

**ARTICLE**

# Effect of Non-Meat Proteins on Storage Characteristics and Amino Acid Composition of Pork Emulsified Sausages

 Sang-Keun Jin<sup>1,†</sup>, Sol-Hee Lee<sup>2,†</sup>, Sung-Ho Kim<sup>3</sup>, Sung-Sil Moon<sup>4</sup>,  
 and Jungseok Choi<sup>2,\*</sup>
<sup>1</sup>Division of Animal Science, Gyeongsang National University, Jinju 52828, Korea

<sup>2</sup>Department of Animal Science, Chungbuk National University, Cheongju 28644, Korea

<sup>3</sup>Kornex Co., Yongin 17115, Korea

<sup>4</sup>Sunjin Co., Seoul 05372, Korea

**OPEN ACCESS**
**Received** April 7, 2024

**Revised** May 3, 2024

**Accepted** May 9, 2024

**\*Corresponding author** : Jungseok Choi  
 Department of Animal Science, Chungbuk National University, Cheongju 28644, Korea  
 Tel: +82-43-261-2551  
 Fax: +82-43-261-2773  
 E-mail: [jchoi@chungbuk.ac.kr](mailto:jchoi@chungbuk.ac.kr)

**\*ORCID**

Sang-Keun Jin  
<https://orcid.org/0000-0002-8983-5607>  
 Sol-Hee Lee  
<https://orcid.org/0000-0003-1124-7095>  
 Sung-Ho Kim  
<https://orcid.org/0009-0004-4191-0841>  
 Sung-Sil Moon  
<https://orcid.org/0000-0003-2734-8931>  
 Jungseok Choi  
<https://orcid.org/0000-0001-8033-0410>

<sup>†</sup> These authors contributed equally to this work.

**Abstract** This study was conducted to confirm the following effects of non-meat binders (NMB) on proximate composition, pH, cooking yield, amino acids, volatile basic nitrogen (VBN), thiobarbituric acid reactive substance (TBARS), and correlation of pork emulsified sausages during refrigerated storage. The following groups of sausage samples were manufactured: Control (non-addition), BBP (1% bovine blood plasma); PBP (1% porcine blood plasma), EWP (1% white egg powder), CPPP (1% commercial porcine plasma powder), ISP (1% isolated soy protein), SP (1% seaweed powder), and SC (1% sodium caseinate). When NMB was added, ISP, SP, and SC showed higher heating yields while PBP showed lower heating yields than the control. As a result of amino acid analysis, PBP, CPPP, and SC showed significantly higher serine content than the control. EWP and SC showed significantly lower TBARS values than the control group, and VBN did not exceed 20 mg% in any treatments until the 5<sup>th</sup> week. These results demonstrate that SC is a NMB that can lower TBARS value while improving heating yield and serine content.

**Keywords** non-meat protein binders, emulsified sausage, amino acid composition, physicochemical property, storage property

## Introduction

The use of binders is essential for emulsified meat products (including frankfurters, sausages, and bologna) and reconstituted meat products (including reconstituted hams, patties, and steaks; Herz et al., 2023) as it can promote high production and cost-effectiveness of meat products, while meeting textural characteristics and consumer preferences (Dekkers et al., 2018). Therefore, producers in the meat processing industry use various non-meat binders (NMBs; protein-based, carbohydrate-based) such as salt, phosphate, transglutaminase, and protein to promote water holding

capacity (Owusu-Ansah et al., 2022). Soluble proteins extracted by salt play an important role as binders in meat products (Herz et al., 2021). However, the addition of large amounts of salt can reduce the food appeal of meat products. In addition, it is not sufficient to produce a complete product in the food industry. As a result, the use of binders is necessary to increase the binding force of meat products.

Recently, safety issues related to chemical additives used in meat products have emerged. Consumers demand safe and nutritious meat products that are tasty, healthy, and functional (Tahmasebi et al., 2016). Therefore, research is being conducted to develop products using natural substances in meat processing. Frequently used natural NMBs are either protein-based or carbohydrate-based (Guedes-Oliveira et al., 2021). There are a variety of NMBs, including soy proteins, milk proteins, gluten proteins, plasma proteins, egg proteins, gelatin, hydrocolloids, dietary fiber, sugars, and starches (Lu et al., 2021; Nasrollahzadeh et al., 2021). These proteins have the functional property of retaining moisture and fat mainly due to hydrophilic (water-loving) and lipophilic (fat-loving) groups of protein molecules and their hydrogen bonds (Vilgis, 2023).

The efficacy of various NMBs, depending on their amount and type, can affect the binding capacity, emulsion capacity, lipid oxidation, microbiology, and nutritional value of meat products (Anzani et al., 2020; Reddy et al., 2023). Animal plasma proteins contain high molecular weight compounds such as albumin, globulin, and fibrinogen with diverse functionalities. They are mainly used as emulsifiers, stabilizers, colorants, fertilizers, or pharmaceuticals (Toldrá et al., 2021). White egg powder (EWP), soy protein isolate, and sodium caseinate (SC) are the three most commonly used proteins in the meat processing industry. Seaweed contains polysaccharides, proteins, and essential fatty acids, especially alginate, the most abundant ionic polysaccharide (Ferrara, 2020).

Research is being conducted on how each NMB affects emulsified sausages. However, there is a lack of research on comparisons between various binders and how they affect storage properties. Therefore, this study investigated effects of adding NMBs on physicochemical and storage properties of pork emulsified sausage.

## Materials and Methods

### Preparation of non-meat binders

Blood samples from cattle and pigs were collected immediately after slaughter and used directly for plasma sampling. Blood samples were immediately transported to the laboratory and stored on ice. It was prepared by adding and mixing ethylenediaminetetraacetic acid at a rate of 2 g/L to the collected blood. Bloods were centrifuged at 8,000×g and 15 min at 4°C using a refrigerated centrifuge (SUPRA 25K, Hanil Science, Gimpo, Korea). The separated plasma was completely frozen at -96°C using a freeze dryer (PVTFD10R, Ilshinlab, Dongducheon, Korea). Bovine blood plasma (BBP) and porcine blood plasma (PBP) powders were ground to a certain size. The EWP, commercial porcine plasma powder (CPPP), isolated soy protein (ISP), seaweed powder (SP), and SC were purchased DONG BANG Food Master (Eumseong, Korea) and used in the experiment.

### Preparation of emulsion-type pork sausage

Fresh pork lean *Biceps femoris* meat and back-fat from LYD (Landrac×Yorkshire×Duroc) pork were purchased from a local market. Pork meat and back-fat were ground twice through a 5-mm plate. Experimental groups included Control (Non-addition), BBP (1% BBP); CPPP (1% CPPP), PBP (1% PP), T4 (1% EWP), SP (1% SP), ISP (1% ISP), and SC (1% SC), respective treatments were prepared (Table 1). The formula consisted of 70% meat, 15% back fat, and 15% ice water. Salt-soluble proteins were extracted from minced meat with 1% salt for 1 min using a bowl cutter (Talsa K30, DSL Food Machinery, Valencia,

**Table 1. Formulation of emulsified pork sausage added with various non-meat protein binders**

Traits (%)	Control	BBP	PBP	EWP	CPPP	ISP	SP	SC
Lean meat	70.0	70.0	70.0	70.0	70.0	70.0	70.0	70.0
Fat	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Ice	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Total	100							
Salt	1	1	1	1	1	1	1	1
Sugar	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Spices	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
BBP		1.0						
PBP			1.0					
EWP				1.0				
CPPP					1.0			
ISP						1.0		
SP							1.0	
SC								1.0

BBP, bovine blood plasma; PBP, porcine blood plasma; EWP, white egg powder; CPPP, commercial porcine plasma powder; ISP, isolated soy protein; SP, seaweed powder; SC, sodium casein.

Spain). Next, 0.4% sugar, 1.2% mixed seasoning, NMBs, and half of ice were added and mixed for 2 min, at this time, the emulsion batter temperature is below 10°C. The emulsion was then filled into a fibrous casing (Nalo Top, Kalle, Wiesbaden, Germany, diameter 70 mm) using a stuffer (IS-8, Sirman, Padova, Italy). The stuffed emulsion samples were heated in a heating chamber (Thematec Food Industry, Seongnam, Korea) to reach an internal temperature of 75°C, and the manufactured sausages were immediately refrigerated and stored for 5 weeks at 4°C before conducting the experiment.

### Proximate composition

Approximate compositions of both treatments were determined using the analytical method of AOAC (2016a), AOAC (2016b), AOAC (2016c), and AOAC (2016d).

### Cooking yield

The weight measured after filling the casing with the emulsion batter was referred to as the initial weight and was then heated to an internal temperature of 75°C. The heated sausages were then cooled for 30 min at 4°C and weighed (final weight). Cooking yield was then calculated using the following formula:

$$\text{Cooking yield (\%)} = (\text{Final weight} / \text{Initial weight}) \times 100 \quad (1)$$

### Volatile basic nitrogen

In this experiment, volatile basic nitrogen (VBN) was measured using a modification of Pearson (1976). To 10 mL of sample (mixed with distilled water), a few drops of 0.5 wt% phenolphthalein indicator (dissolved in 50 wt% ethanol) and 3.5 mL of 20% sodium hydroxide were mixed. The 250 mL of distillate was collected in the flask, which was immediately sealed and collected.

The distillate was collected in a flask containing 20 mL of 4% boric acid and Tashiro indicator (methyl red: methylene blue=2:1). Afterwards, the obtained basic solution (green) was titrated with 0.01M hydrochloric acid until it turned gray. The VBN content was measured after correction: the blank was measured by steam distillation of 6% perchloric acid.

### **2-Thiobabituric acid reactive substance**

The 5 g of each sample and 15 mL of deionized distilled water were homogenized for 10 s at 3,000×g using a homogenizer (CPPP5, IKA Werke, Staufen, Germany). Afterwards, butylated hydroxyanisole (50 µL, 10%) and thiobarbituric acid/trichloroacetic acid (TBA/TCA; 2 mL) were added to 1 mL of the homogenate and mixed. The mixture was reacted in a water bath at 100°C for 15 min to develop color. The colored sample was immediately cooled for 10 min to bring it to room temperature, and then centrifuged at 2,000×g for 15 min to obtain the supernatant. The 1 mL of distilled water and 2 mL of TBA/TCA solution were used as blanks, and absorbance was measured at 531 nm. The amount of 2-thiobabituric acid reactive substance (TBARS) was then expressed as milligrams of malondialdehyde per kilogram of sample.

### **Free amino acids analysis**

Free amino acids were tested transforming the method of Aristoy and Toldra (1991). Samples were extracted with 0.01N HCl, and 300 µL of the extracted sample was mixed with 10 µL internal standard (l-citrulline) and 690 µL acetonitrile. The mixture was reacted at 4°C for 30 min, and then centrifuged at 10,000×g for 15 min at 4°C using a centrifuge. The collected supernatant was filtered through a 0.45 µL membrane filter and used in the experiment. At this time, external standards (amino acid standard: 0.25 nM, Agilent, Santa Clara, CA, USA; glutamine, Sigma-Aldrich, St. Louis, MO, USA) were analyzed for O-phthalaldehyde and 9-fluorenylmethyl chloroformate derivatization using HPLC (Agilent; Herbert et al., 2000). Analysis conditions were conducted according to the method of Henderson et al. (2000), and the conditions were as follows: pH 7.8; Column, Zorbax Eclipse AAA, 4.6×60 mm, 5 µm; DAD detector, 262 nm, 338 nm; Column temperature, 40°C; Mobile phase A, 40 mM sodium phosphate buffer, and mobile phase B, acetonitrile:methanol:water, 45:45:10 (v:v:v).

### **Statistical analysis**

There were a total of 144 samples used in the statistical analysis in the experiment, each consisting of 8 treatments×3 repetitions×2 storage times×3 batches. Batch means conducted at different times in the same place and temperature, and the experiment had a completely randomized design. Data on physicochemical and storage properties of sausages were subjected to one-way analysis of variance (ANOVA) using the general linear model procedure in the SAS program. Physicochemical properties were analyzed based on statistics of the treatment group. Storage characteristics were analyzed using the statistics of eight treatment groups and two storage time. Statistical significance between means was determined at the 95% significance level using Duncan's multiple range test. Mean values and SD are presented. Correlation coefficients between storage parameters (pH, fat and protein contents, TBARS, and VBN) were tested using Pearson's correlation using SAS software version 9.4 (SAS Institute, Cary, NC, USA).

## **Results and Discussion**

### **Proximate composition**

The influence of NMBs on approximate compositions of pork emulsified sausages is shown in Table 2. Moisture content

**Table 2. Proximate compositions and cooking yields of pork emulsified sausages added with non-meat protein binders**

Traits (%) <sup>1)</sup>	Control	BBP	PBP	EWP	CPPP	ISP	SP	SC
Moisture contents	65.28±0.09 <sup>a</sup>	64.67±0.00 <sup>b</sup>	64.22±0.39 <sup>bc</sup>	63.89±0.38 <sup>c</sup>	64.67±0.00 <sup>b</sup>	64.22±0.19 <sup>bc</sup>	64.11±0.38 <sup>c</sup>	64.22±0.19 <sup>bc</sup>
Crude protein contents	15.94±1.63	15.82±0.09	14.70±1.41	15.80±0.06	14.76±0.39	15.65±0.39	14.55±1.89	14.95±1.44
Crude fat contents	13.43±0.61 <sup>cd</sup>	12.24±0.59 <sup>c</sup>	13.48±0.20 <sup>cd</sup>	14.50±0.30 <sup>ab</sup>	13.07±0.55 <sup>d</sup>	14.01±0.18 <sup>bc</sup>	14.77±0.21 <sup>a</sup>	13.88±0.16 <sup>bc</sup>
Cooking yield	92.81±0.22 <sup>ab</sup>	93.17±0.31 <sup>ab</sup>	92.58±0.15 <sup>b</sup>	92.59±0.69 <sup>b</sup>	93.35±0.42 <sup>ab</sup>	93.62±0.19 <sup>a</sup>	93.51±0.53 <sup>a</sup>	93.47±0.55 <sup>a</sup>

<sup>1)</sup> Control, non additive; BBP, bovine blood plasma; PBP, porcine blood plasma; EWP, white egg powder; CPPP, commercial porcine plasma powder; ISP, isolated soy protein; SP, seaweed powder; SC, sodium casein.

<sup>a-c</sup> Means different superscriptions within the same row ( $p < 0.05$ ).

was significantly higher in the control than in other groups. It was significantly higher in BBP and CPPP than in EWP and SP ( $p < 0.05$ ). According to Hsu and Sun (2006), sodium casein consists of 3.9% moisture, 91.7% protein, 0.7% fat, 3.6% ash, and 0.1% carbohydrate, while ISP contains 5%–6% moisture and 84.6% protein, 0.5%–1.0% fat, 4.0%–4.5% ash, and 3.9%–5.0% carbohydrate. EWP consists of 8.8% moisture, 80.2% protein, 0.2% fat, 5.1% ash, and 5.7% carbohydrate. Chemical compositions of dried seaweed are mainly dietary fiber and proteins (Premarathna et al., 2022). Plasma powder is composed mostly of protein. It contains 40%–50% globulin, 1%–3% fibrinogen, and 50%–60% albumin (Nair et al., 2022). Parés et al. (1998) have reported that approximate compositions of spray-dried porcine plasma are 66.45% protein, 14.13% ash, 11.83% moisture, and 3.88% fat. The plasma powder used in this study was lyophilized. Therefore, it was determined that pork sausages with added NMBs had higher solids content or lower moisture content than control sausages. Protein contents of sausages containing NMBs showed no significant difference between control and treatment groups. Fat contents of sausages added with EWP and SP were significantly higher than those of control sausages ( $p < 0.05$ ), which is believed to be due to the relatively low moisture content. These different values are thought to be due to different ingredients of the additive, as shown in the references mentioned above.

### Cooking yield

The influence of NMBs on heating yield of pork emulsified sausages is shown in Table 2. Most studies on NMBs added as meat binders to meat products have reported NMBs can reduce cooking yield and moisture loss while increasing emulsion stability, cohesion, water holding capacity, and hardness (Ismail et al., 2021; Ruther et al., 2020). Results of heating yield revealed that ISP, SP, and SC groups had higher values than the control. This meant that ISP and sodium casein contributed to the increase in thermal stability of frankfurter sausages due to formation of a protein network, consistent with previous research results (Yu et al., 2023). In addition, cooking losses of pork patties containing 1%–5% of seaweed (*Laminaria japonica*) were similar to previous research results showing that cooking loss of pork patties containing seaweed was significantly lower than that of the control due to the presence of alginate and laminarin in seaweed, which had water-holding and binding properties (Choi et al., 2012). Therefore, it was concluded that ingredients of NMBs such as ISP, SP, and sodium casein could affect product yields of emulsified pork sausages.

### Amino acids

Effects of NMBs on amino acid compositions in emulsion type pork sausages are summarized in Table 3. Glutamic acid was known to contain a flavor not affected by the addition of NMBs ( $p > 0.05$ ). Among different sweet taste amino acids (serine, threonine, glycine, and alanine), significant difference was only observed in serine content. Serine contents of PBP,

**Table 3. Amino acid compositions of pork emulsified sausages added with non-meat protein binders**

Amino acids	Control	Treatments <sup>1)</sup>						
		BBP	PBP	EWP	CPPP	ISP	SP	SC
Aspartic acid	10.99±0.12	10.51±1.02	11.44±0.24	11.18±0.05	11.27±0.20	11.33±0.23	11.25±0.20	11.40±0.11
Threonine <sup>2)</sup>	4.82±0.13	4.85±0.28	5.05±0.05	4.91±0.11	5.04±0.08	4.99±0.01	5.04±0.08	5.04±0.01
Serine <sup>2)</sup>	4.01±0.00 <sup>c</sup>	4.15±0.21 <sup>bc</sup>	4.36±0.03 <sup>a</sup>	4.12±0.02 <sup>bc</sup>	4.34±0.03 <sup>a</sup>	4.30±0.01 <sup>ab</sup>	4.15±0.04 <sup>bc</sup>	4.37±0.06 <sup>a</sup>
Glutamic acid <sup>3)</sup>	17.24±0.14	16.70±0.94	16.97±0.10	17.29±0.05	17.14±0.01	17.46±0.03	17.31±0.06	17.66±0.03
Proline	4.91±0.12	4.84±0.20	4.90±0.11	4.36±0.28	4.78±0.28	4.75±0.13	4.74±0.02	4.90±0.18
Glycine <sup>2)</sup>	6.13±0.26	3.69±2.84	5.63±0.13	5.46±0.04	5.66±0.01	5.72±0.09	5.89±0.04	5.41±0.10
Alanine <sup>2)</sup>	6.54±0.04	6.78±0.50	6.48±0.03	6.36±0.15	6.50±0.03	6.38±0.01	6.52±0.01	6.27±0.01
Valine <sup>4)</sup>	0.58±0.13	4.32±5.20	0.62±0.22	0.65±0.01	0.72±0.18	0.73±0.19	0.66±0.14	0.59±0.08
Isoleucine <sup>4)</sup>	5.62±0.05	4.20±2.04	5.58±0.01	5.95±0.04	5.64±0.01	5.74±0.04	5.78±0.06	5.68±0.05
Leucine	8.80±0.04	6.97±2.88	9.01±0.06	9.03±0.09	8.96±0.08	8.90±0.03	8.94±0.01	8.96±0.04
Tyrosine <sup>4),5)</sup>	2.89±0.06	7.54±6.39	3.31±0.10	2.89±0.01	3.11±0.01	3.04±0.07	2.83±0.01	3.21±0.03
Phenylalanine <sup>4),5)</sup>	5.02±0.91	4.01±0.66	4.48±0.03	4.51±0.04	4.45±0.01	4.44±0.00	4.40±0.01	4.43±0.03
Histidine <sup>4)</sup>	5.33±0.09	5.09±0.45	5.27±0.04	5.99±0.70	5.38±0.01	5.35±0.06	5.45±0.06	5.29±0.02
Lysine	9.68±0.06	9.58±3.19	9.69±0.02	10.06±0.23	9.76±0.06	9.62±0.11	9.80±0.01	9.68±0.11
Arginine <sup>4)</sup>	7.47±0.01	6.82±4.16	7.24±0.13	7.27±0.11	7.29±0.06	7.28±0.07	7.27±0.04	7.15±0.06
FAA <sup>3)</sup>	17.24±0.14	16.70±0.94	16.97±0.10	17.29±0.05	17.14±0.01	17.46±0.03	17.31±0.06	17.66±0.03
STAA <sup>2)</sup>	21.49±0.42	19.46±2.83	21.51±0.18	20.84±0.24	21.53±0.04	21.38±0.12	21.59±0.03	21.08±0.13
AAA <sup>5)</sup>	7.91±0.96	11.54±5.73	7.79±0.13	7.40±0.05	7.55±0.01	7.48±0.07	7.22±0.00	7.64±0.05
BAA <sup>4)</sup>	26.91±0.67	34.71 11.64	26.49±0.45	27.26±0.49	26.58±0.09	26.57±0.09	26.38±0.23	26.34±0.11
EAA <sup>6)</sup>	47.31±0.37	45.81±2.09	46.91±0.25	48.37±0.52	47.22±0.13	47.04±0.11	47.33±0.29	46.80±0.22
TAA <sup>7)</sup>	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00

<sup>1)</sup> BBP, bovine blood plasma; PBP, porcine blood plasma; EWP, white egg powder; CPPP, commercial porcine plasma powder; ISP, isolated soy protein; SP, seaweed powder; SC, sodium casein.

<sup>2)</sup> STAA (sweet taste amino acid, threonine, serine, glycine, alanine).

<sup>3)</sup> FAA (flavorous amino acid, glutamic acid).

<sup>4)</sup> BAA (bitter amino acid, valine, methionine, isoleucine, tyrosine, phenylalanine, histidine, arginine).

<sup>5)</sup> AAA (aromatic amino acid, tyrosine, phenylalanine).

<sup>6)</sup> EAA (essential amino acid, threonine, valine, methionine, isoleucine, leucine, phenylalanine, histidine, lysine, arginine).

<sup>7)</sup> TAA (total amino acid).

<sup>a-c</sup> Means different superscriptions within the same row ( $p < 0.05$ ).

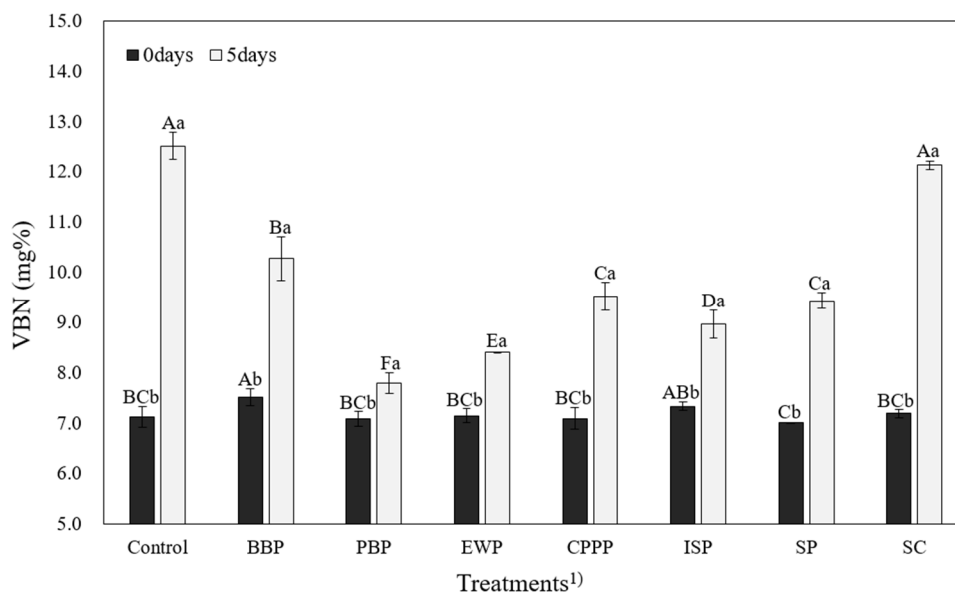
CPPP, ISP, and SC groups were higher than that of the control (all  $p < 0.05$ ). Contents of aromatic amino acids such as tyrosine and phenylalanine had no significant difference among treatments. Contents of amino acids (valine, phenylalanine, isoleucine, histidine, tyrosine, and arginine) with bitter taste had no significant difference among treatments. Contents of essential amino acids (threonine, valine, isoleucine, leucine, phenylalanine, histidine, lysine, and arginine) showed no significant difference either. Most amino acid compositions of pork sausages had no significant difference. This might be due to the fact that the NMB addition level was 1%, which might not be enough to affect amino acid compositions of sausages. Very few research studies have been conducted on amino acid compositions of sausages after adding NMBs. According to Márquez et al. (2005), isoleucine, lysine, and methionine contents in essential amino acids were not significantly different

between bovine and porcine plasma proteins. Vilar et al. (2020) have reported that the addition of seaweed into frankfurter does not affect amino acid profiles. We also found that amino acid compositions of emulsion-type pork sausages were not influenced by the addition of 1% NMBs.

## 2-Thiobabituric acid reactive substance and volatile basic nitrogen

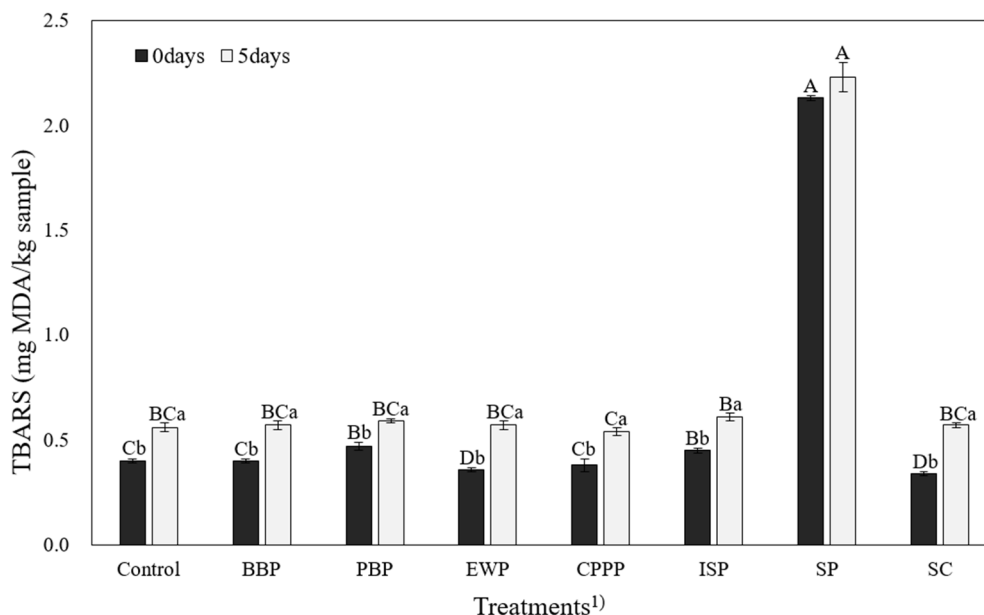
All VBN values of sausages increased significantly during five weeks of storage ( $p < 0.05$ , Fig. 1). BBP and ISP had significantly higher VBN values than SP at week 0 ( $p < 0.05$ ). At the 5<sup>th</sup> week of storage, control and SC had significantly higher VBN values than other sausages ( $p < 0.05$ ). A high VBN value means that protein denaturation occurs during storage. It can be used as an indicator of the freshness of meat products (Lee et al., 2021). VBN values of all pork sausages, including the control group, did not exceed 20 mg% during the 5-week storage period (Cho et al., 2021). Therefore, it was believed that these NMBs did not have any adverse effect on the VBN of emulsion-type pork sausage during storage. These results suggest that EWP and SC, which have the lowest TBARS and VBN, play a positive role as NMBs.

Effects of NMBs on TBARS values of emulsion-type pork sausages during refrigerated storage are shown in Fig. 2. TBARS values for other sausages were all less than 1.00 TBARS values of pork sausages at 0 and 5 weeks were the highest in SP among treatments ( $p < 0.05$ ). Most of the previous studies have reported that adding seaweed to food can reduce lipid oxidation (Harrysson et al., 2021; Munsu et al., 2021). Seaweeds contain polyphenols (e.g., phlorotannins) and carotenoid pigments (e.g., fucoxanthin) with the ability to scavenge free radicals, hydroxyl, and acetate radicals (Airanthi et al., 2011). Additionally, because seaweed has high K, Ca, Mg, and Mn contents, which might promote lipid oxidation (Teets and Were, 2008). Therefore, SP added to sausages in this study might have promoted oxidation. Additional research on this is needed. On the other hand, EWP and SC groups showed significantly lower lipid oxidation values than other treatments ( $p < 0.05$ ). It is believed that these binders might have structures that make it difficult for microorganisms to feed on.



**Fig. 1. VBN (volatile basic nitrogen, mg%) values of pork emulsified sausages added with non-meat protein binders.** <sup>1)</sup> Control, non additive; BBP, bovine blood plasma; PBP, porcine blood plasma; EWP, white egg powder; CPPP, commercial porcine plasma powder; ISP, isolated soy protein; SP, seaweed powder; SC, sodium casein. <sup>A-D</sup> Means different superscriptions within the same days ( $p < 0.05$ ). <sup>a,b</sup> Means different superscriptions within the same treatments ( $p < 0.05$ ).





**Fig. 2. TBARS (thiobarbituric acid reactive substance, malondialdehyde/kg sample) values of pork emulsified sausages with non-meat protein binders.** <sup>1)</sup> Control, non additive; BBP, bovine blood plasma; PBP, porcine blood plasma; EWP, white egg powder; CPPP, commercial porcine plasma powder; ISP, isolated soy protein; SP, seaweed powder; SC, sodium casein. <sup>A-D</sup> Means different superscriptions within the same days (p<0.05). <sup>a,b</sup> Means different superscriptions within the same treatments (p<0.05).

### Relationship between storage parameters

Damage to meat products can be caused by several factors, and by analyzing this, the expiration date of food can be set and quality deterioration can be prevented (Hoa et al., 2023). Therefore, in this study, we attempted to analyze the correlation between indicators related to storage, which are shown in Table 4. It was found that pH showed strong negative correlations with protein and VBN (p<0.01), meaning that pH was more affected by protein than by fat in this study. Yang et al. (2020) have reported that protein and VBN are affected when protein additives are added to meat products. Therefore, an increase in pH due to the generation of ammonia from protein might have a greater effect during the storage period than the generation of acid due to fat rancidity (Bekhit et al., 2021). Fat showed a negative correlation with TBARS and protein showed a strong negative correlation with VBN (p<0.001). Therefore, when manufacturing emulsified sausages by adding NMBs, additional measures appear to be necessary to prevent protein spoilage.

**Table 4. Pearson’s correlations between various storage parameters of pork emulsified sausages and non-meat protein binders**

Traits	pH	Fat content	Protein content	TBARS	VBN
pH	1	-0.15	-0.55**	-0.12	0.60**
Fat content		1	-0.26	0.61**	0.11
Protein content			1	-0.06	0.71***
TBARS				1	0.18
VBN					1

Values are correlation coefficients for n=135.

\*\* p<0.01; \*\*\* p<0.001.

TBARS, thiobarbituric acid reactive substance; VBN, volatile basic nitrogen.



## Conclusion

In this study, effects of fattening protein binders on quality and storage characteristics of emulsified sausages were analyzed. There was no significant difference in protein content. In addition, fat contents of egg white powder and SP were higher than that of the control. The difference in general ingredients is thought to be influenced by general ingredients of added additives. Seaweed, which contains a lot of dietary fiber, ISP, and sodium casein, which contains salt, appears to increase emulsion stability, resulting in high cooking yields in treatments containing these ingredients. The addition of NMBs did not affect amino acid compositions of sausages except serine. VBN showed a higher value at 5 weeks compared to 0 week, although did not exceed 20 mg% in any treatments. During five weeks of refrigerated storage, TBARS showed significantly higher values while egg white powder and SP showed significantly lower values in SP more than other treatments. In conclusion, adding a NMB to tanned pork sausage can improve the binding force and develop healthier meat products that meet consumer demands. In this study, SP was found to be the most suitable natural binder.

## Conflicts of Interest

The authors declare no potential conflicts of interest.

## Acknowledgements

This work was supported by the Regional Animal Industry Center at Gyeongsang National University and by “Regional Innovation Strategy (RIS)” through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (MOE) (2021RIS-001).

## Author Contributions

Conceptualization: Jin SK, Lee SH, Choi J. Data curation: Lee SH, Kim SH, Moon SS. Formal analysis: Lee SH, Choi J. Methodology: Lee SH. Software: Jin SK, Lee SH. Validation: Lee SH, Choi J. Investigation: Jin SK, Lee SH, Choi J. Writing - original draft: Jin SK, Lee SH, Kim SH, Moon SS. Writing - review & editing: Jin SK, Lee SH, Kim SH, Moon SS, Choi J.

## Ethics Approval

This article does not require IRB/IACUC approval because there are no human and animal participants.

## References

- Airanthi MLWA, Hosokawa M, Miyashita K. 2011. Comparative antioxidant activity of edible Japanese brown seaweeds. *J Food Sci* 76:C104-C111.
- Anzani C, Boukid F, Drummond L, Mullen AM, Álvarez C. 2020. Optimising the use of proteins from rich meat co-products and non-meat alternatives: Nutritional, technological and allergenicity challenges. *Food Res Int* 137:109575.
- AOAC. 2016a. AOAC official methods of analysis, official method 920.153 ash in meat by ashing method. 20<sup>th</sup> ed. AOAC International, Rockville, MD, USA.

- AOAC. 2016b. AOAC official methods of analysis, official method 942.05 protein in meat by combustion method. 20<sup>th</sup> ed. AOAC International, Rockville, MD, USA.
- AOAC. 2016c. AOAC official methods of analysis, official method 950.46 moisture in meat by air drying method. 20<sup>th</sup> ed. AOAC International, Rockville, MD, USA.
- AOAC. 2016d. AOAC official methods of analysis, official method 991.36 fat in meat by solvent extraction method. 20<sup>th</sup> ed. AOAC International, Rockville, MD, USA.
- Aristoy MC, Toldra F. 1991. Deproteinization techniques for HPLC amino acid analysis in fresh pork muscle and dry-cured ham. *J Agric Food Chem* 39:1792-1795.
- Bekhit AEDA, Holman BWB, Giteru SG, Hopkins DL. 2021. Total volatile basic nitrogen (TVB-N) and its role in meat spoilage: A review. *Trends Food Sci Technol* 109:280-302.
- Cho J, Kim HJ, Kwon JS, Kim HJ, Jang A. 2021. Effect of marination with black currant juice on the formation of biogenic amines in pork belly during refrigerated storage. *Food Sci Anim Resour* 41:763-778.
- Choi YS, Choi JH, Han DJ, Kim HY, Kim HW, Lee MA, Chung HJ, Kim CJ. 2012. Effects of *Laminaria japonica* on the physico-chemical and sensory characteristics of reduced-fat pork patties. *Meat Sci* 91:1-7.
- Dekkers BL, Boom RM, van der Goot AJ. 2018. Structuring processes for meat analogues. *Trends Food Sci Technol* 81:25-36.
- Ferrara L. 2020. Seaweeds: A food for our future. *J Food Chem Nanotechnol* 6:56-64.
- Guedes-Oliveira JM, Brad Kim YH, Conte-Junior CA. 2021. What are the potential strategies to achieve potentially more healthful meat products? *Int J Food Sci Technol* 56:6157-6170.
- Harrysson H, Krook JL, Larsson K, Tullberg C, Oerbekke A, Toth G, Pavia H, Undeland I. 2021. Effect of storage conditions on lipid oxidation, nutrient loss and colour of dried seaweeds, *Porphyra umbilicalis* and *Ulva fenestrata*, subjected to different pretreatments. *Algal Res* 56:102295.
- Henderson JW, Ricker RD, Bidlingmeyer BA, Woodward C. 2000. Rapid, accurate, sensitive and reproducible HPLC analysis of amino acids: Amino acid analysis using Zorbax Eclipse-AAA columns and the Agilent 1100 HPLC. Agilent Technologies, Santa Clara, CA, USA.
- Herbert P, Barros P, Ratola N, Alves A. 2000. HPLC determination of amino acids in musts and port wine using OPA/FMOC derivatives. *J Food Sci* 65:1130-1133.
- Herz E, Herz L, Dreher J, Gibis M, Ray J, Pibarot P, Schmitt C, Weiss J. 2021. Influencing factors on the ability to assemble a complex meat analogue using a soy-protein-binder. *Innov Food Sci Emerg Technol* 73:102806.
- Herz E, Moll P, Schmitt C, Weiss J. 2023. Binders in foods: Definition, functionality, and characterization. *Food Hydrocoll* 145:109077.
- Hoa VB, Song DH, Seol KH, Kang SM, Kim HW, Bae IS, Kim ES, Park YS, Cho SH. 2023. A comparative study on the meat quality, taste and aroma related compounds between Korean Hanwoo and Chikso cattle. *Foods* 12:805.
- Hsu SY, Sun LY. 2006. Comparisons on 10 non-meat protein fat substitutes for low-fat Kung-wans. *J Food Eng* 74:47-53.
- Ismail MA, Chong GH, Ismail-Fitry MR. 2021. Comparison of the microstructural, physicochemical and sensorial properties of buffalo meat patties produced using bowl cutter, universal mixer and meat mixer. *J Food Sci Technol* 58:1-8.
- Lee S, Kim EJ, Park DH, Ji YR, Kang G, Choi MJ. 2021. Deep freezing to maintain the freshness of pork loin during long-term storage. *Food Sci Biotechnol* 30:701-710.
- Lu W, Li X, Fang Y. 2021. Introduction to food hydrocolloids. In *Food hydrocolloids: Functionalities and applications*. Fang

- Y, Zhang H, Nishinari K (ed). Springer, Singapore. pp 1-28.
- Márquez E, Bracho M, Archile A, Rangel L, Benítez B. 2005. Proteins, isoleucine, lysine and methionine content of bovine, porcine and poultry blood and their fractions. *Food Chem* 93:503-505.
- Munsu E, Mohd Zaini H, Matanjun P, Wahab NA, Sulaiman NS, Pindi W. 2021. Physicochemical, sensory properties and lipid oxidation of chicken sausages supplemented with three types of seaweed. *Appl Sci* 11:11347.
- Nair RP, Lekshmi P, Bhatt A. 2022. Blood-derived products. In *Biomedical product and materials evaluation: Standards and ethics*. Mohanan PV (ed). Woodhead, Sawston, UK. pp 231-257.
- Nasrollahzadeh M, Nezafat Z, Shafiei N. 2021. Proteins in food industry. In *Biopolymer-based metal nanoparticle chemistry for sustainable applications*. Nasrollahzadeh M (ed). Elsevier, Amsterdam, The Netherlands. pp 97-136.
- Owusu-Ansah P, Besiwah EK, Bonah E, Amagloh FK. 2022. Non-meat ingredients in meat products: A scoping review. *Appl Food Res* 2:100044.
- Parés D, Sagner E, Saurina J, Suñol JJ, Carretero C. 1998. Functional properties of heat induced gels from liquid and spray-dried porcine blood plasma as influenced by pH. *J Food Sci* 63:958-961.
- Pearson D. 1976. *The chemical analysis of food*. 7<sup>th</sup> ed. Churchill Livingstone, London, UK. p 575.
- Premarathna AD, Tuvikene R, Fernando PHP, Adhikari R, Perera MCN, Ranahewa TH, Howlader MM, Wangchuk P, Jayasooriya AP, Rajapakse RPVJ. 2022. Comparative analysis of proximate compositions, mineral and functional chemical groups of 15 different seaweed species. *Sci Rep* 12:19610.
- Reddy GVB, Viswa natha Reddy K, Amaravathi P. 2023. Quality characteristics and ultra structural changes of restructured buffalo meat slices with flaxseed flour as binder: A novel value added technology. *Emir J Food Agric* 35:342-350.
- Ruther BL, Dickson JS, Prusa KJ, Tarté R, Sebranek JG. 2020. Effects of processing method and nonmeat binding ingredients on batter stability, yield and texture of frankfurters. *J Food Process Preserv* 44:e14626.
- Tahmasebi M, Labbafi M, Emam-Djomeh Z, Yarmand MS. 2016. Manufacturing the novel sausages with reduced quantity of meat and fat: The product development, formulation optimization, emulsion stability and textural characterization. *LWT-Food Sci Technol* 68:76-84.
- Teets AS, Were LM. 2008. Inhibition of lipid oxidation in refrigerated and frozen salted raw minced chicken breasts with electron beam irradiated almond skin powder. *Meat Sci* 80:1326-1332.
- Toldrá F, Reig M, Mora L. 2021. Management of meat by- and co-products for an improved meat processing sustainability. *Meat Sci* 181:108608.
- Vilar EG, Ouyang H, O'Sullivan MG, Kerry JP, Hamill RM, O'Grady MN, Mohammed HO, Kilcawley KN. 2020. Effect of salt reduction and inclusion of 1% edible seaweeds on the chemical, sensory and volatile component profile of reformulated frankfurters. *Meat Sci* 161:108001.
- Vilgis TA. 2023. Consequences of early industrialization on the molecular composition of food. In *Nutrition biophysics: An introduction for students, professionals and career changers*. Vilgis TA (ed). Springer, Berlin, Germany. pp 107-175.
- Yang F, Cho WY, Lee N, Kim DH, Lee J, Lee HJ, Seo HG, Lee CH. 2020. Effects of *Boswellia serrata* and whey protein powders on physicochemical properties of pork patties. *Foods* 9:334.
- Yu J, Xiao H, Song L, Xue Y, Xue C. 2023. Impact of O/W emulsion stabilized by different soybean phospholipid/sodium caseinate ratios on the physicochemical, rheological and gel properties of surimi sausage. *LWT-Food Sci Technol* 175:114461.