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Emulsion-Type Chicken Sausage Quality with Fat Substitution by Rice Starches during Freeze-Thaw Cycles

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ABSTRACT

The potential of rice starch types was explored to mimic the fat and improve the stability of chicken sausage during multiple freeze-thaw (F-T) cycles. This study investigated the effect of partial fat replacement with non-waxy and waxy rice starch on the physical properties of chicken sausage during multiple F-T cycles. Sausages were manufactured using eight different formulas (standard fat, reduced-fat, and reduced-fat with both starches at addition levels of 3%, 6%, and 9%). Emulsion stability, cooking loss, color, and texture profile were analyzed as initial qualities. Sausages were stored at -18 °C for seven days and thawed at 4 °C for 17 h for each of three F-T cycles, then thawing loss and texture were evaluated. The result showed that the incorporation of non-waxy and waxy rice starch up to 9% increased the emulsion stability, lightness (L*), and the value of hardness, gumminess, and chewiness of chicken sausage ($p<0.05$). Multiple F-T cycles lead to mechanical damage and quality deterioration, including water-holding capacity loss and textural change. The thawing loss value of all treatments significantly increased during the F-T cycle. Utilization of waxy rice starch 3% in low-fat chicken sausage exhibited optimum physical properties and minimum thawing loss and texture change during multiple F-T cycles.

Keywords: chicken sausage; freeze-thaw cycle; meat emulsion; reduced-fat; rice starch

INTRODUCTION

The shift in consumer preference toward clean-label products is significantly affecting the meat industry, driving the development of meat products that prioritize safety, high-quality raw materials, and substituting artificial ingredients with natural resources (Inguglia *et al.*, 2023; Roobab *et al.*, 2021). Fat, in particular, plays a crucial role in affecting meat emulsion product's characteristics, influencing emulsion stability, cooking losses, and sensory profiles, including flavor and texture (Alves *et al.*, 2016; Zhao *et al.*, 2018). Consequently, various approaches, including fat reformulation, have been explored to produce healthier meat options while aligning with clean-label trends. However, reducing fat is difficult without degrading the product quality. Chicken skin, a poultry by-product, has been widely used in chicken sausage manufacturing as a fat source due to its cost-effectiveness and sustainability. Its unique characteristics also enhance the sensory profile and align with customer demand for healthier, religiously approved, and clean-label products (Pena-Saldarriaga *et al.*, 2020). Recent studies have focused on reformulating fat with natural ingredients to improve the nutritional, physicochemical, and sensorial properties of meat-processed products, including cereal flours and starches. Nonetheless, fat type and storage conditions also affect the role of fat in meat products (Pan *et al.*, 2021b).

Storage conditions also affect the quality of meat products, and it is important to ensure thermal stability to maintain quality despite temperature changes. Temperature variation during freezing, frozen storage, transportation, circulation, and thawing induce the freeze-thaw (F-T) cycle which causes concern for both consumers and processors. The formation and melting of ice crystals during multiple freezing and thawing caused mechanical damage and protein oxidation, which induce discoloration, loss of water-holding capacity, and sensory quality decrease in meat and meat products (Fan *et al.*, 2022; Pan *et al.*, 2021b; Zhu *et al.*, 2023). Existing studies showed a degradation in the quality of cereal flour containing sausage, including appearance and texture, dramatic color change, and higher lipid oxidation during refrigerated storage (Xiong *et al.*, 2022).

Rice starch is a promising clean-label ingredient for achieving good product quality and stability during storage. It was reported that rice starch resulted in better F-T stability of pastes, has a wide range of amylose and amylopectin ratios, and was considered hypoallergenic (Mitchell, 2009). Non-waxy and waxy rice starch have gelatinization temperatures in the processing temperature range of poultry products. Additionally, their relatively small granule size contributes to achieving a smooth texture in the final product (Hsieh *et al.*, 2019; Wani *et al.*, 2012). Previous studies have indicated that incorporating cereal flour and starches, including rice flour, can significantly improve sausage quality. Nevertheless, there remains a gap in research concerning the stability of these enhancements under long-term storage conditions and temperature fluctuations. Most research on chicken sausage has used refrigerated temperatures in short-term storage, while the effects of freezing temperatures have not been extensively investigated, particularly in the continuous impact of freezing and thawing as a cycle. Therefore, this study aims to evaluate the effect of partial replacement of chicken skin with non-waxy and waxy rice starch on the physical properties of reduced-fat chicken sausage and its stability during multiple F-T cycles.

MATERIALS AND METHODS

Chicken Sausage Preparation

Skinless chicken breast, chicken skin, salt, sugar, and MSG were purchased from a local wholesale store (CP Axtra Public Co., Ltd, Thailand). Sodium tripolyphosphate and sodium nitrite were obtained from Krungthepchemi Co., Ltd (Thailand). Waxy and non-waxy rice starch were provided by Burapa Prosper Co., Ltd (Thailand). Reduced-fat (20% chicken skin) chicken sausage was used as the control (RF), and standard fat (30% chicken skin) was used as reference (SF). Non-waxy rice starch was treated at 3%, 6%, and 9% substitution levels (R3, R6, R9), along with waxy rice starch (W3, W6, W9). Initially, ground chicken breast (SF: 2100 g, RF: 2400 g), salt 1.67%, sodium nitrite 125 ppm, sodium tripolyphosphate 0.25%, and half of the iced water 16.67% were mixed at 3000 rpm for 1 min in the bowl cutter (Model CM-14, Mainca, Spain). After that, ground chicken skin, rice starch (Burapa Prosper, Thailand), sugar 1.6%, monosodium glutamate 0.33%, and the rest of the iced water were added, followed by mixing for 5 min. The final temperature of the batter was maintained at no higher than 5 °C. The batter was stuffed into a 24 mm collagen casing and cooked in the smoke chamber (CS700EL, Kerres Anlagensysteme GmbH, Germany) until the internal temperature reached 73 °C.

Freeze-Thaw Cycle

The condition used followed the procedures described by Pan *et al.* (2021a). The cooked sausages were frozen at -18 °C and stored for seven days. Then, samples were thawed at 4 °C for 17 h until the internal temperature reached 0–2 °C. The F-T cycles were repeated three times, and analysis were conducted after each cycle.

Sample Analysis

Emulsion stability. The method described by Colmenero *et al.* (2005) was used to evaluate emulsion stability (ES). The batter (50 g) was stuffed into tubes, then centrifuged at 2600 g for 5 min at 2 °C. The batter was heated in a water bath at 40 °C for 15 min followed by 75 °C for 20 min. Water released (WR) was determined from weight loss after evaporation (105 °C for 16h), and fat released (FR) was calculated as a percentage of the fat content from the remaining material.

Cooking loss and thawing loss. Cooking loss and thawing loss were determined by calculating the weight ratio of the sample before and after the process. Both properties are expressed in percentages as follows:

$$\% \text{ Cooking loss} = [(a - b) / a] \times 100\%$$

$$\% \text{ Thawing loss} = [(p - q)/ p] \times 100\%$$

where a is the weight of batter, b is the weight of cooked sausage, p is sausage weight before freezing, and q is sausage weight after thawing.

Color analysis. The cross-section color of samples was measured using a MiniScan EZ Spectrophotometer (Model 4500 L, HunterLab, USA). The lightness, redness, and yellowness (L*, a*, b*) were recorded.

Texture profile analysis. The texture profile analysis (TPA) was performed using Texture Analyzer (TA-TX Plus, Stable Micro System Ltd., UK) with a 36 mm cylindrical probe on a 15 mm height sample. The analysis was conducted at room temperature, and the apparatus was set according to Xiong *et al.* (2022) test conditions. Hardness, springiness, cohesiveness, gumminess, and chewiness were obtained.

Experimental Design and Statistical Analysis

A completely randomized design (CRD) with eight treatments and three replicates was used as an experimental design. Analysis of variance was performed using Minitab 18 (Minitab, LLC, State College, USA) followed by Tukey's test at p<0.05 significance level.

RESULTS

Emulsion Stability and Cooking Loss

Figure 1(A) shows that while the RF sample had greater WR than the SF sample, the addition of starch reduced WR (p<0.05). Waxy rice starch samples (W3, W6, and W9) exhibited lower WR compared to non-waxy rice starch at the same level (p<0.05). The FR in starch-containing samples were lower than RF (p<0.05), though they were insignificant to each other. Figure 1(B) displays that R3 had the highest cooking loss (p<0.05). In contrast, R6 and R9 resulted in lower values than RF and SF. The addition of waxy rice starch resulted in a lower cooking loss than RF and SF (p<0.05).

Color

Table 1 shows sausage prepared with starch exhibited an increasing L* along with the addition levels (R9 > R6 > R3 and W9 > W6 > W3, p<0.05). SF presented

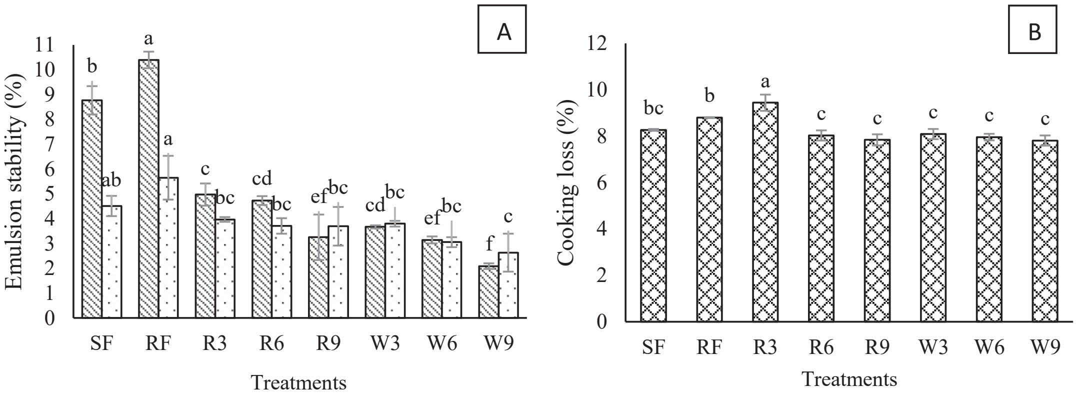

Figure 1. Emulsion stability (A) and Cooking loss (B) of emulsion-type chicken sausage. Different letters within the same parameter differ significantly (p<0.05). SF= standard fat, RF= reduced-fat, R3= rice starch 3%, R6= rice starch 6%, R9= rice starch 9%, W3= waxy rice starch 3%, W6= waxy rice starch 6%, W9= waxy rice starch 9%. ▧= Water released; ⊡= Fat released.

Table 1. Cross section color of reduced-fat chicken sausage with rice starch incorporation

Treatments	L*	a*	b*
SF	75.29±0.37^{d}	1.02±0.09^{b}	14.31±0.16^{a}
RF	74.15±0.08^{e}	1.84±0.07^{a}	13.78±0.31^{ab}
R3	74.78±0.3d^{e}	1.71±0.14^{a}	12.41±0.90^{ab}
R6	77.91±0.25^{bc}	1.59±0.17^{a}	13.14±0.78^{ab}
R9	78.92±0.11^{a}	1.54±0.08^{a}	12.33±1.10^{b}
W3	74.81±0.47^{de}	1.65±0.12^{a}	11.92±0.09^{b}
W6	77.21±0.48^{c}	1.55±0.28^{a}	12.01±0.34^{b}
W9	78.57±0.37^{ab}	1.44±0.20^{a}	12.27±1.00^{b}

Note: Means in the same column with different superscripts differ significantly (p<0.05). SF= standard fat, RF= reduced-fat, R3= rice starch 3%, R6= rice starch 6%, R9= rice starch 9%, W3= waxy rice starch 3%, W6= waxy rice starch 6%, W9= waxy rice starch 9%.

the lowest a* and highest b*; however, they were insignificant among RF and starch treatments.

Thawing Loss and Change in Texture

Figure 2 shows all samples exhibited an increasing trend in thawing loss over F-T cycles (p<0.05). RF had a lower thawing loss than the SF at the F-T3 (after the third freeze-thaw cycle). The substitution of 9% chicken skin resulted in a higher thawing loss at the F-T3. W3 exhibited a lower thawing loss compared to R3. Table 2 shows the hardness, gumminess, and chewiness of R6, W6, R9, and W9 were higher than the RF (p<0.05). However, 3% starch addition did not show a significant difference with RF. The incorporation of non-waxy rice starch presented a decline in hardness, gumminess, and chewiness values after F-T cycles. W3 exhibited the most stable hardness value after three F-T cycles.

DISCUSSION

Emulsion Stability and Cooking Loss

In our study, reducing fat without any replacer resulted in greater liquid release, as also found in Lu *et al.* (2021) and Choi *et al.* (2016) experiments. A lower amount of chicken skin reduced the water-holding ability due to the role of added protein. Chicken skin has a protein content of around 8%-12%, consisting of 10% salt-soluble protein (da Silva Araújo *et al.*, 2021; Lucarini *et al.*, 2020). The presence of salt and phosphate enhanced the functionality of salt-soluble protein to contribute to holding water, forming a gel, and stabilizing emulsion in the food system. Salt induced the expansion of myofibrils and low-concentration phosphate dissociating actomyosin, thereby facilitating easier penetration of water molecules (Xiong, 2014).

Our result was consistent with the other studies that reported incorporation of 2%-6% plant starches into

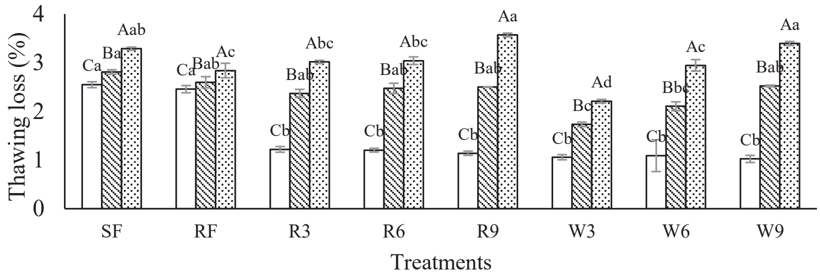

Figure 2. Thawing loss of chicken sausage with starch addition during freeze-thaw cycles. Different uppercase letters (A-C) in the same treatment and lowercase (a-c) in the same F-T cycles differ significantly (p<0.05). SF= standard fat, RF= reduced-fat, R3= rice starch 3%, R6= rice starch 6%, R9= rice starch 9%, W3= waxy rice starch 3%, W6= waxy rice starch 6%, W9= waxy rice starch 9%, F-T1= after first freeze-thaw cycle, F-T2= after second freeze-thaw cycle, F-T3= after third freeze-thaw cycle. □= F-T1; ▧= F-T2; ⊡= F-T3.

Table 2. Change in texture of reduced-fat chicken sausage during three freeze-thaw cycles

F-T Cycle	Treatments	Texture variables				
		Hardness (kg)	Springiness (mm)	Cohesiveness	Gumminess (kg)	Chewiness (kg)
F-T0	SF	4.02±0.20^{d}	0.91±0.03^{A}	0.72±0.04	2.90±0.12^{c}	2.56±0.18^{d}
	RF	4.78±0.01^{Bb}	0.96±0.02^{A}	0.85±0.02	3.43±0.13^{b}	3.06±0.17^{bc}
	R3	4.25±0.10^{Acd}	0.95±0.03	0.85±0.01	3.61±0.07^{Ab}	3.41±0.10^{Abc}
	R6	4.88±0.08^{Aab}	0.96±0.01^{A}	0.86±0.03	4.18±0.10^{Aa}	3.99±0.13^{Aa}
	R9	5.11±0.35^{Aa}	0.93±0.03^{A}	0.84±0.04	4.27±0.29^{Aa}	3.97±0.36^{Aa}
	W3	4.38±0.15^{cd}	0.89±0.08	0.83±0.01	3.09±0.32^{Ac}	2.81±0.30^{Acd}
	W6	4.41±0.18^{cd}	0.92±0.03	0.83±0.02	3.68±0.13^{b}	3.37±0.04^{bc}
	W9	4.89±0.12^{Aab}	0.94±0.01	0.84±0.05	4.10±0.19^{Aa}	3.85±0.23^{Aa}
F-T1	SF	4.11±0.05^{cd}	0.85±0.01^{B}	0.73±0.00	2.91±0.24^{d}	2.45±0.17^{d}
	RF	4.28±0.13^{Bbc}	0.90±0.03^{AB}	0.78±0.02^{AB}	3.12±0.02^{cd}	2.70±0.21^{cd}
	R3	4.28±0.08^{Bbc}	0.87±0.03	0.79±0.01^{B}	3.38±0.10^{BCbc}	2.92±0.16^{BCbc}
	R6	4.53±0.12^{Babc}	0.89±0.01^{B}	0.79±0.01^{B}	3.59±0.02^{Bab}	3.19±0.07^{Bab}
	R9	4.83±0.23^{Ba}	0.89±0.02^{AB}	0.8±0.01^{AB}	3.87±0.32^{Ba}	3.44±0.22^{Ba}
	W3	4.34±0.18^{abc}	0.90±0.03	0.78±0.01^{B}	2.99±0.11^{Bd}	2.48±0.13^{Bd}
	W6	4.73±0.25^{ab}	0.89±0.08	0.81±0.02^{A}	3.81±0.19^{a}	3.40±0.49^{a}
	W9	4.60±0.23^{Babc}	0.91±0.02	0.78±0.02^{AB}	3.61±0.10^{Bab}	3.30±0.13^{Cab}
F-T2	SF	3.94±0.09d	0.86±0.02^{B}	0.71±0.03	2.69±0.05^{d}	2.32±0.04^{e}
	RF	4.15±0.14^{ABcd}	0.87±0.02^{B}	0.77±0.01^{AB}	2.92±0.10^{cd}	2.40±0.21^{de}
	R3	4.37±0.09^{Bc}	0.90±0.05	0.80±0.01^{B}	2.90±0.08^{Bcd}	2.70±0.03^{Bc}
	R6	4.63±0.29^{Bb}	0.89±0.01^{B}	0.76±0.01^{B}	2.99±0.23^{Bcd}	2.77±0.20^{Bbc}
	R9	5.15±0.04^{ABa}	0.91±0.00^{AB}	0.78±0.01^{B}	3.46±0.02^{ABab}	3.24±0.01^{Ba}
	W3	4.03±0.10^{d}	0.89±0.01	0.77±0.01^{BC}	2.66±0.27^{ABd}	2.59±0.12^{ABcd}
	W6	5.27±0.20^{a}	0.94±0.06	0.80±0.00^{A}	3.62±0.16^{a}	3.45±0.16^{a}
	W9	4.82±0.05^{Bb}	0.91±0.00	0.75±0.01^{B}	3.11±0.05^{Bbc}	2.93±0.04^{Cb}
F-T3	SF	4.07±0.14^{cd}	0.86±0.00^{B}	0.71±0.02	2.90±0.04^{c}	2.49±0.04^{c}
	RF	4.21±0.07^{Abc}	0.88±0.05^{AB}	0.74±0.01^{B}	3.06±0.12^{bc}	2.50±0.21^{c}
	R3	3.84±0.01^{Bd}	0.88±0.03	0.75±0.02^{C}	2.86±0.08^{Cc}	2.51±0.13^{Cc}
	R6	4.41±0.14^{Bb}	0.87±0.02^{B}	0.76±0.01^{B}	3.34±0.12^{Bab}	2.90±0.12^{Bb}
	R9	4.45±0.27^{Bb}	0.88±0.01^{B}	0.73±0.00^{C}	3.24±0.17^{Bb}c	2.86±0.13^{Bb}
	W3	4.00±0.16^{cd}	0.87±0.03	0.73±0.03^{C}	2.46±0.13^{ABd}	2.05±0.23^{ABd}
	W6	4.49±0.37^{b}	0.87±0.04	0.75±0.01^{B}	3.38±0.24^{ab}	2.95±0.17^{b}
	W9	4.99±0.15^{Aa}	0.88±0.04	0.74±0.00^{B}	3.71±0.10^{Aa}	3.27±0.15^{Ba}

Note: Means in the same column with different uppercase letters (A-C) in the same treatment and lowercase (a-c) in the same freeze-thaw (F-T) cycles differ significantly ($p<0.05$). SF= standard fat, RF= reduced-fat, R3= rice starch 3%, R6= rice starch 6%, R9= rice starch 9%, W3= waxy rice starch 3%, W6= waxy rice starch 6%,W9= waxy rice starch 9%, F-T0= before undergoing freeze-thaw cycle, F-T1= after first freeze-thaw cycle, F-T2= after second freeze-thaw cycle, F-T3= after third freeze-thaw cycle.

sausage improved ES due to water-binding ability of starch (Pereira *et al.,* 2016; Pietrasik & Soladoye, 2021). Higher amylopectin content in waxy rice promoted higher gelatinization capacity, better hydration, viscosity, and water binding (Cornejo-Ramírez *et al.*, 2018; Pietrasik & Soladoye, 2021). Pereira *et al.* (2019) and Barbut (2018) reported that the fat-binding properties may be related to the sticky character of waxy starch.

The result indicates the cooking loss of chicken sausage was also affected by fat reduction. In the absence of a replacer, fat reduction increased the cooking loss of low-fat sausage, as found in the experiments of Choi *et al.* (2016) and Nacak *et al.* (2021). Chicken skin contributed to raising total protein and its role in binding water. Waxy rice starch addition resulted in a lower cooking loss than the control. Previous studies reported the same effect obtained from the addition of glutinous rice flour, barley flour, and pea starch (Pereira *et al.,* 2019; Pereira *et al.,* 2016; Pietrasik & Soladoye, 2021; Zhu *et al.,* 2022). Figure 3 demonstrate the role of starch in meat emulsion. In the high presence of water and high temperatures during cooking, starch granules take up some water to swell and gelatinize. Our results showed that emulsion stability did not reflect starch behavior in cooking loss. It can be explained that many factors can influence cooking loss, including cooking method, temperature, time, and fat content (Choi *et al.,* 2009; Shin *et al.,* 2020).

Color

Color in meat products plays a significant role in consumer choice, often indicating freshness, quality, and flavor profile. Sausage formulated with vegetable oil, oleogel, and native flour was reported to be brighter in color and less red, resulting in a significant decrease in panelist acceptability, particularly concerning color and appearance attributes (da Silva *et al.,* 2019; Franco *et al.,* 2019; Pereira *et al.,* 2020). Higher starch incorporation

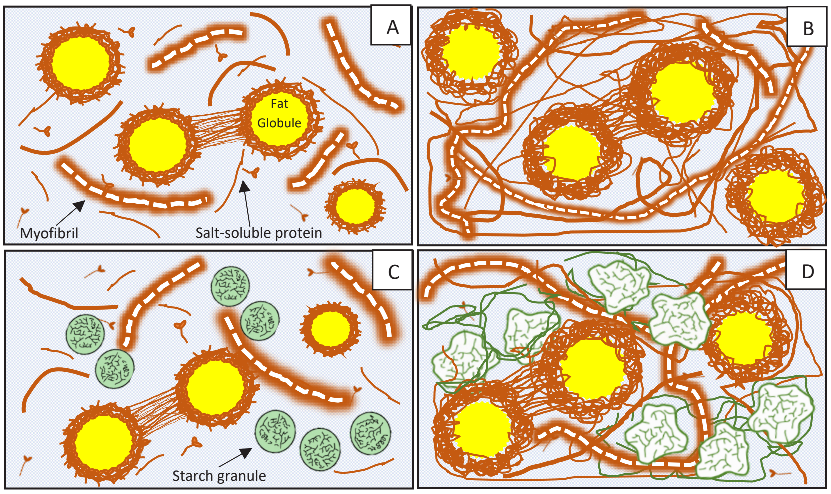

Figure 3. Modified schematic representative of (A) batter of emulsion-type chicken sausage without starch, (B) emulsion-type chicken sausage without starch after cooking, (C) batter of starch-containing emulsion-type chicken sausage, (D) starch-containing emulsion-type chicken sausage after cooking based on Xiong (2014).

resulted in lower gel transparency, which may increase light scattering during observation. Similarly, flour and starch additions were reported to increase the L* value of meat processed products, including rice flour (10%) in chicken sausage (Ali *et al.*, 2011), tapioca (1%-4%) in chicken breast patties (Chatterjee *et al.*, 2019) and various starches in surimi-beef gel (Zhang *et al.*, 2013). The highest a* value observed in RF is attributed to the myoglobin content derived from the higher proportion of chicken breast used in the formulation (SF: 2100 g; RF: 2400 g) without starch inclusion. According to Min & Ahn (2009), each gram of chicken breast contains approximately 1.16 mg of myoglobin, which significantly contributes to the red pigmentation observed in meat products.

Additionally, fat decrement and starch inclusion influenced a* value as well (Choe & Kim, 2019). Approving the results, previous studies revealed starch and cereal flour addition reduced a* of poultry sausage (Garcia-Santos *et al.*, 2019; Yang *et al.*, 2009; Zhang *et al.*, 2013). Conroy *et al.* (2018) confirmed that the b* value of emulsified sausage was unaltered by fat reduction. Similar to our result, many studies stated that native starch addition had a minimum effect on the a* and b* values (Pereira *et al.*, 2016; Zhang *et al.*, 2013).

Thawing Loss and Change in Texture

During F-T cycles, water retention was mainly affected by protein functionality. The reduction of chicken skin in starch-containing samples lowered the emulsion matrix's ability to bind water, which is related to the levels and types of protein present. Instead of collagen, myofibrillar proteins play a more significant role in enhancing water retention in the presence of salt and low concentrations of phosphate by unfolding and aggregating to form a cross-linked gel matrix (Lonergan *et al.*, 2019; Xiong, 2014). Multiple F-T cycles that induced ice crystal formation also caused damage to the muscle fibers, resulting in alterations to the textural properties of chicken sausage (Pan *et al.*, 2021a; Zhu *et al.*, 2023).

The components of meat emulsion, including protein, fat, water, salt, and starch in this study were changing continuously. Higher lipid-protein interaction enhances the gel network formation in meat products (Li *et al.*, 2022; Pereira *et al.*, 2020). Furthermore, the higher protein content in sausages prepared with more chicken skin enhanced water retention, thereby contributing to the stabilization of the textural properties. The addition of non-waxy rice starch showed higher thawing loss compared to the waxy type at the same level. It can be attributed to the composition of amylose that runs into higher retrogradation (Charoenrein & Preechathammawong, 2012; Cornejo-Ramírez *et al.*, 2018). This process might also contribute to the textural change due to the water released from the starch matrix.

This study demonstrated that incorporating non-waxy or waxy rice starch at a 3% level resulted in comparable texture parameters to the reference. Thus, this formula is optimal for reduced-fat chicken sausage. Cereal starches are generally beneficial to meat emulsions, though only limited amounts produce high-quality products. Future studies are suggested to evaluate the suitability of other native starches based on availability, processing conditions, and specific product characteristics.

CONCLUSION

Starch of rice origin has shown the potential as a partial fat replacer in the production of reduced-fat chicken sausage. Replacing animal fat with 3% waxy rice starch effectively improved emulsion stability and preserved the color and texture of chicken sausage. Waxy rice starch at this level also reduced cooking loss and provided optimum stability during the multiple F-T cycles by lowering thawing loss and maintaining textural properties.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organizations related to the material discussed in the manuscript.

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