

## Reduction of pork content in emulsion sausage by substituting with *Pangasius hypophthalmus*

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### Abstract

This research aimed to develop an emulsion sausage with reduced pork content by replacing *Pangasius hypophthalmus*. The experimental design involved varying formulations at different levels (0, 10, 20 and 30%) with pork substituting with *P. hypophthalmus*. This study examined the effects of *P. hypophthalmus* substitution on sensory, physicochemical, texture, and nutritional values of emulsion sausages to determine as a high-quality alternative for healthier meat products. Sensory evaluations determined consumer acceptance, indicating that the 30% substitution formula achieved the highest overall acceptance. Consumer perception was influenced by mechanical properties of sausages such as hardness, cohesiveness, and chewiness, which were assessed through texture profile analysis. The nutritional analysis demonstrated the effect of modified formulation for health improving. These studies contributed to the developed meat products providing improved nutritional and environmental benefits with a desirable taste.

**Keywords:** emulsion sausage, *Pangasius hypophthalmus*, pork reduction, sensory evaluation, nutritional analysis

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## Introduction

The fresh lean pork offers vital calories, macronutrients, and micronutrients as low-cost and healthful diets, that support growth and development in all age groups (Poinso et al., 2023). It complements a balanced diet and helps individuals control their blood pressure, diabetes, and weight. Furthermore, lean pork has a positive impact on mood, vitality, overall quality of life, sleep quality, and cognitive function, encouraging a balanced and healthy lifestyle (Pluske et al., 2024). Both fresh and processed meats contain high-quality proteins and essential micronutrients that contribute significantly to the food chain, promoting balanced diets through adequate nutrient supply. Various production strategies can increase meat's nutritional content, enhancing its role in a healthy diet (De Smet & Vossen, 2016).

Despite these benefits, the International Agency for Research on Cancer (IARC) has classed processed meat as carcinogenic and red meat as possibly carcinogenic, especially for colorectal cancer (De Smet & Vossen, 2016). Strong evidence suggests that reducing meat consumption, particularly processed and red meat, has significant public health benefits. Even a 30% reduction in meat consumption could prevent hundreds of thousands of cases of type 2 diabetes, cardiovascular disease, and colorectal cancer, while also lowering overall mortality. These results indicate that reducing food intakes lead to overall healthiness with the goals of determining body weight, restricting body fat percentages, and prolonged life span (Kennedy et al., 2024). In addition, much more land and water were used to produce meat than to produce the plant foods they replace, and this destruction is immense in environmental and climate terms. Intensive production of meat has increasingly negative effects on human health and the welfare of animals while meat consumption is high and increasing (Parlasca & Qaim, 2022).

In response to such concerns, food scientists have started to search for alternatives that may acceptable by consumer and also be health and sustainability centered. Sausages are a popular and versatile food category made by stuffing ground meat (usually pork) with seasonings and casings. Puree the meat into a smooth paste that contains moisture (Gisslen, 2018) and these are made into emulsion sausages. The products usually contain 30% of animal fat which is emulsified with water and meat proteins to give the desired texture and stability (Choi et al., 2013). Given their popularity and high meat content, sausages are an excellent opportunity to introduce healthier protein alternatives.

Fish presents a promising alternative protein source for processed meat products. The Sutchi catfish (*P. hypophthalmus*), which is widely farmed in Southeast Asia, particularly Vietnam, has suggested to be a potential meat substitute. The mild flavor and firm white flesh of this fish also makes it a market favorite, which is sold as frozen or thawed fillets internationally. The nutritional composition of the fish meat includes low cholesterol, and moderate polyunsaturated fatty acids (PUFA), especially linoleic acid (Orban et al., 2008). Based on these features, it is possible that fish meat might be compatible with processed meat applications. The following table presents a nutritional comparison between pork and *P. hypophthalmus* as shown in (Table 1).

**Table 1** Nutritional comparison between pork and *P. hypophthalmus*.

nutritional attribute	pork	<i>P. hypophthalmus</i>
protein content	20-21%	20%
fat content	6-10%	3-6%
DHA	negligible	2.70mg
EPA	negligible	0.36mg
saturated fatty acids (SFA)	high	moderate
PUFA content	low	moderate
EAA/NEAA ratio	high	high
vitamin B12	high	moderate
iron content	high	lower bioavailability

note: data adapted from Van Mierlo et al., (2022) and Chakma et al., (2022).

In this study, pork meat was partially substituted with *Pangasius* fish at 0, 10, 20 and 30% to evaluate the impact on emulsion sausage quality. Skatecki et al., (2021) previously demonstrated that replacing pork with 20% fish meat in pork pâtés and found to result in an improved nutritional profile with consumer acceptance. According to the findings, this study extended the substitution level up to 30% to investigate whether a higher proportion of fish meat would further enhance product characteristics or introduce challenges, particularly in oxidative stability and sensory quality.

Furthermore, previous research has shown that manufacturers may successfully produce an emulsion sausage with fish, having good textural properties and palatability and increasing nutritional value. This approach provides not only healthier alternatives but also greater sustainability, as fish production typically consumes fewer natural resources than livestock production (Parlasca & Qaim, 2022).

Several studies have looked into different binding agents to improve product stability and lower fat content. Soy protein isolate can be used to increase protein content and improve texture firmness of fish sausages. However, it has flavor limitations. Lago et al., (2017) investigated the effect of minced fish (MF) inclusion in tilapia sausages and found that a 50% substitution level achieved the highest consumer acceptance across sensory attributes such as appearance, aroma, flavor, texture, and overall impression. Their findings confirmed that MF can be effectively used in fish sausage production without compromising consumer appeal, especially when supported by appropriate emulsification and formulation control. However, most existing research has focused exclusively on fish-based sausages, leaving a critical gap in exploring hybrid meat products. Despite the dominance of pork-based emulsion sausages in the processed meat industry, there is limited investigation into formulations that blend pork and fish to enhance nutritional profiles while retaining consumer familiarity. This gap presents a valuable opportunity for innovation within a widely accepted product format.

The purpose of this study was to fill this gap by producing an emulsion sausage with less pork content by partially substituting with *P. hypophthalmus* meat. The study was examined the impact of this substitution on sensory attributes, physical properties, chemical composition, textural properties and nutritional value of the end products. Consumer acceptance of these products was determined through sensory evaluation, and critical parameters (i.e., color, moisture content, pH and oxidation indices) were evaluated. Mechanical properties such as hardness, cohesiveness and chewiness were assessed by texture profile analysis. The modified formulation was subjected to a nutritional analysis and quantitatively compared with traditional pork sausages.

### Methodology

#### 1. Development of standard sausage recipe

##### 1.1 Preparation of ingredients

For this research, pork tenderloin and pork fat were purchased from Freshket (<https://freshket.co/> household), an online supplier specializing in food and household products. The pork is cleaned with tap water, vacuum-packed, and immediately stored in the refrigerator based on Dusit Thani College's procedure. The Sutchi catfish (*P. hypophthalmus*) fillet was bought from a supermarket in Bangkok and delivered in an icebox. It was washed thoroughly, vacuum-sealed, and placed in a refrigerator. Paprika, onions powder, garlic powder, salt, and pepper were bought and kept at dry storage. Lean meat, fat, ice, salt and some spices were mixed in the ratio as shown in (Table 2).

##### 1.2 Preparation of emulsion sausage

According to Choi et al., (2009), with slight modifications, the sausage production process was outlined as following. Firstly, the lean meat was trimmed to remove excess connective tissues and unwanted particles. The meat was ground using a 5-millimeter plate to form a stable emulsion. For formulas that include fish, the fish must also be ground using a 5-millimeter plate and mixed with the pork according to the specified ratio in the recipe.

It was then transferred to a food processor. Salt and ice were added, and the mixture was blended for 6 minutes while maintaining the temperature below 2°C. Once an emulsion was formed, paprika, garlic powder, onion powder, and ground pork fat were incorporated, and the mixture was blended until smooth.

For filling, collagen casings with a 30-millimeter diameter were soaked in cold water for 5 minutes, cut into 30-centimeter lengths, and knotted at one end. Three small holes were made near the knot to allow air to escape during filling. The sausage mixture was filled into the casing using a sausage syringe, and the second knot was tied once filled.

For cooking, the sausages were hung on racks and baked in a convection oven under steam mode at 73°C with 50% relative humidity for 40 minutes. After baking, the sausages were cooled at ambient temperature, then packed and chilled to set and retain moisture.

**Table 2** Formulations of sausages with different ratio of pork and *P. hypophthalmus*.

ingredients	formulas			
	F0 (100:0)	F1 (90:10)	F2 (80:20)	F3 (70:30)
	----- (%) -----			
pork tenderloin	46.86	42.17	37.49	32.80
fish meat	0.00	4.69	9.37	14.06
fat	18.74	18.74	18.74	18.74
ice	28.12	28.12	28.12	28.12
corn starch	1.87	1.87	1.87	1.87
baking powder	0.94	0.94	0.94	0.94
paprika	0.94	0.94	0.94	0.94
onion powder	0.94	0.94	0.94	0.94
salt	0.94	0.94	0.94	0.94
garlic powder	0.47	0.47	0.47	0.47
pepper	0.19	0.19	0.19	0.19

note: F0=0% fish, F1=10% fish, F2=20% fish, F3=30% fish.

Although the fat content in the control formula (F0) was 18.74%, which may appear high for health-oriented products, it was necessary to ensure emulsion stability, desirable texture, and flavor typical of emulsion-type sausages (Choi et al., 2009). Maintaining a consistent fat level across all formulas allowed accurate evaluation of the effects of pork reduction and fish substitution.

## 2. Sensory evaluation

Sensory analysis is an important part of understanding the consumers' acceptance and enhancing the food products' compositions. Six sensory attributes, namely springiness, appearance, taste, texture, aroma and overall liking were measured on a 9-point hedonic scale with 1 being dislike extremely and 9 being like extremely as suggested by Lawless & Heymann (2010). These assessments are useful when alongside instrumental measurement including the texture profile analysis (TPA) and color evaluation as noted by Fiorentini et al., (2020). A total of thirty untrained panelists assessed the emulsion sausages. The samples were randomly assigned to codes in three-digit and were served at temperatures of  $40\pm 2^{\circ}\text{C}$ . To avoid contamination, the panelists were allowed to take water in between sausage samples. In conducting the research, the study adhered to the guidelines for ethical research and human subjects and received clearance from Rangsit University's Ethics Review Committee for Research Involving Human Subjects (RSUERB2024-049) and all the participants gave their consent to participate in the sensory evaluation.

## 3. Physical and chemical analysis

The proximate composition and selected chemical properties determined the effect of various ratios of pork and *P. hypophthalmus* as formulation and storage conditionss. The samples were stored at  $4\pm 1^{\circ}\text{C}$  in ziplock

bags and analyzed at days 1, 3 and 7 to assess quality changes during refrigerated storage. Color characteristics ( $L^*$ ,  $a^*$ ,  $b^*$ ), moisture content, pH, and oxidative stability were considered as the quality parameters of the product. Color was determined with a colorimeter (Spectraflash SF600 Plus-CT spectrophotometer, Datacolor, Switzerland) using the CIELab system with three replications. Thus, the total color difference ( $\Delta E$ ) was monitored as it changed over time. Moisture content which affects texture and shelf life was analyzed by the oven-drying method (AOAC, 2023). A digital pH meter was used to determine the pH after preparing the sample by mixing 5g of the sample with 45ml of distilled water. Lipid oxidation which influences the flavor and color stability of the meats was determined by peroxide value (PV) and thiobarbituric acid reactive substances (TBA) for primary and secondary products. The PV was determined using Titration method (AOAC, 2023) and TBA values were measured using spectrophotometer (UV-Vis UV-1900i, SHIMADZU, Japan) and it was expressed in mg malondialdehyde/kg sample. These analyses provided information on formulation stability and the potential shelf life under controlled storage conditions

#### 4. Texture profile analysis

Texture plays a crucial role in consumer preference and product acceptability. Texture Profile Analysis (TPA) is a double mechanical compression test that simulates how food behaves during chewing and measures key mechanical properties influencing mouthfeel (Rahman & Al-Farsi, 2005). A universal testing machine (TA-Xt plus; Stable Micro Systems; UK) equipped with a cylindrical probe (P/50, 50mm diameter) was used to analyze the mechanical properties of emulsion sausages through TPA. Seven key texture parameters were measured: hardness (firmness), adhesiveness (stickiness), springiness (elastic recovery), cohesiveness (structural integrity), gumminess (energy required for mastication), chewiness (effort needed for chewing) and resilience (ability to regain shape). Combining these instrumental measurements with sensory evaluation provides a thorough assessment to optimize product texture and enhance consumer appeal.

#### 5. Nutritional analysis

Nutritional analysis was performed by the Food Quality Assurance Service Center (FQA LAB), Institute of Food Research and Product Development, Kasetsart University. The procedures followed the standards specified in Thai nutrition labeling guidelines and in-house analytical methods based on official AOAC methods (AOAC, 2023; Institute of Food Research and Product Development, 2024). This encompassed the measurement of various parameters including total energy, total carbohydrate (calculation method), total fat, and protein, which were determined using the AOAC methods. Sugars were quantified using High-Performance Liquid Chromatography (HPLC) techniques. In addition, sodium content was measured by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) methods (Institute of Food Research and Product Development, 2024).

#### 6. Statistical analysis

Sensory evaluation data were analyzed using a Randomized Complete Block Design (RCBD), with panelists serving as blocks to control for individual differences. Two-way ANOVA and Duncan's Multiple Range Test (DMRT,  $p < 0.05$ ) were then applied to compare the four formulations (F0, F1, F2, F3). For physicochemical

and texture analyses, a balanced 2×3 factorial design was implemented, in which the two factors were formulation (F0 and F3), representing the original and the most accepted formulations, and storage day (day 1, 4 and 7). Each physicochemical measurement was performed in triplicate. This factorial design primarily focused on presenting the main effects of formulation and storage day, which are critical determinants of sausage quality. Two-way ANOVA was used to partition the total variance into main effects and their interaction, and DMRT ( $p < 0.05$ ) was used for post hoc comparisons. This integrated approach ensures robust and interpretable results by clearly elucidating both individual and interactive influences on product quality.

## Results and Discussion

### 1. Sensory acceptance test

(Table 3) shows the sensory acceptance test results of consumer acceptability of sausage formulation based on springiness, appearance, taste, texture, aroma, and overall liking for sausage with different ratio of pork and *P. hypophthalmus*. Overall, formulation F3 had the best overall scores in terms of sensory attributes, indicating that consumers had a preference for sausages containing 30% *P. hypophthalmus*. Regarding springiness, F3 was scored of  $6.23 \pm 1.91$ , which was considered as better than other formulations, while F0 and F2 did not significantly difference in their scores. In terms of appearance, F3 had the highest score ( $7.00 \pm 1.51$ ), indicating that the higher proportion of *P. hypophthalmus* improved the sausage's outlook.

In taste, F3 had the best score of  $6.90 \pm 1.45$  ( $p < 0.05$ ) since the highest fish content gave the best taste. This means that the texture of the fish paste containing 30% fish meat was satisfactory and the texture of F3 was not significantly different from that of F2. In terms of aroma, all the formulations received nearly equal scores (6.27-6.47), implying that the aroma of fish meat had no effect. On overall preference, F3 recorded the highest score ( $6.93 \pm 1.44$ ), which was significantly different ( $p < 0.05$ ) from F1 and therefore suggested the highest acceptability of the formulation containing 30% fish meat which tallies with the results of the taste and appearance. The consistent aroma scores may result from the mild odor of *P. hypophthalmus* and the use of spices like garlic, pepper, and paprika in the base formula, which helped mask fishy notes. The formulation aimed to maintain sensory familiarity and ensure consumer acceptance rather than highlight fish aroma.

From the sensory acceptance, the formulation F3 with 30% *P. hypophthalmus* showed a highly significant acceptability for appearance, taste, and overall liking. The texture ( $6.37 \pm 1.75$ ) and springiness ( $6.23 \pm 1.91$ ) of F3 were acceptable, and it can be concluded that fish protein has the ability to offer a texture that is preferred by the consumers. Research on fish-based meat products has also established that the inclusion of fish will enhance both sensory and nutritional attributes of the products without posing any threat to consumer acceptability (Skatecki et al., 2021). The high acceptability of F3 in appearance ( $7.00 \pm 1.51$ ) and taste ( $6.90 \pm 1.45$ ) indicated that fish meat may have improved on these factors. Based on the results, it may be concluded that the 30% fish blend could serve as a viable substitute for pork sausages because of the high overall liking score ( $6.93 \pm 1.44$ ). This is in line with the study by Skatecki et al., (2021), who noted that fish roe and meat additions enhanced nutritional and sensory characteristics while still being acceptable by the consumers.

**Table 3** Sensory evaluation scores of emulsion sausages with different pork- *P. hypophthalmus* ratios.

formula	sensory test					
	springiness	appearance	taste	texture	aroma	overall liking
F0	5.67±1.65	6.37±1.38	5.80 <sup>b</sup> ±1.56	6.00 <sup>a</sup> ±1.68	6.33±1.86	6.27 <sup>ab</sup> ±1.55
F1	6.03±1.19	6.47±1.25	5.23 <sup>b</sup> ±1.55	4.90 <sup>b</sup> ±1.63	6.27±1.60	5.73 <sup>b</sup> ±1.34
F2	5.77±1.61	6.70±1.26	6.03 <sup>b</sup> ±1.50	5.97 <sup>a</sup> ±1.52	6.47±1.78	6.53 <sup>a</sup> ±1.28
F3	6.23±1.91	7.00±1.51	6.90 <sup>a</sup> ±1.45	6.37 <sup>a</sup> ±1.75	6.43±1.77	6.93 <sup>a</sup> ±1.44

note: different superscript letters within the same column indicate significant differences ( $p < 0.05$ ). sample size,  $n=30$ .

These results indicated that the acceptance of F3 in appearance, taste, and overall liking implies that the inclusion of *P. hypophthalmus* improves the sensory characteristics of emulsion sausages. This characteristic of fish protein is desirable in the sense that it enhances mouthfeel, thus supporting its use as a source of protein. Therefore, F3 was selected for further comparative testing against F0. Skatecki et al., (2021) also revealed that fish-based proteins enhanced the texture and springiness of meat products, contributing positively to both sensory and nutritional qualities, and were well accepted by consumers. This aligns with the high texture (6.37±1.75) and springiness (6.23±1.91) scores obtained in F3, confirming that the inclusion of *P. hypophthalmus* improved the mouthfeel of the emulsion sausages. Therefore, F3 was selected for further comparative testing against F0.

## 2. Physical and chemical analysis

The physicochemical characteristics of the formulated emulsion sausages made from different levels of *P. hypophthalmus* were determined the effect of formulation and storage day on color, texture, and oxidative stability of the product. The statistical analysis results are presented in (Table 4), the mean values of each formulation on each storage day presented in (Tables 5-6).

### 2.1 Color attributes

The color of meat products is one of the main factors that define consumer attitude towards the product in terms of acceptability. The  $L^*$ ,  $a^*$  and  $b^*$  values of the emulsion sausages were determined at different storage days. As shown in (Table 3), there was a significant difference for the formula on  $a^*$  ( $p < 0.01$ ),  $b^*$  ( $p < 0.01$ ) and  $C$  ( $p < 0.01$ ), but not on the  $L^*$  ( $p = 0.19$ ). The storage period affected  $L^*$  ( $p < 0.01$ ),  $a^*$  ( $p < 0.01$ ),  $b^*$  ( $p < 0.01$ ) and  $C$  ( $p < 0.01$ ) significantly, while the interaction between formulation and storage period had a significant effect on  $b^*$  ( $p < 0.01$ ) and  $C$  ( $p < 0.01$ ). These results indicate that both formulation and storage time influence the color characteristics of sausages. This observation is consistent with previous studies, which reported that the oxidation of oxymyoglobin to metmyoglobin during storage leads to changes in meat color, particularly the reduction of  $a^*$  value and alterations in color attribute, thereby affecting overall color stability (De Smet & Vossen, 2016).

Mean values of each formulation (Table 4) show that F3 had a significantly higher  $a^*$  value (10.43±0.48) than F0 (8.96±0.47), indicating that the addition of *P. hypophthalmus* improved the redness of the sausages. The  $b^*$  and  $C$  values were also significantly higher in F3 than in F0, showing that fish incorporation had a positive



effect on the yellowness and chroma intensity of the product. Besides, the hue angle ( $h^\circ$ ) was also used to establish the direction of color of the sausages. It was deduced that F0 was of hue angle 71.54, while F3 was of hue angle 70.56 as shown in (Table 5). A lower hue value means that the color is closer to red may due to the increase in fish protein. This trend indicates that the use of *P. hypophthalmus* slightly improved the color of sausage with a reddish tinge.

For storage effects (Table 6),  $L^*$  values fluctuated across days, with day 7 ( $68.51 \pm 0.13$ ) being significantly higher than day 4 ( $67.67 \pm 0.18$ ), possibly due to moisture loss and protein oxidation affecting surface reflectance. The  $a^*$  values were highest on day 4 ( $10.25 \pm 0.83$ ) and lowest on day 7 ( $9.25 \pm 0.74$ ), likely reflecting pigment oxidation over time. The  $b^*$  and C values followed a similar trend, with day 4 having significantly higher yellowness than day 7, reinforcing the impact of oxidation on color degradation. The total color difference ( $\Delta E$ ) between formulations was also examined. The ANOVA results (Table 4) revealed that formulation significantly affected  $\Delta E$  ( $p < 0.01$ ), with F0 showing a significantly higher  $\Delta E$  ( $1.02 \pm 0.24$ ) compared to F3 ( $0.65 \pm 0.23$ ). Nevertheless, storage day did not significantly affect  $\Delta E$ , indicating that overall color stability was maintained during storage.

## 2.2 Oxidative stability

The lipid oxidation is one of the most critical factors that determine the shelf-life and acceptability of processed meat products. The peroxide value (PV) and thiobarbituric acid reactive substances (TBA) were utilized as primary and secondary measures of oxidation. (Table 4) shows that formulation had a significant effect on PV ( $p < 0.01$ ), and F3 had a higher PV ( $2.33 \pm 0.56$ ) than F0 ( $1.87 \pm 0.45$ ), as shown in (Table 5). This means that fish fat can undergo oxidation than pork fat. Storage day also significantly affected PV ( $p < 0.01$ ), ranging from  $1.53 \pm 0.17$  on day 1 to  $2.43 \pm 0.19$  on day 4, indicating that lipid peroxidation continues during storage (Table 6).

In the same way, TBA values were significantly different due to formulation ( $p < 0.01$ ) as well as storage period ( $p < 0.01$ ). TBA value of F3 ( $0.20 \pm 0.03$ ) had a higher than F0 ( $0.17 \pm 0.04$ ), which suggested that it was more prone to secondary oxidation. Duration also affected TBA values, day 7 ( $0.22 \pm 0.03$ ) had higher value than day 4 ( $0.15 \pm 0.04$ ), indicating the effect of time in lipid peroxidation. Based on the oxidative stability analysis, formulation F3, which incorporates *P. hypophthalmus* meat, exhibited greater susceptibility to lipid oxidation compared to the pork-only formulation (F0). Both PV and TBA were significantly higher in F3 across all storage days, indicating that fish-derived lipids are more prone to oxidation. Moreover, PV and TBA values increased significantly over time, particularly on days 4 and 7, suggesting continuous oxidative degradation during storage. Although the total color difference ( $\Delta E$ ) remained relatively stable, indicating minimal visual change, chemical deterioration was evident. Therefore, while F3 offers nutritional advantages, its shelf life may be shorter, unless antioxidant strategies are applied to mitigate lipid oxidation.

To address this issue, future formulations should consider incorporating natural antioxidants such as tocopherols, rosemary extract, or plant-derived polyphenols. Essential oils like oregano, rosemary and sage (classified as GRAS) have shown strong antioxidant effects in meat products. Ünal et al., (2014) found that oregano essential oil significantly reduced lipid oxidation and extended shelf life in minced beef, indicating its potential for improving oxidative stability in fish-based emulsion sausages.

**Table 4** p-values for physicochemical properties.

source of variation	L <sup>*</sup>	a <sup>*</sup>	b <sup>*</sup>	C	h	ΔE	PV	TBA
formula	0.190	<0.01 <sup>***</sup>	<0.01 <sup>**</sup>	<0.01 <sup>**</sup>	<0.01 <sup>**</sup>	<0.01 <sup>**</sup>	<0.01 <sup>**</sup>	<0.01 <sup>**</sup>
day	<0.01 <sup>**</sup>	<0.01 <sup>**</sup>	0.062	<0.01 <sup>**</sup>	<0.01 <sup>**</sup>	0.608	<0.01 <sup>**</sup>	<0.01 <sup>**</sup>
formula×day	0.390	0.610	<0.01 <sup>**</sup>	<0.01 <sup>**</sup>	0.570	<0.01 <sup>**</sup>	<0.01 <sup>**</sup>	<0.01 <sup>**</sup>

note: <sup>\*</sup> p<0.05, <sup>\*\*</sup> p<0.01. results are based on a factorial design (n=2×3×3=18).

**Table 5** Physicochemical properties of emulsion sausages by formulation.

variable	F0	F3
L <sup>*</sup>	68.17±0.54	67.98±0.35
a <sup>*</sup>	8.96 <sup>b</sup> ±0.47	10.43 <sup>a</sup> ±0.48
b <sup>*</sup>	26.85 <sup>b</sup> ±0.44	29.56 <sup>a</sup> ±0.14
C	28.31 <sup>b</sup> ±0.52	31.35 <sup>a</sup> ±0.22
h (°)	71.54 <sup>a</sup> ±0.76	70.56 <sup>b</sup> ±0.83
ΔE	1.02 <sup>a</sup> ±0.24	0.65 <sup>b</sup> ±0.24
PV (mEq/kg)	1.87 <sup>b</sup> ±0.45	2.33 <sup>a</sup> ±0.56
TBA (mg MDA/kg)	0.17 <sup>b</sup> ±0.04	0.20 <sup>a</sup> ±0.03

note: different superscript letters within the same row indicate significant differences (p<0.05). sample size, n=9.

**Table 6** Physicochemical properties of emulsion sausages by storage day.

variable	day 1	day 4	day 7
L <sup>*</sup>	68.06 <sup>b</sup> ±0.47	67.67 <sup>c</sup> ±0.18	68.51 <sup>a</sup> ±0.13
a <sup>*</sup>	9.59 <sup>b</sup> ±0.89	10.25 <sup>a</sup> ±0.84	9.25 <sup>c</sup> ±0.74
b <sup>*</sup>	28.11 <sup>ab</sup> ±1.77	28.40 <sup>a</sup> ±1.21	28.10 <sup>b</sup> ±1.52
C	29.70 <sup>ab</sup> ±1.95	30.19 <sup>a</sup> ±1.41	29.59 <sup>b</sup> ±1.67
h (°)	71.19 <sup>ab</sup> ±0.71	70.18 <sup>b</sup> ±0.76	71.79 <sup>a</sup> ±0.45
ΔE	0.78 <sup>a</sup> ±0.52	0.85 <sup>a</sup> ±0.08	0.89 <sup>a</sup> ±0.15
PV (mEq/kg)	1.53 <sup>b</sup> ±0.17	2.43 <sup>a</sup> ±0.19	2.33 <sup>a</sup> ±0.59
TBA (mg MDA/kg)	0.18 <sup>b</sup> ±0.01	0.15 <sup>c</sup> ±0.04	0.22 <sup>a</sup> ±0.03

note: different superscript letters within the same row indicate significant differences (p<0.05). sample size, n=6.

The higher redness (a) in F3 means that the addition of *P. hypophthalmus* improved the color of the sausage, in accordance with Skatecki et al., (2021), who stated that fish proteins increase the redness and chroma due to the presence of natural pigments. The decrease in a and b values with storage time is in agreement with the typical oxidation changes that occur in processed meat products due to the instability of pigments. Although pigment composition was not directly analyzed, the higher a<sup>\*</sup> value in F3 may reflect better color retention from

fish-derived antioxidants. Hamzah et al., (2021) showed that protein hydrolysate from silver catfish preserved  $a^*$  by delaying lipid oxidation. Likewise, Intarasirisawat et al., (2014) found that skipjack roe hydrolysate enhanced redness and reduced oxidative degradation. These findings suggest that the increased  $a^*$  in F3 may result from both natural pigments and improved oxidative stability. The lower hue angle (h) in F3 also supports fish protein's ability to produce a redder appearance, which is more appealing to consumers because the color red is associated with freshness. The low  $\Delta E$  values show that color did not change significantly with oxidation as preferring for the stability of the product.

The higher PV and TBA in F3 supported the higher lipid oxidation susceptibility due to the fact that PUFAs oxidize faster than pork fats (Kawecki et al., 2021). The increase in storage prolongs the effect of oxidation on shelf life and thus the use of antioxidants such as tocopherols or rosemary extract. Although the fat content in all formulations was standardized at 18.74%, the measured total fat in F3 (14.21g/100g) was slightly higher than F0 (13.88g/100g). However, this increase was not accompanied by a rise in saturated fat or cholesterol levels, suggesting that the difference may be attributed to higher levels of unsaturated fatty acids, particularly MUFA and PUFA, naturally present in fish meat such as *P. hypophthalmus*. Since trans fats are rarely found in unprocessed animal tissues, they are unlikely to account for the increase. The results support the nutritional profile improvement by replacing pork with fish, as unsaturated fats are considered more beneficial to health.

### 3. Texture profile analysis

In order to examine the effect of formulation and storage day on hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness and resilience of the texture properties of emulsion sausages, the sausages were formulated with different levels of *P. hypophthalmus*. The overall findings revealed that hardness, cohesiveness, gumminess, chewiness, and resilience were affected by formulation and/or storage day, while adhesiveness and springiness were not affected (Table 7). The mean values for each formulation and storage day are as shown in (Tables 8-9).

#### 3.1 Hardness and adhesiveness

Hardness is another attribute of meat texture and it characterizes sausage by the force required to change its shape. Two-way ANOVA indicated that formulation played a role in affecting hardness ( $p=0.047$ ) and storage day ( $p=0.003$ ). Furthermore, there was a significant interaction between formulation and storage ( $p=0.017$ ), indicating that both factors affected sausage firmness. The hardness of F0 ( $43.19\pm5.31$ ) had higher than F3 ( $40.77\pm5.20$ ), indicating that incorporation of *P. hypophthalmus* slightly reduced product firmness. This reduction could be attributed to the higher moisture content and the inherently softer texture of fish proteins compared with pork proteins.

For storage effects, the hardness of the composites increased with time and the composites at day 7 ( $43.93\pm5.42$ ) were harder than those at day 1 ( $39.03\pm4.05$ ). This change might be attributed to the loss of moisture and cross-linkage of proteins that lead to an increase in firmness of the sausage over time. However, adhesiveness, which was the other parameter evaluated, was not influenced by formulation ( $p=0.146$ ), storage day ( $p=0.131$ ),

or their interaction ( $p=0.433$ ). The adhesiveness was not significantly affected by either formulation or storage duration; therefore, these factors had no significant effect on sausage stickiness.

### 3.2 Springiness and cohesiveness

Springiness refers to the capacity of the sausage to regain its shape after being compressed while cohesiveness is the internal structure of the sausage product. ANOVA results indicated that formulation ( $p=0.869$ ), storage ( $p=0.109$ ), and their interaction ( $p=0.506$ ) did not significantly affect springiness, which means that the elasticity of the sausages was not influenced by formulation or storage period. On the other hand, cohesiveness was significantly influenced by formulation ( $p<0.01$ ), storage ( $p<0.01$ ), and their interaction ( $p<0.01$ ). The cohesiveness of F3 ( $0.78\pm0.01$ ) was higher than that of F0 ( $0.73\pm0.03$ ), clearly depicting that the inclusion of *P. hypophthalmus* enhanced the binding force of the final product. This could be attributed to the fact that fish proteins improve the formation of gel structures, hence improving the structural characteristics of the product. Cohesiveness also rose with time, the cohesiveness of day 7 ( $0.78\pm0.01$ ) was higher than day 1 and day 4 (both at  $0.75\pm0.04$ ). This indicates that protein restructuring during storage likely enhanced internal binding strength through further protein–protein interactions and water redistribution.

### 3.3 Gumminess and chewiness

Gumminess is the energy needed to cause the food to break down, while chewiness is a similar characteristic that also includes springiness as a parameter; both are affected by several factors. ANOVA showed that formulation ( $p<0.01$ ), storage ( $p<0.01$ ) and their interaction ( $p<0.01$ ) had significant effects on gumminess. Likewise, chewiness was significantly affected by formulation ( $p<0.01$ ) and storage ( $p<0.01$ ), although their interaction was non-significant ( $p=0.083$ ). These results imply that both formulation and storage contribute to changes in texture, although chewiness was more influenced by the main effects than by their interaction. The gumminess ( $33.99\pm3.47$ ) and chewiness ( $33.32\pm4.12$ ) of F3 were higher than those of F0 ( $30.38\pm3.56$  and  $26.06\pm4.18$ , respectively), indicating that the addition of fish proteins increased the hardness and cohesiveness of the product. The gumminess and chewiness increased over the storage period. The gumminess ( $33.29\pm3.97$ ) and chewiness ( $32.63\pm5.61$ ) at day 7 were significantly higher than those on day 1 ( $30.43\pm4.62$  and  $26.49\pm5.21$ , respectively). These changes could be due to moisture loss and protein–protein interactions that continually enhance sausage firmness throughout storage. The gumminess and chewiness of the products increased during the storage period, which day 7 having the highest values for gumminess ( $33.29\pm3.97$ ) and chewiness ( $32.63\pm5.61$ ), which were significantly different from those on day 1 ( $30.43\pm4.62$  and  $26.49\pm5.21$ , respectively). These changes could be due to loss of moisture and protein–protein interactions that continually enhance the firmness of the sausages throughout storage.

### 3.4 Resilience

Resilience is the deformation from which the sausage can recover, indicating its performance under mechanical stress. ANOVA indicated that formulation ( $p<0.01$ ), storage ( $p<0.01$ ), and their interaction ( $p=0.012$ ) all significantly affected resilience. The resilience for F3 ( $0.27\pm0.03$ ) was higher than for F0 ( $0.22\pm0.02$ ),

suggesting that fish proteins improved the sausage's ability to regain its original shape. Storage effects indicate that resilience improved over time; a resilience of day 7 ( $0.26 \pm 0.04$ ) was compared to day 1 ( $0.23 \pm 0.02$ ). Further, day 4 ( $0.25 \pm 0.03$ ) also showed a significant rise versus day 1, implying gradual enhancement in structural restoration of the stored sausage samples during storage. This aligns with observations for cohesiveness and chewiness and supports the role of protein restructuring and moisture redistribution in improving resilience during storage.

The lower hardness in F3 indicates that the firmness of *P. hypophthalmus* decreased due to increased moisture content and softer fish proteins as confirmed by Maulu et al., (2021), who stated that fish proteins increase water retention and decrease hardness of the emulsified products. The increase in hardness over storage is in agreement with the loss of moisture content and protein covalent bonding, which is typical of processed meat products. F3 sample had higher cohesiveness due to stronger protein matrix interaction possibly from fish proteins forming a structural network, which is in support of Lee et al., (2020), who found that fish proteins increase structural stability in emulsified meat. The increase in cohesiveness over storage indicates the restructuring of the proteins and change in the distribution of moisture. The increased gumminess and chewiness of the F3 sample mean that fish proteins enhance the textural properties of the final product, as reported by Skatecki et al., (2021). The textural characteristics of processed meats improved with the addition of fish proteins. The increase over storage is as a result of the continual protein-protein interactions, supporting storage as a way of improving the texture stability.

The higher resilience in F3 indicated the improved structural recovery of the product, which was evident from similar trends of cohesiveness and chewiness. This is in agreement with other research works done on protein reformation at long intervals and the ability of fish proteins to enhance the textural properties of the product during storage. The increased gumminess and chewiness observed in F3, caused by the incorporation of fish proteins, reflect a denser and more elastic product structure. These changes, while indicating a firmer texture, were well received by panelists, as evidenced by the highest texture score in the sensory evaluation ( $6.37 \pm 1.75$ ). This alignment between instrumental measurements and consumer acceptance confirms that the textural modifications enhanced rather than compromised the palatability of the final product.

**Table 7** p-values for texture profile analysis.

source of variation	hardness	adhesiveness	springiness	cohesiveness	gumminess	chewiness	resilience
formula	0.047*	0.146	0.869	<0.01**	<0.01**	<0.01**	<0.01**
day	<0.01**	0.131	0.109	<0.01**	<0.01**	<0.01**	<0.01**
formula×day	0.017*	0.433	0.506	<0.01**	<0.01**	0.083	0.012*

note: \* $p < 0.05$ , \*\* $p < 0.01$ . results are based on a factorial design ( $n = 2 \times 3 \times 10 = 60$ ).

**Table 8** Mean texture properties of emulsion sausages by formulation.

variable	F0	F3
hardness (N)	43.19 <sup>a</sup> ±5.31	40.77 <sup>b</sup> ±5.20
adhesiveness (N.sec)	-0.67±0.40	-0.81±0.34
springiness (sec/sec)	0.84±0.06	0.84±0.08
cohesiveness (N.sec/N.sec)	0.73 <sup>b</sup> ±0.03	0.78 <sup>a</sup> ±0.01
gumminess (N)	30.38 <sup>b</sup> ±3.56	33.99 <sup>a</sup> ±3.47
chewiness (N)	26.06 <sup>b</sup> ±4.18	33.32 <sup>a</sup> ±4.12
resilience	0.22 <sup>b</sup> ±0.02	0.27 <sup>a</sup> ±0.03

note: different superscript letters within the same row indicate significant differences ( $p < 0.05$ ). sample size,  $n = 30$ .

**Table 9** Mean texture properties of emulsion sausages by storage day.

variable	day 1	day 4	day 7
hardness (N)	39.03 <sup>b</sup> ±4.05	42.98 <sup>a</sup> ±5.37	43.93 <sup>a</sup> ±5.42
adhesiveness (N.sec)	-0.60±0.29	-0.79±0.40	-0.82±0.41
springiness (sec/sec)	0.83±0.06	0.82±0.08	0.87±0.06
cohesiveness	0.75 <sup>b</sup> ±0.04	0.75 <sup>b</sup> ±0.04	0.78 <sup>a</sup> ±0.01
gumminess (N)	30.43 <sup>b</sup> ±4.62	32.83 <sup>a</sup> ±2.47	33.29 <sup>a</sup> ±3.97
chewiness (N)	26.49 <sup>c</sup> ±5.21	29.95 <sup>b</sup> ±3.93	32.63 <sup>a</sup> ±5.61
resilience	0.23 <sup>b</sup> ±0.02	0.25 <sup>a</sup> ±0.03	0.26 <sup>a</sup> ±0.04

note: different superscript letters within the same row indicate significant differences ( $p < 0.05$ ). sample size,  $n = 20$ .

#### 4. Nutritional analysis

The proximate composition as macronutrients and minerals, and energy value of the emulsion sausages prepared with various proportions of pork and *P. hypophthalmus* were determined. Among all formulations under sensory acceptance test, formulations F0 and F3 were chosen for further nutritional analysis as indicated in (Table 10). This formulation F0 made from pork only was among the most preferred by the consumers. F3 with 30% *P. hypophthalmus* had the highest overall liking scores, thus making it to be considered as a nutritionally improved and accepted product in the sausage market.

##### 4.1 Macronutrient composition

Both formulations offered a good amount of energy; F0 with 195.60kcal/100g and F3 with 191.05 kcal/100g. The total fat content of the F3 was 14.21g/100g and was similar to that of F0 at 13.88g/100g to provide a rich taste to the final product in accordance to consumer preferences. It was also observed that the saturated fatty acid content was not significantly affected by the formulation type (F3: 5.88g/100g and F0: 5.80g/100g). Protein content decreased from F0 (12.61g/100g) to F3 (11.37g/100g), this may be attributed to the difference in the structure of pork and fish. Nevertheless, the presence of *P. hypophthalmus* might be suggested as a good protein source due to the essential amino acids. The moisture content of the control sample F3 was significantly higher (67.96g/100g) when compared to the initial sample F0 (66.38g/100g) that enhanced the juiciness and

tenderness of the product. This is in concordance with sensory analysis where F3 received a better score with regard to texture and mouthfeel.

#### 4.2 Mineral content and sodium levels

Cholesterol content of F3 (31.16mg/100g) was reduced compared to F0 (35.69mg/100g) and thus, F3 could be more suitable for consumers who are concerned with low cholesterol diet. Sodium of F3 was 565.48mg/100g, which was slightly higher than F0 (523.88mg/100g) because of the presence of sodium in fish proteins or formulation changes to improve the taste. While sodium levels rose, they were not in the high amount, and the flavors were well balanced savory without going overboard with sodium. However, the potassium content in F3 (235.27mg/100g) was significantly lower than that in F0 (280.96mg/100g) to show that fish incorporation might have affected potassium solubility in the formulation. However, both formulations contained ingredients that were needed in the body to carry out some functions of electrolytes.

Total carbohydrate content in F3 (4.42g/100g) was slightly reduced as compared to F0 (5.06g/100g) and both formulations did not have total sugars, which make them suitable for consumers in low-sugar or carbohydrate diets. In addition, the ash content which depicts the total mineral content of the samples was relatively the same in both formulations (F0=2.07g/100g and F3=2.04g/100g). There was no much change in the mineral levels but fluctuation was observed in specific minerals like sodium and potassium.

#### 4.3 Nutritional implications

*P. hypophthalmus* in formulation F3 may contribute essential polyunsaturated fatty acids (PUFAs), particularly EPA and DHA, which support cardiovascular and anti-inflammatory health. This is consistent with Maulu et al., (2021), highlighting the dietary benefits of fish-derived PUFAs. Similarly, Howe et al., (2002) found that supplementing pig diets with tuna fishmeal significantly increased long-chain n-3 PUFA, especially DHA, without compromising sensory quality. Skatecki et al., (2021) also demonstrated that replacing 20% of pork with fish meat or roe in pâtés improved the fatty acid profile and nutritional value while maintaining consumer acceptability. These studies support the potential of fish-based ingredients to enhance PUFA content in meat products.

In conclusion, Formulation F3 has a favorable nutritional composition and can be considered as a pork sausage with some positive effects of fish protein. This differentiation brings value to the product by making it more appealing to consumers while also solving the problem of consumers' health needs regarding their diet. F3 enhances the palatability of the product due to enhanced moisture content and lower cholesterol levels and it would be suitable for those consumers with different diets. The slightly lower energy content in F3 suggests that *P. hypophthalmus* contributed to a leaner nutritional profile without compromising taste, aligning with Maulu et al., (2021), highlighting fish-based products as lower-calorie alternatives in processed meats. The higher moisture content in F3 supports enhanced juiciness, consistent with Lee et al., (2020), who reported improved water retention in fish-protein-based emulsified products. This aligns with the sensory evaluation, where F3 was rated favorably for texture. The lower cholesterol in F3 reinforces its health benefits, aligning with Parlasca & Qaim (2022), who noted that fish protein incorporation reduces dietary cholesterol levels. Despite a slightly higher sodium content, the values remain

within an acceptable range, supporting a balanced savory profile. The potential presence of PUFAs in F3, common in fish-derived proteins, suggests cardiovascular benefits, as emphasized by Maulu et al., (2021). Although PUFA content was not directly analyzed, its inclusion may enhance nutritional quality and functional health benefits. Overall, F3 presents a health-conscious alternative to traditional pork sausages, offering lower cholesterol, higher moisture content, and potential PUFA benefits, making it suitable for consumers seeking a nutritionally balanced yet flavorful product.

**Table 10** Nutritional composition of sausage samples (F0 and F3).

analysis Item	unit	result		test method	remark
		F0	F3		
energy	kcal/100g	195.60	191.05	methods of analysis for nutrition labeling (1993) Ch.6, p.106	*
total fat	g/100g	13.88	14.21	in-house method WI-TMC-100 based on AOAC (2023) 2003.05	
saturated fat	g/100g	5.80	5.88	in-house method WI-TMC-126 based on AOAC (2023) 996.06, 969.33	
cholesterol	mg/100g	35.69	31.16	in-house method WI-TMC-125 based on AOAC (2023) 994.10	
protein (factor 6.25)	g/100g	12.61	11.37	in-house method WI-TMC-03 based on AOAC (2023) 991.20	
total carbohydrate	g/100g	5.06	4.42	methods of analysis for nutrition labeling (1993) Ch.6, p.106	*
total sugars	g/100g	not detected	not detected	in-house method WI-TMC-07 based on AOAC (2023) 982.14	
sodium	mg/100g	523.88	565.48	in-house method WI-TMC-19 based on AOAC (2023) 984.27	
potassium	mg/100g	280.96	235.27	in-house method WI-TMC-133 based on AOAC (2023) 984.27	*
ash	g/100g	2.07	2.04	AOAC (2023) 923.03	*
moisture	g/100g	66.38	67.96	AOAC (2023) 925.10	*

\* indicates test items that are not certified under ISO/IEC 17025 standards.

### Conclusion

This study developed an emulsion sausage with 30% *Pangasius hypophthalmus* meat substitution, which improved texture, appearance, and overall liking with consumer acceptability. Although it had lower cholesterol, higher moisture, moderate fat and sodium levels, so its health advantage should be interpreted with caution. Higher lipid oxidation was observed, indicating stability issues. The findings support the potential of *P. hypophthalmus* as a partial pork substitute for more sustainable meat products. Future studies should investigate natural antioxidants and advanced packaging to enhance shelf life, and conduct broader consumer testing to inform product positioning.



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