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INTERACTION BETWEEN PORCINE MYOFIBRILLAR PROTEIN AND FOUR ANIONIC POLYSACCHARIDES: EFFECTS ON THE HEAT-INDUCED GELATION AND DIGESTIVE BEHAVIOR OF PROTEINS IN VITRO

Soeun Kim[1*], Kyung Jo[1], Minkyung Woo[1], Seul-Ki-Chan Jeong[1], Hayeon Jeon[1], Yun-Sang Choi[2], Samooel Jung[1]*, and Seonmin Lee[1]*

[1] Department of Animal Science and Biotechnology, Chungnam National University, Daejeon 34134, Korea

[2] Research Group of Food Processing, Korea Food Research Institute, Wanju 55365, South Korea.

*Corresponding author email: samooel@cnu.ac.kr, seonminlee@cnu.ac.kr

I. INTRODUCTION

Polysaccharides have been considered as the ingredients for animal fat substitutes and natural quality improvers in meat products [1]. Since ionic polysaccharides exhibit different interactions with proteins depending on their chain characteristics and surface charge, they can affect the gel properties by altering the structure and gel-forming behavior of myofibrillar proteins (MP) [2]. Changes in gel properties, such as gel strength, can affect the diffusion of digestive fluids and particle breakdown, ultimately altering proteins' in vitro digestibility [3]. Therefore, this study investigated the interaction mechanisms between four types of anionic polysaccharides (kappa-carrageenan (CAR), gum Arabic (GA), xanthan gum (XT), and sodium alginate (SA)) and MPs. The focus was on how the physicochemical properties of MP gels, influenced by different types of polysaccharides, affect in vitro digestion.

II. MATERIALS AND METHODS

The polysaccharides used in this study were named CAR, GA, XT, and SA. MP was extracted using pork hind legs described by Lee et al. [4]. Each polysaccharide was mixed into the MP solution at an equal concentration of 0.02%, resulting in a final protein concentration of 5 mg/mL. Mixtures of MP (MPE) and each polysaccharide were named MPE-CAR, MPE-GA, MPE-XT, and MPE-SA for CAR, GA, XT, and SA, respectively. Intrinsic tryptophan fluorescence intensity and surface hydrophobicity were analyzed to confirm protein structure. After settling the MPEs for 2 h, confocal laser scanning microscopy images were obtained in the upper and precipitated fractions. For heat-induced gelation, mixtures of the MP solution and hydrated polysaccharide were heated until the core temperature reached 75 °C in a water bath. The protein and polysaccharide contents of the final MP gels were 50 mg/mL and 0.2%, respectively. In vitro digestion proceeded using the INFOGEST protocol [5] with two compartments of gastric and intestinal phases. To analyze the degree of protein digestion, the content of α-amino groups using O-phthaldialdehyde assay, protein content of the <3 kDa fraction, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) were monitored in the digesta. The MP gels' digesta (MPD) were named MPD-CAR, MPD-GA, MPD-SA, and MPD-XT for MPE-CAR, MPE-GA, MPE-SA, and MPE-XT, respectively. In all analyses, three batches were used with three experimental iterations (nine samples per treatment).

III. RESULTS AND DISCUSSION

In the tryptophan fluorescence intensity curve of MPE-CAR and MPE-GA (Figure 1A), the maximum tryptophan fluorescence intensity (FI_{max}) significantly decreased ($P<0.05$), and slight red shifts were observed ($P<0.05$), indicating a change in the λ_{max} from that of MPE. In addition, MPE-CAR and MPE-GA exhibited increased surface hydrophobicity compared to MPE ($P<0.05$, Figure 1B). Thus, it seems that CAR and GA could interact with MPs through hydrophobic interactions; phase separation behavior was evident after 2 h of settling (Figure 1C). MPE-XT and MPE-SA significantly reduced FI_{max} compared to MPE ($P<0.05$, Figure 1A). However, there was no change in the surface hydrophobicity ($P>0.05$, Figure 1B). Also, Figure 1C shows similar sizes of protein particles in MPE-XT and MPE-SA with MPE. The strong negative charges of XT (-80.83 mV) and SA (-90.67 mV) (data not shown) likely lead to electrostatic repulsion between these polysaccharides and MPs. In contrast, the weaker negative charges of CAR (-55.70 mV) and GA (-30.80 mV) appear to result in less electrostatic repulsion with MPs.

After gastric and intestinal digestion in vitro, MPD-CAR showed no significant difference from MPD in the α-amino group content ($P>0.05$, Figure 2A) and had a lower protein content in the <3 kDa fraction (Figure 2B); especially, the presence of undegraded protein fragments was observed (Figure 2C). After gastric digestion, MPD-GA showed no significant difference in α-amino group content compared to MPD ($P>0.05$), but after the intestinal phase, the α-amino group content was higher in MPD-GA than in MPD ($P<0.05$, Figure 2A). Nevertheless, the protein

contents for the <3 kDa fraction of MPD-GA were similar to MPD. MPD-XT and MPD-SA exhibited higher α-amino group contents after gastric and gastrointestinal digestion than MPD (P<0.05, Figure 2A). In addition, the protein content in the <3 kDa fraction of MPD-SA was significantly higher than that of MPD (P<0.05, Figure 2B). Also, thicker bands of digestion products were observed compared to MPD (Figure 2C). These different protein digestion behaviors suggest potential impacts of polysaccharides on the nutritional value of meat products.

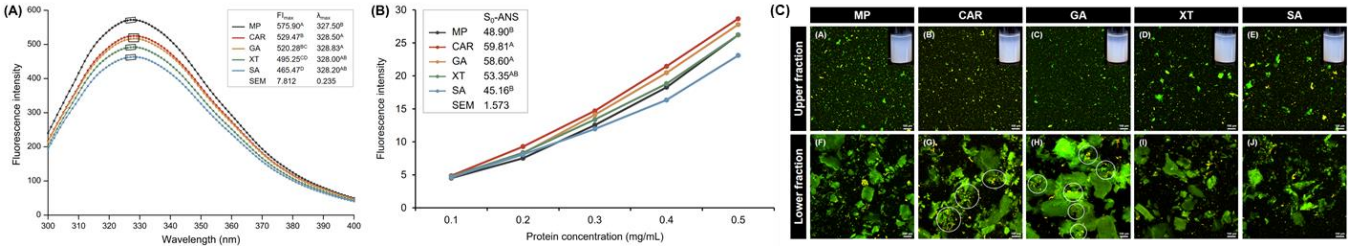


Figure 1. Intrinsic tryptophan fluorescence spectra (A), surface hydrophobicity (B, S_0-ANS), and upper fraction and lower fraction after settling for 2 h (C, scale bar = 100 μm) of confocal laser scanning microscopy images of myofibrillar protein extracts
[A-D] Different uppercase letters indicate significant differences between treatments (P<0.05).

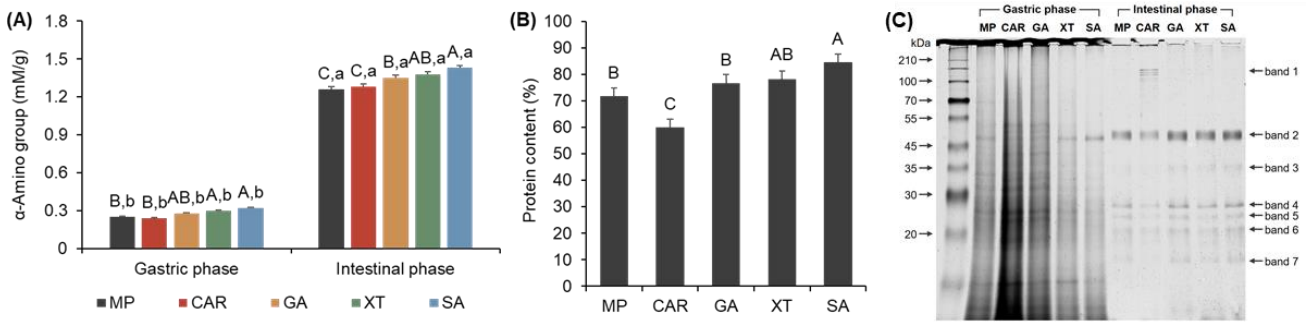


Figure 2. The α-amino group contents (A, mM/g) of the 10% trichloroacetic acid-soluble fractions, protein content in the fraction <3 kDa (B, %), and SDS-PAGE electrophoretogram of digesta (C) after in vitro gastrointestinal digestion of the myofibrillar protein gels
[A-C] Different uppercase letters indicate significant differences between the treatments (P<0.05).
[a-b] Different lowercase letters indicate significant differences between the digestion phase (P<0.05).

IV. CONCLUSION

The interaction between polysaccharides and MP differed depending on the types of polysaccharides, leading to different gel formation and protein digestion behavior in vitro. Our findings highlight the need to carefully select and regulate polysaccharide types and concentrations based on their structure and charge intensity to effectively modify the physicochemical and nutritional properties of meat products.

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