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Research Article

Effect of Flaxseed Mucilage, Sodium Chloride and Their Combination on Some Functional Properties of Sodium Caseinate Solution

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Abstract

Background and Objective: Proteins-polysaccharides interaction in the presence of other constituents play an essential role in stabilizing food formulations. In the present study, the functional properties of sodium caseinate (NaCas) solution containing different amounts of flaxseed mucilage (FM), sodium chloride (NaCl) and their combinations were studied. **Materials and Methods:** The dried FM and NaCl were added separately or in combination to the NaCas solution at ratio of 0.0, 0.05, 0.10 and 0.20% (w/w) and 0.0, 0.3, 0.6 and 1.0 M, respectively. The zeta-potential, viscosity, foaming properties, emulsion activity and emulsion stability of all NaCas solutions were determined. **Results:** The zeta-potential of NaCas increased on the addition of FM increased, up to 0.1% and then declined on further addition of FM. Addition of NaCl, alone or in combination with FM, decreased the zeta-potential of NaCas. The viscosity of NaCas solution increased with the addition of FM or NaCl, but the increases were more pronounced in case of FM addition. The viscosity of NaCas solution containing 0.05% FM increased on addition of NaCl, up to 1 M. The formed foam of NaCas solution was more stable in the presence of FM or NaCl, but the foam stability decreased with combined addition of NaCl and FM. The emulsion activity increased on addition of FM, up to 0.1% or NaCl, up to 0.6 M and then decreased on further addition of either additive. **Conclusion:** Addition of NaCl, up to 0.6 M to NaCas solution containing 0.05% FM enhanced the emulsion stability while the addition of NaCl, up to 1 M to NaCas solution containing 0.1% FM had no significant effect on its emulsion stability.

Key words: Sodium caseinate solution, flaxseed mucilage, sodium chloride, functional properties, casein, milk protein, dairy products

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The use of flaxseed components such as oil, mucilage or the whole flaxseed meal in formulations of some foods is becoming more attractive due to their technological properties and specific health advantages¹. The flaxseed mucilage can affect the viscosity and water-binding, foaming and emulsifying properties of protein products². Recently, dairy industries have employed protein-polysaccharide interaction to improve the stability of dairy products, such as dairy beverages, fermented milk, ice cream and whipped cream³. These products should be stable to phase separation during processing and storage. Certain food biopolymers such as proteins and polysaccharides possess effective stabilizing properties in multi-phase food systems, where they commonly exist in their native forms. The structural state of different biopolymers can be altered by physical or chemical processing⁴. In this respect, protein-polysaccharide interactions have been studied in recent years^{5,6}. The attraction or repulsion can occur between proteins and polysaccharides present in an aqueous solution. The attraction may result in the formation of a protein-polysaccharide complex while repulsion can lead to a phase separation due to thermodynamic incompatibility⁷. These interactions are sensitive to the nature of the protein and polysaccharide used and to the solution conditions as pH, temperature and ionic composition⁸.

Food hydrocolloids are high molecular weight hydrophilic biopolymers used as functional ingredients to control of microstructure, texture, flavor and shelf-life of food products⁹. Proteins and polysaccharides classes are biopolymer that contribute to the structural and textural properties of foods through their aggregation and gelation behavior. Proteins are known for their emulsification and foaming properties and polysaccharides for their water-holding and thickening properties. Large differences in functional properties exist between the various food biopolymers depending on the detailed chemical structure and sensitivity to solution conditions (pH, ionic strength, specific ions)¹⁰.

Sodium caseinate is widely used as an ingredient in the food industry due to its unique functional properties such as emulsification, water and fat-binding, thickening, gelation and whipping⁴. The structure, charge and state of aggregation of casein molecules depend also on the sodium chloride concentration. Sodium ions act as counter ions of negative charge of phosphoserine residues and carboxylic groups. At low ionic strength, the electrostatic repulsions can lead to the formation of monomeric casein molecules in their solution. In

the presence of sodium chloride, the casein molecules tend to associate together to form aggregates¹¹. The flaxseed mucilage contained less carbohydrate, more minerals and more protein than locust bean and guar gums. Its solubility, however, was higher than locust bean and guar gums. It also exhibited good foam stability properties in aqueous solutions¹² at 1.0%. Flaxseed mucilage appears to resemble gum arabic more closely than any other of the common gums and it can replace gum arabic in emulsions^{13,14}. Therefore, this work was aimed to study the effect of flaxseed mucilage (FM) alone or in combination with sodium chloride on the viscosity, foaming properties, emulsion stability and zeta-potential of sodium caseinate solution for to be used as a healthy alternative to commercial hydrocolloids in the dairy industry.

MATERIALS AND METHODS

Materials: Bovine sodium caseinate was obtained from Sigma/Aldrich (St. Louis, MO, USA). Flaxseeds (*Linum usitatissimum*) and soybean oil were purchased from local markets, Cairo, Egypt. NaCl was obtained from El-Naser Pharmaceutical Chemicals Co., Egypt.

Methods: This study was conducted in the laboratories of Dairy Department, National Research Centre from January to March, 2019.

Flaxseed mucilage extraction: Flaxseeds (100 g) were washed in tap water for 1 min to remove the surface dust and then mixed with 900 mL distilled water. The seeds and water were stirred for 5 h at a speed of 300 rpm min⁻¹ at 60°C in a water bath as described by Cui¹⁵. The slimy suspension of seeds was filtered through a wire mesh (40 mesh) to separate the seeds and precipitated with ethanol absolute (1:2, respectively). The precipitate was separated by centrifugation (Sigma 301, Western Germany) at 3000 g for 10 min and dried at 55°C in a hot air oven overnight.

Sodium caseinate solution: Sodium caseinate (NaCas) solution was prepared by dispersing the required amount of protein (2.0% protein) in distilled water under continuous stirring at room temperature¹⁶ for 4 h. The dried flaxseed mucilage (FM) and sodium chloride (NaCl) were added separately or in combination to the NaCas solution at ratio of 0.0, 0.05, 0.10 and 0.20% (w/w) and 0.0, 0.3, 0.6 and 1.0 M, respectively. All solutions were heated to 75°C, cooled to room temperature (25 ± 2°C) and kept at 5 ± 2°C overnight to achieve equilibrium.

Zeta-potential: The zeta-potential measurement was performed using nano ZS/ZEN3600 Zetasizer Malvern instruments Ltd., UK with a He/Ne laser ($\lambda = 633 \text{ nm}$), scattering angle 90° scattering optics and refractive index of 1.35. The control of this device was provided by the software Zeta Mode v 1.6. The samples were diluted (1:10) before measurement. The zeta-potential represents the electrostatic charge that particles acquire when they are suspended in liquid containing ions.

Apparent viscosity: The apparent viscosity of all NaCas solutions was measured using a coaxial cylinder viscometer (Bohlen V88, Sweden) attached to a workstation loaded with V88 viscosity program. The system C30 was filled with the solution at the measurement temperature of 20°C . All solutions were then, subjected to a programmed shear rate which increased linearly from $37\text{-}847 \text{ sec}^{-1}$ over a 3 min period. The apparent viscosity was expressed as millipascal (mPa.s).

Foaming properties: Foaming capacity of NaCas solution was determined using the methods proposed by Shahidi *et al.*¹⁷. The solution of 100 mL was whipped at 15,000 rpm for 2 min with a high-speed stirrer (IKA Dispersers, T 25 digital ULTRA-TURRAX®, Staufen, Germany). Foaming capacity was measured immediately after whipping and calculated using the following equation⁹:

$$\text{Foaming capacity (\%)} = \frac{V - V_0}{V} \times 100$$

Where:

V = Volume immediately after whipping

V_0 = Volume before whipping

The foaming stability was measured as described by Mangino *et al.*¹⁸. A 100 mL of whipped NaCas solution was left in a graduated cylinder at $25 \pm 2^\circ\text{C}$ and the decrease in the volume was measured at 10 min intervals for a total of 50 min.

Emulsion activity and stability: Emulsion activity and emulsion stability were measured by the method of Wu¹⁹. An aliquot of 100 mL NaCas solution was mixed with 100 mL soybean oil and homogenized at 10000 rpm for 1 min using high-speed homogenizer (OV5 homogenizer, ULTRA-TURRAX®, Velp, Italy) and then centrifuged at $1300 \times g$ for 5 min. The emulsion obtained was divided evenly into 2 portions. The first portion was centrifuged directly at

1300 g for 5 min to measure emulsion activity. The second portion was heated at 80°C for 30 min before centrifugation to measure emulsion stability. Emulsifying activity and stability were calculated as:

$$\frac{\text{Height of the emulsified layer}}{\text{Total height of mixture in centrifugation tube}} \times 100$$

Statistical analysis: Data was analyzed by analysis of variance (MANOVA) using the GLM procedure with SAS software²⁰. Significant differences were determined as $p \leq 0.05$) using Duncan's multiple range tests.

RESULTS AND DISCUSSION

Zeta-potential: The high zeta-potential is considered as an index for the stability of the colloidal systems. As shown in Table 1, the zeta-potential of sodium caseinate (NaCas) solution increased from -13.30 to -19.40 mV as the added FM increased from 0.0-0.10% which can be attributed to the formation of a complex between NaCas molecules and FM molecules through electrostatic interaction²¹. However, the zeta-potential of NaCas solution gradually decreased from -13.30 to -6.33 mV as NaCl increased from 0.0-1.0 M which can be attributed to the of the surface charge of NaCas with Na^+ ions and increased thickness the electrical double layer^{22,23}. The formation of soluble complexes between polyelectrolytes is mostly caused by the electrostatic attractions between them²¹. The combined addition of NaCl and FM decreased the zeta-potential of NaCas solution from -17.30 to -6.75 mV and from -19.40 to -7.90 mV by the addition of NaCl, up to 1 M, to NaCas solutions containing 0.5 and 0.1% FM, respectively.

Table 1: Effect of flaxseed mucilage, sodium chloride and their combination on zeta-potential of sodium caseinate solution

Solutions (w/w)	Zeta-potential (mV)
NaCas	-13.30
NaCas+0.05% FM	-17.30
NaCas+0.10% FM	-19.40
NaCas+0.20% FM	-14.00
NaCas+0.3 M NaCl	-10.60
NaCas+0.6 M NaCl	-9.09
NaCas+1.0 M NaCl	-6.33
NaCas+0.05% FM+0.3 M NaCl	-11.94
NaCas+0.05% FM+0.6 M NaCl	-9.85
NaCas+0.05% FM+1.0 M NaCl	-6.75
NaCas+0.10% FM+0.3 M NaCl	-13.55
NaCas+0.10% FM+0.6 M NaCl	-10.74
NaCas+0.10% FM+1.0 M NaCl	-7.90

NaCas: 2.0% sodium caseinate solution, FM: Flaxseed mucilage, NaCl: Sodium chloride

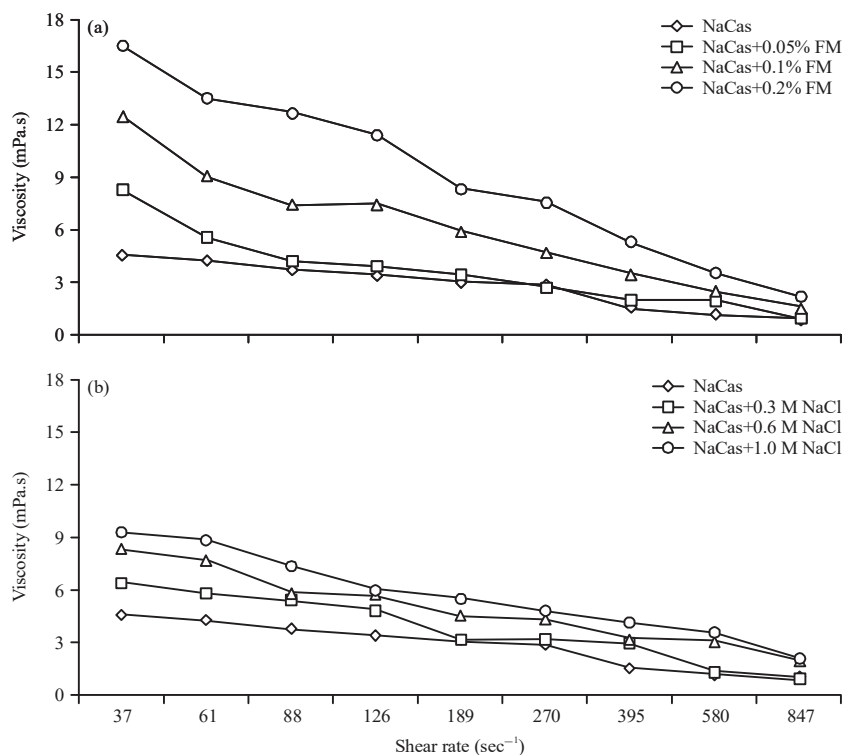


Fig. 1(a-b): Apparent viscosity of 2% sodium caseinate solutions containing different concentrations of (a) Flaxseed mucilage and (b) Sodium chloride

Apparent viscosity: Figure 1 and 2 show that the addition of FM (up to 0.20%), NaCl (up to 1.0 M), or their combination had variable effects on the viscosity of NaCas solution. Generally, all solutions showed pseudoplastic behavior in which the fluid exhibited shear thinning, decreasing apparent viscosity, over the shear rate range studied (37-847 sec^{-1}). Changes in the apparent viscosity over the shear rates were similar in NaCas solution of low concentration²⁴ (1-3%). The apparent viscosity of NaCas solution increased as the FM increased from 0.00-0.2%, the increase was more pronounced ($p < 0.05$) in the NaCas solution containing 0.2 followed by 0.1% FM (Fig. 1a). Such an effect was found in both yoghurt and ice cream containing FM or cress seed mucilage^{25,26}. The increase of the apparent viscosity could be due to the hydrophilic interactions between NaCas and FM. Behnia *et al.*²⁷ reported the gum can bind water and increase the viscosity of protein solution. A similar, but less marked, slightly increase in the apparent viscosity of the NaCas solution was observed as the NaCl increased (Fig. 1b), due to possible competition between the NaCas molecules for water²⁸.

The apparent viscosity of NaCas solution with different combinations of FM and NaCl is presented in Fig. 2. Addition of NaCl, up to 1 M, increased the apparent viscosity of NaCas solution containing 0.05% FM (Fig. 2a), but slightly decreased the apparent viscosity of NaCas solution containing 0.1%

FM (Fig. 2b). Addition of NaCl probably caused noticeable changes in the conformation of polysaccharides and accelerated the formation of sub-micelles from NaCas molecules resulting in decreases of the viscosity²⁹.

Foaming properties: Figure 3 shows that the addition of FM or NaCl enhanced ($p < 0.05$) the foaming capacity of NaCas solution. The highest foaming capacity ($p < 0.05$) was obtained for NaCas solution containing low FM concentration (0.05%) and decreased slightly on a further increase of the added FM. The difference was more pronounced after two min (Fig. 3a). A similar trend was found by Abd El-Aziz *et al.*²⁶ for ice cream containing FM. Proteins and polysaccharides contain simultaneously polar and non-polar regions, which give them surface-active properties. During the foaming processes, they are rapidly adsorbed on the surface of the air bubbles to form a film³⁰. At high concentrations, hydrocolloids can cause kinetic hindrance and partial inhibit the foaming properties of milk proteins³¹. However, the foaming capacity of NaCas solution increased gradually on addition of NaCl, up to 1 M (Fig. 3b). The rate of increase was similar in all NaCas solution until 2 min. When NaCl was added to the NaCas solution containing FM, the foaming capacity linearly increased, being more pronounced than that of NaCas solution containing only FM or NaCl (Fig. 4a, b).

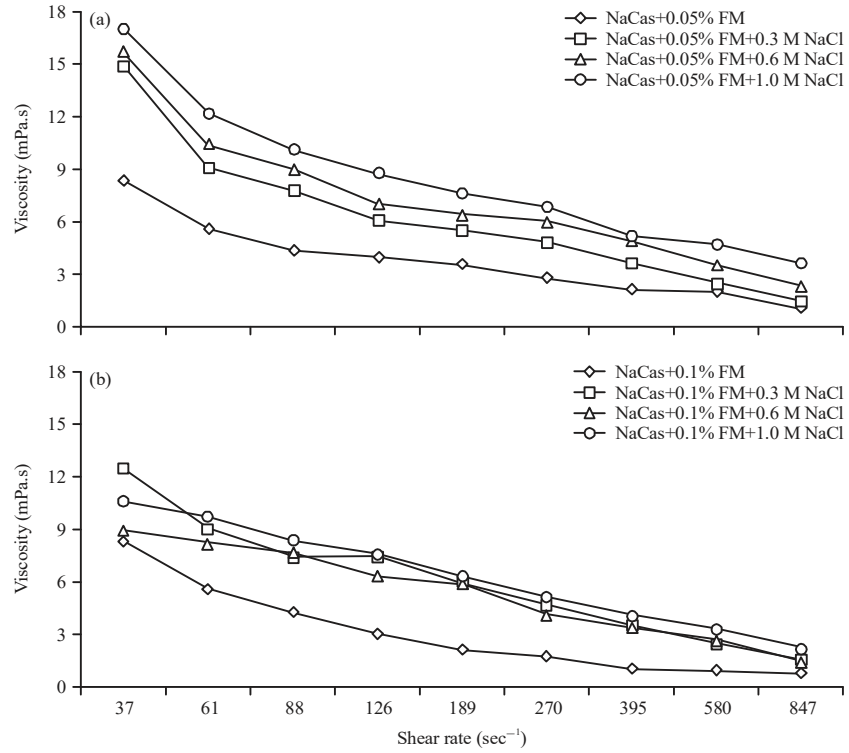


Fig. 2(a-b): Apparent viscosity of 2% sodium caseinate solutions containing, (a) 0.05% and (b) 0.1% flaxseed mucilage in the presence of different concentrations of sodium chloride

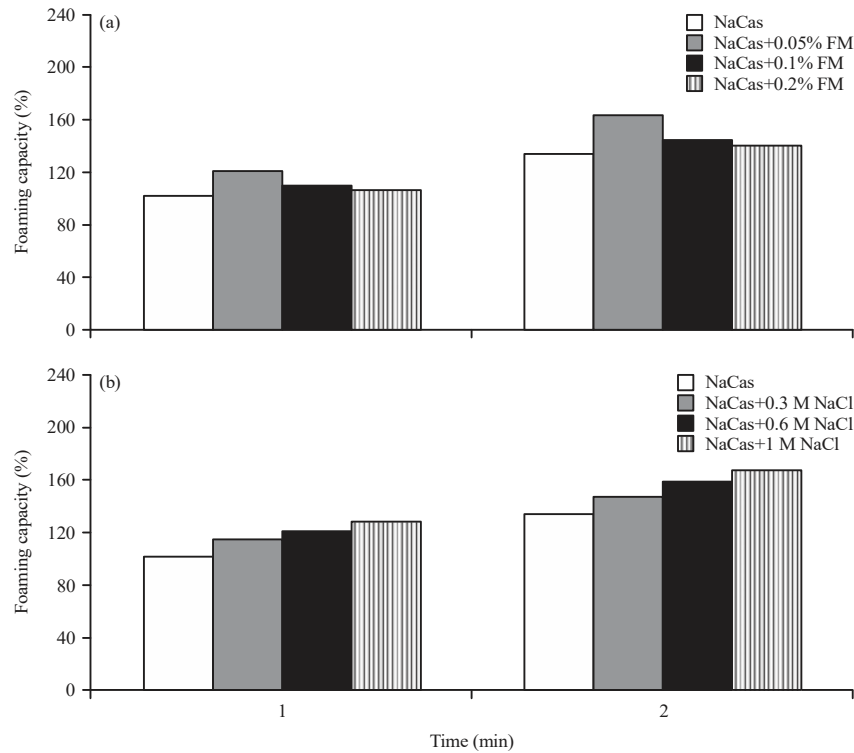


Fig. 3(a-b): Foaming capacity of 2% sodium caseinate solutions containing different concentrations of (a) Flaxseed mucilage and (b) Sodium chloride

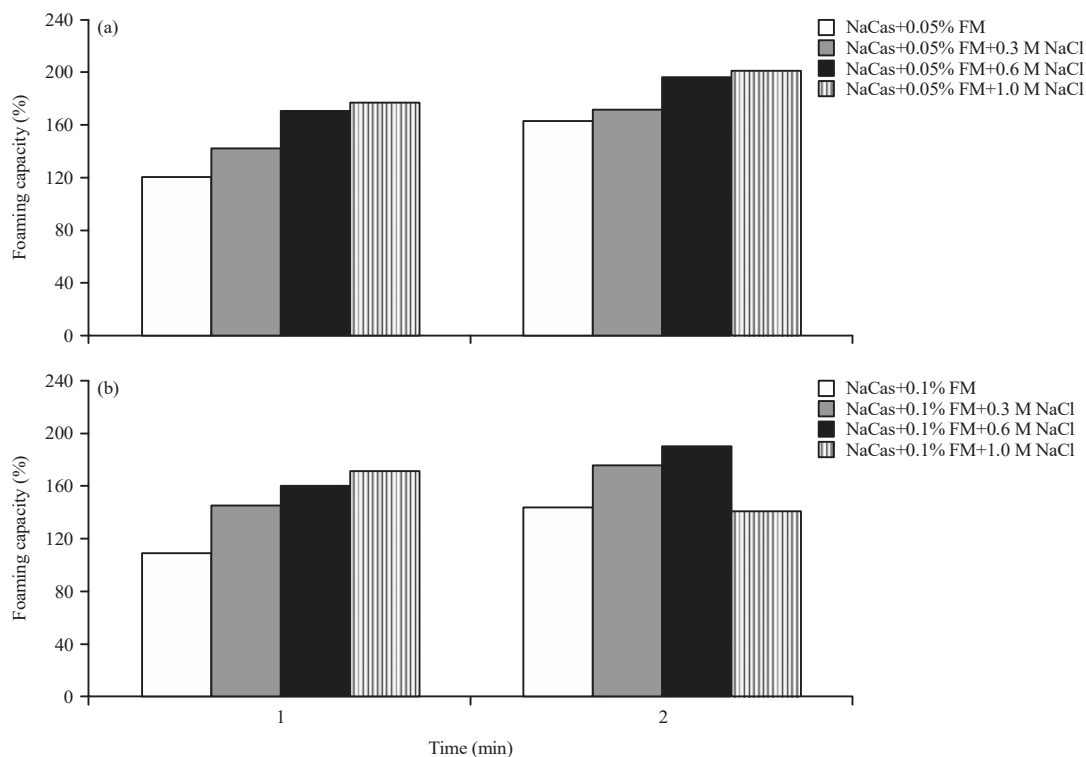


Fig. 4(a-b): Foaming capacity of 2% sodium caseinate solutions containing (a) 0.05% and (b) 0.1% flaxseed mucilage in the presence of different concentrations of sodium chloride

The foam stability is a measure the decay of foam height over times. The slope of the curves shows the decreasing rate of foam with time. Figure 5 and 6 show that the foam was most stable in the presence of FM and that the stability increased as the concentration of FM increased from 0.05-0.2% ($p < 0.05$) which related to the increase of the solution viscosity. Adapa *et al.*³² reported that the increase of the mix viscosity could be the primary reason for the decrease of foaming capacity but could favor foam stability. Also, the foam of NaCas solution was more stable in the presence of NaCl. The foam stability during the first 30 min increased ($p < 0.05$) as the concentrations of NaCl increased from 0.3-1 M ($p < 0.05$) but no significant differences were found thereafter. When NaCl was added to NaCas solution containing FM, the foam stability was reduced compared to those containing only FM. The improvement of foam stability of NaCas solution on adding NaCl may be due to the formation of more dense protein film³³. Mita *et al.*³⁴ reported that addition of NaCl up to 1 M to gluten solution (1%) resulted in a more protein compact film at air-water interface and increased foam stability. The NaCl also could affect the electrostatic interactions between chains of mucilage polymers resulting in the reduction in the viscosity and foam stability³⁵.

Emulsions activity and stability: As shown in Fig. 7a and b, the emulsion activity of NaCas solution was dependent on the concentration of FM and NaCl. The emulsion activity increased from 69.34-82.97% and increased from 69.34-80.0% as FM increased from 0.0-0.1% ($p < 0.05$) and NaCl increased from 0.0-0.6 M ($p < 0.05$), respectively but decreased on further addition of either. At higher added hydrocolloid concentrations (0.1%), creaming was inhibited due to the viscoelastic changes in interconnected regions of the emulsion droplets leading to flocculation and formation of a gel-like network³⁶. The emulsion stability of NaCas solution increased linearly from 67.19% at 0.0% FM to 77.78% at 0.2% FM, the difference was significant only at 0.1 and 0.2% FM ($p < 0.05$). When NaCl was added to NaCas solution, the emulsion stability slightly increased from 67.19% at 0.0 M NaCl to 71.36% at 0.3 M NaCl ($p > 0.05$). However, addition of further NaCl decreased emulsion stability to 66.67% at 1 M NaCl. Shao *et al.*³⁷ found that Na^+ concentration did not have a significant effect on emulsions stability.

When NaCl was added at the concentration of 0.3 and 0.6 M to the NaCas solution containing 0.05% FM, the emulsion activity increased linearly, the increase was better than that of NaCas solution containing only 0.05 FM or 0.3% M NaCl (Fig. 8a). Addition of more than 0.6 M NaCl to

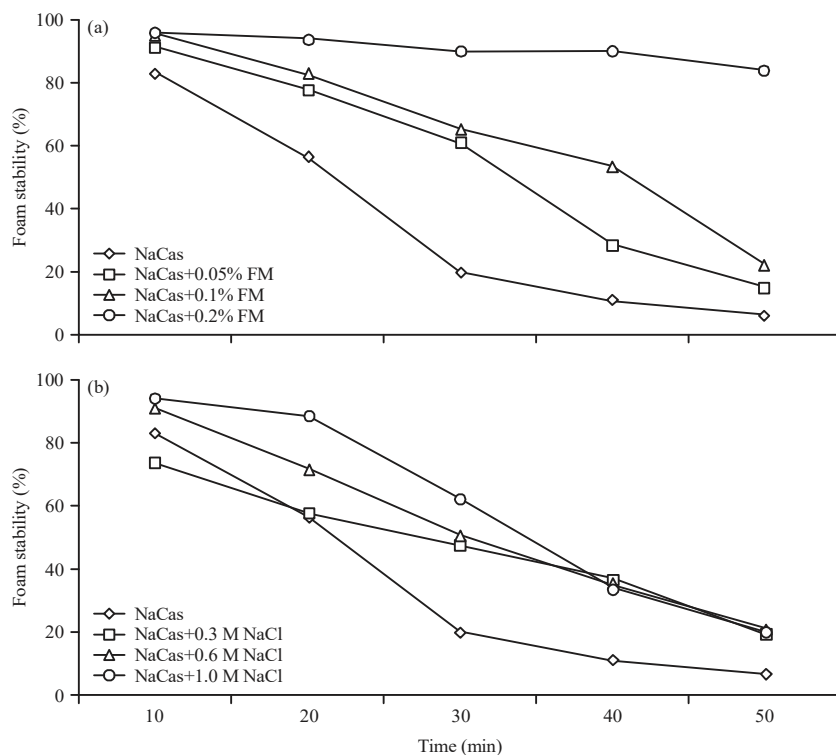


Fig. 5(a-b): Foam stability of 2% sodium caseinate solutions containing different concentrations of (a) Flaxseed mucilage and (b) Sodium chloride

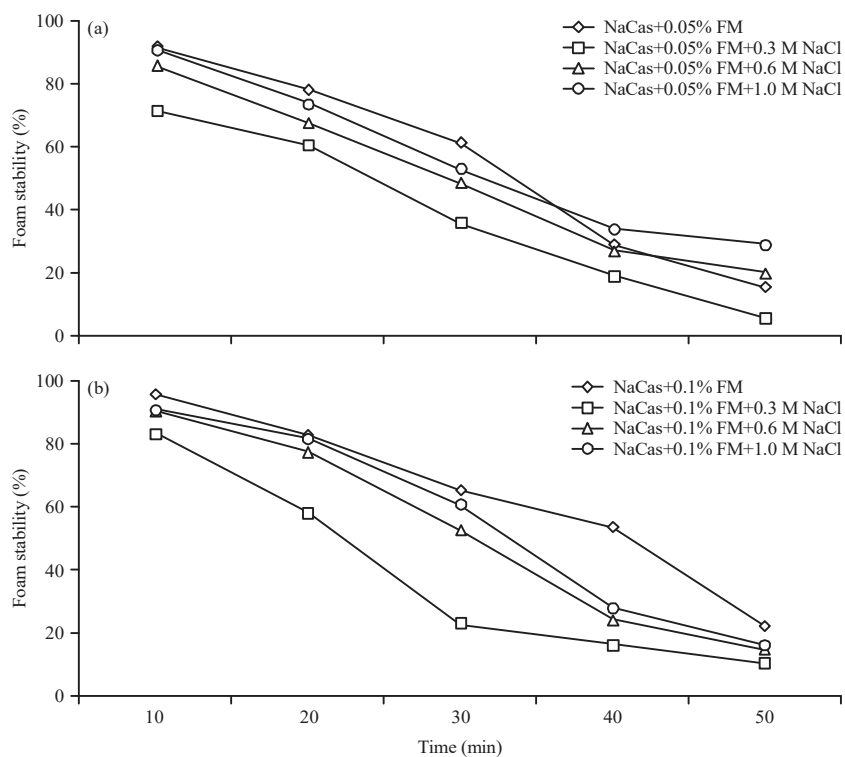


Fig. 6(a-b): Foam stability of 2% sodium caseinate solutions containing, (a) 0.05% and (b) 0.1% flaxseed mucilage in the presence of different concentrations of sodium chloride

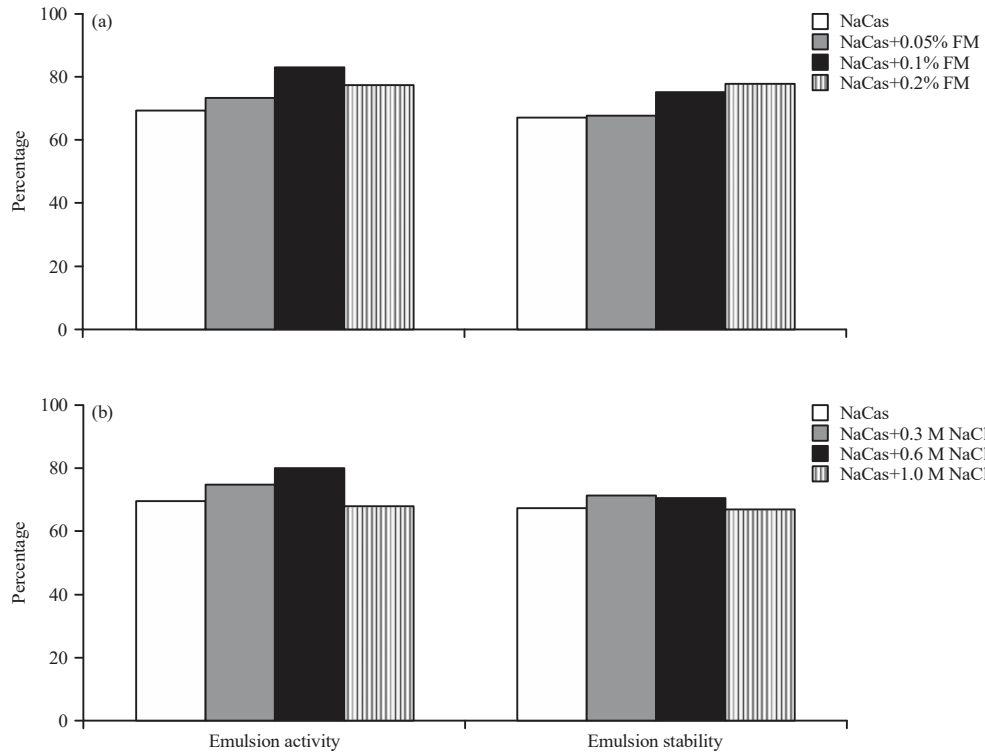


Fig. 7(a-b): Emulsions activity and stability of 2% sodium caseinate solutions containing different concentrations of (a) Flaxseed mucilage and (b) Sodium chloride

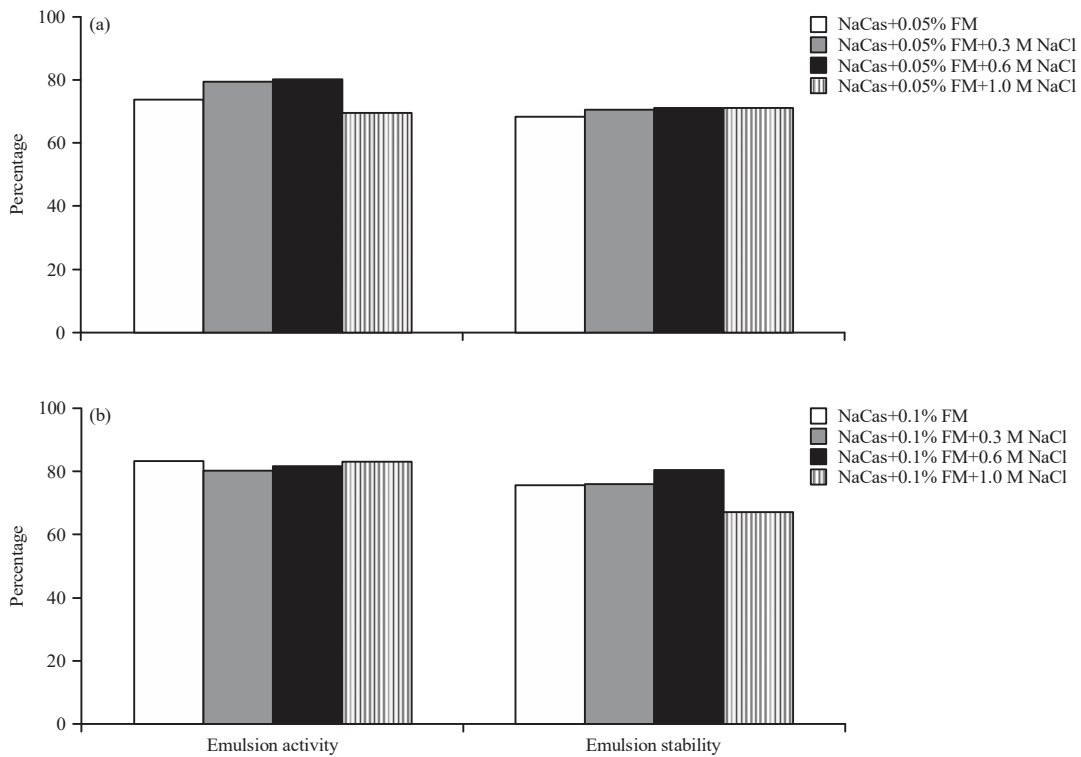


Fig. 8(a-b): Emulsions activity and stability of 2% sodium caseinate solutions containing, (a) 0.05% and (b) 0.1% flaxseed mucilage in the presence of different concentrations of sodium chloride

NaCas solution containing 0.05% FM reduced emulsion activity. However, the addition of NaCl, up to 1 M, to NaCas solution containing 0.1% FM had no significant effect ($p>0.05$) on emulsion activity (Fig. 8b). Addition of NaCl, up to 1 M or 0.6 M, to NaCas solution containing 0.05 or 0.1% FM, respectively had no significant effect on emulsion stability (Fig. 8a, b). When NaCl was added more than 0.6 M to the NaCas solution containing 0.1% FM decreased emulsion stability (Fig. 8b).

CONCLUSION

Addition FM alone or in combination with NaCl affected variably the functional properties of 2.0% NaCas solution. The viscosity and non-Newtonian behavior of NaCas solution (2.0%) increased with FM concentration, up to 0.2%, but decreased in the presence of NaCl, especially at high concentration of FM ($\geq 0.1\%$). The high concentration of FM ($>0.1\%$) or NaCl (>0.6 M) would not be a suitable emulsifying agent in NaCas solutions. Therefore, FM can be used in the dairy industry at low concentrations (≤ 0.1) to improve its functional properties. The addition of low FM increased the viscosity, foaming properties and emulsion activity and stability of the NaCas solution. Also, the FM would not be a suitable emulsifying agent in food systems with high concentrations of NaCl.

SIGNIFICANCE STATEMENT

This study discovers the Addition FM alone or in combination with NaCl affected variably the functional properties of 2.0% NaCas solution. The viscosity and non-Newtonian behavior of NaCas solution (2.0%) increased with FM concentration, up to 0.2%, but decreased in the presence of NaCl, especially at a high concentration of FM ($\geq 0.1\%$). The high concentration of FM ($>0.1\%$) or NaCl (>0.6 M) would not be a suitable emulsifying agent in NaCas solutions. That can be beneficial for the dairy industry by using FM at low concentrations (≤ 0.1) to improve its functional properties. The addition of low FM increased the viscosity, foaming properties and emulsion activity and stability of the NaCas solution. Also, the FM would not be a suitable emulsifying agent in food systems with high concentrations of NaCl.

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