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ARTICLE

Effect of NaCl Concentration on the Emulsifying Properties of Myofibrilla Protein in the Soybean Oil and Fish Oil Emulsion

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Abstract

The aim of the present work was to investigate the effect of NaCl concentration on the emulsifying and rheological properties of porcine myofibrillar protein (MF)-stabilized soybean oil and fish oil emulsion (SO-EMs and FO-EMs). Emulsions (EMs) were prepared from 1% MF with 10% SO or FO at various NaCl concentration (0-0.5 M). The emulsifying ability index (EAI) of the EMs increased with increasing NaCl concentration for both oil types. Conversely, increasing NaCl manifested decrease in the emulsion stability index (ESI). In addition, creaming index (CI) also increased with NaCl concentration. From the microscopic observation, droplets of the EMs were more aggregated at relatively higher NaCl concentrations, especially for FO-EMs. All EMs had a gel-like structure owing to G' > G" from the rheological analysis. Comparing the oil types, the emulsifying capacity of SO-EMs was more stable than that of FO-EMs at all NaCl concentrations as determined from the CI value and microscopic observation. Therefore, it can be concluded that SO-EMs and FO-EMs are more stable at relatively lower concentrations of NaCl. In addition, the dispersed stability of SO-EMs was better than that of FO-EMs at the same concentration of NaCl.

Keywords: myofibrillar protein, emulsion, fish oil, soybean oil, NaCl

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Introduction

Muscle consists of two major proteins, sarcoplasmic protein and myofibrillar protein (MP), and the MP plays a key role in the various functional properties of meat products. The functional properties of MP include gel forming (protein-protein interactions), emulsifying (protein-lipid interactions), and water binding (protein-water interactions). Gel forming has been extensively investigated for a broad range of ionic strengths (NaCl concentration) and pH owing to its importance in the texturization of the final products (Tornberg, 2005). According to the salt-soluble nature of MP, ionic strength has been recognized as the most important factor in terms of regulating the functional properties of MP. It was reported that the enhancement of protein-protein interactions resulted in weakening of protein-water or protein-lipid interactions (Chin *et al.*, 2009).

In regular emulsion-type meat products, meat batter is formulated with ~20% lipids (mostly pork backfat) and additional water, hence emulsification followed by thermal gelation accounts for the mechanisms involved in meat product formation. During the chopping process, MP is extracted by NaCl and phosphate, and the MP is adsorbed onto the surface of fat globules. Based on the proximate composition of meat products, meat batter was traditionally considered as an oil-in-water (O/W) emulsion system in which the extracted and solubilized MP acted as an emulsifier (Hansen, 1960). More recently, however, the importance of the functional properties of MP has been focussed on the coagulating network formation, in which the melted fat and added water are immobilized (Hamm, 1986). Consequently, protein-protein interactions are considered an important factor in terms of water binding and fat binding in emulsion-type meat products (Ziegler and Acton, 1984). Despite numerous investigation of MP functionality in meat products, little investigation regarding the role of MP in highly diluted system such as meat sauces, soups or stews is available. The action of the MP in these systems might be distinguished from the semi-solid products and detail investigation on the emulsifica-

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tion and rheological properties of the liquid products are necessary.

The consumption of meat products has a long history throughout the world, but it is currently associated with a high saturated fat and sodium diet. In terms of the consumption of high saturated fat, research has been conducted to replace animal fat with plant or fish oils (Jiménez-Colmenero *et al.*, 2013; Josquin *et al.*, 2012; Ospina-E *et al.*, 2010). It is widely reported that the consumption of unsaturated fatty acids from plant or fish sources provides many advantages to human physiology. However, lipid oxidation, textural modification, and low sensorial preference (particularly from fish oil) limit their application in meat product formulation (Josquin *et al.*, 2012; Marchetti *et al.*, 2014).

Based on the nutritional value of meat, researchers have focused on rheological and physicochemical properties in model studies resulting from variations of pH and NaCl concentration (Chin *et al.,* 2009). However, these model studies were carried out using emulsion-type sausage products or patties. Actually, meat components are also used in sauces, soups, and stews. Therefore, in this study, our ultimate goal was to confirm the effect of NaCl in soybean oil or fish oil emulsions (SO-EMs or FO-EMs) when MF was used as an emulsifier and to investigate their emulsifying and rheological properties.

Materials and Methods

Materials

Fresh pork, *M. longissimus dorsi*, was obtained from a local meat market (24 to 48 h post-mortem, pH 5.6 to 5.9). All visible fat and connective tissue was trimmed out, cut into 1 cm cubes, and then mixed randomly. The meat cubes were packaged in polyethylene bags (approximately 200 g) and stored at 80°C prior to use. To prepare the emulsions, soybean oil (SO) and fish oil (FO) were purchased from Sajo Haepyo (Korea) and Sigma-Aldrich Chemical Company (USA), respectively. All chemical products were of analytical grade.

Extraction of myofibrillar protein (MP)

Myofibrillar protein isolate was prepared in a previous study (Hong *et al.*, 2012; Xiong, 1992), with all preparation steps performed at 4°C. The meat (200 g) was washed two times with 4 vol% (w/v) of 0.1 M NaCl and 50 mM sodium phosphate buffer (pH 6.5), followed by washing with 8 vol% (w/v) of 0.1 M NaCl. The MP suspension was filtered using gauze in order to remove the impurities and the pH was adjusted to 6.5 using 1 M HCl or NaOH. Centrifugation was conducted for 15 min at 1,000 *g* at 4°C between each wash. After extraction, the concentration of MP was determined by the Biuret method (Gornall *et al.*, 1949).

Emulsion preparation

MF was diluted with 50 mM sodium phosphate buffer at 1% protein concentration. For the preparation of the EMs, 3 mL of soybean oil or fish oil were emulsified with 27 mL of MF (10 mg/mL) containing 0, 0.1, 0.3, and 0.5 M NaCl. The solutions were homogenized with an Ultra-Turrax (T25 basic, IKA Works Inc., USA) system at 13,000 rpm (217 s^{-1}) for 3 min. The samples were stored in a walk-in cooler maintained at 4°C.

Emulsion properties

The emulsifying activity index (EAI) and emulsion stability index (ESI) were measured by the turbidimetric technique (Pearce and Kinsella, 1978). The EMs (50 μL) were diluted with 5 mL of 0.1% (w/w) sodium dodecyl sulfate (SDS) and the absorbance was measured at 500 nm. The EAI was mathematically calculated as follows:

$$EAI\ (\mathrm{m}^2/g) = \frac{2 \times 2.303 \times A_{500} \times D}{\varnothing \times C \times 10{,}000}$$

where A_{500} is the absorbance at 500 nm, Ø the volume of oil, C the protein concentration (g/mL), and D the dilution factor. The ESI was determined in accordance with the following equation:

$$ESI(\%) = \frac{A_t}{A_0} \times 100$$

where A_0 and A_t are the absorbance (500 nm) at 0 and 3 h, respectively.

Creaming index

The creaming index (CI) of EM-stabilized MF was determined by the method reported by Ionescu *et al.* (2008). To evaluate the long-term stability of SO-EMs and FO-EMs as a function of salt levels, 15 g EM samples were transferred to a test tube and maintained at ambient temperature overnight. The creaming index was determined as follows:

$$CI\ (\%) = \frac{H_c}{H_t} \times 100$$

where H_c is the height of the cream and H_t is the total height of the EMs.

Analysis of ζ-potential

The ζ-potential of SO- or FO-EMs at various salt levels was determined by dynamic light scattering using a Zetasizer Nano-ZS90 (Malvern Instruments, UK) system. All samples were diluted with distilled water prior to each measurement in order to avoid multiple light-scattering effects. All measurements were taken in triplicate.

Optical microscopy

After EM preparation, a drop of each EM was placed on a slide glass and covered with a cover glass. The microstructures of the EMs as a function of salt levels were observed directly using an optical microscope (Olympus CX31RTSF, Japan).

Rheological properties

The rheological properties of the EMs as a function of salt level were measured using a Rheometer® (Anton Paar, Austria) with a CP50-2 plate and a probe in small-amplitude oscillatory sweep mode. 1.25 mL of SO-EMs or FO-EMs with various salt levels were loaded onto the lower plate. The upper plate (50 mm diameter) was slowly lowered to close the gap between the two parallel plates to 1 mm. The frequency sweeps of the EMs oscillated from 0.01 to 10 Hz at 20°C, and all measurements were preformed within the identified linear viscoelastic region and fixed at 2% strain. The storage modulus (G'), loss modulus (G"), and Tan δ values (the ratio of G"/G') are presented to demonstrate viscoelastic changes.

Statistical analysis

The data were analyzed by ANOVA using the SAS statistical program 9.2 (SAS Institute, USA). Differences among the means were compared using Duncan's Multiple Range test, and the correlations between independent variables and measured values were calculated as Pearson's correlation coefficients. All measurements were performed on at least three preparation samples and are reported as means and standard deviations.

Results and Discussion

Emulsifying capacity and creaming stability

The effects of NaCl concentration (0-0.5 M) on the EAI and ESI values of EMs prepared with MF and SO or FO mixtures are presented in Table 1. The EAI values of SO-EMs and FO-EMs increased with increasing NaCl concentration. This means that a relatively high concentration of NaCl can stabilize the MF after EM formation regardless of oil type. Jang and Chin (2011) reported that a mixture of MF and SO showed the highest EAI value at pH values of 6.0 and 6.5 when mixed with 0.3 M NaCl. According to Zhang *et al.* (2009), the salts played an important role in EM properties in the meat products in that the salt reduces electrostatic repulsion between the droplets. High salt levels caused structural unfolding of the MP thereby altering the hydrophobic interactions between non-polar groups. Additionally, Hong and Chin (2010) reported that high salt levels (0.6 M NaCl) may promote electrostatic interaction by neutralization above pH 6.0. They have reported that the structure of MF was changed from a globular to a linear form by neutralization. The EAI values of the FO-EMs were higher than those of the SO-EMs. The ESI values showed similar trends, although there was statically no significant difference ($p>0.05$). Emulsifying activity is dictated by protein-protein and protein-lipid interactions (Das and Kinsella, 1990).

Table 1. Effects of NaCl concentration on emulsifying characterization of myofibrillar protein stabilized at different oil type

NaCl (M)	SO-EMs[3)]	FO-EMs[4)]
EAI (m^2/g)[1)]		
0	$17.05\pm0.397^{Cb,*}$	18.53 ± 0.300^{Da}
0.1	16.86 ± 0.815^{Cb}	19.83 ± 0.192^{Ca}
0.3	24.89 ± 0.687^{Bb}	29.40 ± 0.359^{Ba}
0.5	26.42 ± 0.683^{Ab}	34.16 ± 0.467^{Aa}
ESI (%)[2)]		
0	97.42 ± 4.416^{Aa}	98.42 ± 7.060^{Aa}
0.1	73.69 ± 5.040^{Ba}	83.85 ± 5.054^{Ba}
0.3	47.49 ± 8.952^{Ca}	61.04 ± 3.483^{Ca}
0.5	46.91 ± 3.951^{Cb}	63.33 ± 3.673^{Ca}

[1)]EAI, emulsifying activity index; [2)]ESI, emulsifying stability index; [3)]SO-EMs, soybean oil emulsions; [4)]FO-EMs, fish oil emulsions.
[*]Mean±standard deviation of triplicate determinations (n=3).
[A-D]Means with different superscripts within the same column are significantly different ($p<0.05$). [a-b]Means with different superscripts within the same row are significantly different ($p<0.05$).

The ESI values of the SO-EMs and FO-EMs were the highest at 0.0 M NaCl, and the values decreased with increasing NaCl concentration. Our results showed opposite trend that the MF at the high concentration of cationic ion stabilizes the EMs at meat product since MF can be dissolved in a saline solution, resulting in an increase in the viscosity of the EM. Moreover, the ESI value without the addition of NaCl showed the highest value. Although the additional NaCl was not mixed into the EM with MF, Na ions were present in the MF solution from the extraction of pork.

The effect of NaCl concentration on the CI of the SO-

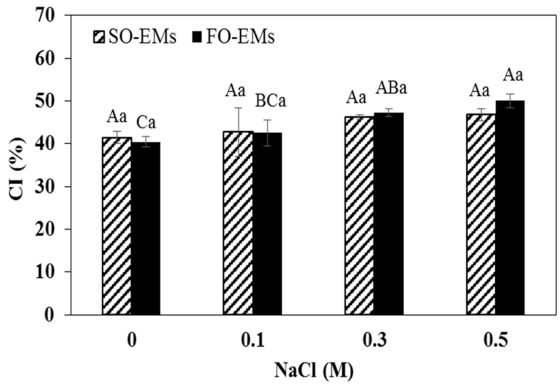

Fig. 1. Effects of NaCl concentration on creaming index (CI) of myofibrillar protein-stabilized SO-EMs and FO-EMs. Mean±standard deviation of triplicate determinations (n=3). $^{A-D}$Mean with different capital letters are significantly different (p<0.05) when comparing the salt levels in the same oil type. $^{a-b}$Mean with different small letters are significantly different (p<0.05) when comparing oil types in each formulation.

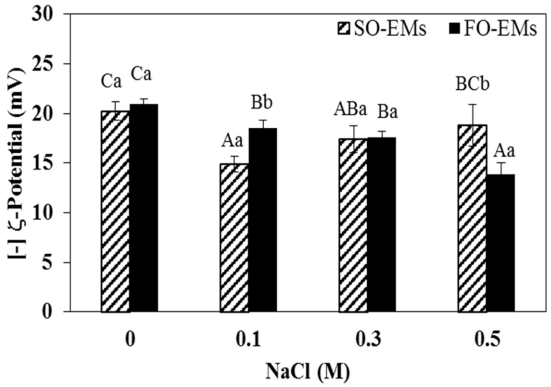

Fig. 2. Effects of NaCl concentration on ζ-potential of myofibrillar protein-stabilized SO-EMs and FO-EMs. Mean ±standard deviation of triplicate determinations (n=3). $^{A-D}$ Mean with different capital letters are significantly different (p<0.05) when comparing the salt levels in the same oil type. $^{a-b}$Mean with different small letters are significantly different (p<0.05) when comparing oil types in each formulation.

EMs or FO-EMs with MF is shown in Fig. 1. There was no significant difference in the CI of the SO-EMs with increasing NaCl concentration (p>0.05). Nonetheless, the CI of the FO-EMs tended to increase with increasing NaCl concentration, whereas the CI did not significantly change as a function of oil type at each NaCl concentration (p>0.05). Creaming contributes to the migration of the dispersed phase of an EM under the influence of buoyancy. In general, a high concentration of NaCl with MF in a meat product can maintain the long-term stability of EMs owing to the improved viscosity of the continuous phase (Jang and Chin, 2011). However, the CI value increased with increasing NaCl concentration, similar to the increase of ESI value regardless of oil type.

From these results, we supposed that MF can be aggregated by three-dimensional cross-linking of Na ions at the protein (MF)-protein (MF) interface, namely, at the interface between the MF on the droplet surface and the Na ion. Similar trends in ESI values were reported by Zhang *et al.* (2009), in which ESI values were unaffected by high salt levels. They suggested that this may be attributable to the collision of particles at high salt levels (>0.3 M). In contrast to our results, Hong *et al.* (2012) showed that ESI values increased with increasing NaCl concentration from 0 M to 0.3 M at pH 6.5 in gel-type samples. Wu *et al.* (2009) studied the rheological and microstructural properties of porcine MF-lipid EM composite gels. They reported that the EAI values were higher for peanut oil than for lard. In our results, SO-EMs with MF can form more stable EMs than FO-EMs with increasing NaCl concentration. The results seem to indicate that MP could disperse vegetable oils more efficiently than liquid animal fats, even though both were in a liquid state when homogenized. It can be suggested that unsaturated fatty acids, which are structurally more flexible than saturated fatty acids, could more strongly interact with proteins at the oil/water interfaces. Therefore, the values of CI and ESI for the FO-EMs were higher than those of the SO-EMs at the same concentration of NaCl.

ζ-potential

The ζ-potential of the SO-EMs and FO-EMs at various NaCl concentrations is presented in Fig. 2. At 0.0 M NaCl concentration, the EMs showed the highest ζ-potential value, -20.2 mV and -21.0 mV for the SO-EMs and FO-EMs, respectively. For the SO-EMs, the ζ-potential increased with increasing NaCl concentration. However, the ζ-potential of the FO-EMs consistently decreased with increasing NaCl concentration, from -21.0 mV to -13.9 mV. The emulsion droplets have an electrical charge depending on surfactant type on the oil surface, and a pH of the aqueous phase (McClements, 2005). Generally, the intensity of ζ-potential could be decided the concentration of oil or electrical intensity of surfactant. It is an indirect measurement of the electrical charge of the colloidal droplets and indicates moderate stability of EMs or dispersions when they are over ±30 mV (McClements, 2005). The lower value of the ζ-potential means that the EM

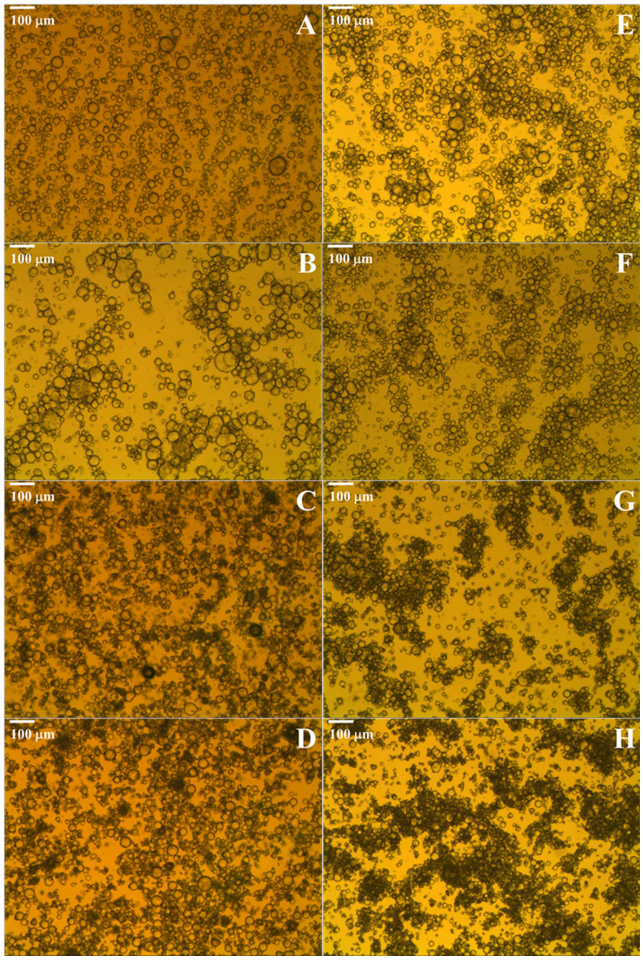

Fig. 3. Effects of NaCl concentration (A and E, 0 M; B and F, 0.1 M; C and G, 0.3 M; D and H, 0.5 M) on optical microstructure of myofibrillar protein-stabilized oil types: (A-D) SO-EMs and (E-F) FO-EMs.

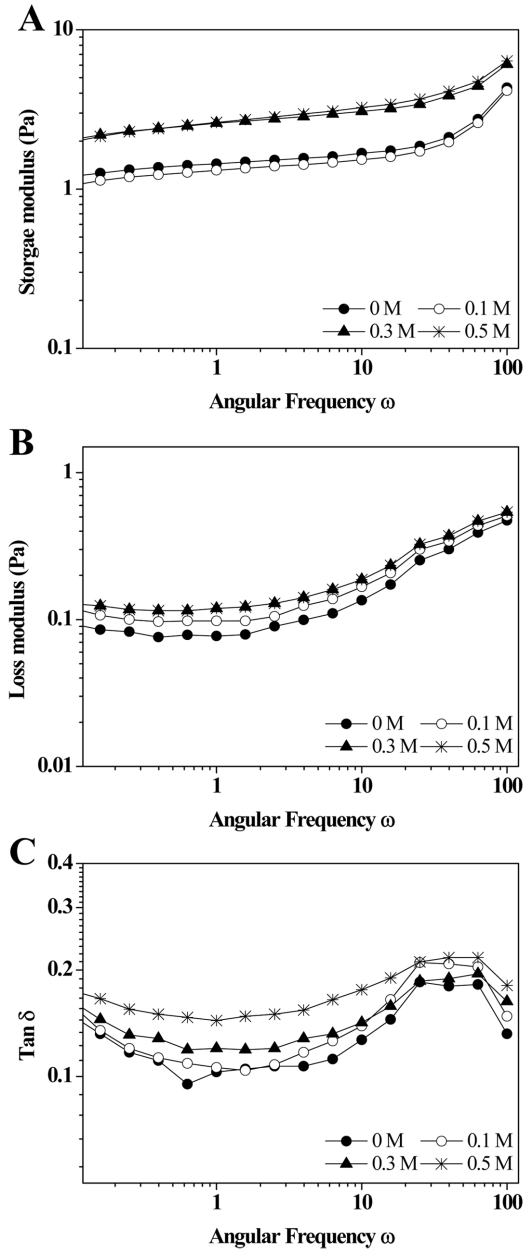

Fig. 4. Effects of NaCl concentration on (A) shear storage modulus, (B) shear loss modulus, and (C) Tan δ of myofibrillar protein stabilized at SO-EMs.

could be destroyed over time. From our results, the addition of NaCl caused the neutralization of the EM system by the interaction between Na ions and MF on the surfaces of the droplets.

Microstructure of emulsions

Fig. 3 shows the degree of coalescence and flocculation as a function of the NaCl concentration for the different oil type. The SO-EMs and FO-EMs formed stable droplets at low NaCl concentrations (0.0-0.1 M). With increasing NaCl concentration, aggregates appeared. Observation of the microstructure showed that the droplets of SO-EM (0.0 M NaCl) were homogenously dispersed without coalescence and flocculation. On the other hand, the SO-EM droplets at 0.1 M NaCl were larger than those of 0.0 M NaCl (Fig. 3A and B), which can be explained by droplet coalescence and flocculation, thereby resulting in low EM stability. The microstructures of the SO-EMs at 0.3 M and 0.5 M NaCl showed that the droplets were entrapped by the aggregation between MF and MF (Fig. 3C and D). The microstructures of the FO-EMs were similar to those of the SO-EMs. In particular, the FO-EMs were flocculated more than the SO-EMs at high NaCl concentrations (above 0.3 M). Namely, the FO-EMs showed intensive aggregation and excessive flocculation among EM droplets at 0.3 M and 0.5 M NaCl. These images support our claim that higher concentrations of NaCl can interact

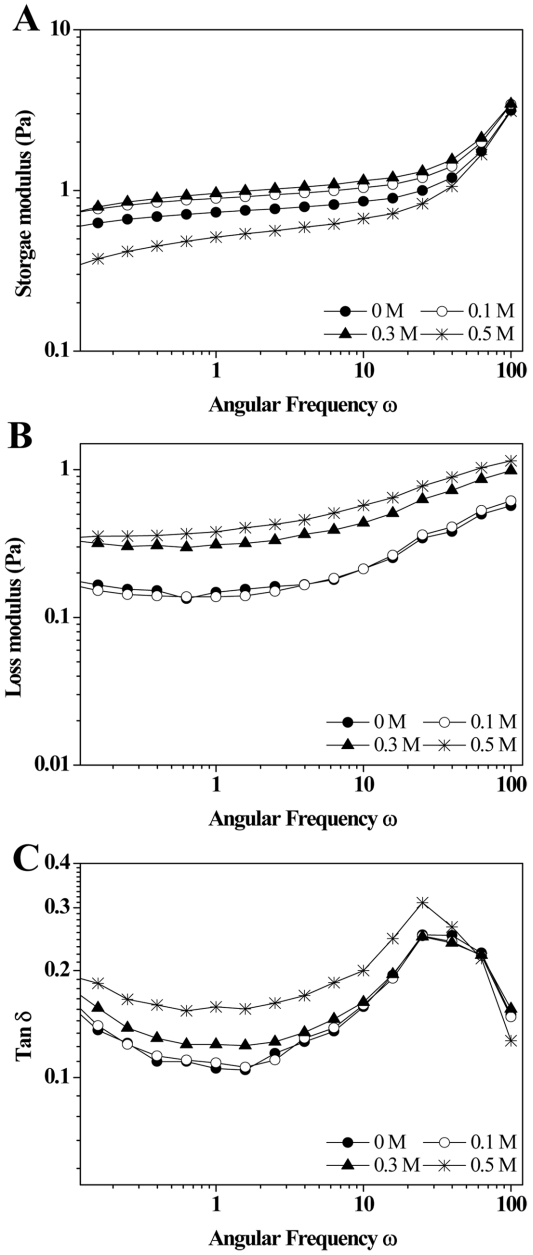

Fig. 5. Effects of NaCl concentration on (A) shear storage modulus, (B) shear loss modulus, and (C) Tan δ of myofibrillar protein stabilized at FO-EMs.

with proteins at the oil/water interface. Consequently, the oil droplet was entrapped the aggregates of MFs at the high component of unsaturated fatty acid.

Rheological properties

The changes in dynamic viscoelastic properties of the SO-EMs and FO-EMs with various NaCl concentrations under oscillating frequency are indicated by monitoring the storage modulus (G'), loss modulus (G"), and Tan δ = (G"/G'). The changes in G' and G" as a function of frequency of all EMs are shown in Fig. 4A and 5A, respectively. No cross-point was observed and the changes in G' and G" both increased as the frequency increased, indicating that the EMs displayed a weak gel-like structure. Especially, the G_n values of the SO-EMs at high concentrations of NaCl (0.3 M and 0.5 M) were lower than those at low concentrations (0 M and 0.1 M). Namely, the rheological properties of the EMs prepared at high NaCl concentrations showed more elastic behavior. The EM rheology also can be expressed by Tan δ. All samples showed Tan δ < 1, as indicated by G' > G". Tan δ is the relative distribution of "viscosity" as compared with "elasticity" during the EM formation, namely, the higher the value, the more viscous or less elastic the material (Ferry, 1980). Therefore, MF emulsified with SO or FO shows elastic behaviour. According to study of Wu *et al.* (2009), although different oil types had a strong influence on the physical properties (emulsifying and microstructure properties) of the MF, their rheological properties were similar regardless of oil type.

Conclusion

From our results, higher concentrations of NaCl can affect the aggregation of oil droplets in O/W emulsions owing to the interaction between MF (protein) and Na ions. Despite that NaCl increased emulsifying capacity of MP, the NaCl also attributed to decrease in stability of MP-stabilized emulsions. Comparing the oil types, the SO-EMs were more stable than the FO-EMs at higher concentrations of NaCl owing to the fact that they contain fatty acids. In terms of using MF as an emulsifier, it can be used in liquid products such as sauces and soups with low salt content.

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