ISSN: 2347-467X, Vol. 10, No. (3) 2022, Pg. 1007-1019

Current Research in Nutrition and Food Science

www.foodandnutritionjournal.org

Effects of Physicochemical Characteristics and Storage Stability of Porcine Albumin Protein Hydrolysates in Pork Sausage

GYUTAE PARK[1†], SANGKEUN JIN[2†] and JUNGSEOK CHOI[1*]

[1]Dept. of Animal Science, Chungbuk National University, Cheongju, Republic of Korea.
[2]Dept. of Animal Resources Technology, Gyeongsang National University, Jinju, Republic of Korea.

Abstract

In this study, the physicochemical characteristics and storage stability of porcine albumin protein hydrolysates (PAPH) in sausage were evaluated. Four concentrations of PAPH were added to pork emulsion sausage (T1, 0.3%; T2, 0.6%; T3, 0.9%; T4, 1.2%) and compared to the control (CON, 0%). On day 0, proximate composition, cooking loss, and sensory evaluation were compared. Purge loss, color, texture profile analysis, shear force, free amino acids, lipid oxidation, microbial counts, and volatile basic nitrogen (VBN) were compared on day 0 and after 4 weeks of refrigeration. The content of essential amino acids and redness (a*) increased as the level of PAPH added increased ($p<0.05$). Also, the cooking loss was improved ($p<0.05$). However, lipid oxidation, microbial counts, and VBN were increased significantly during storage for 4 weeks ($p<0.05$). The findings indicated that the addition of PAPH improved cooking loss and the protein composition of sausages, but negatively affected storage stability.

Article History
Received: 22 July 2022
Accepted: 14 september 2022

Keywords
Albumin;
Free amino acid;
Hydrolysates;
Pork sausage;
Storage stability.

Introduction

Animal blood accounts for up to 4% of the weight of live animals or 6-7% of the meat content of carcasses as a by-product.[1] A variety of removal techniques are commonly used, such as drying and incineration, as animal blood produced in slaughter houses is problematic due to its very high contamination load when disposed of directly into the environment.[2,3] However, blood proteins show excellent functional properties, making them suitable for use in processed foods for human consumption. And blood proteins are potentially valuable resources that can provide nutrients such as peptides, amino acids, and heme proteins.[4] Blood is separated into a plasma fraction and a red blood cell fraction after centrifugation. The red blood cell fraction is problematic due to its characteristic dark color and strong taste, but the former shows excellent emulsifying and thermal

CONTACT Jungseok Choi Jchoi@chungbuk.ac.kr Dept. of Animal Science, Chungbuk National University, Cheongju, Republic of Korea.

Doi: http://dx.doi.org/10.12944/CRNFSJ.10.3.17

coagulation properties and is, therefore, a protein material of interest in food applications.[5,6,7] Since the manufacture of many foods, such as mayonnaise and sausages, involves an emulsification process, the properties of blood proteins that form or stabilize emulsions are very important from an industrial point of view.[8] The plasma showed good emulsifying properties in several meat products.[9] Plasma protein is composed of 50-60% albumin, 40-50% globulin, and 1-3% fibrinogen.[10] The functional features of plasma proteins can be changed and improved by enzymatic hydrolysis. Protein hydrolysates are composed of various peptides with short sequences of 2 to 20 amino acids, regardless of the origin, and exert various biological activities such as antioxidant, antibacterial, immunomodulatory, and cholesterol-lowering effects when ingested.[11,12] Lipid peroxidation is a major problem in meat and the meat industry, causing quality deterioration due to discoloration, drip loss, off-odor and off-flavor, and the generation of potentially toxic compounds.[13,14] The blood protein hydrolysates showed emulsification and antioxidant activity in meat emulsions.[15] When porcine plasma protein hydrolysates are used in frankfurters, their emulsification and binding capabilities are improved.[16] The above studies showed that blood protein hydrolysates could be used for meat processing. However, those studies, uncooked emulsions were tested, or hydrolysates were used as a substitute for fat when making sausages. Therefore, this study prepared sausages by replacing the meat part with porcine albumin protein hydrolysates (PAPH). And the effects of antioxidant and antibacterial properties during storage were investigated and determined the appropriate amount of PAPH to be added to sausage.

Materials and Methods

Preparation of Porcine Albumin Protein Hydrolysates

Porcine blood was got from a commercial abattoir, anticoagulated with 0.2% EDTA(BD Vacutainer K2 EDTA tube, Novolab, Belgium) transferred to the laboratory, and centrifuged (Super R12, Hanil Science, Korea) at 8,000 rpm and 2° C for 15 minutes to separate blood cells and plasma. Antihemophilic factors and fibrinogen were removed by centrifugation using ethanol according to the method of Cohn.[17] The obtained protein fraction was lyophilized and diluted in distilled water (DW) to a concentration of 2%. For enzymatic hydrolysis, the protein was hydrolyzed at 60° C. for 1 hour using 5% enzyme mixture(Protamex (Protamex, Novozymes, Denmark): flavozyme (Flavozyme, Novozymes, Denmark) 4:1). Then, it was concentrated using afreeze-dried (HyperCOOL, Hanil Science, Korea).

Table 1: Pork sausages formulations treated with PAPH

Ingredients (%)	CON	T1	T2	T3	T4
Pork	61.5	61.2	60.9	60.6	60.3
Fat	21.52	21.52	21.52	21.52	21.52
Ice	13.08	13.08	13.08	13.08	13.08
N2	3.5	3.5	3.5	3.5	3.5
Handel spices	0.4	0.4	0.4	0.4	0.4
PAPH	0	0.3	0.6	0.9	1.2
Total	100	100	100	100	100

- N2 (NaCl 36%, granulated sugar 15%, isolated soy protein 15%, and binder 34%)

Treatments Preparation

Five treatments were classified with dissimilar concentrations of PAPH added: control (CON): 0%, T1:0.3%, T2, 0.6%; T3, 0.9%; T4, 1.2%. PAPH is a protein source, so the amount of meat was replaced in the same way. Jin reported that albumin hydrolysate had an antibacterial effect *in vitro* and had a better effect than plasma hydrolysate.[18] Therefore, we designed it to increase the concentration and add it to the sausages and check the effect by level. The design of the sausage is shown in Table 1. The bones were removed, and the meat and fat

chopped into 5mm thick pieces. The raw meat was put into a silent cutter bowl and cut in the first stage, salting agent (N2) and PAPH were added, and then, the meat was cut in the second stage until it became stiff. After 3 minutes, add 1/2 of ice and cut. After 6 minutes, add the remaining ice and cut. Fat and Handel spices (M2 Co. Ltd., Seongnam, Korea) were added at about 5°C and cut. The cutting time was 9 minutes in total, and the final temperature of the emulsion was less than 14°C. The mixed emulsion was used to fill a PVDC (polyvinylidene chloride)(Ø 4.14cm) casing and heated in an 80 °C boiling tank for 55 minutes (80°C/80min based on a diameter of 6 cm (increased and decreased by 25 minutes according to a diameter of 2 cm). The cooked sausage was cooled in running water for at least 30 minutes so that the surface temperature of the product was 10 °C or lower. The sausages in a vacuum werestored at 4±1°C for 4 weeks.

Proximate Composition

The moisture, protein, and fat content (%) of 0-day sausages was perfomed according to Association of Official Analytical Chemists (AOAC).[19] For crude fat, a 0.5 g sample was homogenized in 25 ml of Folch solution (chloroform: methanol, 2:1) and left in a cold space at 4° C for 24 h. Filter through whatman No.2 paper and clean with 5ml of Folch solution. After mixing 10 ml of DW with the filtrate, it was centrifuged at 3000 rpm at room temperature for 20 minutes. After removing the separated upper layer consisting of water and ethanol using a pipette, the chloroform was evaporated overnight in a hood, and the weight was measured. Proteins were measured using the Kjeldahl method. Briefly, 0.5 g of sample and 25 mL of 98% sulfuric acid (12080.100, Merck, USA) were heated together in a flask, and then the flask was connected to a distillation apparatus, and the ammonia component of the sample was adsorbed using boric acid in the flask. After titration with 0.1N sulfuric acid, the nitrogen content and protein were calculated using the following formula. N(%) = ((Sulfuric acid standard solution (mL) – Sulfuric acid standard solution (mL) used in the blank) / sample weight (g)) × (14mg /mmole) × N (H_2SO_4) × 100% × (1g / 1000mg). Protein (%) = N(%) x 6.25

pH

All samples (3g sample + 30 mL DW) were homogenized for 40 seconds with a homogenizer (Stomacher® 400 Circulator, Seward, UK), and then measured with a pH meter (Orion Star™ A211 pH Benchtop Meter, Thermo scientific™, USA) calibrated in phosphate buffer at pH 4 and 7.

Color

Color was measured with a Spectro Colorimeter (CM-26d, Konica Minolta, Japan) calibrated on a zero plate and white plate. At this time, the light source was used a white fluorescent lamp (D65). Color values were expressed as lightness (L*), redness (a*), yellowness (b*), whiteness (W), chroma, and hue values.Whiteness was calculated using the following formula: W = L* – 3b*. And the chroma and hue values were calculated using the following

formula: $C = (a^{*2} + b^{*2})^{1/2}$, h = arctag (b*/a*).[20]

Texture Profile and Shear Force

Texture profile was measured with a rheometer (Model Compac-100, Sun Scientific Co., LTD., USA). Before the measurement, the temperature of the sample was equilibrated to room temperature. Force versus time curves were obtained from two compression cycle measurements. The weight of the load plate was 10kg and the speed of the cross-head was measured to be 20 cm/min. The parameters ofgumminess; springiness; cohesiveness; hardness; adhesiveness and chewiness were measured based on the Bourne's curves.[21] For the shear force, the sample was cut so that the width × length × height was 1 × 2 × 1 cm, respectively, and the max weight was determined by shearing tests with a rheometer. The program used was the Rheology Data System (R.D.S) version 2.01. The load plate max weight was 10 kg, The platerate was 11cm/min, and the graph interval was 20 m/sec.

Sensory Evaluation

For the sensory evaluation were performed for sausage with different amounts of PAPH added, which were heated and cooked by seven trained panelists. To standardize each sensory attribute, emulsified pork sausages were prepared with different levels of lean:fat ratio (flavor and chewiness), sodium nitrite (color), and cooking time (juiciness), respectively. Six sessions were conducted by six panels, who were randomly allocated to each sensory session.[22] Using a 9-point scale, color (1 = pale, 9 = deep red), chewiness (1:highly soft, 9:highly chewy), juiciness

(1:highly dry, 9:highly juicy), flavor (1:highly bad, 9:highlygood),aroma (1: none, 9:highly strong), and overall palatability (1 :highly bad, 9:highly good) were evaluated.

Lipid Oxidation

For lipid oxidation analysis,2-thiobarbituric acid-reactive substances (TBARS) and the peroxide value (POV) were measured. To obtain the POV, lipids were extracted from sample according to the method of Folch.[23] The lipids were mixed with 10 mL chloroform and 15mL acetic acid , and then 1 mL of potassium iodide solution (99% potassium iodide:DW, 7:3) was added. Place the mixture in the dark for 15 minutes, then 30 mL of ultra-pure water was added and mixed. Add 1 mL of indicator of 1% starch solution and titrate with 0.01N $Na_2S_2O_3$ solution until colorless. The POV was calculated as the mg amount of iodine liberated per 1,000g of lipid. TBARS was measured by modifying the extraction method of Witte[24] 3.75% perchloric acid (20 mL) were added to 5 g of the sample and homogenized at 11,000 rpm for 15 seconds using a mixer. The mixture was filtered with Whatman No. 2 filter paper. The filtered mixture (5mL) and 5 mL of 0.02 M TBA solution were mixed thoroughly, and left in a dark, cool place for 16 hours. The absorbance was measured at a wavelength of 529 nm using a spectrophotometer (Mobi, Microdigital, Korea). Blank was used with ultra-pure water. TBA levels were measured as mg malonaldehyde (mg malonaldehyde/kg) per 1,000 g of sample. The standard curve used at this time was x = (y+0.0011) / 0.1975 (r = 0.999), and was calculated as x = TBA value, y = absorbance.

2,2-Diphenyl-1-Picrylhydrazyl Hydrate (DPPH) Radical Scavenging Activity

DPPH radical scavenging ability was measured based on the method of Brand-Williams[25] After homogenizing 5 g of sample and 45 mL of 99% MeOH, the mixture was filtered using Whatman No. 2 filter paper. DPPH solution (3mL) was mixed with 2 mL of the filtrate. The mixture was left in the dark for 30 minutes. The same amount of Me OH and DPPH as for the sample was used for the blank. The solution was measured at an absorbance at 517 nm. DPPH inhibition was calculated using the following formula.

DPPH inhibition (%) = {1-(sample absorbance/blank absorbance)} × 100

Purge Loss and Cooking Loss

Cooking loss was measured as the ratio (%) of the weight of the initial sample to the weight after heating the sample. For the purge loss, the weight of the sausage was measured before packaging, and after storage, the package was opened to remove moisture from the surface of the sausage, and then the weight was measured to calculate the loss of juice after storage as a percentage.

Purge loss (%) = [weight before packaging (g)-weight after opening (g)]/weight before packaging (g) × 100

Volatile Basic Nitrogen

The method of Pearson[26] was used to measure the volatile basic nitrogen (VBN) content. Ultra-pure water (45 mL) was added to 5 g of the sample and homogenized at 11,000 rpm for about 40 seconds. The mixture was filtered with Whatman No. 2 filter paper. Conway unit outer plate was put 3mL filtrate, and 3 drops of the indicator (0.066% bromocresol green+0.066% methyl red) and1 mL of 0.01 N boric acid were put into the inner plate. After apply white vaseline to the conway unit to ensure good coverage, 1 mL of 50% K_2CO_3 was put intos the outer plate, immediately sealed, and the vessel was incubated at 37 °C for 2 hours. After incubation, the inner plate was titrated with 0.02 N H_2SO_4. The VBN value was caculated in terms of mg per 100 g sample.

VBN value= ((a-b)×F×28.014×100)/amount of sample

a= amount of H_2SO_4 put into the sample (μL)

b= amount of H_2SO_4 put into the blank (μL)

F= 0.02 N H_2SO_4 standardized index

28.014= Amount of N required to titrate 1 mL of 0.02N H_2SO_4

Total Microbial Count

The total microbial count (TMC) was calculated using a serial dilution method. A 0.1% peptone solution (45 mL) was added to 5 g of the sample and homogenized for 30 seconds. The diluted sample was placed in a plate count agar (PCA) and incubated at 37 °C for 48 hours. After the incubation was completed, the colonies were counted with a colony counter. The total number of microorganisms was caculated as log cfu/g.

Free Amino Acid Analysis

The sample was put into a bottle, 40 mL of 6N HCL was added, and then, nitrogen gas was injected. HCL was removed by placing it in an evaporation flask at 50 °C. Upon the completion of the evaporation, the contents were transferred to an evaporation flask, and the DW bottle was washed. The evaporation process was repeated 3 times. After dissolving the amino acids by adding a buffer solution (pH 2.2) or DW to the evaporated final flask, the sample was filtered with No. 5B filter paper to make a volume of 50 mL. The absorbance of the sample was measured at 570 nm using an amino acid analyzer.

Statistical Analysis

All measurements were repeated at least 3 times, and the statistical processing program SAS (9.4 for Windows, USA) was used to test the significance of the results. To compare significant differences between the measured values, a significance test ($p< 0.05$) was performed with the Duncan multiple range test.

Results and Discussion

Proximate Composition and Cooking Loss

Table 2 shows the proximate composition of the sausage. The moisture content was range from 59.56% (CON) to 58.44% (T4), showing the significantly highest value in the CON group. It was considered that the water content was lower due to the addition of lyophilized PAPH. The crude fat content ranged from 19.87% (CON) to 15.39% (T4), with the highest value in the CON group. The crude protein was 18.12% (T3) ~12.62% (CON), which showed a significantly lower value in CON group among samples. Cavalheirod reported that the addition of protein hydrolysates could be an alternative to improving the protein content of meat products.[27] Pork is generally 71% water, 21.8% protein, 4% fat, and 1.5% ash. Therefore, it is considered that the amount of protein was lower than that of T3 because T4 decreased the pork while increasing the PAPH content the most.[28] Cooking loss was significantly higher in the CON group, and the value tended to decrease as the amount of PAPH added increased. Peña-Ramos considered that the increase in polar amino groups and carboxyl groups due to peptide cleavage from hydrolysis induced protein-water interactions.[29] This result found that the higher the PAPH addition level, the lower the cooking loss.

Table 2: Proximate composition and cooking loss of pork sausages treated with PAPH

	Treatments[1)]					SEM
	CON	T1	T2	T3	T4	
Moisture (%)	59.56^{A}	59.00^{B}	59.11^{B}	58.22^{C}	58.44^{C}	0.13
Fat (%)	19.87^{A}	18.44^{B}	18.06^{B}	17.55^{B}	15.39^{C}	0.49
Protein (%)	12.62^{C}	17.88^{AB}	18.11^{A}	18.12^{A}	17.63^{B}	0.71
Cooking loss (%)	5.27^{A}	4.82^{B}	4.53^{BC}	4.50^{BC}	4.30^{C}	0.08

$^{A-C}$ Different superscripts on the same row are statistically different ($p< 0.05$).

[1)]Treatments: CON (no addition), T1 (albumin hydrolysates 0.3%), T2 (albumin hydrolysates 0.6%), T3 (albumin hydrolysates 0.9%), T4 (albumin hydrolysates 1.2%).

pH and Color

Table 3 shows results pH and the color of the sausage. The pH values in the CON group were the highest at week 0 and the pH decreased as the level of added PAPH increased. After 4 weeks of storage, the pH of the CON and T1 groups decreased, but the rest of group increased ($p < 0.05$). Changes in the pH during the storage of meat products and meat are affected by microbial growth, the deamination of meat proteins, and enzyme production.[30,31] In this study, it was thought to be affected by the proliferation of microorganisms as the storage length increased. In the case of yellowness (b*) and whiteness (W), there was

no significant difference at week 0. Lightness (L*) and hue values (h) tended to decrease as the amount of PAPH added increased, but the redness (a*) and chroma (c) values tended to increase. Verma reported that the change in L* and a* was thought to be due to the interaction between the red color characteristic of blood hydrolysates and the macromolecules and lipid peroxide in pork emulsion.[31] The hue value is a characteristic that is greatly affected by a*, and the value decreases when the degree of redness is high. At the fourth storage week, T1 and T2 showed a significant difference in L*, W, and c compared to 0 weeks, and the CON group showed a significant difference in L*. In the case of a*, b*, and h, there was no significant difference according to the storage period. T4 showed no significant difference even after 4 weeks of storage.

Table 3: pH and color of pork sausages treated with PAPH

	Storage (weeks)	Treatments[1)]					SEM
		CON	T1	T2	T3	T4	
pH	0	6.78^{Aa}	6.68^{Ba}	6.61^{Cb}	6.46^{Db}	6.30^{Eb}	0.03
	4	6.50^{Eb}	6.56^{Db}	6.63^{Ca}	6.66^{Ba}	6.86^{Aa}	0.03
	SEM	0.05	0.02	0.00	0.03	0.09	
L*	0	81.60^{ABa}	81.81^{Aa}	81.12^{ABCa}	80.77^{C}	80.89^{BC}	0.14
	4	81.01^{Ab}	81.04^{Ab}	80.37^{Bb}	80.37^{B}	80.98^{A}	0.10
	SEM	0.16	0.19	0.19	0.17	0.17	
a*	0	4.68^{D}	4.86^{CD}	5.36^{BC}	5.86^{B}	6.52^{A}	0.19
	4	4.35^{C}	4.85^{BC}	5.17^{B}	6.28^{A}	6.64^{A}	0.24
	SEM	0.10	0.15	0.13	0.15	0.06	
b*	0	8.44	8.38	8.56	8.55	8.33	0.06
	4	8.78	8.86	8.95	8.70	8.54	0.07
	SEM	0.12	0.15	0.11	0.10	0.10	
W	0	56.29	56.68^{a}	55.45^{a}	55.13	55.91	0.26
	4	54.67^{AB}	54.47^{ABb}	53.51^{Bb}	54.26^{AB}	55.36^{A}	0.25
	SEM	0.50	0.60	0.48	0.46	0.36	
c	0	9.65C	9.70^{Cb}	10.10^{B}	10.37^{Ab}	10.58^{A}	0.10
	4	9.80D	10.10^{Ca}	10.35^{B}	10.74^{Aa}	10.82^{A}	0.11
	SEM	0.07	0.11	0.10	0.09	0.07	
h	0	60.97^{A}	60.30^{A}	57.93^{AB}	55.55^{BC}	51.95^{C}	0.98
	4	63.66^{A}	61.29^{A}	59.97^{A}	54.18^{B}	52.16^{B}	1.24
	SEM	0.79	1.09	0.87	0.87	0.49	

$^{A-B}$Different superscripts on the same row are statistically different ($p< 0.05$).
$^{a-b}$Different superscripts on the same column are statistically different ($p< 0.05$).
[1)]Treatments: CON (no addition), T1 (albumin hydrolysates 0.3%),
T2 (albumin hydrolysates 0.6%), T3 (albumin hydrolysates 0.9%), T4 (albumin hydrolysates 1.2%).
L*(lightness), a* (redness), b* (yellowness), W (whiteness), c (chroma), h (hue value).

Texture Profile Analysis, Shear Force, and Sensory Evaluation

Table 4 shows the texture profile analysis and shear force changes of sausages during storage. The hardness at week 0 was not significantly different between the samples, but there was a significant difference between the CON and T2 groups. Cohesiveness, springiness, gumminess, and chewiness were not different between the samples. Adhesiveness showed a significant difference in T4 among samples. After 4 weeks of storage, there was no significant difference compared to

week 0 in the T1, T2, and T4 groups, and the CON group showed an increase in gumminess and chewiness. The T3 group showed a significant decrease in hardness. Shear force was the highest in the CON at week 0, and there was no significant difference between the remaining samples. After 4 weeks of storage, the CON group showed no significant change compared to week 0, but showed a significant increase in the T1, T2, T3, and T4 groups. Lundreported that protein oxidation during storage also affected meat tenderness.[32] In this study, it was thought that the increase in shear force at week 4 was due to protein oxidation. Table 5 shows the sensory evaluation of the sausages. The color was not significantly different between the treatments. The aroma value was significantly lower in the T4 group among samples. In flavor, juiciness, chewiness, and overall acceptability, the T3 and T4 groups showed significantly lower values than the other treatments. Fu reported that plasma hydrolysates produced from Protamex showed strong sour and salty tastes.[33] The PAPH used in this study was hydrolyzed using Protamex and Flavorzyme, which is thought to have affected the sensory evaluation.

Table 4: Texture profile analysis and shear force of pork sausages treated with PAPH

	Storage (weeks)	Treatments[1)]					SEM
		CON	T1	T2	T3	T4	
Hardness (kg)	0	0.24^{A}	0.22^{AB}	0.21^{B}	0.22^{ABa}	0.22^{AB}	0.00
	4	0.27^{A}	0.22^{B}	0.20^{BC}	0.19^{Cb}	0.22^{B}	0.01
	SEM	0.01	0.00	0.01	0.01	0.00	
Surface hardness (kg)	0	0.23^{A}	0.22^{AB}	0.21^{B}	0.22^{ABa}	0.22^{AB}	0.00
	4	0.26^{A}	0.22^{B}	0.20^{BC}	0.19^{Cb}	0.22^{B}	0.01
	SEM	0.01	0.00	0.01	0.01	0.00	
Cohesiveness (%)	0	0.56	0.62	0.61	0.51	0.56	0.02
	4	0.63^{AB}	0.54^{B}	0.55^{B}	0.72^{A}	0.55^{B}	0.02
	SEM	0.02	0.03	0.04	0.06	0.01	
Springiness (mm)	0	1.11	1.14	1.07	1.01	1.00	0.02
	4	1.17^{AB}	1.03^{AB}	1.01^{AB}	1.20^{A}	1.00^{B}	0.03
	SEM	0.02	0.04	0.03	0.07	0.00	
Gumminess (kg)	0	0.13^{b}	0.14	0.13	0.11	0.12	0.00
	4	0.17^{Aa}	0.12^{B}	0.11^{B}	0.13^{B}	0.12^{B}	0.01
	SEM	0.01	0.01	0.01	0.01	0.00	
Chewiness (kg, mm)	0	0.15^{b}	0.16	0.14	0.11	0.12	0.01
	4	0.20^{Aa}	0.12^{B}	0.12^{B}	0.16^{AB}	0.12^{B}	0.01
	SEM	0.01	0.01	0.01	0.02	0.00	
Adhesiveness (kgf)	0	0.08^{B}	0.08^{B}	0.09^{B}	0.09^{B}	0.10^{A}	0.00
	4	0.08	0.08	0.09	0.08	0.09	0.00
	SEM	0.00	0.00	0.00	0.00	0.00	
Shear force (kg/cm2)	0	1.05^{A}	0.85^{Bb}	0.88^{Bb}	0.90^{Bb}	0.83^{Bb}	0.02
	4	1.09^{B}	1.06^{Ba}	1.25^{Aa}	1.29^{Aa}	1.22^{Aa}	0.03
	SEM	0.02	0.05	0.08	0.09	0.09	

$^{A-B}$ Different superscripts on the same row are statistically different ($p< 0.05$).

$^{a-b}$ Different superscripts on the same column are statistically different ($p< 0.05$).

[1)]Treatments: CON (no-addition), T1 (albumin hydrolysates 0.3%), T2 (albumin hydrolysates 0.6%), T3 (albumin hydrolysates 0.9%), T4 (albumin hydrolysates 1.2%).

Table 5: Sensory evaluation of pork sausages treated with PAPH

	Treatments[1)]					SEM
	CON	T1	T2	T3	T4	
Color	7.42	7.58	7.67	7.67	8.00	0.09
Aroma	7.83^{A}	8.00^{A}	7.58^{AB}	7.75^{AB}	7.33^{B}	0.07
Flavor	8.08^{AB}	8.25^{A}	7.92^{B}	7.42^{C}	7.08^{D}	0.09
Juiciness	8.00^{AB}	8.25^{A}	8.00^{AB}	7.67^{B}	7.58^{B}	0.08
Chewiness	8.25^{A}	8.08^{A}	7.92^{A}	7.50^{B}	7.25^{B}	0.09
Overall acceptability	8.08^{AB}	8.33^{A}	7.92^{B}	7.50^{C}	7.25^{C}	0.08

$^{A-B}$Different superscripts on the same row are statistically different ($p< 0.05$).
$^{1)}$Treatments: CON (no addition), T1 (albumin hydrolysates 0.3%),
T2 (albumin hydrolysates 0.6%), T3 (albumin hydrolysates 0.9%),
T4 (albumin hydrolysates 1.2%).

Table 6: Lipid oxidation, volatile basic nitrogen, and total microbial counts of pork sausages treated with PAPH

	Storage (weeks)	Treatments[1)]					SEM
		CON	T1	T2	T3	T4	
Purge loss (%)	0	0.16^{Cb}	0.18^{BCb}	0.20B^{Cb}	0.22^{ABb}	0.25^{Ab}	0.01
	4	2.22^{ABa}	2.10^{Ba}	2.08^{Ba}	2.13^{ABa}	2.27^{Aa}	0.03
	SEM	0.46	0.43	0.42	0.43	0.45	
TBARS (mg MA/kg)	0	0.46^{D}	0.52^{Ca}	0.61^{Ba}	0.65^{Aa}	0.68^{Aa}	0.02
	4	0.43^{C}	0.44^{BCb}	0.48^{BCb}	0.49^{Bb}	0.55^{Ab}	0.01
	SEM	0.02	0.02	0.02	0.03	0.03	
POV	0	0.06^{Cb}	0.05^{Cb}	0.17^{Bb}	0.17^{Bb}	0.30^{Ab}	0.03
	4	1.21^{Da}	1.56^{Ca}	1.85^{Ba}	2.39^{Aa}	2.44^{Aa}	0.13
	SEM	0.26	0.34	0.38	0.48	0.48	
DPPH	0	95.59	93.96	98.68^{a}	98.68^{a}	96.41^{a}	1.03
	4	88.20^{A}	75.57^{AB}	55.08^{BCb}	39.80^{Cb}	37.68^{Cb}	6.31
	SEM	3.33	6.78	10.75	12.38	13.50	
VBN (mg%)	0	7.42^{Bb}	8.05^{Ab}	8.05^{Ab}	8.36^{Ab}	8.30^{Ab}	0.08
	4	8.79^{Ca}	8.96^{Ca}	9.69^{Ba}	9.80^{Ba}	12.43^{Aa}	0.27
	SEM	0.25	0.18	0.31	0.26	0.69	
Total plate count	0	-	-	-	-	-	-
	4	2.96^{D}	3.08^{D}	3.51^{C}	3.98^{B}	4.49^{A}	0.19
	SEM	-	-	-	-	-	

$^{A-B}$Different superscripts on the same row are statistically different ($p< 0.05$).
$^{a-b}$Different superscripts on the same column are statistically different ($p< 0.05$).
$^{1)}$Treatments: CON (no addition), T1 (albumin hydrolysates 0.3%), T2 (albumin hydrolysates 0.6%),
T3 (albumin hydrolysates 0.9%), T4 (albumin hydrolysates 1.2%).

Storage Stability

As shown in Table 6, 4-week changes in purge loss, TBARS, POV, DPPH, VBN, and TMC was analyzed to determine the storage stability of sausages with added PAPH. The purge loss value was increased as the PAPH addition level increased at week 0 and showed a significantly higher value at T4. At 4 weeks, all treatments increased significantly compared to 0 weeks. The TBA values were significantly higher in the T3 and T4 groups at week 0. After 4 weeks of storage, the CON group showed significantly lower values. The values increased significantly as the amount added increased, but the T1, T2, T3, and T4 groups showed decreased values compared to the 0-week values. The POV value increased significantly as the amount of PAPH added increased, and the T4 group showed a significantly higher value. After 4 weeks of storage, the value increased significantly compared to week 0 in all samples and tended to increase as the amount of PAPH added increased previous studies have reported that the addition of protein hydrolysates inhibited lipid oxidation with high antioxidant activity.[29,34,35] However, in this study, the addition of porcine albumin hydrolysate did not show any effect on POV and TBARS. This result was due to the difference between the various types of hydrolysates and the treatment processes.[22] The DPPH values were significantly lower in the CON and T1 groups at week 0. At 4 weeks of storage, the value in the CON group was significantly higher, and with higher amounts of PAPH added, the values decreased significantly compared to those at 0 weeks. In the case of VBN, the CON group showed significantly lower values at week 0. After 4 weeks of storage, the values in all treatment groups increased significantly compared to 0 weeks, and the values tended to increase significantly as the amount of added PAPH increased. Wang reported an increase in VBN due to protein degradation by microorganisms according to the storage period.[36] TMC showed a significantly lower value in the CON and T1 groups at the fourth week of storage, and the number of microorganisms increased significantly as the amount of added PAPH increased, with a significantly higher value in the T4 group. The added albumin protein has a high affinity for fatty acids, which affects the growth of microorganisms.[37] and Jin reported that PAPH has antibacterial activity only in Bacillus cereus.[18] Therefore, it was considered that the addition of PAPH had an effect growth of microorganisms in this study as well.

Free Amino Acids Analysis

Table 7 shows the free amino acid composition of the sausage to which PAPH was added. Glycine, alanine, leucine, tyrosine, phenylalanine, lysine, and arginine showed significantly lower values in CON than in another group, and significantly higher values as the amount of PAPH added increased. Among the amino acid composition at week 0, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, histidine, tryptophan, lysine, and arginine are essential amino acids.[5] Among them, except for tryptophan, the other amino acids showed either significantly higher values in the treatment group or no difference. When phenylalanine is exposed to hydroxyl radicals, it is converted to tyrosine, and tyrosine shows antioxidant activity by scavenging hydroxyl radicals.[38,39] After 4 weeks of storage, serine showed a significant increase in all treatment groups except for the CON group, and lysine showed a significant decrease in all samples. Tyrosine, r-aminobutyric acid, and arginine were not detected after 4 weeks of storage. Triki found that in meat storage, arginine was reduced due to the formation of agmatine and putrescine, which could subsequently trigger the production of spermidine and spermine.[40] Therefore, it was thought that arginine decreased due to the formation of agmatine and putrescine in this study. In general, biogenic amines in food are formed by the decarboxylation of free amino acids by microorganisms.[41] It was thought that lysine was a precursor of cadaverine and was converted to cadaverine by microorganisms during storage. The biogenic amines content in meat can be used as an indicator of freshness and spoilage. The presence of putrescine, cadaverine, spermidine, and spermine indicates early spoilage. Cadaverine is a detectable indicator in both red meat and white meat.[42] The results of this study suggested that the addition of PAPH could partially replace the meat protein portion nutritionally at the initial stage of storage. However, it was thought to have a negative effect due to the formation of biogenic amine during storage.

Table 7: Free amino acids analysis of pork sausages treated with PAPH

Amino acids	Storage (weeks)	Treatments[1)]					SEM
		CON	T1	T2	T3	T4	
Serine	0	0.82^{AB}	0.81^{ABb}	0.87^{Ab}	0.76^{ABb}	0.72^{Bb}	0.02
	4	0.93^{C}	0.96^{Ba}	1.21^{Aa}	0.91^{Ca}	1.18^{Aa}	0.04
	SEM	0.04	0.05	0.10	0.05	0.13	
Taurine	0	4.25^{ABa}	4.43^{Aa}	4.06^{BCa}	3.79^{D}	3.97^{CDa}	0.08
	4	3.72^{Bb}	3.83^{Ab}	3.22^{Db}	3.35^{C}	3.15^{Db}	0.09
	SEM	0.15	0.17	0.24	0.14	0.24	
Proline	0	1.22	0.96	0.57^{b}	1.11	1.02	0.11
	4	0.31^{E}	1.26^{A}	1.21^{Ba}	0.91^{C}	0.79^{D}	0.12
	SEM	0.32	0.13	0.18	0.08	0.09	
Glycine	0	1.61^{Ba}	1.73^{AB}	1.88^{Aa}	1.89^{Aa}	1.96^{Aa}	0.05
	4	1.24^{Cb}	1.59^{A}	1.21^{Cb}	1.52^{Bb}	1.58^{Ab}	0.06
	SEM	0.11	0.05	0.19	0.11	0.11	
Alanine	0	3.91^{C}	4.26^{BCb}	4.55^{ABa}	4.62^{AB}	4.84^{Ab}	0.11
	4	4.33^{CD}	5.10^{Ba}	4.43^{Cb}	4.26^{D}	5.51^{Aa}	0.17
	SEM	0.14	0.25	0.04	0.13	0.20	
Valine	0	1.31^{Ca}	1.22^{Cb}	1.52^{Bb}	1.48^{Ba}	1.80^{Ab}	0.07
	4	0.93^{Eb}	1.59^{Ca}	2.01^{Ba}	1.22^{Db}	2.36^{Aa}	0.17
	SEM	0.11	0.11	0.14	0.08	0.16	
Methionine	0	1.05	1.08	0.92^{a}	0.87^{b}	0.97	0.04
	4	0.62^{D}	1.28^{A}	0.40^{Eb}	0.91^{Ba}	0.79^{C}	0.10
	SEM	0.15	0.07	0.15	0.01	0.05	
Isoleucine	0	1.15^{a}	1.28	1.25^{a}	1.34^{a}	1.47	0.05
	4	0.62^{Eb}	0.96^{B}	0.80^{Db}	0.91^{Cb}	1.58^{A}	0.11
	SEM	0.15	0.11	0.13	0.13	0.05	
Leucine	0	2.67^{Ca}	2.96^{C}	3.44^{Ba}	3.72^{Ba}	4.28^{A}	0.19
	4	1.55^{Eb}	3.19^{B}	2.82^{Cb}	2.44^{Db}	3.94^{A}	0.26
	SEM	0.33	0.08	0.18	0.38	0.11	
Tyrosine	0	1.50^{B}	1.61^{B}	1.72^{AB}	1.73^{AB}	1.93^{A}	0.05
	4	-	-	-	-	-	-
	SEM	-	-	-	-	-	
Phenylalanine	0	1.75^{C}	1.82^{BCa}	2.11^{Ba}	2.11^{Ba}	2.59^{A}	0.10
	4	1.24^{E}	1.59^{Cb}	2.01^{Bb}	1.52^{Db}	2.76^{A}	0.18
	SEM	0.16	0.07	0.03	0.17	0.07	
r-Aminobutyric acid	0	0.24^{AB}	0.33^{AB}	0.16^{B}	0.32^{AB}	0.37^{A}	0.03
	4	-	-	-	-	-	-
	SEM	-	-	-	-	-	
Histidine	0	0.59^{D}	0.59^{D}	1.23^{Aa}	0.73^{Ca}	0.84^{B}	0.08
	4	0.62^{C}	0.64^{B}	0.80^{Ab}	0.61^{Cb}	0.79^{A}	0.03
	SEM	0.02	0.02	0.12	0.03	0.01	
Tryptophan	0	58.80^{Aa}	58.98^{Aa}	54.83^{Ba}	56.18^{ABa}	53.35^{Ba}	0.79
	4	46.28^{Ab}	45.14^{Bb}	44.06^{Cb}	43.53^{CDb}	43.30^{Db}	0.37
	SEM	3.66	4.00	3.11	3.66	2.92	
Carnosine	0	16.16^{b}	14.58^{b}	17.04^{b}	15.43^{b}	15.48^{b}	0.46
	4	37.31^{Aa}	30.62^{Da}	32.60^{Ca}	36.99^{Ba}	30.31^{Ea}	1.01

	SEM	6.10	4.69	4.49	6.26	4.29	
Ornithine	0	0.17^{Bb}	0.11^{Cb}	0.25^{Ab}	0.12^{Cb}	0.08^{Db}	0.02
	4	0.31^{Ea}	0.64^{Ba}	0.80^{Aa}	0.61^{Ca}	0.39^{Da}	0.06
	SEM	0.04	0.15	0.16	0.14	0.09	
Lysine	0	1.58^{D}	1.74^{Ca}	2.01^{B}	2.06^{Ba}	2.32^{Aa}	0.09
	4	-	0.64^{Bb}	2.01^{A}	0.30^{Db}	0.39^{Cb}	0.23
	SEM	-	0.32	0.00	0.51	0.56	
Arginine	0	1.19^{C}	1.51^{BC}	1.59^{B}	1.76^{AB}	2.03^{A}	0.10
	4	-	-	-	-	-	-
	SEM	-	-	-	-	-	

[A-C]Different superscripts on the same row are statistically different ($p < 0.05$).
[1)]Treatments: CON (no addition), T1 (albumin hydrolysates 0.3%), T2 (albumin hydrolysates 0.6%), T3 (albumin hydrolysates 0.9%), T4 (albumin hydrolysates 1.2%).

Conclusion

This study was conducted to investigate the physicochemical properties and storage effects of the addition of PAPH to sausages and determine the appropriate amount of PAPH to add to sausages. The addition of PAPH showed an increase in protein, a decrease in cooking loss, an increase in essential amino acid content, and an increase in redness, but did not show a significant difference in texture profile analysis. After storage, the higher the amount of PAPH that had been added, the more the shear force was significantly increased compared to the control group, but the antioxidant effect was insignificant. In addition, the formation of biogenic amines such as cadaverine, putrescine, spermidine, and spermine in free amino acids adversely affected the sausages during storage. The results showed that the addition of PAPH to sausage had a good effect on cooking loss, essential amino acid content, but had a not good effect on lipid oxidation as the amount of PAPH added increased. So 3g/kg was considered an appropriate amount to add. Additionally, although free amino acids were analyzed in this study, additional studies on biogenic amines produced after storage are needed.

Author Contribution

† These authors contributed equally to this work.

Acknowledgements

We are grateful to Regional Animal Industry Center at Gyeongsang National Universityand and the research team for helping with this study.

Funding

This work was supported by the Regional Animal Industry Center at Gyeongsang National University.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Wismer-Pedersen J. Use of haemoglobin in foods–a review. *Meat Sci.* 1988;24:31–45.10.1016/0309-1740(89)90005-3.
2. Del Hoyo P, Rendueles M, Díaz M. Effect of processing on functional properties of animal blood plasma. *Meat Sci.* 2008;78:522–8.10.1016/j.meatsci.2007.07.024.
3. Rendueles M, Moure F, Fernández A, Díaz M. Preliminary studies on the processing of slaughter houses blood for protein recovery. Res Environ Biotechnol. 1997;1:193-206.
4. Kim S.H. A multi-omics approach to assess production of the valuable peptides and amino acids in porcine blood protein hydrolsate. LWT. 2022;163:113593.10.1016/j.lwt.2022.113593.
5. Ramos-Clamont G, Fernández-Michel S, Carrillo-Vargas L, MartinezCalderón E, Vázquez-Moreno L. Functional properties of protein fractions isolated from porcine blood. *J Food Sci.* 2003;68(4):1196–1200.10.1111/j.1365-2621.2003.tb09624.x.

6. Dàvila E, Saguer E, Toldrà M, Carretero C, Parés D. Surface functional properties of blood plasma protein fractions, Eur Food Res Technol. 2007;226:207–214.10.1007/s00217-006-0527-2.
7. Autio K, Lyytikäinen H, Mälkki Y, Kanko S. Penetration studies of blood globin gels *J Food Sci.* 1985;50:615-617.10.1111/j.1365-2621.1985.tb13757.x.
8. Silva V.D.M., Silvestre M.P.C. Functional properties of bovine blood plasma intended for use as a functional ingredient in human food. *LWT-Food Sci Technol.* 2003;36(7):709-718.10.1016/S0023-6438(03)00092-6.
9. Caldironi H.A., Ockerman H.W. Incorporation of blood proteins into sausage. *J Food Sci.* 1982;47(2):405-408. 10.1111/j.1365-2621.1982.tb10091.x.
10. Dàvila E, Parés D, Cuvelier G, Relkin P. Heat-induced gelation of porcine blood plasma proteins as affected by pH. *Meat Sci.* 2007;76(2):216-225.10.1016/j.meatsci.2006.11.002.
11. Bernardini R.D., Harnedy P., Bolton D., Kerry J., O'Neill E., Mullen A.M., Hayes M. Antioxidant and antimicrobial peptidic hydrolysates from muscle protein sources and by-products. *Food Chem.* 2011;124(4):1296–1307. 10.1016/j.foodchem.2010.07.004.
12. Nasri M. Protein hydrolysates and biopeptides: Production, biological activities, and applications in foods and health benefits. A review. *Adv Food Nutr Res.* 2017;81:109–159.10.1016/bs.afnr.2016.10.003.
13. Min B., Ahn D.U. Mechanism of lipid peroxidation in meat and meat products - A review. *Food Sci Biotechnol.* 2005;14(1):152-163.
14. Morrisey P.A., Sheehy P.J.A., Galvin K., Kerry J.P., Buckley D.J. Lipid stability in meat and meat products. *Meat Sci.* 1998;49(1):S73-S86.10.1016/S0309-1740(98)90039-0.
15. Karakaya M., Bayrak E., Ulusoy K. Use of natural antioxidants in meat and meat products. *J Food Sci Engin.* 2011;1:1–10. 10.17265/2159-5828/2011.01.001.
16. Chen Y., Jia X., Sun F., Jiang S., Liu H., Liu Q., Kong B. Using a stable pre-emulsified canola oil system that includes porcine plasma protein hydrolysates and oxidized tannic acid to partially replace pork fat in frankfurters. *Meat Sci.* 2020;160:107968.10.1016/j.meatsci.2019.107968.
17. Cohn E.J., Strong L.E., Hughes W.L., Mulford D.J., Ashworth J.N., Melin M., Taylor H.L. Preparation and properties of serum and plasma proteins. IV. A system for the separation into fractions of the protein and lipoprotein components of biological tissues and fluids. *J Am Chem Soc.* 1946;68(3):459–475.10.1021/ja01207a034.
18. Jin S.K., Choi J.S., Yim D.G. Hydrolysis conditions of porcine blood proteins and antimicrobial effects of their hydrolysates. *Food Sci Anim Resour.* 2020;40(2):172-182.10.5851/kosfa.2020.e2.
19. Association of Official Analytical Chemists (AOAC). Official methods of analysis. 2000;17th ed. Gaithersburg, MD: AOAC.
20. Mclellan, M. R., Lind, L. R. Kime, R. W. Hue angle determinations and statistical analysis for multiquadrant Hunter L, a, b data. Journal of food quality, 1995;18(3): 235-240. 10.1111/j.1745-4557.1995.tb00377.x.
21. Bourne M.C. Texture profile analysis. *Food Technol.* 1978;32(7):62–66
22. Jin S.K., Choi J.S., Kim G.D. Effect of porcine plasma hydrolysate on physicochemical, antioxidant, and antimicrobial properties of emulsion-type pork sausage during cold storage. *Meat Sci.* 2021;171:108293.10.1016/j.meatsci.2020.108293.
23. Folch J., Lees M., Sloane-Stanley G.H. A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem.* 1957;226(1):497–509.
24. Witte V.C., Krauze G.F., Bailey M.E. A new extraction method for determining 2-thiobarbituric acid values of pork and beef during storage. *J Food Sci.* 1970;35(5): 582-585.10.1111/j.1365-2621.1970.tb04815.x.
25. Brand-Williams W., Cuvelier M.E., Berset C. Use of a free-radical method to evaluate antioxidant activity. *LWT-Food Sci Technol.* 1995;28(1):25–30.10.1016/S0023-6438(95)80008-5.
26. Pearson D. Application of chemical methods for the assessment of beef quality. II. Methods related to protein breakdown. *J Sci Food Agric.* 1968;19(7): 366-369.10.1002/jsfa.2740190703.

27. Cavalheiro C.P., Lüdtke F.L., Stefanello F.S., Kubota E.H., Terra N.N., Fries L.L.M. Replacement of mechanically deboned chicken meat with its protein hydrolysate in mortadella-type sausages. *Food Sci Technol.* 2014;34(3):478–484.10.1590/1678-457x.6370.
28. Okrouhla, M., Stupka, R., Čítek, J., Okrouhlá, M., Stupka, R., Čítek, J., Šprysl, M., Trnka, M., Kluzáková, E. Effect of lean meat proportion on the chemical composition of pork. *Czech Journal of Food Sciences,* 2009;26(6):464-469. 10.17221/18/2008-CJFS.
29. Peña-Ramos E.A., Xiong Y.X. Whey and soy protein hydrolysates inhibit lipid oxidation in cooked pork patties. *Meat Sci.* 2003;64(3):259-263.10.1016/S0309-1740(02)00187-0.
30. Jay J.M., Loessner M.J., Golden D.A. Intrinsic and extrinsic parameters of foods that affect microbial growth. In Modern food microbiology (7th ed.). New York: Springer. 2005;39–59.10.1007/978-1-4615-7476-7_3.
31. Verma A.K., Chatli M.K., Mehta N., Kumar P. Assessment of physico-chemical, antioxidant and antimicrobial activity of porcine blood protein hydrolysate in pork emulsion stored under aerobic packaging condition at 4 ± 1 °C. LWT-Food Sci Technol. 2018;88:71–79.10.1016/j.lwt.2017.10.002.
32. Lund M.N., Heinonen M., Baron C.P., Estévez M. Protein oxidation in muscle foods: A review. *Mol Nutr Food Res.* 2011;55(1):83–95.10.1002/mnfr.201000453.
33. Fu Y., Liu J., Hansen E.T., Bredie W.L.P., Lametsch R. Structural characteristics of low bitter and high umami protein hydrolysates prepared from bovine muscle and porcine plasma. *Food Chem.* 2018;257:163–171.10.1016/j.foodchem.2018.02.159.
34. Xu X., Cao R., He L., Yang N. Antioxidant activity of hydrolysates derived from porcine plasma. *J Sci Food Agric.* 2009;89(11):1897–1903.10.1002/jsfa.3670.
35. Park S.Y., Lee, J.-S., Baek H.-H., Lee H.G. Purification and characterization of antioxidant peptides from soy protein hydrolysate. *J Food Biochem*. 2010;34(s1):120–132.10.1111/j.1745-4514.2009.00313.x.
36. Wang S., Xu F.B., Zhu L.F., Yu Y.Z., Na C.Z. Antimicrobial and Antioxidant Activities of Deer Blood Hydrolysate on Fresh Beef during Refrigerated Storage. Adv Mater Res. 2013;634-638:1417–1422.10.4028/www.scientific.net/AMR.634-638.1417.
37. Davis B.D., Dubos R.J. The binding of fatty acids by serum albumin, a protective growth factor in bacteriological media. *J Exp Med.* 1947;86(3):215–228.10.1084/jem.86.3.215.
38. Kapiotis S., Hermann M., Held I., Mühl A., Gmeiner B. Tyrosine: An inhibitor of LDL oxidation and endothelial cell cytotoxicity initiated by superoxide/nitric oxide radicals. FEBS Lettrs. 1997;409(2):223–226.10.1016/S0014-5793(97)00513-9.
39. Togashi S., Takahashi N., Kubo Y., Shigihara A., Higashiyama K., Watanabe S., Fukui T. Purification and identification of antioxidant substances in human-placenta extracts. *J Health Sci.* 2000;46(2):117–125.10.1248/jhs.46.117.
40. Triki M., Herrero A., Jiménez-Colmenero F., Ruiz-Capillas C. Quality assessment of fresh meat from several species based on free amino acid and biogenic amine contents during chilled storage. *Foods.* 2018;7(9):132.10.3390/foods7090132.
41. Liu Z-F, Wei Y-X, Zhang J-J, Liu D-H, Hu Y-Q, Ye X-Q. 2011. Changes in biogenic amines during the conventional production of stinky tofu. *Int J Food Sci Technol* 46: 687–694.doi 10.1111/j.1365-2621.2011.02545.x10.1111/j.1365-2621.2011.02545.x.
42. Vinci G., Antonelli M. Biogenic amines: quality index of freshness in red and white meat. *Food Control.* 2002;13(8):519–524.10.1016/S0956-7135(02)00031-2.-