Effects of Salt, Blood Concentration and Cooking Temperature on the Quality of Edible Blood Gel

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***Abstract*—Blood gel is traditional product in Thailand. The gel-forming ability is upon the concentrations of major ingredients and cooking temperature. Therefore, the effects of salt, blood concentration and cooking temperature on the quality of blood gel were investigated. Blood gel samples were prepared using different concentrations of salt (3.0%, 3.5% and 4.0%), of porcine blood (20%, 30% and 40%) and cooking temperature (80 ℃, 90 ℃ and 100 ℃). The correlative influences on gelling quality were evaluated by texture, color and microbiology. The results showed that the addition of salt as well as rising cooking temperature significantly increased breaking force of gel. The correlations between factors demonstrated that salt content and cooking temperature are highly correlated with texture of gel. The concentration of blood and cooking temperature had slightly effect on the lightness and yellowness of gels. The microbiological quality of prepared gels was better than the control. The sensory test demonstrated that the consumer panels preferred 6 gel samples that prepared from 3.0%-4.0% salt, 30% blood and cooking with 90 ℃.**

***Index Terms*—animal blood proteins, blood proteins functional properties, edible animal blood, heat-induced gelation, porcine blood**

I. Introduction

In the past of years, blood from slaughterhouse was well known as a potential source of edible proteins and essential amino acids especially lysine [1], [2]. Blood is typically composed of two fractions of plasma and red blood cells. Plasma, a straw color liquid, accounts for 55% of blood's volume. Approximately 90% of plasma is water, 8% is the protein components which primary are albumin, globulin and fibrinogen, and the remaining division is inorganic salts. Red blood cells account for 45% of blood's volume. Hemoglobin (Hb) is the most abundant protein found in red blood cells, accounts for more than 90% [3]-[5]. Blood proteins level in whole blood account for same as lean meats (18% by average). Table I presents levels of different proteins in blood derived from cattle, pig and sheep [6]. Therefore, many applications of animal blood and animal blood proteins for human food had been reported [1], [2], [5], [7]-[11]. These may be contributed to their functional properties such as water-holding, water-binding foaming, emulsifying and heat-induced gelling properties. However, only a small part of total blood collected from slaughterhouse has been used in food manufactures. Most of blood went to animal feed manufactures. Therefore, a number of researches conducting on the utilization of blood and blood proteins for human food are still required.

TABLE I. Levels of Blood Protein Constitutes in Different Animals

Species	Levels in whole blood (%)				
	Hemo-globin	Albumin	Globulin	Fibrinogen	Total proteins
Cattle	10.30	3.61	2.90	0.60	17.41
Pig	14.20	3.83	2.96	0.65	22.25
Sheep	9.30	3.83	3.00	0.46	16.59

There has been growing interest in the utilization of animal blood to food products as functional protein and nutritional supplementary compound. Many researches have attempted to incorporate blood and blood proteins into food products. Reference [12] incorporated porcine blood in bread and they found that bread with blood showed small loaf volume, coarse texture and dark color. Blood plasma was used as a replacement for egg in cakes [13], [14], as an emulsifying agent in sausage [15] and as a surimi gel enhancement [16], [17]. Currently, animal blood has been incorporated successfully in blood sausage which is growing in popularity. Blood sausage, black pudding or blood pudding is a sausage which is made by incorporating animal blood into meat, fat and seasoning. The products are available in the market of UK, Ireland, France, Spain and Germany [11], [18].

Blood gel is traditional product in Thailand. It is used as an ingredient for many foods especially with soup. Bloods from pig, chicken and duck are commonly used for preparing blood gel. Although in some Thai people, blood gel or other productions from animal blood may not be accepted overall, attending to religious considerations in some cases. Actually, blood is not an alien ingredient in meat products because it is naturally found in meat. Moreover, it is worth pointing out that blood proteins do not show allergenic potential [11]. Blood gel is prepared from three ingredients; animal blood, salt, and water. The gel-forming ability is upon the concentrations of major ingredients and cooking temperature. Therefore, these main factors could be recognized to significantly affect on textural properties of blood gel. Under appropriate conditions, a good network may be formed. Functional properties such as texture, color and odor of blood gel are the major factors responsible for the final acceptance by consumers. Blood

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gel usually prepared with the traditional style and sold without packaging in local markets or vacuum-packaged for retail sale. Therefore, the problems of this product are the texture and shelf-life. Elastic gel, not too hard or too soft gel, tends to be desirable texture. Shelf-life of blood gel without packaging is quite short only 2-3 days while with vacuum-packaged is around 5-7 days. However, the shelf-life depends on initial contamination levels, transportation and storage conditions (pre-study). Therefore, this research aimed to study the effects of salt, blood concentration and cooking temperature on the quality of animal blood gel.

II. Materials and Methods

A. Preparation of Porcine Blood

Porcine blood was collected from local slaughterhouse. A whole blood was mixed immediately with an anticoagulant of NaCl after being derived from slaughtered pig. The concentration of NaCl was adjusted to three different levels, 3.0%, 3.5% and 4.0%. Each blood sample was cooled on ice and immediately taken to the laboratory. The temperature of blood was controlled lower than 10 ℃. The blood samples must be used within 12h.

B. Preparation of Blood Gels

TABLE II. Experimental Formula of Blood Gels

Treatments (n = 3×3×3)		
NaCl content (%)	Blood content (%)	Cooking temperature (℃)
3.0	20	80
3.5	30	90
4.0	40	100

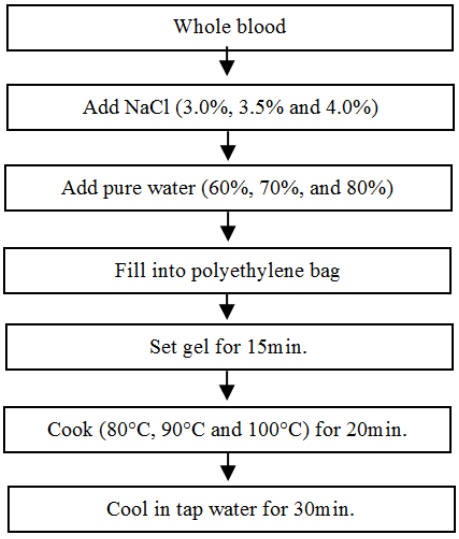


Figure 1. Main steps for preparing blood gel.

Formulations of blood gels are shown in Table II. The scheme followed for preparing blood gel is shown in Fig. 1. Each blood samples containing with various NaCl concentrations of 3.0%, 3.5% and 4.0% (S3, S3.5, S4) were diluted with pure water to have final concentration of 20%, 30% and 40% of blood (B20, B30, B40). Then, the samples were filled into polyethylene bag of 35mm diameter and sealed. The samples were left for 15min at room temperature in order to set gel before heating at various temperatures of 80 ℃, 90 ℃ and 100 ℃ (T80, T90, T100) for 20min. After heating, the gel samples were cooled immediately by immersing in tap water for 30min. The gel samples were kept at room temperature until used.

C. Textural Analysis of Blood Gel

Textural properties of blood gel were evaluated after cooking for 12h using Texture Analyzer (TA.XT plus, Stable Micro System Ltd., Surry UK) with a 25kg load cell. The blood gel was removed from the polyethylene bag before cutting in cylinder of 35mm diameter × 25mm length. Each cylinder was compressed axially in two consecutive cycles of 25% compression, 5s apart, with a flat plunger 50mm in diameter. The cross-head moved at a constant speed of 1mm/s. The measured data were analyzed using the software (TPA) developed for the instrument. The results were expresses as breaking force (g) and breaking distance (mm). The texture analysis was carried out for three independent batches. At least 20 cylinders were taken for each batch.

D. Color Measurement

Color measurements of gel samples were evaluated in color meter (Mini Scan XE Plus, USA). The results were reported as L*a*b*, where L* is the lightness from black to white, a* is the colors from green (-a*) to red (a*) and b* is the color from blue (-b*) to yellow (b*).

E. Microbiological Evaluation

Microbiological evaluation was performed 24 h after preparation blood gel. Total viable count (TVC) was enumerated in pour plates of plate count agar (Biomark, India) after incubation at 35±2 ℃ for 48h. Microbiological level of blood gel from the local market near laboratory was evaluated as the control.

F. Sensory Evaluation

Sensory test was conducted following by Ref. [18]". The tests assessed texture, color, odor and overall acceptability using a 9-point hedonic scale ranging from 1 (extremely disliked) to 9 (extremely liked). Thirty panelists, age between 20-45 years (60% females and 40% males), who usually consumed blood gel were selected.

G. Statistical Analysis

All analyses were run in triplicate for each batch. Results are reported as mean values ± standard deviation (SD). Analysis of variance was performed by ANOVA procedures (SPSS 17.0 for Windows). Difference among the mean values of the various treatments was determined by the least significant difference (LSD) test, and the significance was defined at $P < 0.05$.

III. Results and Disscussion

A. Textural Analysis of Blood Gel

The textural property of gel is the most important factor that responsible for the final acceptance by consumers. Elastic and soft gel is preferred by consumer. According to their resistance against compression force

(breaking force) and their ability to retain conformation (breaking distance), large-deformation were performed. The effects of concentrations of salt, blood and cooking temperature on breaking force (g.) of gels were shown in Fig. 2. The concentration of salt and cooking temperature had strongly effect on breaking force of gels in all treatments (P<0.05). Therefore, the addition of salt as well as rising cooking temperature improved hardness of gels. As stated by ref. [19] that protein concentrations could influence the gelation of blood plasma and a solution of 30% proteins of blood serum albumen got gel when the temperature exceeded 30 ℃. Fig. 3 demonstrated the effects of concentrations of salt, blood and cooking temperature on breaking distance (mm.) of gels. Breaking distance is an indicative parameter of elasticity of gel. Large scale of breaking distance implied that gels could retain to deformation. The cooking temperature at 90 ℃ and 100 ℃ brought about a more consistent increase in breaking distance in all treatments (P<0.05). However, no significant difference was found between gels containing 3% salt at the same cooking temperature. This result is consistent with the results described previously by ref. [20] that the gelling capacity of plasma proteins was related to the heating temperature and the percentage of inclusion of plasma. In comparison between samples with different blood content, it was found that blood content had no effect on breaking distance. Breaking distance of all samples tended to increase as salt content was increased, indicating that addition of salt enhanced gel elasticity. NaCl has been reported to use in food industries to increase the water absorption and solubility of protein, as well as to improve the flavor of several products [21]. Furthermore, the salting out effect could be explained these results since the effect normally observed for salt concentration over 1mol/L [8]. Heat-induced gelation is a two-step process, the first step is considered to be denaturing and the second aggregation, resulting in a gel network [18]. Actually, blood proteins are a complex mixture with different proteins being hemoglobin, albumin, globulin and fibrinogen (Table I). Therefore, the gel-forming ability upon heating is probably attributed of blood proteins such as hemoglobin and albumin. Under appropriate conditions, a three-dimensional network may be formed, contributing to the development of the internal structure gel and improved properties like water holding capacity [9]. A number of studies have suggested that blood proteins formed a gel at temperature above 60 ℃ [2], [22].

The improvement of gelling properties prepared from blood proteins was previously observed [21], [23]. Ref. 20 reported that gelling properties of blood plasma could be improved using microbial transglutaminase under specific condition. High-pressure processing was used to improve the water holding capacity of heat-induced gel prepared from porcine blood plasma [22].

The statistical analysis indicated that salt content and cooking temperature were significantly affected on all textural properties of gel, while, blood content significantly affected only on breaking force as shown in Table III. There are some researches reported either an advantageous or a harmful effect of salt on the solubility depending on the protein and pH condition, such as, the addition of 0.25mol/L of NaCl decreased the solubility of bovine globine at pH 6.0 [18]. Although the exact mechanism involved in blood gel formation is unknown, the most likely mode of action will be according to the following sequence of events: protein denaturation and unfolding followed by protein interactions and aggregation to form a three-dimensional protein network. The interactions between blood proteins-water-NaCl, and the attracting and repelling forces between peptide chains, lead to the formation of the gel. Some of the attraction forces which may contribute to gel formation arise from hydrophobic and electrostatic interactions, hydrogen, covalent and disulfide bridges. Given the important role played by intermolecular protein interactions in gelling [20].

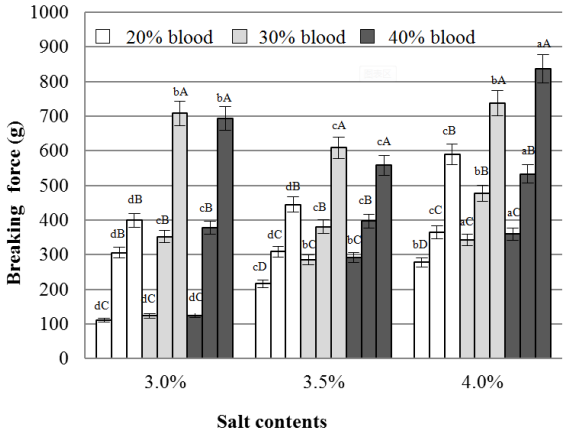


Figure 2. The effect of concentrations of salt (3.0%, 3.5% and 4.0%), blood (20%, 30% and 40%) and cooking temperature (80 ℃, 90 ℃ and 100 ℃) on breaking force (g) of gel. The first, second and third column with the same color means cooking temperature at 80 ℃, 90 ℃ and 100 ℃, respectively. Capital letters refer to difference between samples at the same salt content and lowercase letters refer to differences between samples at the same cooking temperature.

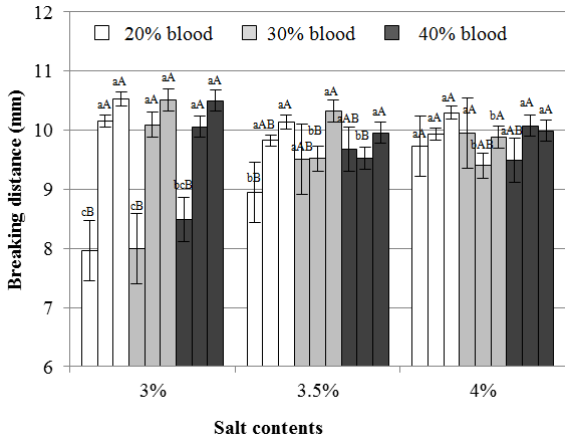


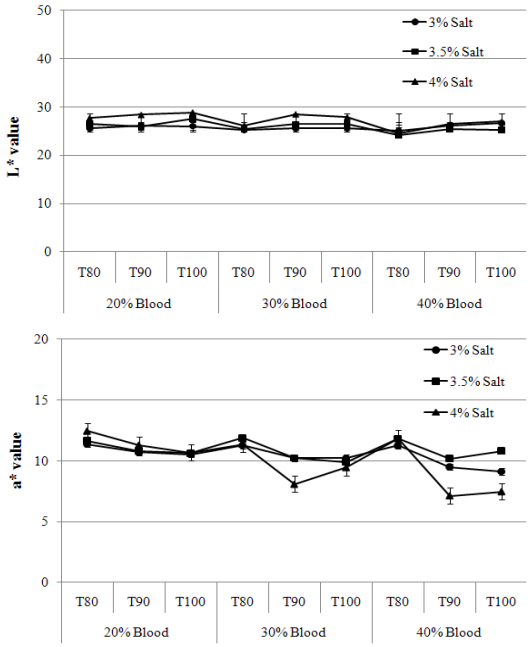
Figure 3. The effect of concentrations of salt (3.0%, 3.5% and 4.0%), blood (20%, 30% and 40%) and cooking temperature (80 ℃, 90 ℃ and 100 ℃) on breaking distance (mm) of blood gel. The first, second and third column with the same color means cooking temperature at 80 ℃, 90 ℃ and 100 ℃, respectively. Capital letters refer to difference between samples at the same salt content and lowercase letters refer to differences between samples at the same cooking temperature.

TABLE III. CORRELATION BETWEEN FACTORS ON TEXTURAL PROPERTIES OF BLOOD GELS. ALPHABET "S" REFERS TO SIGNIFICANT DIFFERENCE BETWEEN FACTORS AND "NS" REFER TO NOT SIGNIFICANT DIFFERENCE BETWEEN FACTORS.

Factors	Breaking force (g)	Breaking distance (mm)
Salt content (S)	s	s
Blood content (B)	s	ns
Cooking Temp. (T)	s	s
S ×B	s	ns
S ×T	s	s
B ×T	s	ns
S ×B ×T	s	ns

B. Color Measurement of Blood Gel

Gel color is generally considered one of the main criteria in assessing overall quality of the product. Fig. 4 presents L*a*b* colors of gel samples. As was expected, the addition of blood significantly decreased the L* and a* values of gels, indicating a reduction in brightness and redness of gels. Furthermore, increasing cooking temperature showed slightly reduction in a* and b* values, reflecting a decrease in redness and yellowness, respectively. However, the addition of salt did not affect color of gel. The color change of gel was due to heme containing in blood. Consequently, the containing heme in blood decreased the brightness of gel samples. These results were similar to those reported by ref. [18] in sausage containing goat blood. According to ref. [18], the brightness (L*) of product containing blood depended on the concentration of blood; a higher blood concentration is associated with lower brightness values, which made the product darker. In addition, heme in samples could be an effective iron source, benefiting for consumers. Ref. [24] suggests that iron from Hb, derived from animal blood, is more easily absorbed than iron from the more common source, ferrous salts.



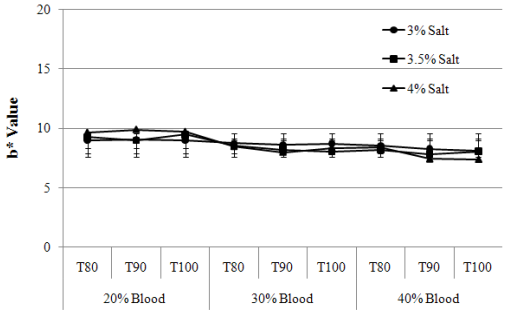
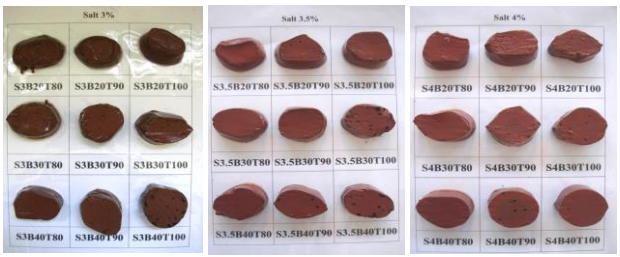


Figure 4. The effect of concentrations of salt (3.0%, 3.5% and 4.0%), blood (20%, 30% and 40%) and cooking temperature (80 ℃, 90 ℃ and 100 ℃) on the color of blood gel. The results were presented by L* value (4 a), a* value (4 b) and b* value (4 c), respectively.

C. Sensory Evaluation

The sensory tests were assessed including texture, color, odor and overall acceptability using a 9-point hedonic scale with thirty panelists. The results showed that 6 formulations (S3B30T80 S3.5B30T80 S4B30T80 S3B30T90 S3.5B30T90 and S4B30T90) of blood gels were highest scored, achieving above 7.1 points for overall acceptability, 7.5 points for texture, 6.3 points for odor and 6.9 points for color. These were evidence that the proper temperature, salt and blood protein contents influenced the structural matrix necessary for holding water to provide desirable texture and mouthfeel [18]. An increase in hardness and chewiness was previously observed by ref. [25] in meat products containing porcine blood plasma that is due to thermogelling ability of plasma to form an internal network-like structure in which fat and water can be retained. The picture of all gel samples were shown in Fig. 5.



a) 3% salt b) 3.5% salt c) 4% salt

Figure 5. Porcine blood gels; gel samples containing with 3% salt (a), 3.5% salt (b) and 4% salt (c), respectively.

TABLE IV. MICROBIAL EVOLUTION OF TOTAL VIABLE COUNT (CFU/G) OF GEL SAMPLES.

Gel samples	Total Viable Count (CFU/g)
Control	1.67×10^{7a}
S3B30T80	8.65×10^{5b}
S3.5B30T80	5.22×10^{5b}
S4B30T80	4.90×10^{5b}
S3B30T90	7.12×10^{4c}
S3.5B30T90	5.35×10^{4c}
S4B30T90	6.83×10^{4c}

D. Microbiological Evaluation

The gel samples with highest scored (6 formulations) of overall acceptability were evaluated for total viable count (TVC). The test was performed 24h after preparation blood gel. In the study, porcine blood gel from the local market near laboratory was evaluated as the control. The results were shown in Table IV.

The TVC of gels were lower than the control. The high microbes level of samples can be attributed to the use of low cooking temperature (T80). Normally, the microbiological contamination is greatly reduced by heating process and using good quality of raw materials.

IV. Conclusion

The addition of salt as well as rising cooking temperature could enhance gel strength—the strengthening gel network, resulting for firm and elastic gels. The correlations between factors demonstrated that salt content and cooking temperature are significantly correlated with texture of gel. The concentration of blood and cooking temperature had slightly effect on the lightness and yellowness of gels. The microbiological quality of gels was better than the control. The sensory test demonstrated that the consumer panels preferred the 6 gel samples.

Acknowledgment

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References

[1] C. W. Dill and W. A. Landmann, *Edible Meat By-Products, Advance in Meat Research*, London: T. R. Elsevier Applied Sci., 1988, pp. 127-145.
[2] S. Hayakawa, Y. Matsuura, R. Nakamura, and Y. Sato, "Effect of heat treatment on preparation of colorless globin from bovine hemoglobin using soluble carboxymethyl cellulose," *J. Food Sci.*, vol. 51, pp. 786-790, Nov. 1986.
[3] G. Ramos-Clamont, S. Fernandez-Michel, L. Carrillo-Vargas, E. Martinez-Calderon, and L. Vazquez-Mopeno, "Functional properties of protein fractions isolated from porcine blood," *J. Food Sci.*, vol. 68, pp. 1196-1200, May 2003.
[4] C. S. Bizzotto, M. Capobiango, and M. P. C. Silvestre, "Evaluation of functional properties of a blood protein," *Pakistan J. Nutr.*, vol. 4, pp. 11-16, Jun. 2005.
[5] D. Parés, E. Saguer, and C. Carretero, "Blood by-products as ingredients in processed meat," in *Processed Meat*, U.K.: Woodhead Publishing Ltd., 2011, pp. 218-242.
[6] V. M. Gorbatov, "Collection and utilization of blood and blood proteins for edible purpose in the USSR," in *Edible Meat By-Products, Advances in Meat Research*, London, UK, 1988, pp. 167-195.
[7] H. C. Kee and S. H. Kil, "Utilization of bovine blood plasma obtained from a slaughterhouse for economic production of probiotics," *J. of Fermentation and Bioengineering*, vol. 86, pp. 34-37, Apr. 1998.
[8] V. D. M. Silva and M. P. C. Silvestre, "Functional properties of bovine blood plasma intended for use as a functional ingredient in human food," *Lebensm.-Wiss. U.-Technol.*, vol. 36, pp. 709-718, Mar. 2003.
[9] D. Eduard, P. Dolars, C. Gerard, and R. Perla, "Heat-Induced gelation of porcine blood plasma proteins as affected by pH," *Meat Science*, vol. 76, pp. 216-225, Jun. 2007.
[10] T. Fidel, A. M. Concepcion, M. Leticia, and R. Milagro, "Innovations in value-addition of edible meat by-products," *Meat Science*, vol. 92, pp. 290-296, Nov. 2012.
[11] M. D. Ana, M. S. Eva, J. Isabel, and R. Jordi, "Application of organic acid salts and high-pressure treatments to improve the preservation of blood sausage," *Food Microbiology*, vol. 25, pp. 154-161, Feb. 2008.
[12] R. P. Bates, L. C. Wu, and B. Murphy, "Use of animal blood and cheese whey in bread: Nutritive value and acceptance," *J. Food Sci.*, vol. 39, pp. 585-587, May 1974.
[13] L. A. Johnson, E. F. Havel, and R. C. Hoseney, "Blood plasma a replacement for egg in cakes," *Cereal Chem.*, vol. 56, pp. 339-342, Sep. 1979.
[14] C. C. Lee, J. A. Love, and L. A. Johnson, "Sensory and physical properties of cakes with bovine plasma products substituted for egg," *Cereal Chem*., vol. 70, pp. 18-21. Jan. 1993.
[15] H. A. Caldironi and H. W. Ockerman, "Incorporation of blood into sausage," *J. Food Sci.*, vol. 47, pp. 405-408, Mar. 1982.
[16] S. Benjakul, W. Visessanguan, and C. Chantarasuwan, "Effect of porcine plasma protein and setting on gel properties of surimi produced from fish caught in Thailand," *Lebensm.-Wiss. U.-Technol.*, vol. 37, pp. 177-185, 2004.
[17] S. Rawdkuen, S. Benjakul, W. Visessanguan, and T. C. Lanier, "Chicken plasma protein affects gelation of surimi from bigeye snapper (priacanthus tayenus)," *Food Hydrocolloids*, vol. 18, pp. 259-270, Mar. 2004.
[18] F. A. P. Silva, D. S. Amaral, I. C. D. Guerra, P. S. Dalmas, N. M. O. Arcanjo, *et al*., "The chemical and sensory qualities of smoked blood sausage made with the edible by-products of goat slaughter," *Meat Science*, vol. 94, pp. 34-38, Jan. 2013.
[19] S. Dailloux, G. Djelveh, A. Peyron, and C. Oulion, "Rheological behaviour of blood plasmas concentrated by ultrafiltration and by evaporation in relation to liquid-gel transition temperature," *J. of Food Engineering*, vol. 55, pp. 35-39, Nov. 2002.
[20] J. Polo, C. Rodriguez, N. Saborido, and J. Rodenas, "Functional properties of spray-dried animal plasma in canned petfood," *Animal Feed Science and Technology*, vol. 122, pp. 331-343, Mar. 2005.
[21] E. Saguer, N. Fort, D. Pares, M. Toldra, and C. Carretero, "Improvement of gelling properties of porcine blood plasma using microbial transglutaminase," *Food Chem*., vol. 101, pp. 49-59, 2007.
[22] K. Autio, M. Kiesvaara, Y. Malkki, and S. Kanko, "Chemical and functional properties of blood globin prepared by a new method," *J. Food Sci.*, vol. 49, pp. 859-863, May 1984.
[23] D. Pares and D. A. Ledward, "Emulsifying and gelling properties of porcine blood plasma as influenced by high-pressure processing," *Food Chem*., vol. 74, pp. 139-145, 2001.
[24] M. Toldra, A. Elias, D. Pares, E. Saguer, and C. Carretero, "Funtional properties of a spray-dried porcine red blood cell fraction treated by high hydrostatic pressure," *Food Chem*., vol. 88, pp. 461-468, Sep. 2004.
[25] S. Hurtado, I. Daga, E. Espigule, D. Pares, E. Saguer, M. Toldra, and C. Carretero, "Use of porcine blood plasma in phosphate-free frankfurters," in *Proc. 11th International Congress on Engineering and Food*, 2011, vol. 1, pp. 477-482.

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