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ARTICLE

# Emulsifying and Gelling Properties of Pork Myofibrillar Protein as Affected by Various NaCl Levels and pH Values

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**Abstract**

The effects of various NaCl levels (0, 0.3, and 0.6 M) and pH values (pH 5.0, 5.5, 6.0, and 6.5) on the emulsifying and gelling properties of pork myofibrillar protein (MP) were assessed. The emulsion stability index (ESI), emulsifying activity index (EAI), and creaming index were measured at a 1:20 ratio of MP to corn oil. The EAI and ESI of pork MP showed maximum values at pHs 6.0 and 6.5 and at 0.3 M NaCl, resulting in better emulsion properties. Additionally, the cooking yield (CY) and gel strength (GS) of emulsified MP gel were measured at an MP: corn oil ratio of 1: 2; GS increased with increasing levels of salt. At 0.6 M NaCl, GS decreased with decreasing pH from 5.5 to 6.5. GS and gelling properties were optimal at pH 5.5 in 0.6 M salt. The highest CY was observed at 0.6 M NaCl, regardless of the pH value. However, increasing pH increased CY at salt levels of 0 and 0.3 M. These results indicate that NaCl and pH profoundly affected the emulsified MP system. Future work will be conducted on the rheological properties of the pork emulsified system as affected by adding non-meat protein.

**Key words:** emulsion, myofibrillar protein, salt level, pH value, gelling properties

## Introduction

Myofibrillar proteins (MPs) contribute to the characteristics of muscle proteins. In particular, they improve the texture of heat-induced gels and play an important role in meat processing (Ionescu *et al*., 2008). Emulsion is an important technology in meat processing, and affects product quality, such as flavor and texture (Ramirez-Suarez and Xiong, 2003). It has been previously reported that the hydrophobic myosin head combined with fat, whereas the hydrophilic myosin tail combined with water (Park *et al*., 2003). Thus, MP would be expected to contribute to emulsifying properties – in particular, emulsifying activity and emulsion stability. Currently, consumers tend to prefer emulsified meat products that contain as low a fat content as possible, although fat is an important factor in food palatability, and also stabilizes emulsion systems. Destabilization of emulsion can occur through sequential mechanisms – generally, creaming, sedimentation, flocculation, coalescence, and phase separation (McClements, 1994). Changes in pH and salt levels have been implicated as possible factors that affect emulsion stability (Thakur *et al*., 2006). It has also been previously reported that changes in pH affected gelling properties, emulsifying properties, and water holding capacity (Bertram *et al*., 2004; Manoi and Rizvi, 2009). Moreover, the addition of salt improves the solubility of meat batter, resulting in improvements in gelling and emulsifying properties (Guzey and McClements, 2006). Therefore, this study was carried out to evaluate the effects of various NaCl levels (0, 0.3, and 0.6 M) and pH values (pH 5.0, 5.5, 6.0, and 6.5) on the emulsifying and gelling properties of pork MP.

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## Materials and Methods

### Materials

Pork loin (crossbreed of Landrace × Yorkshire × Duroc, 6 months old hogs), at pH 5.5-5.7 were collected from the *M. longissimus dorsi* carcasses at 24 h post-mortem. All fat and connective tissues were trimmed out and cut into a cubic shape (1 $cm^3$). The meat cubes were portioned at approximately 200 g before stored at -70°C under vacuum until analyzed.

**Extraction of myofibrillar protein (MP)**

Myofibrillar protein isolate (MPI) was prepared by the previous method (Hong and Chin, 2010) from the pork loin by washing three times with 4 volumes (v/w) of 0.1 M NaCl, 50 mM $NaH_2PO_4$ buffer (pH 6.25), followed by washing with 8 volumes (v/w) of 0.1 M NaCl (pH 6.25). The MP was adjusted to targeted pH values (5.0, 5.5, 6.0, and 6.5) using 1 N HCl or NaOH (Hong and Chin, 2010) and centrifuged for 15 min at 3,000 g at 4°C (Beckman, USA). Protein concentrations were determined by the Biuret method (Gornall *et al*., 1949).

**Emulsion preparation and properties**

The emulsion was prepared at different pH values (5.0, 5.5, 6.0, and 6.5) and NaCl levels (0, 0.3, and 0.6 M) using an MP : corn oil ratio of 1:20. The mixtures were homogenized for 2 min using an Ultra-Turrax (T25 Basic, Ika Works, Inc., USA) at 13,000 rpm. The emulsion stability index (ESI) and emulsifying activity index (EAI) were measured by the turbidimetric method (Pearce and Kinsella, 1978). The homogenates (2.7 mL) were mixed with 0.3 mL of 0.1% sodium dodecyl sulfate (SDS). The absorbance of diluted emulsion was measured at 500 nm and reported as the initial absorbance ($A_0$). Thereafter, the samples were maintained for 3 h at 20°C prior to determining the incubated absorbance ($A_t$). The emulsifying activity index was calculated in accordance with the equation shown below:

$$\text{EAI } (m^2/g) = \frac{2 \times 2.303}{\Phi \times C \times 10.000} \times A_{500} \times D \quad (1)$$

$\Phi$ is the volume of oil, C is the protein concentration (g/mL), and D is the dilution factor

$$\text{ESI } (\%) = \frac{A_t}{A_0} \times 100 \quad (2)$$

$A_0$ and $A_t$ are the absorbance values at 500 nm at 0 and 3 h, respectively.

**Creaming index**

The creaming index confirms emulsion stabilized of MP by the method (Ionescu *et al*., 2008). Emulsions were transferred into test tubes and stored for 24 h at room temperature. The creaming index was determined as in equation (3):

$$\text{Creaming index } (\%) = \frac{H_s}{H_t} \times 100 \quad (3)$$

in which $H_t$ is the height of the initial emulsion, and Hs is the height of the separated emulsion.

**Cooking yield and gel strength**

The emulsified MP was prepared at different pH values (5.0, 5.5, 6.0, and 6.5) and NaCl concentrations (0, 0.3, and 0.6 M) at 3% MP. The ratio of MP and corn oil was controlled at 1: 2. The mixture was cooked in a water bath heated from 5 to 80°C for about 30 min. The cooking yield was calculated from the equation:

$$\text{Cooking yield} = (W_a/W_I) \times 100$$

in which $W_{a=}$ the weight of the remaining gels.

$W_I$ is the weight of the initial gels.

In order to measure the gel strength of the emulsified MP samples (12 mm diameter and 20 mm height), a puncture test was conducted using an Instron (model 3344, Instron Corporation, USA) equipped with a stainless probe (diameter = 9 mm); the head speed was controlled at 50 mm/min.

**Statistical analysis**

Data were analyzed by two-way ANOVA using the SPSS statistical software package, version 17.0. The main factors included pH values (5.0, 5.5, 6.0, and 6.5) and NaCl concentrations (0, 0.3, and 0.6 M). Differences among the means were compared using Duncan's multiple range test. Each measurement was conducted in duplicate and the entire experiment was replicated three times.

## Results and Discussion

**Emulsifying properties**

The effects of salt concentrations and pH values on the EAI of emulsions prepared with myofibrillar protein (MP) and corn oil mixtures are shown in Table 1. The EAI of emulsified pork MP did not differ among various pH values at 0 M salt ($p$>0.05). EAIs at 0.3 M were increased when pHs were increased, and the highest value was found at the pH values of either 6.0 or 6.5 (Table 1). It has been reported that the isoelectric point (PI) of MP was approximately pH 5.2 (Huff-Lonergan and Lonergan, 2005; Westphalen *et al*., 2005). Additionally, EAI seems to have the lowest value at the pI of meat protein, due to the repulsion of proteins induced by reduced charges. At pH 5.0, the EAI of emulsified MP increased with increasing salt concentrations (Table 1). These results suggest that the increased ionic strength at pH 5.0 and 5.5 could change the charge of the proteins, thereby the results may

improve emulsifying properties. However, the MP and corn oil mixtures at pH 6.0 and 6.5 showed the highest EAI values at a 0.3 M salt, when compared to values observed at 0 or 0.6 M salt. High salt levels (0.6 M salt) may promote electrostatic interaction by charge neutralization at pH higher than 6.0. Thus, the EAI was affected by salt and pH which they were interacted. Agyare *et al*. (2009) reported that an increase in ionic strength did not influence emulsion properties. Zhang *et al.* (2009) concluded that NaCl played an important role in emulsion properties, by two mechanisms. First, NaCl reduces electrostatic repulsion. Second, high salt levels may alter the hydrophobic interactions between non-polar groups. These results explain why EAI varied, depending on various NaCl and pH combinations in this study.

Although ESI values did not differ at lower pH values (pH 5.0 and 5.5), regardless of three different salt levels (Table 1), those were values increased at pH 6.0 and 6.5 in 0.3 M salt; this suggests that the optimal conditions would be a pH higher than 6.0 at a salt concentration of 0.3 M. Thus, ESI values were unaffected by high salt levels. This may be attributable to the collision of particles at high salt levels (>0.3 M). An increased cohesiveness of protein did not allow the protein to envelop fat globules and accelerate the phase separation by gravity (McClement, 1999).

The creaming index (CI) tended to be higher with increased pH and salt level, resulting in an optimal creaming index at both pH 6.0 and 6.5 at a salt level of 0.6 M salt level (Table 1). These results indicated that the optimal conditions for EAI and ESI were at pH 6.0 or 6.5 at a salt level of 0.3 M. A combination of higher pH and salt proved better for the CI. Creaming identified the separation of oil droplets by different densities, after which the fat globules have to rise to the surface. Creaming also contributes to the cohesive properties of protein particles and then droplets stable against coalescence maintain integrity for better emulsion stability (Robins *et al*., 2002). Thus, emulsion stability affects creaming and cohesion.

**Table 1. Emulsifying properties of pork myofibrillar protein as affected by various pH and salt levels**

| EAI ($m^2$/g)* | 0 M | 0.3 M | 0.6 M |
|---|---|---|---|
| pH 5.0 | 5.5±0.8$^{xB}$ | 5.5±0.5$^{yB}$ | 7.9±0.9$^{xA}$ |
| pH 5.5 | 6.0±0.3$^{xA}$ | 7.8±0.5$^{yA}$ | 6.5±2.5$^{xyA}$ |
| pH 6.0 | 6.1±0.6$^{xB}$ | 12.4±0.2$^{xA}$ | 3.7±0.3$^{yC}$ |
| pH 6.5 | 6.7±0.6$^{xB}$ | 10.8±2.4$^{xA}$ | 4.4±1.3$^{yB}$ |
| ESI (%)** | 0 M | 0.3 M | 0.6 M |
| pH 5.0 | 39.2±2.6$^{xA}$ | 34.4±2.8$^{zA}$ | 40.6±4.9$^{xA}$ |
| pH 5.5 | 38.6±1.5$^{xA}$ | 44.8±2.2$^{yA}$ | 42.0±8.4$^{xA}$ |
| pH 6.0 | 37.6±2.6$^{xB}$ | 56.3±0.6$^{xA}$ | 34.7±6.3$^{xB}$ |
| pH 6.5 | 41.0±2.7$^{xB}$ | 53.9±7.5$^{xA}$ | 43.1±2.4$^{xB}$ |
| CI (%)*** | 0 M | 0.3 M | 0.6 M |
| pH 5.0 | 62.7±3.2$^{xA}$ | 69.3±4.8$^{yA}$ | 67.4±7.3$^{yA}$ |
| pH 5.5 | 62.0±1.8$^{xB}$ | 58.4±2.4$^{zB}$ | 92.9±12.1$^{xA}$ |
| pH 6.0 | 57.9±0.4$^{yB}$ | 59.7±2.0$^{zB}$ | 100 $^{xA}$ |
| pH 6.5 | 54.9±1.9$^{yB}$ | 97.0±5.1$^{xA}$ | 100$^{xA}$ |

*EAI, emulsion activity index; **ESI, emulsion stability index; ***CI, creaming index

$^{A-C}$Means±SD with same superscript in a same raw are not different ($p$>0.05).

$^{x-z}$Means±SD with same superscript in a same column are not different ($p$>0.05).

**Cooking yield and gel strength**

The gel strength of emulsified pork MP was acceptable at most pH levels with salt level at 0.6 M NaCl, except at pH 5.0 (Fig. 1). Thus, the increased salt level improved the gelling properties of emulsified pork MP. ESI and EAI values at a salt level of 0.6 M were lower than those at a salt level of 0.3 M, whereas gel strength at 0.6 M salt was higher than that at 0.3 M salt. These results indicated that the maximum CY was achieved at pH 5.5 in 0.6 M salt or at pH 6.0 and 6.5 at salt level higher than 0.3 M. The acceptable gel strength was noted at higher salt level (0.6 M salt), regardless of pH values (pH >5.5).

CYs of the pork emulsified MP were improved at pH 6.0 and 6.5 at salt levels of either 0.3 or 0.6 M (Fig. 2). Additionally, the CY at pH 5.5 was also improved with higher salt level (0.6 M). Although WHC was lowest at the isoelectric point (PI) of protein, increased salt levels improved WHC (Westphalen *et al*., 2005). Thus, CY at high salt level (0.6 M) improved, regardless of the pH value. However, that value was higher at pH 6.5 than at pH

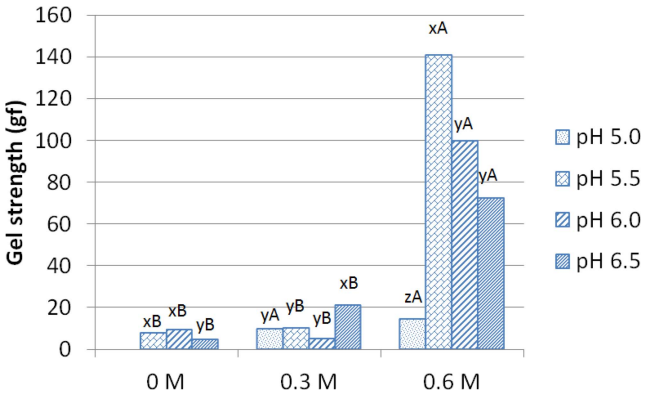


**Fig. 1. Gel strength (gf) of pork myofibrillar protein as affected by various pH and salt levels.** $^{A,B}$Same superscript in a same row are not different ($p$>0.05). $^{x-z}$Same superscript in a same column are not different ($p$>0.05).

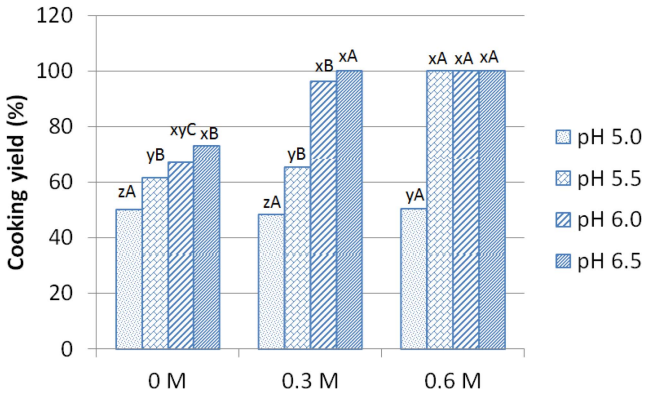


**Fig. 2. Cooking yield (%) of pork myofibrillar protein as affected by various pH and salt levels.** $^{A-C}$Same superscript in a same row are not different ($p$>0.05). $^{x-z}$Same superscript in a same column are not different ($p$>0.05).

5.5 in a salt value of 0.3 M. This was because high pH levels increased the negative charges of proteins (Guzey and McClements, 2006).

The optimum condition to obtain acceptable EAI and ESI of emulsified pork MP was pH values of 6.0 or 6.5 in a salt level of 0.3 M NaCl. However, higher pH and salt levels were better for the creaming index. The best combination for higher CY was at pH 6.5 and 0.3 M salt, and pH values higher than pH 5.5 and 0.6 M salt. Acceptable gel strength was observed at pH 6.5 and 0.3 M or higher salt level (pH >5.5), regardless of pH values.

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