase (olive oil) to 80% of the aqueous phase containing the NaCn/XG complex under stirring at 10,000 rpm for 5 min using a homogenizer (Ultra-turrax T-25, IKA, Germany). To evaluate the effect of the NaCn:XG ratio on emulsion stability, samples with different biopolymer ratios were prepared. The pH of the aqueous phase is that of the NaCn/XG complex solution (pH 4). The final composition of the emulsions was 20% olive oil, 0.16%–0.96% NaCn, 0.32% XG, and 0.016% sodium azide.

Determination of turbidity

Schmitt et al. (1999) noticed that any significant increase in turbidity results from an increase in particle number and size. The biopolymer mixing ratio is also important for controlling the balance of the electrical load in mixed systems. Thus, the turbidity of the NaCn/XG complexes was measured directly after preparation at a wavelength of 600 nm using a UV–visible spectrophotometer (Shimadzu UV-1800, Japan). The samples were placed into a plastic cuvette (1 cm in length), and the absorbance was measured at room temperature. Distilled water was used as a blank reference.

Surface tension measurements

The surface tensions (ST) of the pure NaCn solution and aqueous NaCn/XG mixtures were measured at 25°C by the ring method using a CSC-Du Nouy Tensiometer (CSC, Fairfax, VA, USA).

FTIR spectroscopy

FTIR spectroscopy was performed to investigate the possible interactions that can occur between biopolymers in solution. Vacuum-dried complexes (40°C, 30 mbar, 12 h) as well as, NaCn and XG powders, were characterized by FTIR using a Bruker spectrophotometer (Germany). A sample (~10 mg) was placed on the diamond square of the spectrophotometer. The spectra were recorded over a wavenumber range between 4000 and 400 cm^{-1} at room temperature, by the data processing software (OPUS 6.0).

Scanning electron microscopy

Scanning electron microscopy (SEM) is a technique capable of producing high-resolution images of the surface of a sample and provides information about its morphology and particle size. Therefore, the morphologies of vacuum-dried biopolymers and complexes were characterized by SEM (FEI Quanta 650, USA).

Emulsion stability study

The stability of the different emulsions formulated by the NaCn/XG complexes was monitored for 4 weeks. A sample of each formulation was transferred into a glass test tube immediately after preparation and sealed to prevent evaporation. Periodic changes in the creaming limit were noted during storage at 25°C. The emulsions were separated into the upper cream and lower aqueous phase layers over time, and the total emulsion height (H_T) and aqueous phase height (H_{aq}) were measured. The creaming index (CI %) was calculated as a percentage of the ratio of the measured values (Sabri & Moulai-Mostefa, 2020):

$$CI\,(\%) = \left(H_{aq}/H_T\right) \times 100. \quad (1)$$

Optical microscopy and droplet size distribution

Microscopic observations of the emulsions were made with an Olympus® BX-51 phase contrast microscope (Germany), equipped with an oil immersion lens (×100). It is connected to a digital camera for image capture. A minimum of 100 droplets is counted for each test. The sample is placed between slide and lamella, and observations were made directly after formulation and after 28 days of storage at 25°C. The ImageJ software (USA) was utilized to calculate the diameter of emulsion droplets. The droplet diameter ($d_{3,2} = \Sigma n_i d_i^3/\Sigma n_i d_i^2$) of the emulsion was calculated with the ImageJ software (USA) and over 100 droplets in every scope were measured.

Rheological analysis

Rheological tests were carried out on dispersions and emulsions after 24 h of their preparation using the imposed stress rheometer (Anton Paar Modular Compact Rheometer MCR 302, Germany), with the cone-plane geometry of 50 mm in diameter and 1° angle. The measurements were achieved at 25°C by a Peltier effect system. The collection of information was realized by the Anton Paar Rheo Compass software.

Conductivity and pH measurements

The conductivity of the emulsions was measured with a LF 191 conductimeter (WTW, Germany). The pH was measured directly in the solutions and emulsions using a microprocessor pH/ion Meter pMX 2000 (WTW, Germany).

Statistical analysis

All experimental measurements were repeated in triplicate, and the data are presented as the mean ± standard deviation (SD). Data were examined by one-way analysis of variance (ANOVA), followed by the Tukey test with significance defined at $p < 0.05$, to confirm the difference in the mean value between samples using OriginPro.8 software (2019).

RESULTS AND DISCUSSION

Characterization of NaCn/XG complexes

Determination of turbidity

All NaCn/XG dispersions showed an opalescent (white) appearance just after their preparation. After 24 h, the majority of the dispersions were found stable, except the formulation prepared with 1.2% of NaCn which became transparent with a white precipitate (Figure 1). The degree of opalescence is also a function of NaCn:XG ratio. By increasing the concentration of PR, the transparency of the dispersions decreases due to the complexation between the two biopolymers. Moreover, turbidity was used for the determination of the degree of aggregation and stability of particles in solution due to the attenuation of the transmitted light or sample absorbance (Espinal-Ruiz et al., 2014). Generally, an electrostatic complex of PR and PS forms in solution at a pH near the pI of the protein (Xiong et al., 2016). The PR:PS ratio is an important parameter in mixed biopolymer systems, because it controls the charge balance of macromolecules, therefore, the intensity of electrostatic interactions

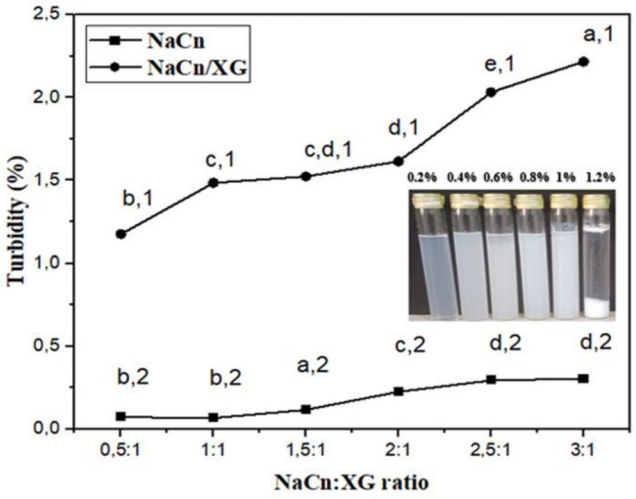


FIGURE 1 Effect of NaCn:XG ratio on the turbidity of NaCn solutions (pH 7.59) and NaCn/XG dispersions (pH4); Appearance of NaCn/XG dispersions after 24 h of storage. ($^{a-e}$) Means within the same curve without a common letter are significantly different ($p < 0.05$); (^{1,2}) Means within different curve without a common number are significantly different ($p < 0.05$).

(Schmitt et al., 1999; Yan et al., 2021). To explore if there was an electrostatic interaction between NaCn and XG, the turbidity of NaCn/XG solution was measured directly after preparation. From the results obtained (Figure 1), it was noticed that the turbidity of the complexes increases with the increase of NaCn concentration. The tendency to modify the turbidity of the composite solution compared to NaCn used alone was different; it was statistically improved ($p < 0.05$). The addition of XG to NaCn solution induced a considerable increase in turbidity. This is due to the formation of a complex between the two biopolymers. The complexation could be related to the pH 4 of the solution which is between the pI of PR and the pka of PS (Rajabi et al., 2019). NaCn in this case is positively charged (pI ~4.6) while XG (pka ~2) is negatively charged, so the formation of NaCn/XG complexes is owing to the electrostatic interactions. For mixture solutions prepared with (0.2%, 0.4%, 0.6%, 0.8%, and 1%) of NaCn, the complexes formed are soluble with a single homogeneous phase containing both biopolymers. In contrary for the solution prepared with 1.2% NaCn, the complex formed is insoluble; it is characterized by the associative phase separation, the solvent (water) is exempt from biopolymer which precipitates. This precipitation is due to the neutralization of charges (Archut et al., 2023; Gorji et al., 2014).

Surface properties

ST measurements give important information about the system behavior in the bulk, as well as at the interface

(Milanović et al., 2014). So, this technique was utilized to study the interactions between NaCn and XG in solutions. The variations in ST of the solutions of NaCn/XG complexes and of pure NaCn solution as a function of NaCn are shown in Figure 2.

Examination of these curves showed that the ST of NaCn decreases from 27.7 to 22.8 mN.m^{-1} ($p < 0.05$) by increasing NaCn concentration from 0.2% to 1.2%. This PR is flexible and can be easily unfolded and adsorbed at the air/water or oil/water interface forming an interfacial film (Liu et al., 2012). By adding XG to NaCn, the ST increases, meaning that the surface activity decreased. XG is not surface active and replacing NaCn with XG will increase surface tension. For the NaCn/XG mixture solutions, their ST decreases from 37.7 to 30.9 mN.m^{-1} ($p < 0.05$) by increasing NaCn concentration from 0.2% to 1.2%. The interactions between NaCn and XG promote the formation of a protective layer on the surface of the liquid. The complex thus formed between proteins and polysaccharides has a surface activity that can reduce ST. Analysis of these curves also showed that the addition of XG molecules and their binding induced some conformational changes of NaCn film resulting in higher surface tension. The effect of XG and NaCn molecules on the surface tension is the result of surface activity of the formed complex between the two substances. In addition, the improvement in surface activity depends on the NaCn:XG mixing ratio. For example, for a NaCn:XG ratio of 1:1 and 2:1, the value of ST is 35.43 and 32.7 mN.m^{-1}, respectively ($p < 0.05$).

Chemical structures

Interactions of functional groups at the molecular level can lead to the creation of new bands and changes in the intensity or location of absorption bands (i.e., displacement) in FTIR spectra. The NaCn spectrum (Figure 3) showed N—H stretching absorption bands at 3287 cm^{-1}. The characteristic peaks of NaCn are attributed to the amide I and amide II at 1639 and 1528 cm^{-1} for the stretching of the carbonyl group C═O and for the symmetrical stretching of the N—C═O bonds, respectively. In addition, native XG had the bands at 3304 and 2883 cm^{-1}, which are relative to the O—H stretching vibrations with the intermolecular H bond and C—H stretching. These results are identical to those reported in another study (Pawlicka et al., 2019).

At pH below pI of PR, two main types of non-covalent interactions, namely hydrogen bond and attractive electrostatic interactions occurred between NaCn and XG (Figure 3). At this pH, PR is positively charged and is able to attract a negatively charged anionic gum like XG. However, there were differences between FTIR bands for samples of complexes containing 0.4% XG and different amounts of NaCn (0.2%, 0.4%, 0.6%, 0.8%, 1%, and 1.2%) with those for PR and PS utilized alone. For the NaCn/XG sample (0.6%/0.4%), the adsorption band at 3304 cm^{-1} (OH group in XG) shifted to 3282 cm^{-1} (N—H stretching absorption band of NaCn) which may be a sign of hydrogen bond formation (Figure 3). This shifting reflects the formation of a hydrogen bond between N—H of NaCn and COO^- of XG. These results are in agreement with those of Rahmati et al. (2018). A hydrogen bond occurs between an electron acceptor (such as NH_3^+ or NH_2) and an electron donor group (such as COO^- or OH), so NH_3^+ as much as an electron acceptor and COO^- as well as an electron donor. These two groups are favorable to the formation of a hydrogen bond. Also, Figure 3 shows a shift of the NaCn peak from 1639 cm^{-1} (amide I) to 1633 cm^{-1} while the peak at 1528 cm^{-1} (amide II) is not out of place. In addition, the two characteristic peaks of NaCn have become less intense. A shift of the characteristic peak of NaCn

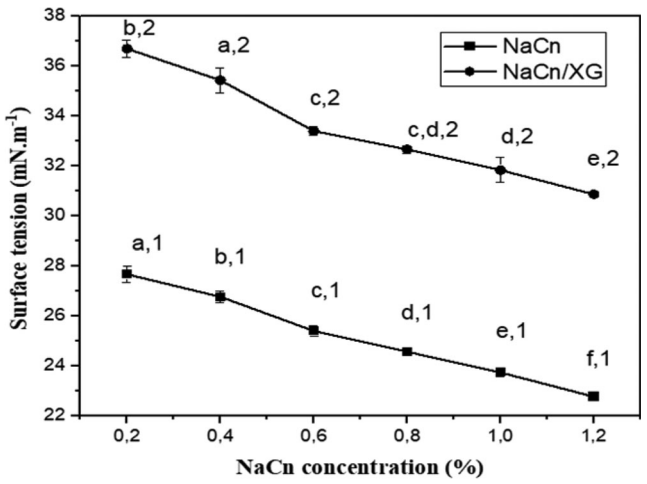


FIGURE 2 Surface tension of NaCn solutions (pH 7.59) and NaCn/XG dispersions (pH 4) as a function of NaCn concentration. Means with different letters ($^{a–f}$) are significantly different ($p < 0.05$); (^{1,2}) Means within different curve without a common number are significantly different ($p < 0.05$).

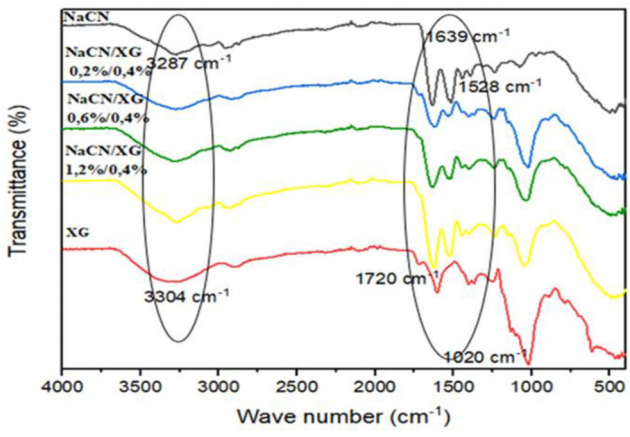


FIGURE 3 FTIR spectra of NaCn/XG dispersions (pH 4).

(amide III) from 1240 to 1243 cm^{-1} with the same intensity was also noted. The shift of the peaks and the large changes in the shape and slopes of the bands for amides I, II, and III proved that there is a formation of new interactions between NaCn and XG. The shoulder at 1720 cm^{-1} was associated with acid groups or free ester of XG and it had almost disappeared. The mean intensity band relative to the asymmetrical C═O stretching vibration at 1600 cm^{-1} has completely disappeared with a significant reduction in the symmetrical C═O stretching at 1406 cm^{-1}, from the carboxylate anion (COO^-) of XG. A band at 1243 cm^{-1}, originally present in pure XG, has disappeared (Figure 3). This band is identical to that reported in the literature (Pawlicka et al., 2019). This is relative to the —OH band of the primary hydroxyl groups present in the XG backbone. So, the disappearance of this peak was assumed as further evidence of the establishment of certain interactions between the two biopolymers.

As shown in Figure 3 as the NaCn concentration increases, the band at 3304 cm^{-1} (OH group in XG) becomes less pronounced. In its place, a new peak was observed at about 2385 cm^{-1} (N—H stretch absorption band) of NaCn. The shoulder at 1720 cm^{-1} (XG) has almost completely disappeared. The intensity of the bands at 1630 and 1553 cm^{-1} increased while the intensity of the band at 1020 cm^{-1} decreased. This can be explained by an excess of NaCn above (0.8%). In conclusion, this analysis proves the formation of a soluble complex between NaCn and XG.

Morphological structures

The particle morphology and surface area of vacuum-dried NaCn/XG dispersions were studied. For this, the vacuum-dried XG and NaCn were analyzed firstly by SEM at two magnifications (×100 and ×800). The examination of the results obtained (Figure 4) revealed that the particles of all samples have an irregular morphology and surface.

Pure NaCn particles (Figure 4a–d) have a polyhedral shape characterized by a porous surface. However, the pure XG (Figure 4b–e) presents a smooth and wrinkled

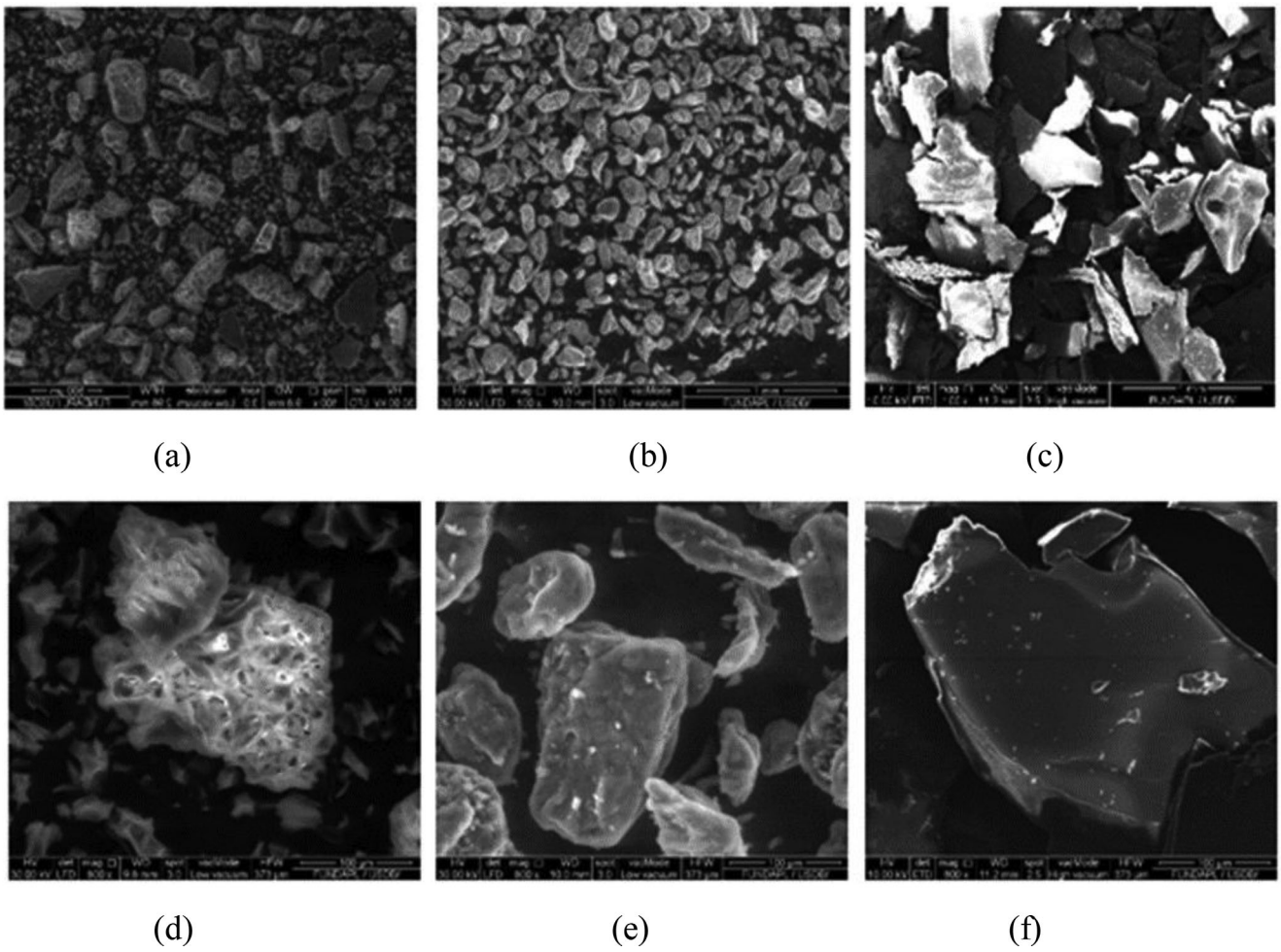


FIGURE 4 SEM of particles of: NaCn (a: ×100 and d: ×800), XG (b: ×100 and e: ×800), NaCn/XG 0.2%/0.4% (c: ×100 and f: ×800).

surface and the granules have a polyhedral shape characteristic of amorphous biopolymers (Yahoum et al., 2021). The particles of the NaCn/XG complex (0.2%/0.4%) have a totally different morphology compared to both biopolymers (Figure 4c–f). This modification in morphology and surface area of the pure particles can be explained by the formation of a new product, which is the complex. This result confirmed those obtained by FTIR spectroscopy.

Rheological properties

To determine the effect of the NaCn:XG ratio on the rheological properties of the complex solutions prepared at pH 4, the variations of the viscosity as a function of the shear rate were established (Figure 5). The rheological behavior of the dispersions is sensitive to the variation of NaCn. In addition, the apparent viscosity varies with the PR concentration, and there are two regions: a first one with very low shear rates, corresponding to the Newtonian plateau, and a second one corresponding to the shear-thinning behavior (Figure 5). These results demonstrated that the mechanical behavior obtained is typical of pseudoplastic fluids (Reinoso et al., 2019; Yahoum et al., 2016). On the other hand, the apparent viscosity was almost identical for all dispersions except for solutions prepared by 1% and 1.2% in NaCn where a slight decrease was observed. It should be noted that the viscosity of XG alone is significantly higher than that of NaCn/XG dispersions (Figure not shown), indicating strong interactions between these two biopolymers at pH 4. This can be explained by the fact that the amino acids of NaCn are protonated at pH 4. Similar variations of apparent viscosity were reported in the work of Pawlicka et al. (2019) using the same complex as well as in the work of Albano et al. (2019) studying the complex formed by the whey protein isolate and XG.

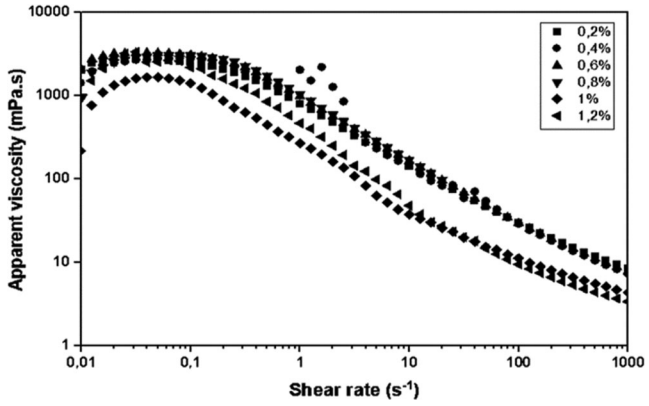


FIGURE 5 Effect of NaCn concentration on the flow curves of NaCn/XG complexes on the apparent viscosity versus shear rate.

The mechanical behavior of the studied complexes was evaluated by fitting the shear stress–shear rate data using the model of Herschel-Bulkley (Equation 2).

$$\tau = \tau_0 + k\dot{\gamma}^n, \quad (2)$$

where $\dot{\gamma}$ is the shear rate, τ is the shear stress, k is the consistency coefficient, n is the flow behavior index (dimensionless), and τ_0 is the yield stress.

The parameters obtained from this model (Table 1) showed that the relationship between the shear stress and shear rate of NaCn/XG complexes was not linear, indicating that these complexes exhibited a shear-thinning behavior. In addition, the higher values of the correlation coefficient ($R^2 = 0.9$) obtained for most dispersions confirmed the reliability of the rheological parameters. The yield stress (τ_0) was an indication of the ability of biopolymer particles in dispersions to resist to the sedimentation. Low values of τ_0 for NaCn/XG complexes, prepared with 1% and 1.2% of NaCn, could indicate a tendency to precipitation. The values of n were related to the degree of non-Newtonian behavior and all dispersions had a value of n less than 1, confirming the pseudoplastic character of the complexes.

Properties of emulsions based on NaCn/XG complexes

Stability of emulsions

Emulsions formulated by different NaCn/XG complexes have a milky and homogeneous appearance directly after their formulation. The electrical conductivity values of samples were found high indicating the nature of emulsions, oil-in water (O/W).

TABLE 1 Rheological parameters of NaCn/XG dispersions deduced from the Herschel-Bulkely model.

NaCn concentration	0.2%	0.4%	0.6%	0.8%	1%	1.2%
K (Pa.s^n)	0.15817 ± 0.00976	0.02196 ± 0.04168	0.12728 ± 0.00645	0.1886 ± 0.03932	0.03943 ± 0.00201	0.02098 ± 0.00161
n	0.55666 ± 0.00853	0.7954 ± 0.27114	0.56878 ± 0.00702	0.5567 ± 0.0288	0.67147 ± 0.0078	0.71746 ± 0.01089
τ_0 (Pa)	0.89399 ± 0.03974	2.10438 ± 0.49009	1.20584 ± 0.02757	1.7109 ± 0.16004	0.21937 ± 0.01329	0.37701 ± 0.01312
R^2	0.9996	0.9474	0.9997	0.9951	0.9997	0.9994

Note: R^2 is the correlation coefficient, k is the consistency coefficient, n is the flow behavior index (dimensionless), and τ_0 is the yield stress.

Figure 6a,b show the macroscopic views of the emulsions fabricated by the NaCn/XG dispersions directly after their formulation and after 28 days of storage at 25°C. The values of the corresponding creaming index (CI%) were also tracked and calculated.

The temporal evolution of CI for emulsions formulated with 1% and 1.2% of NaCn showed that the creaming is obvious and begins from the first day of storage. For all other emulsions, no instability phenomenon was noticed during the 4 weeks of storage.

For complexes prepared by 0.2%, 0.4%, 0.6%, and 0.8% of NaCn, all the PR macromolecules are attached to the PS ones, which lead to the formation of a homogeneous aqueous solution containing a stable PR/PS complex. This product plays the role of emulsifier during the preparation of O/W emulsions which improves their stability.

The concentrations of proteins and polysaccharides determine the PR-PS ratio found in the solution. The importance of this ratio is due to the combined effect of the type of preferred interactions (PR/PR, PS/PS, or PR/PS) and the net charge ratio of the biopolymers in the case of attractive interactions. A higher PR:PS ratio favors PR-PR interactions over PR-PS interactions. In the case of the complexation phenomenon, the charge ratio controls the solubility of the complexes (Gentile, 2020). Thus, the instability of the formulations prepared by the complexes containing 1% and 1.2% NaCn (CI% = 41.18% and 63.16%) may be due to the higher PR:PS ratio and to the excess of PR which was not related to PS. This phenomenon of instability has been observed previously for the solutions containing both biopolymers. In addition, the stability of the emulsions depends on the NaCn/XG mixing ratio. For example, by increasing of NaCn/XG ratio from 2:1 to 3:1, the value of CI increases from 0% to 63.16%, after 28 days of storage. Similar results were obtained by Cheng and McClements (2016) using pectin-caseinate to stabilize oil-in-water emulsions.

In conclusion, the stability of emulsions prepared by NaCn/XG dispersions (pH 4) is largely influenced by the NaCn:XG ratio. It directly affects the nature of the interactions between PR and PS and therefore the nature of the complex that constitutes the interfacial film covering the oil droplets. The best ratios were respectively, 0.5:1, 1:1, 1.5:1, and 2:1.

Microscopic analysis and droplet size distribution

The microscopic and particle size analyses of the different samples were carried out just after formulation (Figure 7a) and after 28 days of storage at 25°C (Figure 7b). It was shown that the formulations have a monodisperse droplet size distribution.

In addition, emulsions based on NaCn/XG dispersions prepared with 0.2% to 0.8% NaCn resulted in an O/W emulsion with a uniform surface and spherical form while emulsions formulated with 1% and 1.2% of NaCn have non-uniform spherically shaped and much larger droplets.

After 28 days of storage at 25°C (Figure 7b), the droplet sizes of all emulsions stabilized with NaCn/XG complexes at different amounts of NaCn except for the emulsion prepared with NaCn/XG at 0.4% of NaCn where there was fewer change of the droplets distribution.

The variation in the average diameter ($d_{3,2}$) of oil droplets as a function of time and NaCn concentration is illustrated in Figure 8. It remained almost constant with the increase of NaCn (26.47 μm) up to 0.8%

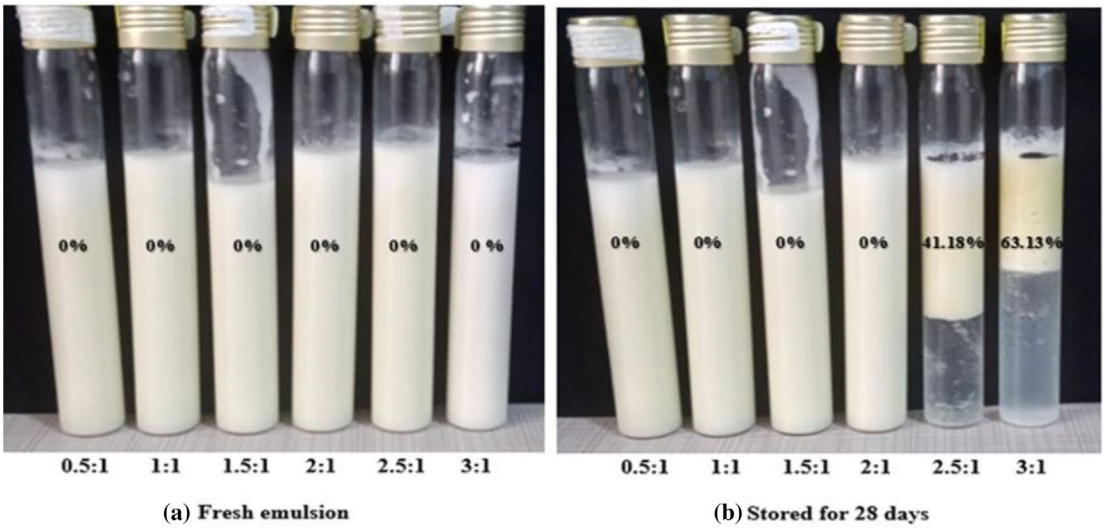


FIGURE 6 Appearance and CI (%) of NaCn/XG based emulsions for different NaCn:XG ratio: (0.5:1, 1:1, 1.5:1, 2:1, 2.5:1, 3:1), (a) freshly prepared, (b) after 28 days of storage at 25°C.

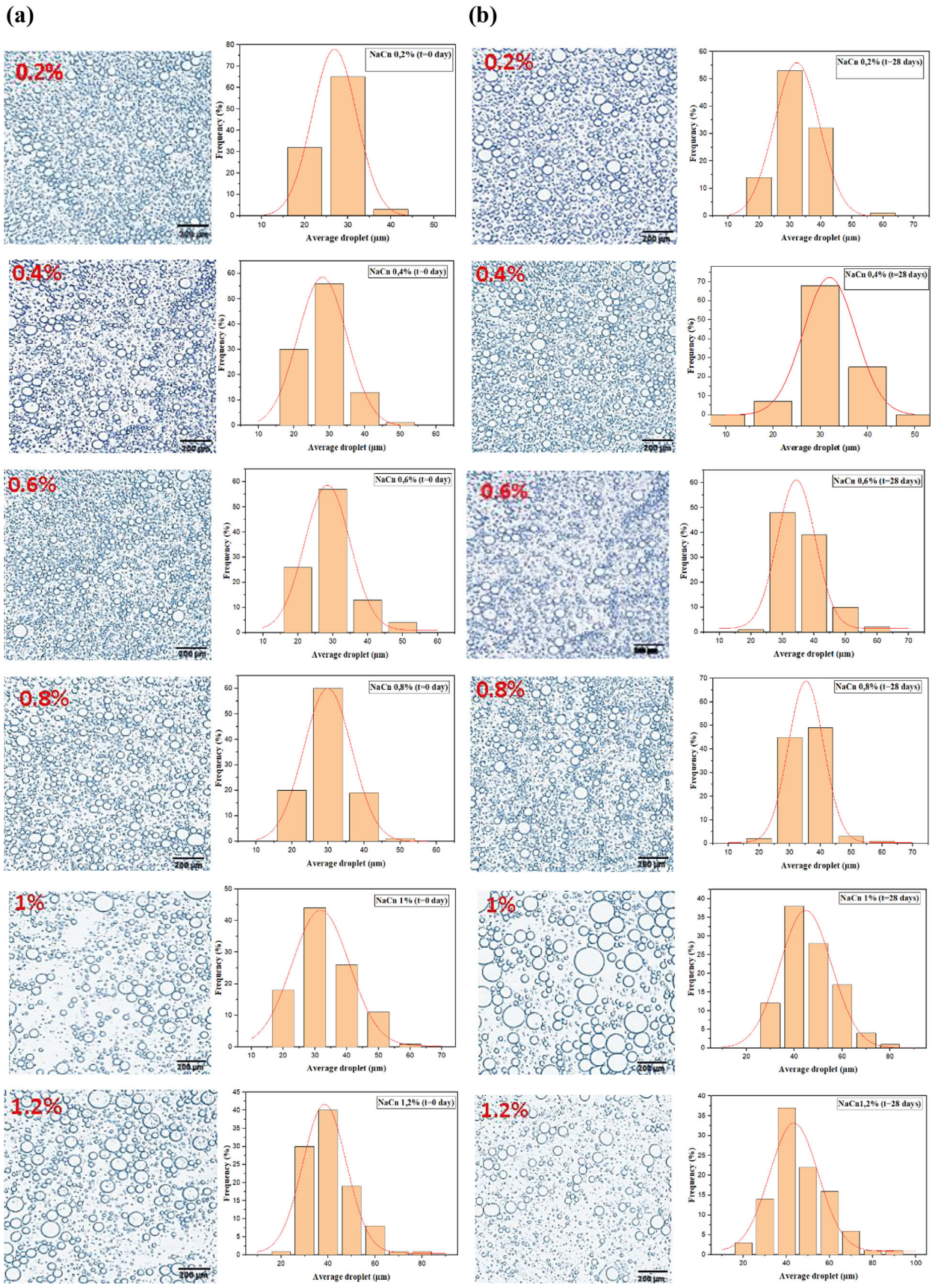


FIGURE 7 Emulsion microstructure (×10) and droplet size distribution for different NaCn concentrations: (a) directly after preparation, (b) after 28 days of storage at 25°C. (Scale bars = 200 µm).

(28.81 μm) and then, it increases sharply for emulsions prepared with 1% and 1.2% (33.86 and 36.25 μm, respectively) ($p < 0.05$). This may be due to the depletion flocculation and coalescence of droplets. Furthermore, the average droplet sizes after days 0 and 28 were statistically significantly different ($p < 0.05$), except for the emulsion with 0.4% NaCn ($p > 0.05$). Thus, this emulsion was considered kinetically-stable. The droplet size of emulsions depends on the NaCn/XG mixing ratio. For example, for a NaCn/XG ratio of 2:1, the value of the average droplet size increases from 28.81 to 35.61 μm, and for a NaCn/XG ratio of 3:1 the value of the average droplet size increases from 36.25 to 46.88 μm. These findings confirmed the importance of the PR:PS ratio on the stability of emulsions, and are in agreement with those reported by several studies (Barbosa et al., 2019; Boonlao et al., 2020; Kumar et al., 2021; Tang et al., 2021). The best average diameter (29.026 μm) is that of the emulsion stabilized by the dispersion with NaCn:XG = 1:1 ratio.

During the emulsification step, the complex formed between the two biopolymers adsorbed at the oil–water interface to form a rigid interfacial film which ameliorated emulsion stability. In addition, emulsion prepared with this ratio presented the higher viscosity (Section: Rheological properties).

Therefore, the increase in viscosity prevents the movement of droplets and consequently their flocculation by depletion. Consequently, stable emulsions could be created by the NaCn/XG complexes through electrostatic molecular associations between both components in acidic condition. The emulsification performance of the NaCn/XG complexes is driven by the effect of the NaCn:XG ratio. Similar results were obtained by Matsuyama et al. (2021) using whey protein isolate/xanthan gum complex to formulate food emulsions under acidic conditions. Also, the preparation of stable emulsions using complex PR/PS was reported in the work of Bahraseman et al. (2023).

Based on the results obtained in this study, emulsions can be prepared at pH 4 using a relatively simple method with natural ingredients, which can serve as a useful strategy for the development of novel food products.

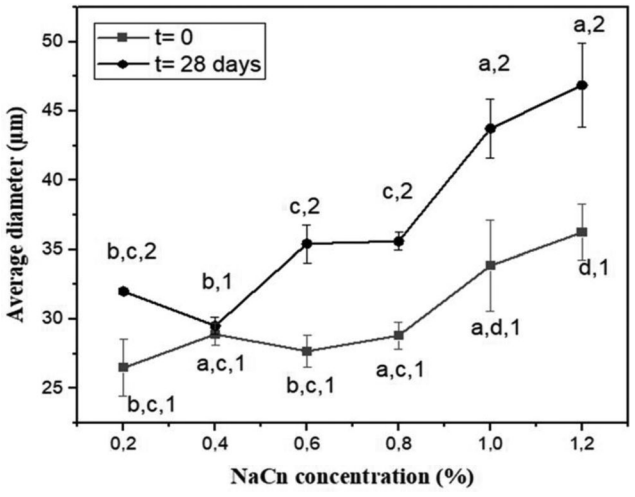


FIGURE 8 Effect of NaCn on the droplet sizes of emulsion stabilized with NaCn/XG complex. ($^{a–d}$) Means within the same curve without a common letter are significantly different ($p < 0.05$); (^{1,2}) Means within different curve without a common number are significantly different ($p < 0.05$).

Rheological behavior of emulsions

Figure 9 displays the flow curves of emulsions-based NaCn/XG complexes. The curve plots are nonlinear and similar to that of complexes studied alone. Also, there are two regions: the first one in the range of low shear rates, corresponding to the Newtonian plateau, and the second region, corresponding to a shear-thinning (pseudoplastic) behavior. So, the rheological behavior of emulsions is that of their aqueous phases. On the other hand, the samples prepared with 0.2% and 0.4% of NaCn presented the greatest values of viscosity compared to those prepared with 0.6% and 0.8% of NaCn. This can be explained by the fact that by increasing the concentration of NaCn, the amount of XG that interacts with NaCn increases so. The complex formed is then positioned at the interface of the oil droplets and the aqueous phase is depleted in XG resulting in a decrease in the viscosity of the aqueous phase. As a result, the rheological behavior of the formulated emulsions follows that of the aqueous phase which is a function of the nature of the complex formed based on both biopolymers at pH 4.

To model the results, the flow curves were fitted to the Herschel-Bulkley model (Table 2). Higher values R^2, ranged between 0.95 and 0.99, confirmed the reliability of the rheological parameters. In addition, the very low value of τ_0 for emulsion stabilized by NaCn/XG dispersion at 0.8% of NaCn indicates a tendency to separation. The value of n was related to the degree of

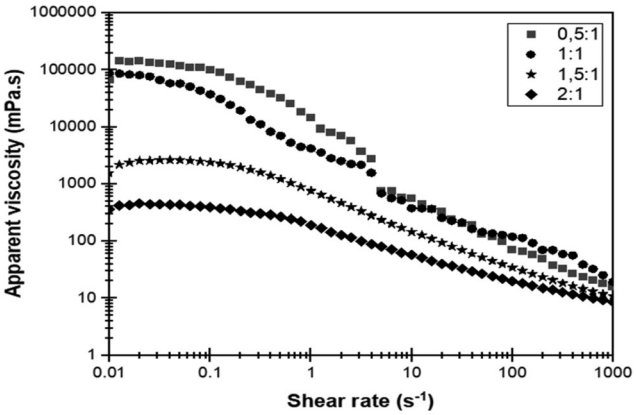


FIGURE 9 Apparent viscosity of O/W emulsions (20%/80%) prepared by NaCn/XG dispersions at different NaCn:XG ratio.

TABLE 2 Rheological parameters of emulsions deduced from Herschel-Bulkely model.

NaCn concentration	0.2%	0.4%	0.6%	0.8%
K (Pa s^n)	0.95457 ± 0.10335	2.24983 ± 0.48193	0.35669 ± 0.02469	0.10367 ± 0.00345
n	0.40089 ± 0.01613	0.35148 ± 0.03142	0.48556 ± 0.01044	0.63651 ± 0.00503
τ_0 (Pa)	0.71597 ± 0.13742	1.2788 ± 0.56531	0.22739 ± 0.04293	0.05714 ± 0.01094
R^2	0.9868	0.9447	0.9956	0.9993

Note: R^2 is the correlation coefficient, k is the consistency coefficient, n is the flow behavior index (dimensionless), and τ_0 is the yield stress.

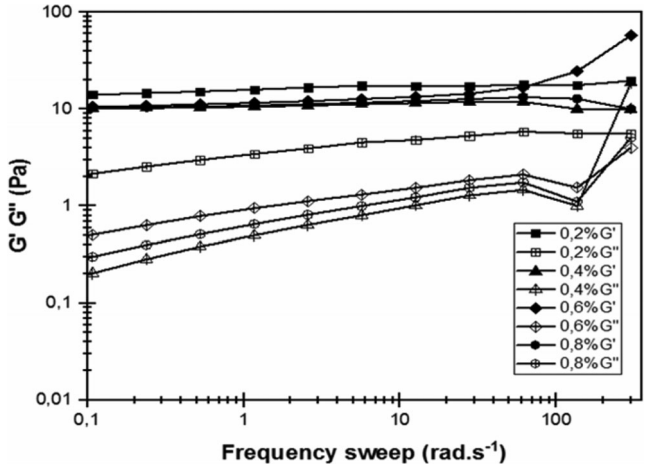


FIGURE 10 Storage modulus (G') and loss modulus (G'') of NaCn/XG-based emulsions at different NaCn concentrations.

non-Newtonian behavior and all emulsions had a value of *n* less than 1, confirming the pseudoplastic character of the emulsions. It was also observed that the value of K for the emulsion stabilized by NaCn/XG dispersion with 0.4% NaCn was found higher than for other formulations, which confirms that the rheological parameters are sensitive to NaCn.

The plots of dynamic frequency sweep test of emulsions stabilized by NaCn/XG complexes are illustrated in Figure 10. For all samples, it was observed that G' is greater than G'', which corresponds to an elastic behavior characteristic of a pseudo-gel. Furthermore, the values of G' are inversely proportional to the concentration of NaCn. As shown in Figure 10, the values of G' were found relatively independent of frequency, whereas those of G'' showed ascending tendency when the frequency increases. This is probably due to intermolecular interactions (Sweedman et al., 2013). According to Torres et al. (2007), these findings confirmed that the emulsions displayed gel-like structures leading to stable properties.

CONCLUSION

In this work, NaCn/XG complexes were prepared at acidic pH and their use as natural stabilizers for O/W emulsions was tested. The complexation between NaCn and XG at pH 4 via electrostatic interactions was proven by spectrophotometry (increase in turbidity compared to NaCn alone) and the formation of a new product identified by FTIR and SEM. The rheological behavior of the dispersions was found sensitive to the variation of NaCn concentration. The O/W emulsions formulated on the basis of these complexes (0.5:1, 1:1, 1.5:1, 2:1 NaCn/XG ratio) showed good stability during 28 days of storage at 25°C. The stability is a function of NaCn concentration and NaCn:XG ratio. These two parameters have also a remarkable effect on the average droplet diameter of emulsions. The best stability and the best average diameter (29.026 μm) is that of the emulsion stabilized by the dispersion with NaCn:XG = 1:1 ratio. Their average diameter remained constant during the storage. Emulsions formulated with higher ration PR/PS (2.5:1, 3:1) were unstable and have larger droplets, possibly due to more depletion and more flocculation leading to coalescence. Furthermore, it was found that the rheological behavior of the formulated emulsions follows that of the aqueous phase which depends on the nature of the complex formed with the two biopolymers at pH 4. These results demonstrated that stable emulsions could be formulated by NaCn/XG complexes under acidic (pH 4) conditions due to the electrostatic molecular associations between the two biopolymers. The emulsions thus prepared can be used for the encapsulation and administration of active compounds.

AUTHOR CONTRIBUTIONS

Soumia Seddari: Conceived and designed the study; analyzed the data; and wrote the draft of the manuscript. **Houria Bouziane:** Carried out the research; analyzed the data; and wrote the draft of the manuscript. **Fatiha Boudjema:** Analyzed the data. **Nadji Moulai-Mostefa:** Conceived and designed the study; analyzed the data; and wrote the draft of the manuscript. All authors contributed to and approved the final draft of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

ETHICS STATEMENT

No human or animal subjects were used in the research for this study.

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