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# THE IMPACTS OF PORK FAT SUBSTITUTION WITH AGAR-BASED EMULSION GEL IN THE SAUSAGE ON THE DIGESTIVE PROPERTIES OF LIPIDS AND PROTEINS *IN VITRO*

Seonmin Lee[1*], Kyung Jo[1], Soeun Kim[1], Minkyung Woo[1], Yun-Sang Choi[2], and Samooel Jung[1]

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

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

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

## I. INTRODUCTION

Animal fats enhance sensory qualities of meat products, but their high saturated fatty acid (SFA) content has driven efforts to replace them with polyunsaturated fatty acid (PUFA)-rich plant oils [1]. Structuring liquid oils into emulsion gels is a strategy to improve the stability and incorporation of oils into food. Building on our previous findings that emulsion gels can reduce lipid digestibility and prevent phase separation during *in vitro* digestion [2], this study explored their potential as a pork fat substitute in sausages while ensuring desirable lipid and protein digestion. Emulsion gels with agar (AG), sodium alginate (SA), and xanthan gum (XT) were optimized to encapsulate canola oil and the lipid digestive behavior of the AG-SA-XT emulsion gel was compared to canola oil and pork fat in an *in vitro* model. Their effects on sausage physicochemical properties and digestion were then evaluated.

## II. MATERIALS AND METHODS

Canola oil was structured using AG, SA, and XT as gelling agents, with isolated soy protein (ISP) and soy lecithin as emulsifiers. The final emulsion gel composition (w/w) was 2.37% AG, 0.59% SA, 0.16% XT, 0.39% ISP, 0.22% soy lecithin, and 20% canola oil. Sausages were prepared in three treatments: CS (conventional sausage with no pork fat replacement), RS50 (50% pork fat replaced with emulsion gel), and RS100 (100% pork fat replaced with emulsion gel). Cooking loss and hardness of the sausages were evaluated. *In vitro* digestion was conducted using INFOGEST protocol [3]. Free fatty acid (FFA) release (%) was recorded during the intestinal digestion using a titrator (TitroLine 7000, SI Analytics GmbH, Mainz, Germany). The degree of proteolysis was measured using the contents of α-amino groups and proteins <3 kDa in the digesta. Confocal laser scanning microscopic images were acquired from the digesta (Olympus FV4000, Olympus Optical Co. Ltd., Tokyo, Japan).

## III. RESULTS AND DISCUSSION

Structuring canola oil in the AG-SA-XT emulsion gel significantly reduced FFA release from 48.70% to 12.76% ($P<0.05$), making it comparable to pork fat (14.60%, $P>0.05$) (Figure 1A). Additionally, the emulsion gel prevented phase separation in gastric digesta (Figure 1B), ensuring even lipid droplet distribution (Figure 1C). This suggests that oil encapsulation within the emulsion gel improved intragastric stability and delayed lipid digestion.

In sausages, cooking loss decreased in RS50 and RS100 than in CS ($P<0.05$) (Table 1). In addition, gel hardness increased as pork fat was replaced with emulsion gel (Table 1), with RS100 showing the highest hardness and CS the lowest ($P<0.05$). During *in vitro* intestinal digestion, FFA release was similar among RS50, RS100, and CS ($P>0.05$) (Figure 2A), but their gel dissociation behaviors differed. Microscopic images revealed that RS100 digesta had smaller particle sizes (Figure 2B), indicating gel fragmentation, a characteristic of high-strength protein gels, whereas CS digesta exhibited surface erosion with bead-shaped particles, typical of softer protein gels [4].

Notably, a higher α-amino group release in RS50 and RS100 than in CS ($P<0.05$) (Figure 2C) was observed, likely due to the increased surface area from gel fragmentation. However, no significant differences were observed in protein content <3 kDa among treatments ($P>0.05$) (Figure 2D), suggesting that protein digestibility did not considerably increase. Nevertheless, fast protein degradation and rapid release of small peptides and amino acids, could still be beneficial for stimulating muscle protein synthesis, particularly in the elderly [5].

Table 1 – Cooking loss (%) and hardness (g) of the pork sausages

| | Cooking loss (%) | Hardness (g) |
|---|---|---|
| CS | $4.76^{A}$ | $6235.40^{C}$ |
| RS50 | $3.37^{B}$ | $8233.79^{B}$ |
| RS100 | $3.09^{B}$ | $9439.56^{A}$ |
| SEM[1] | 0.307 | 549.49 |

[1] Standard error of the least square means

[A-C] Different letters indicate significant differences between means.

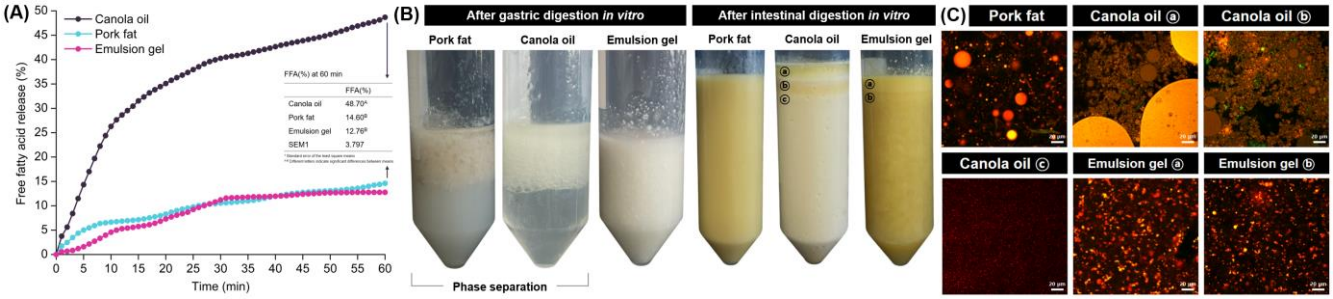



Figure 1. Free fatty acid release (A, %) during the intestinal digestion, the appearance (B) and confocal microscopic images (C, scale bar=20 μm) of the digesta after the *in vitro* digestion of the fat sources

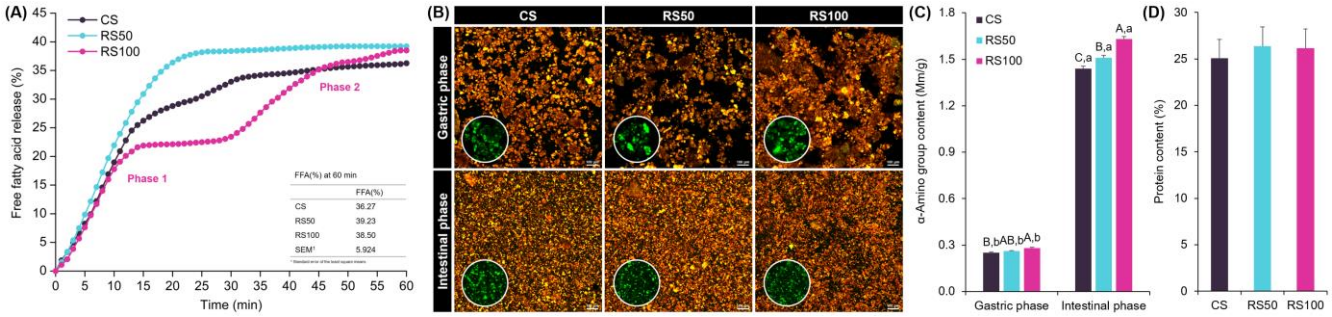



Figure 2. Free fatty acid release of the pork sausages during digestion *in vitro* (A, %), confocal microscopic images of the digesta (B, scale bar=100 μm), the content of α-amino groups (C, mM/g) in the 10% trichloroacetic acid-soluble fraction in the digesta, and the content of proteins (D, %) in the digesta after the *in vitro* digestion of the sausages
[A-C] Different uppercase letters indicate significant differences in means between the treatments in the same phase.
[a-b] Different lowercase letters indicate significant differences in means between the phases in the same treatment.

## CONCLUSION

In this study, incorporating an emulsion gel reduced cooking loss and improved gel strength of the sausages. *In vitro* digestion showed that while canola oil had higher FFA release than pork fat, its encapsulation within the AG-SA-XT gel matrix delayed lipid release, resulting in FFA levels comparable to pork fat in sausage. Sausages with complete pork fat replacement had smaller particle sizes in digesta with delayed oil release. Notably, the altered particle disintegration mechanism due to emulsion gel addition enhanced protein degradation during digestion, likely due to increased protein gel surface area. These improved lipid and protein digestion behaviors, along with the functional benefits of the emulsion gel, suggest its potential for enhancing the nutritional value of meat products while maintaining or improving functional properties.

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