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# Effect of Ratios of Tilapia Trim Meat and Fermentation Periods on Quality Characteristics of Fermented Fish Sausage

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**Abstract**

Using tilapia trim meat (TTM), a by-product from industrial-scale tilapia fillet manufacturing, for production of fermented fish sausage (FFS) may increase the benefit to the tilapia filleting industry by selling this new product to consumers. Six ratios of TTM:tilapia fillet meat (100:0, 80:20, 60:40, 40:60, 20:80 and 0:100) were used for sausage production. FFS samples prepared with each ratio were collected on days 0, 2, 4 and 6, and analyzed for quality parameters including lactic acid bacteria (LAB), total plate count (TPC), yeast and mold (YM), texture profile analysis (TPA), CIE color values (L*, a* and b*), pH, titratable acidity and sensory acceptability. The ratio of TTM to tilapia fillet meat had no effect on YM, b*, pH and titratable acidity. The 0:100 ratio produced lower LAB, TPC and L* values compared to 100:0, 80:20 and 60:40. The ratio also yielded a higher a* than 100:0 and 80:20. The 0:100 ratio generated the best TPA, followed by 20:80. However, these two ratios were not significantly different concerning sensory acceptability. The 20:80 ratio, giving similar sensory quality to 0:100, is recommended for the production of FFS. The results also reveal that FFS ripened on day 2 and ratios of TTM:tilapia fillet meat did not affect the ripeness of the product. The best quality of FFS was found on day 4.

***Keywords:*** Tilapia; Trim meat; Fermented sausage; Quality; Fermentation period; By-product

## 1 Introduction

Fermented sausages are highly popular in several countries around the world, especially in Thailand. Fermented sausage, known in the Thai language as Nham (Suteebut et al., 2017). It is a mixture of ground/minced meat (pork, beef or fish), cooked rice, garlic and salt. Fermented fish sausage (FFS), known in the Thai language as Nham Pla, is mostly produced from freshwater fish such as great white sheatfish (*Wallago attu* Block), Pangas catfish (*Pangasius pangasius* Hamilton), whisker sheatfish (*Kryptopterus bleekeri* Günther), featherback (*Notopterus chitala* Hamilton, *N. blanci, N. borneensis*, and *N. chitala* Buchanan), silver carp (*Puntius* sp.), giant snake-head fish (*Ophicephalus micropeltes*), barb (*Cyclocheilichthys* sp.) and rohu (*Labeo rohita*) (Srisawad & Gawborisut, 2018).

To produce FFS, minced fish meat, cooked rice, minced garlic and salt at a ratio by weight of 60:10:3:3 are mixed and hand-kneaded or mechanically massaged at low temperature until becoming a gel-like sticky paste with elastic texture (Krusong, 2004; Srisawad & Gawborisut, 2018). The paste is then shaped into patties, wrapped tightly with banana leaves or plastic sheets, or stuffed into plastic casing. After that, it is left to

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ferment in the shade at ambient temperature for 3–5 days. The ripened FFS has a firm texture and sour taste with a desirable unique fermented flavor. FFS is usually deep-fried or steamed and consumed with rice and fresh vegetables as a main dish or snack.

Tilapia trim meat (TTM), an underutilized by-product from industrial-scale tilapia fillet manufacturing, is small pieces of meat trimmed from the belly flaps and the edges of tilapia fillets (Srisawad & Gawborisut, 2018). The trimming process gives the fillets a desirable appearance and eliminates the fatty tissue at the edges of the fillets causing rancid off-flavor when oxidized with atmospheric oxygen. TTM collected from the trimming process is washed, drained, packed in plastic bags (1 kg/bag) and kept frozen. Homhong (2014) found that TTM contains 17.41% protein and 22.12% fat. TTM is considered a low-price meat, costing around 70 Baht (2 USD/kg) compared to tilapia fillet costing around 150 Baht (4 USD/kg).

The amount of TTM produced from tilapia processing plants in Thailand can be estimated from the percentage of TTM and that of fillet, which account for 1.45% and 31.48% of the whole fish weight, respectively (Srisawad & Gawborisut, 2018). In 2016, Thailand produced at least 1191 tonnes of tilapia fillet for export, which came from 3873 tonnes of whole fish weight of 3783 tonnes and left 54.85 tonnes of TTM. Srisawad and Gawborisut (2018) pioneered the use of TTM in the production of FFS. However, the sensory texture scores of the product barely passed the cut-off score of 5 out of 9 points. The low texture score was caused by the soft texture. It was concluded that using solely TTM could not yield a good quality FFS. TTM should be used in combination with tilapia fillet meat. This study aims to investigate the use of TTM in combination with tilapia fillet meat for the preparation of FFS. It also aims to investigate quality changes occurring in FFS produced from TTM and tilapia fillet meat during fermentation.

## 2 Materials and Methods

### 2.1 TTM and Tilapia Fillet Meat

TTM (Figure 1) was purchased from Grobest Frozen Co. Ltd. (Nakhon Phanom, Thailand). Ten bags of frozen TTM (1 kg/bag) were delivered by a frozen food delivery van to the Fish Processing Laboratory, Khon Kaen University, Thailand. Upon arrival, they were kept in a freezer at -20 $^{o}$C and used within 3 weeks. Prior to use, frozen TTM was thawed overnight in a refrigerator at 4 $^{o}$C, hand-mixed homogenously and subjected to microbial analyses, and used for the experiment immediately. TTM contained LAB, TPC and YM of $4.34\times10^2$, $7.32\times10^4$ and $< 1.50\times10^2$ CFU/g, respectively.

Tilapia fillets were purchased from a local market (Bang Lamphu Market, Khon Kaen, Thailand). The fillets were placed in plastic bags, iced and delivered to the laboratory. Upon arrival, the skin was removed. The skinless fillets were then washed twice in iced water and ground for 3 minutes. The ground tilapia fillet meat was stored in plastic bags, iced, subjected to microbial analyses and used within 2 h. This ground tilapia fillet meat contained LAB, TPC, and YM of $1.81\times10^2$, $6.16\times10^3$ and $< 1.50\times10^2$ CFU/g, respectively.

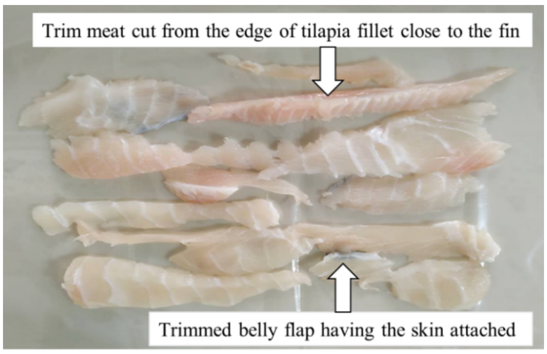


Figure 1: Tilapia trim meat

## 2.2 Ratios of TTM to Tilapia Fillet Meat

Fish meat used in the production of FFS was composed of different ratios of TTM to tilapia fillet meat. Six different TTM:tilapia fillet meat ratios (100:0, 80:20, 60:40, 40:60, 20:80 and 0:100) were prepared. Three kilograms of fish meat for each ratio was prepared and used immediately.

## 2.3 Production of FFS

FFS was produced according to the method of Srisawad and Gawborisut (2018). It was composed of fish meat, cooked non-glutinous white rice, peeled garlic, salt and sugar at the ratio by weight of 5:1:1:0.15:0.15. This composition was equivalent to 3000 g of fish meat, 600 g of cooked rice, 600 g of chopped garlic, 90 g of salt and 90 g of sugar. To produce FFS, firstly, cooked rice and peeled garlic were combined and blended in a food processor (MCM 640660, Bosch, Bratislava, Slovakia). Fish meat was then minced for 10 min in a bowl cutter machine (Cuttex M11, NMH, Hohentengen, Germany). Salt was gradually added into the meat during mincing. After that, the mixture of cooked rice and garlic was added into the meat and briefly mixed. Sugar was then combined and mixed for another 5 min. Finally, FFS paste was stuffed into 35 × 180 mm (diameter × length) polyethylene plastic casings. The casing ends were tied with cotton strings. The FFS stuffed in plastic casings was split into four portions. Four fermentation periods (0, 2, 4 and 6 days) were randomly assigned to these four portions. The portions were then kept separately in an incubator at 28 ± 1 $^{o}$C. When FFS reached the end of each fermentation period, an assigned portion was removed from the incubator. FFS samples were collected, the casings were aseptically removed from the samples, and their microbial contents, texture profile analysis (TPA), pH, titratable acidity and International Commission on Illumination (CIE) color values were analyzed. Raw and baked FFS were also evaluated for sensory acceptability. The experiments were done in triplicate using three different lots of TTM.

## 2.4 Microbial Contents

Lactic acid bacteria (LAB), total plate count (TPC) and yeast and mold (YM) were determined. Twenty-five grams of sample were weighed into 225 ml of 0.1% peptone water and mixed thoroughly for 60 s using a 3500 Jumbo Stomacher (Seward Laboratory Systems, Inc., Bohemia, NY, USA). Serial 10-fold dilutions were then performed and 1 ml aliquots of appropriate dilutions ($10^{-2}$–$10^{-8}$) were pipetted into sterile petri dishes. Fifteen milliliters of molten sterilized de Man, Rogosa and Sharpe (MRS) agar (BBL, Sparks, MD, USA) was poured into the dishes. After that, the dishes were swirled 20 times, allowed to cool, and incubated at 30 ± 0.1 $^{o}$C for 72 h (Palavecino Prpich et al., 2015). TPC was determined by pour plate techniques using standard plate count agar (BBL, Spark, MD, USA) (Qiu et al., 2014). Appropriate dilutions ($10^{-2}$–$10^{-8}$) were transferred to sterilized dishes. Molten agar was combined with the aliquot as previously described. The dishes were incubated at 30 ± 0.1 $^{o}$C for 48 h. YM was determined using appropriate dilutions ($10^{-1}$–$10^{-8}$) spread onto acidified potato agar with a pH of 3.5 ± 0.1 (Özpolat & Patir, 2016). The agar plates were incubated at 23 ± 1 $^{o}$C for 5 days. Microbiological examinations were performed two times/treatment. The enumerated LAB, TPC and YM colonies were counted and expressed as the logarithm of colony-forming units (log CFU/g).

## 2.5 Texture Profile Analysis

TPA of FFS was determined using a procedure modified from Suteebut et al. (2017), which employed a texture analyzer (TA-XT2i, Stable Micro Systems LTD., Vienna, Austria). A dissected FFS sample with a height of 30 mm was pressed with two compression cycles using a cylindrical probe with diameter of 50 mm. Testing conditions were a crosshead speed of 5.0 mm/s, 50% strain, surface sensing force of 99.0 g, threshold of 30.0 g and time interval between first and second strokes of 1 s. TPA was expressed as the values of hardness, adhesiveness, springiness, cohesiveness, chewiness and resilience.

## 2.6 pH

The pH was determined by mixing FFS with recently boiled deionized water in a 1:10 ratio according to Chiou and Huang (2004). Ten grams of FFS were homogenized with 100 ml of recently boiled deionized water. The pH of the homogenized sample was measured by a desktop Sartorius PP150 pH meter (Sartorius Corp., Edgewood, NY, USA) equipped with a rounded glass probe. pH monitoring was performed on days 0, 2, 4 and 6.

## 2.7 Titratable Acidity

Titratable acidity expressed as the percentage of lactic acid was determined by using the indicator method (942.15) of the Association of Official Analytical Chemists (2005). Three grams of FFS was combined with 50 ml of recently boiled deionized water, homogenized, and stirred gently for 1 h using a magnetic stirrer. After that, 0.5 ml of 1% phenolphthalein solution was added into the sample, and it was titrated with 0.1 N sodium hydroxide until the pink endpoint persisting for 30 s. Titratable acidity was calculated using equation 1.

$$\text{Titratable acidity (\%)} = (V \times N \times 90 \times 100)/g \times 1000 \quad (1)$$

where V is the volume (ml) of 0.1 N sodium hydroxide, N is the normality of sodium hydroxide and g is the weight of sample.

## 2.8 CIE Color Values

CIE color values (L*, a* and b*) expressing the surface color of FFS were measured using a Konica Minolta CM2600d spectrophotometer (Konica Minolta, Inc., Japan). The sample was illuminated by a D65 artificial daylight bulb and observed at $10^o$ standard angle. The L* value (0–100) indicates lightness, while a* and b* are the red/green and yellow/blue coordinates, respectively.

## 2.9 Sensory Evaluation

Sensory acceptability of raw and cooked FFS was evaluated using a 9-point hedonic scale (1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely) according to Meilgaard et al. (1999). The attributes evaluated of raw samples were meat homogeneity, color, odor, hand-feel texture and overall acceptability. Those of cooked samples evaluated were meat homogeneity, color, odor, mouth-feel texture, flavor and overall acceptability. Prior to sensory evaluation of cooked FFS, the sausage was baked in an oven at 200 $^o$C for 15 min until the internal temperature reached 70 $^o$C according to the method 976.16 (35.1.04) of the Association of Official Analytical Chemists (2005). Sensory attributes of raw and cooked samples were scored separately by two different groups of untrained panelists. Raw samples were scored by 45 panelists, 23 females and 22 males aged 19–45 years. Cooked samples were determined by 45 panelists, 24 females and 21 males aged 19–47 years. All panelists were acquainted with FFS and not allergic to it. Prior to each evaluation, the samples were randomly assigned a three-digit number. Then, they were randomly presented to the panelists. All sensory evaluations were conducted in sensory booths located inside an air-conditioned room at 25 $^o$C. The booths had the illuminance levels of 503-512 lx on the sensory tables. A score of 5 was considered the cut-off limit of acceptability for all sensory attributes.

## 2.10 Statistical Analysis

The experimental design was a 6 × 4 split plot arrangement in a randomized complete block design (RCBD). The main plot was the six ratios of TTM:tilapia fillet meat (100:0, 80:20, 60:40, 40:60, 20:80 and 0:100). The sub plot was the four fermentation periods (0, 2, 4 and 6 days). Three different lots of TTM used for the preparation of FFS were considered the blocks of this experiment. The quality parameter and sensory acceptability data of FFS were evaluated using analysis of variance (ANOVA) as described by Montgomery (2017). Statistical analysis was conducted using the IBM SPSS Statistics 21 program (IBM, Armonk, NY, USA) at a 95% confidence level. Mean values were compared using the least significant difference (LSD) test.

## 3 Results and Discussion

Statistical analysis showed that interactions between TTM:tilapia fillet meat ratio and fermentation period were not significant for all quality parameters ($p > 0.05$). Therefore, the effect of TTM:tilapia fillet meat ratio and that of fermentation period are reported separately.

### 3.1 Microbial Contents

FFS with a high ratio of TTM tended to have high LAB loads (Figure 2a). Samples made with ratios of 100:0, 80:20, 60:40 and 40:60 had significantly higher LAB counts compared to those with a ratio of 0:100 ($p < 0.05$). The microbial analyses show that TTM contain more LAB ($4.34 \pm 0.13 \times 10^2$ CFU/g) compared to ground tilapia fillet meat ($1.81 \pm 0.21 \times 10^2$ CFU/g). LAB in fermented sausage originate from raw materials added into the sausage and/or environment in contact with the sausage (Belleggia et al., 2022; Sallan et al., 2023). Multiple steps used during the trimming process at the factory may allow LAB to contaminate or proliferate in TTM thus containing more LAB than tilapia fillets exposed to the short meat extraction and grinding processes. Therefore, using TTM (100:0, 80:20, 60:40 and 40:60) produced higher LAB in FFS than using no TTM (0:100).

The LAB count in FFS was affected by the fermentation period (Table 1). LAB on day 0 was equivalent to $3.71 \pm 0.55$ log CFU/g. This level was close to that reported by Kongkiattikajorn (2015) who found LAB of around 4 log CFU/g in FFS, but the bacteria can propagate rapidly during fermentation. The results reveal that, on day 2, LAB rapidly increased to the maximum level of $8.65 \pm 0.38$ log CFU/g. Based on the level of LAB, ripening of FFS may occur on day 2. Maximum LAB counts in the ranges of 7-9 were found in Thai FFS on days 1-3 (Kongkiattikajorn, 2015; Nooniam, 2010; Suteebut et al., 2017). However, in Chinese FFS, LAB reached 11 log CFU/g on day 2 (Nie et al., 2014). After day 2, LAB counts in FFS tended to decline slowly. Although LAB population showed a declining trend, LAB on day 4 at the level of $8.47 \pm 0.38$ log CFU/g was not significantly different from day 2 ($p > 0.05$) (Table 1). Notable reduction of LAB was found on day 6 in which the count of $8.36 \pm 0.27$ log CFU/g was significantly lower than day 2 ($p < 0.05$) (Table 1). However, the results show that LAB counts on days 4 and 6 were not significantly different ($p > 0.05$) (Table 1). The decrease in experimental LAB after day 2 may be caused by a pH reduction progressing during FFS fermentation (Table 2). pH levels of lower than 4.5 found on days 2-6 may not be suitable for LAB species in FFS. de De Macedo et al. (2012) stated that acidity is considered the most important deleterious factor that affects the viability and growth of LAB, since its growth is greatly inhibited at pH lower than 4.5.

TPC was affected by the TTM:tilapia fillet meat ratio (Figure 2b). The ratios of 100:0, 80:20, 60:40, 40:60 and 20:80 were not significantly different in terms of TPC ($p > 0.05$). However, 0 :100 samples contained a significantly lower TPC compared to those with other ratios ($p < 0.05$). TTM contained a higher load of TPC ($7.32 \pm 0.02 \times 10^4$ CFU/g) compared to ground tilapia fillet meat ($6.16 \pm 0.06 \times 10^3$ CFU/g) as previously described. TPC levels in FFS were affected by the fermentation period (Table 1). A dramatic increase in TPC to the level of $8.91 \pm 0.34$ log CFU/g was found on day 2 ($p < 0.05$). This number is higher than the TPC of 6 log CFU/g found in FFS on day 2 reported by Suteebut et al. (2017). After day 2, TPC in FFS decreased significantly ($p < 0.05$). Reduction of TPC was also reported by Kongkiattikajorn (2015). The researcher found that TPC in FFS increased to the maximum level of around 8 log CFU/g on day 3. After that, the bacteria decreased gradually to the level of 7 log CFU on day 5. The reduction of experimental TPC after day 2 may be caused by pH reduction as previously described.

YM was unaffected by the TTM:tilapia fillet meat ratio ($p > 0.05$) (Figure 2c). However, it was affected by the fermentation period ($p < 0.05$) (Table 1). YM increased as the fermentation period increased and reached the level of $8.28 \pm 0.46$ log CFU/g on day 6. Nie et al. (2014) reported that YM in Chinese FFS increases during fermentation. YM may favor the low pH condition occurring in FFS on days 2–6 (Table 2), thus proliferating in the product. YM in Thai fermented fish products are usually acid-tolerant

Table 1: Lactic acid bacteria, total plate count and yeast and mold in fermented fish sausage as affected by fermentation period.

| Microbial content (log CFU/g) | Fermentation period (days) 0 | 2 | 4 | 6 |
|---|---|---|---|---|
| Lactic acid bacteria | 3.71 ± 0.55a | 8.65 ± 0.38c | 8.47 ± 0.38ab | 8.36 ± 0.27b |
| Total plate count | 5.03 ± 0.44a | 8.91 ± 0.34c | 8.59 ± 0.26 b | 8.46 ± 0.35b |
| Yeast and mold | 3.02 ± 0.47a | 6.32 ± 0.47b | 7.83 ± 0.52c | 8.28 ± 0.46d |

Results are mean ± standard deviation (n = 18). Different letters in the same row within each parameter indicate significant differences at a confidence level of 95%.

Table 2: Texture profile analysis, CIE color values, pH and titratable acidity of fermented fish sausage as affected by fermentation period.

| Parameters | | Fermentation period (days) 0 | 2 | 4 | 6 |
|---|---|---|---|---|---|
| Texture profile analysis | Hardness (g) | 377.84 ± 59.22d | 1758.32 ± 81.96a | 1417.87 ± 95.45b | 1242.38 ± 47.76c |
| | Adhesiveness (g.s) | -4.12 ± 1.49d | -10.43 ± 1.93c | -21.46 ± 2.28a | -15.72 ± 1.99b |
| | Springiness | 0.23 ± 0.03c | 0.53 ± 0.02a | 0.52 ± 0.01ab | 0.50 ± 0.06b |
| | Cohesiveness | 0.21 ± 0.03c | 0.35 ± 0.01a | 0.30 ± 0.02b | 0.29 ± 0.07b |
| | Chewiness (g) | 19.41 ± 2.65d | 413.45 ± 32.60a | 325.79 ± 26.32b | 269.86 ± 26.69c |
| | Resilience | 0.06 ± 0.01c | 0.10 ± 0.02a | 0.08 ± 0.01b | 0.07 ± 0.02b |
| pH | | 6.36 ± 0.06a | 4.39 ± 0.13b | 4.18 ± 0.05c | 4.01 ± 0.12d |
| Titratable acidity (%) | | 1.23 ± 0.32d | 2.87 ± 0.8c | 4.33 ± 0.53b | 5.32 ± 0.31a |
| CIE color value | L* | 70.2 ± 2.9a | 73.83 ± 2.34b | 74.08 ± 2.1b | 74.62 ± 1.55b |
| | a* | -1.09 ± 0.18d | -0.49 ± 0.13c | 0.12 ± 0.13b | 0.61 ± 0.15a |
| | b* | 12.95 ± 1.11c | 13.04 ± 0.83bc | 14.01 ± 1.73ab | 14.36 ± 1.73a |

Results are mean ± standard deviation (n = 18). Different letters in the same row within each parameter indicate significant differences at a confidence level of 95%.

and therefore proliferate in acidic condition. Nutrients such as vitamins and amino acids generated by microorganisms during fermentation processes may also assist the proliferation of YM.

## 3.2 Texture Profile Analysis

Hardness, springiness, cohesiveness, chewiness and resilience decreased as the ratio of TTM increased ($p < 0.05$) (Figure 3a, c, d, e and f). However, adhesiveness increased as the ratio of TTM increased ($p < 0.05$) (Figure 3b). Strydom et al. (2015) stated that texture forming is one of functional properties of muscle protein. The more TTM used, the poorer were the TPA values observed. TTM may have poor meat functional properties. These poor properties may be caused by the action of muscular proteases breaking down the muscle during filleting and trimming processes at the factory. In addition to that, TTM exposed to multiple washing steps may absorb some water. This water may increase gradually as the ratio of TTM in FFS increased. The increasing amount of water may cause softer texture in FFS when TTM was used at the high ratios. Hardness is a key indicator reflecting the maturation and textural quality of FFS (Nooniam, 2010). The results indicate that the combination of TTM and tilapia fillet meat should be limited to 20:80, which gives hardness not signif-

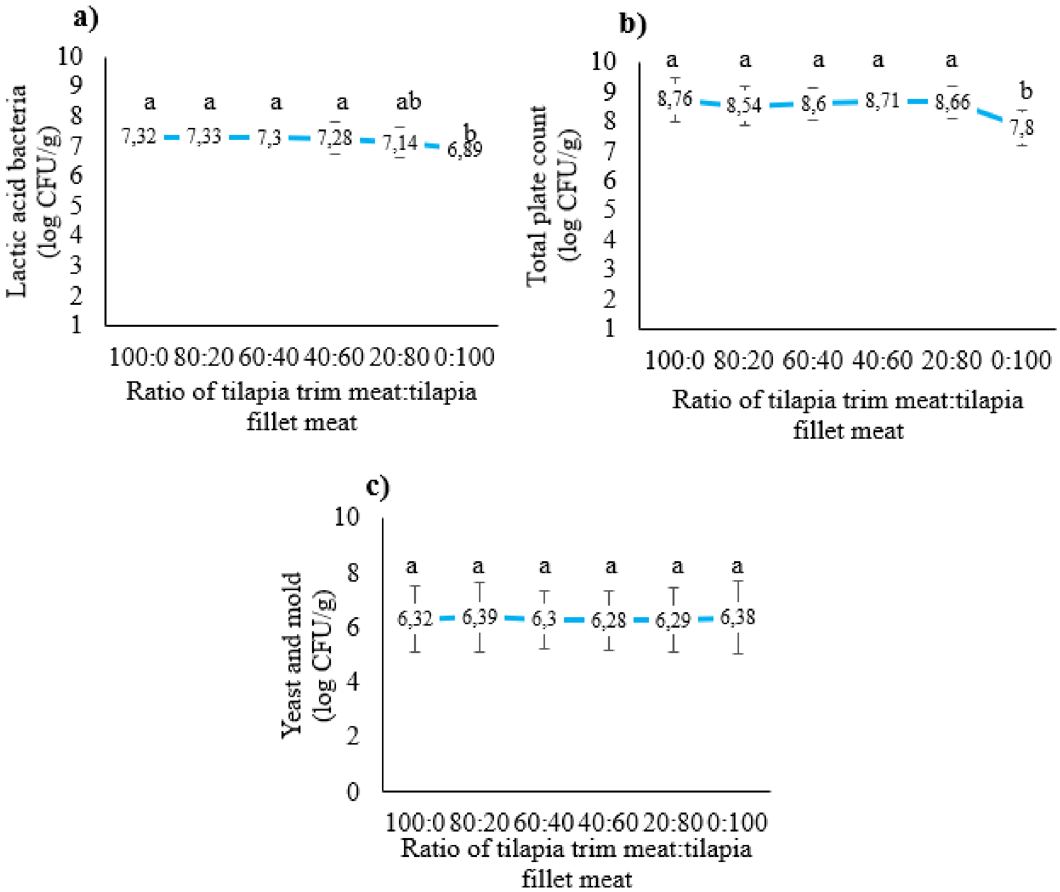


Figure 2: Lactic acid bacteria (a), total plate count (b) and yeast and mold (c) in fermented fish sausage as affected by the ratio of tilapia trim meat to tilapia fillet meat.

Different letters above the line within each parameter indicate values that are significantly different at a confidence level of 95% (n = 12).

icantly different from that of 0:100 ($p > 0.05$) (Figure 2a).

TPA attributes were affected by the fermentation period ($p < 0.05$). The results in Table 2 show that hardness, springiness, cohesiveness, chewiness and resilience increased significantly on day 2 ($p < 0.05$). After that, the values declined on days 4–6. Adhesiveness decreased significantly on days 2–4 ($p < 0.05$) (Table 2) with the lowest point reached on day 4 and increased significantly thereafter to the level of -15.72 ± 1.99 g.s. Organic acids produced by LAB, leading to a pH reduction on day 2 (Table 2), may cause protein denaturation in FFS. This denaturation may reduce the water holding capacity of fish muscle and consequently cause loss of water from the muscle. The water lost from the muscle can form an exudate commonly found in ripened FFS. This loss may further increase internal bonding in the gel network of muscle protein, thus producing a firmer and denser texture on day 2. Afifah et al. (2023) found that the hardness of matured fermented mackerel sausage increased on day 2. Nooniam (2010) found that acids produced by LAB during fermentation of FFS may induce the gradual aggregation of pro-

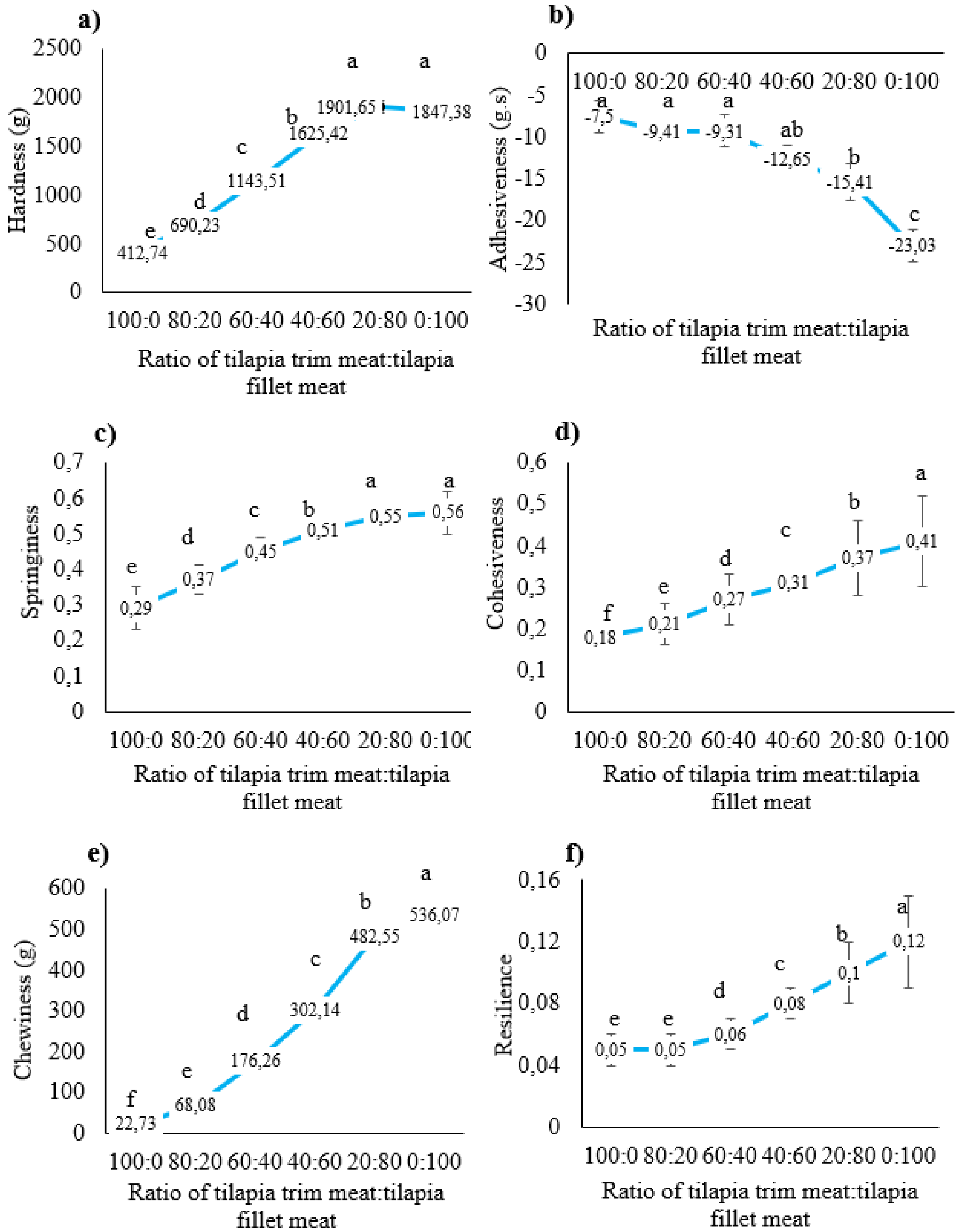


Figure 3: Hardness (a), adhesiveness (b), springiness (c), cohesiveness (d), chewiness (e) and resilience (f) of fermented fish sausage as affected by the ratio of tilapia trim meat to tilapia fillet meat.

Different letters above the line within each parameter indicate values that are significantly different at a confidence level of 95% (n = 12).

teins, leading to the formation of a protein matrix which increases hardness in the sausage.
Decreasing trends in hardness, springiness, cohesiveness, chewiness and resilience in FFS were found after day 2 (Table 2). However, an increasing trend in adhesiveness was found after day 4 (Table 2). The decline in texture attributes may be caused by the action of proteases indigenous in fish meat and those produced by LAB. Lougovois and Kyrana (2005) stated that cathepsin D primarily responsible for hydrolysis of fish meat can work effectively at a pH of around 4. The reduction in pH progressing during FFS fermentation to the level of 4.01 ± 0.12 on day 6 may assist the work of cathepsin D, thus rapidly breaking down meat structures and consequently reducing TPA values after day 2.

## 3.3 pH

The pH of FFS samples (4.68–4.80) was not affected by the TTM:tilapia fillet meat ratio ($p > 0.05$) (Figure 4a). However, it was affected by the fermentation period ($p < 0.05$). The results show that pH of FFS decreased as the fermentation period increased (Table 2). Nooniam (2010) and Belleggia et al. (2022) explained that LAB converting the starch in rice into organic acids is responsible for the pH reduction in FFS during fermentation. The initial pH of FFS was equivalent to 6.36 ± 0.06. This pH was in the normal range of 6.0–6.5 found in fresh fish as reported by Adrah and Tahergorabi (2021). On day 2, the pH of FFS decreased to the level of 4.39 ± 0.13. The reduction of pH after fermentation agrees with Nie et al. (2014) who found that the pH of FFS decreased to around 4.6 within 2 days of fermentation. The Ministry of Industry, Ministry of Industry (2012) requires the pH of FFS to be 4.6 or lower. The results reveal that a 2-day fermentation is sufficient to make FFS which meets the standard.

## 3.4 Titratable Acidity

Titratable acidity is a volumetric method using a standard solution of sodium hydroxide to react with organic acids present in food samples (Nielsen, 2017). The titratable acidity of FFS was not affected by the TTM:tilapia fillet meat ratio ($p > 0.05$) (Figure 4b). However, it was affected by the fermentation period ($p < 0.05$) (Table 2). Titratable acidity increased as the fermentation period increased. Organic acids converted from sugar and the starch in rice by LAB may increase the titratable acidity of FFS during fermentation. An increase in titratable acidity during fermentation of FFS was reported by Nooniam (2010). Riebroy et al. (2004) reported that the acid content is a very important factor determining the acceptability of FFS. The most acceptable FFS contains 2.2–2.5% lactic acid. The experimental result shows that the titratable acidity of FFS of 2.87 ± 0.8% found on day 2 was close to this range. It is concluded that 2-day fermentation may be sufficient to ripen the sausage and may produce an acceptable FFS.

## 3.5 CIE Color Values

L* values of FFS were affected by the TTM:tilapia fillet meat ratio ($p < 0.05$) (Figure 5a). A darker color (low L*values) was detected in samples containing a higher ratio of TTM. TTM may contain a high amount of dark color meat, especially the meat trimmed from the edges of the fillets close to the fins (Figure 1). In addition to that, the skin commonly found in TTM (Figure 1) could have contributed to the reduction of L* values in 80: 20 and 100:0 samples. a* values increased as the ratio of TTM increased ($p < 0.05$) (Figure 5b). Dark color meat trimmed from the edges of the fillets close to the fins in TTM as previously described may also cause the increase in a* values. b* values were not affected by the TTM:tilapia fillet meat ratio ($p > 0.05$) (Figure 6c).
CIE color values were affected by the fermentation period ($p < 0.05$) (Table 2). L*, a* and b* values increased as the fermentation period increased. A change in the muscle structures of FFS caused by protein denaturation and exudate loss which was reported by Nooniam (2010) may contribute to the increase in color values of the product.

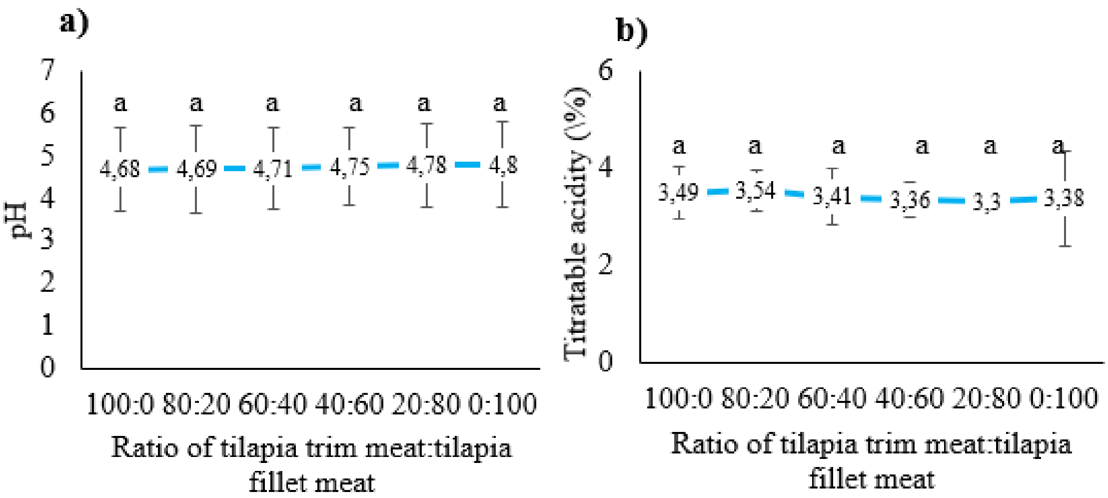


Figure 4: pH (a) and titratable acidity (b) of fermented fish sausage as affected by the ratio of tilapia trim meat to tilapia fillet meat.

Identical letters above the line within each parameter indicate values that are not significantly different at a confidence level of 95% (n = 12).

Table 3: Sensory acceptability scores of raw fermented fish sausage as affected by fermentation period.

| Sensory attribute | Fermentation period (days) | | | |
|---|---|---|---|---|
| | 0 | 2 | 4 | 6 |
| Meat homogeneity | 7.17 ± 0.31a | 7.65 ± 0.47b | 7.78 ± 0.63b | 7.55 ± 0.47b |
| Color | 7.11 ± 0.4a | 7.74 ± 0.35b | 7.82 ± 0.29b | 7.93 ± 0.24b |
| Odor | 7.09 ± 0.32a | 7.75 ± 0.34b | 7.67 ± 0.39b | 7.11 ± 0.43a |
| Hand-feel texture | 7.09 ± 0.46a | 7.67 ± 0.69b | 7.57 ± 0.69b | 7.15 ± 0.81a |
| Overall acceptability | 7.12 ± 0.38a | 7.78 ± 0.50b | 7.66 ± 0.55b | 7.26 ± 0.50a |

Results are mean ± standard deviation (n = 18). Different letters in the same row within each parameter indicate significant differences at a confidence level of 95%.

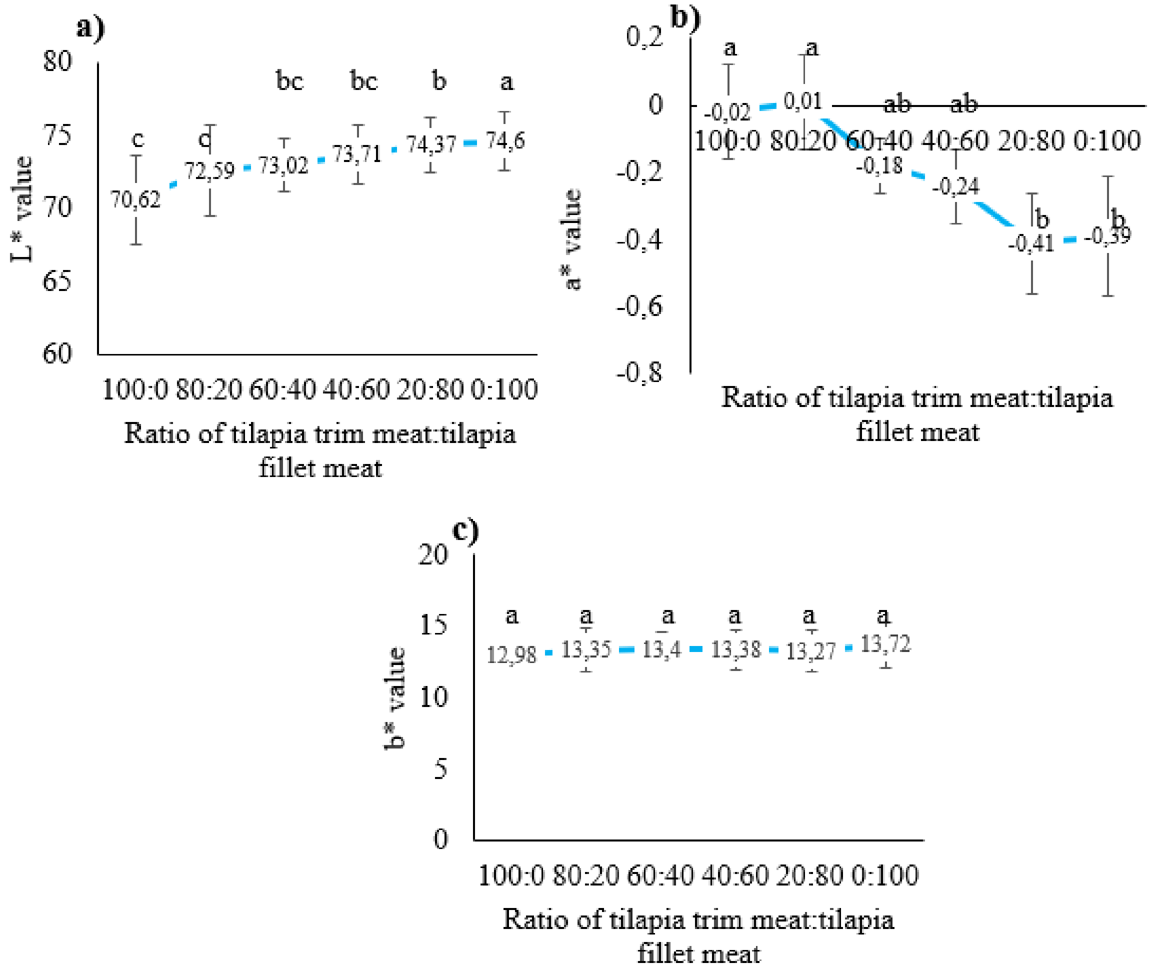


Figure 5: L* (a), a* (b) and b* (c) values of fermented fish sausage as affected by the ratio of tilapia trim meat to tilapia fillet meat.

Different letters above the line within each parameter indicate values that are significantly different at a confidence level of 95% (n = 12).

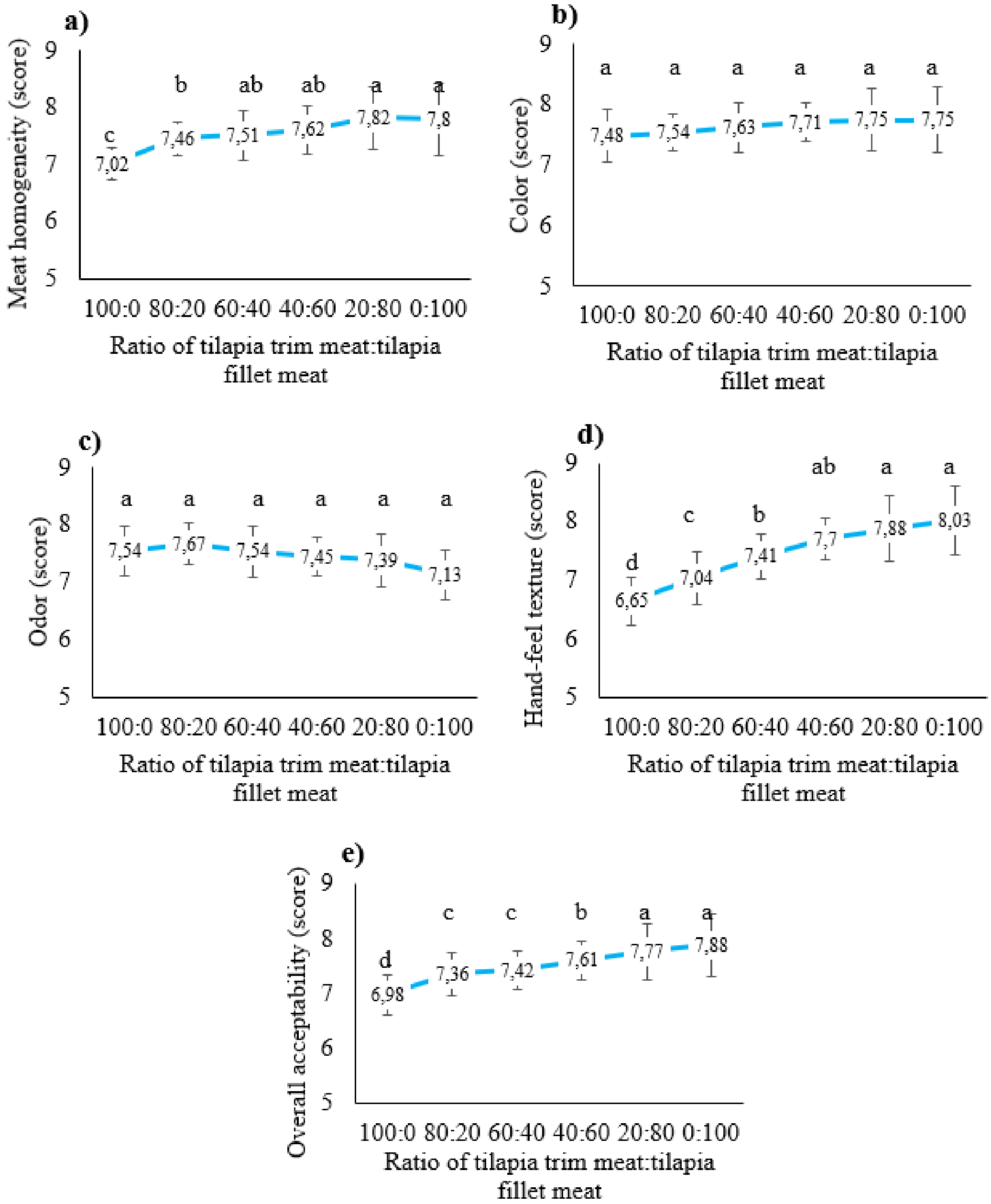


Figure 6: Meat homogeneity (a), color (b), odor (c), hand-feel texture (d) and overall acceptability (e) of raw fermented fish sausage as affected by the ratio of tilapia trim meat to tilapia fillet meat.

Different letters above the line within each parameter indicate values that are significantly different at a confidence level of 95% (n = 12).

Table 4: Sensory acceptability scores of cooked fermented fish sausage as affected by fermentation period.

| Sensory attribute | Fermentation period (days) | | | |
|---|---|---|---|---|
| | 0 | 2 | 4 | 6 |
| Meat homogeneity | 7.62 ± 0.54a | 7.6 ± 0.39a | 7.5 ± 0.47a | 7.38 ± 0.51a |
| Color | 7.64 ± 0.55a | 7.69 ± 0.32a | 7.8 ± 0.34a | 7.56 ± 0.31a |
| Odor | 7.37 ± 0.36a | 7.82 ± 0.30b | 7.9 ± 0.37b | 7.45 ± 0.38a |
| Mouth-feel texture | 7.58 ± 0.63a | 7.65 ± 0.54a | 7.64 ± 0.50a | 7.06 ± 0.60b |
| Flavor | 6.76 ± 0.40b | 7.74 ± 0.36c | 7.79 ± 0.34c | 5.37 ± 0.55a |
| Overall acceptability | 7.26 ± 0.5b | 7.77 ± 0.39c | 7.71 ± 0.47c | 6.16 ± 0.59a |

Results are mean ± standard deviation (n = 18). Different letters in the same row within each parameter indicate significant differences at a confidence level of 95%.

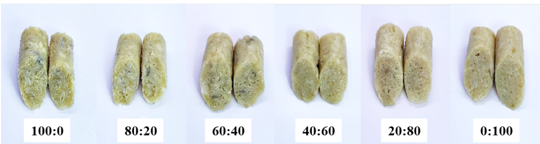


Figure 7: Appearance of fermented sausage as affected by ratios of tilapia trim meat and tilapia fillet meat

### 3.6 Sensory Evaluation

The TTM:tilapia fillet meat ratio affected meat homogeneity, hand-feel texture and overall acceptability of raw FFS ($p < 0.05$) (Figure 6a, d and e). However, the ratio did not affect the color and odor of the product ($p > 0.05$) (Figure 6b and c). The results indicate that using a high ratio of TTM (100:0 and 80:20) does not benefit the homogeneity of FFS (Figure 6a). Panelists' records show that samples with a high TTM ratio contained a significant amount of thread-like fibrous tissue and skin strips causing a non-homogenous appearance (Figure 7). Hand-feel texture scores decreased as the ratio of TTM increased (Figure 6d). Poor texture functional properties of TTM as previously described may have caused the poorer hand-feel texture found in 100:0, 80:20 and 60:40 samples. Higher overall acceptability scores were found in samples containing a low TTM ratio (Figure 6e); 20:80 and 0:100 samples were given significantly higher overall acceptability scores than 100:0, 80:20, 60:40, and 40:60 samples ($p < 0.05$). Based on this information, a 20:80 ratio might be employed for the production of FFS because it produced results not significantly different to those for 0:100. The sensory scores of raw FFS were affected by the fermentation period ($p < 0.05$). Meat homogeneity scores increased significantly during days 2–6 ($p < 0.05$) (Table 3). Denaturation of protein and loss of water from protein structure in FFS may increase internal bonding in the gel network of muscle protein, thus producing a more homogenous appearance on days 2–6. Color scores also increased significantly on days 2–6 ($p < 0.05$) (Table 3). Panelists' records show that a desirable opaque off-white color found on days 2–6 can promote the color scores of FFS. The increase in color scores correlates with the L* values which improved significantly on days 2–6. Odor, hand-feel texture and overall acceptability scores also increased significantly on days 2–4 ($p < 0.05$) (Table 3). However, the scores decreased significantly on day 6. Panelists' records show that an unpleasant smell was found on day 6, leading to the reduction of odor score. Zhao et al. (2021) found that odorants in fermented tilapia sausage are 30 aldehydes, 13 alcohols, 13 hydrocarbons, 9 ketones, 5 furans, 3 sulphur compounds, 3 aromatic compounds, 3 esters, 1 nitrogenous compound and 1 organic acid. Most odorants are produced by actions of microorganisms, mainly LAB, and chemical reactions. Aldehydes are generally produced by lipid oxidation and mostly have a pleasant odor description. Alcohols generally result from the oxidation and degradation

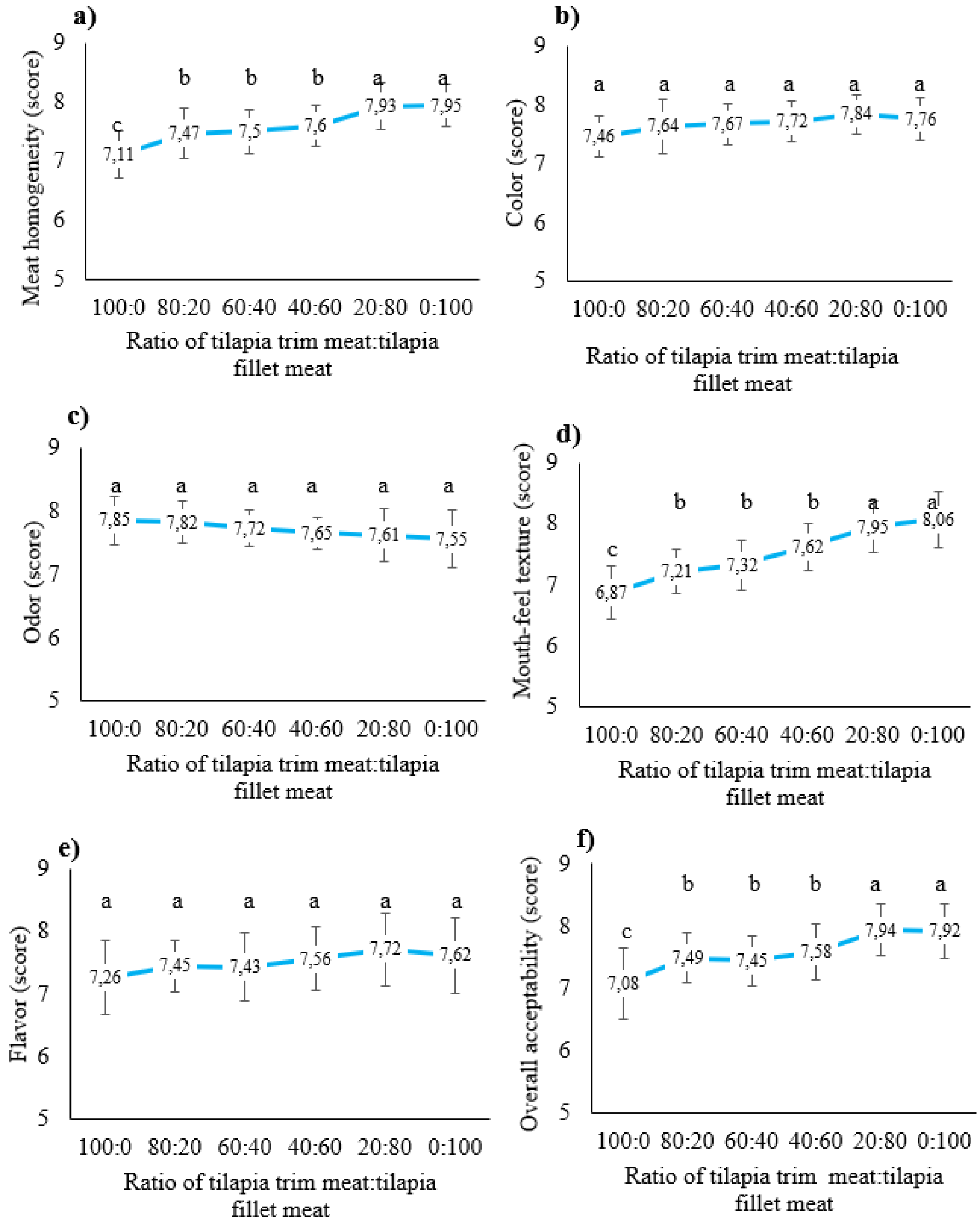


Figure 8: Meat homogeneity (a), color (b), odor (c), mouth-feel texture (d), flavor (e) and overall acceptability (f) of cooked fermented fish sausage as affected by the ratio of tilapia trim meat to tilapia fillet meat.

Different letters above the line within each parameter indicate values that are significantly different at a confidence level of 95% (n = 12).

of polyunsaturated fatty acids. Gao et al. (2019) found that several species of yeast can produce alcohol during fermentation of fish sauce, a fermented fish product. YM in FFS may comprise yeasts capable of producing alcohols from sugar and rice added to the sausage. These yeasts may contribute to an alcohol odor in the product, especially at the end of the fermentation period in which the YM level was as high as 8.28 ± 0.46 log CFU/g (Table 1). Zhao et al. (2021) stated that ketones were mainly produced from amino acid degradation or unsaturated fatty acid oxidation. Furans are generated by Strecker degradation, Maillard reaction and thermal degradation of thiamine. Esters are generally formed through non-enzymatic esterification of alcohols and organic acids as well as the enzymatic catalysis by microorganisms. Hydrocarbons generally have a strong pungent odor and usually give rise to a poor flavor in fermented tilapia sausage (Zhao et al., 2021). A high concentration of hydrocarbons may also accumulate at the end of the fermentation period, thus causing an unpleasant smell on day 6. Aromatic and sulphur compounds are generally formed through the catabolism of aromatic and sulphur-containing amino acids, respectively. A nitrogenous compound, trimethylamine, and acetic acid were detected after 30-hour fermentation (Zhao et al., 2021). Trimethylamine has a pungent fishy smell (Li et al., 2023). Acetic acid gives a pungent smell, associated with vinegar (Zhou et al., 2017). Accumulation of trimethylamine and acetic acid in FFS may also contribute to the unpleasantness of FFS on day 6.

Hand-feel texture score increased significantly on days 2-4 and decreased thereafter ($p < 0.05$) (Table 2). Panelists' records show that a firmer texture which developed on days 2–4 could produce a desirable hand-feel texture. After that, the score was lower on day 6 due to a perceived mushy texture. The reduction of hand-feel texture score agrees with TPA values which were lower on day 6. Proteolytic enzymes originating from fish meat and those produced by microorganisms during fermentation may break down FFS, causing a reduction of the sensory texture score on day 6. Overall acceptability gained a significant increase on days 2–4. After that, the score decreased significantly on day 6 ($p < 0.05$) (Table 2). The combination of unpleasant smell and mushy texture found on day 6 may have contributed to the low overall acceptability score.

Sensory scores of cooked FFS relate to the eating quality of the product. Meat homogeneity, mouth-feel texture and overall acceptability were affected by the TTM:tilapia fillet meat ratio ($p < 0.05$) (Figure 8). However, color, odor and flavor were not affected by the ratio ($p > 0.05$). Meat homogeneity, mouth-feel texture score and overall acceptability decreased as the ratio of TTM increased (Figure 8a, d and f); 0:100 samples containing only tilapia fillet meat produced the highest score. However, scores for these samples were not significantly different from those for 20:80 samples ($p > 0.05$). The results suggest that it is possible to combine TTM with tilapia fillet meat and use it for production of FFS. The ratio of 20:80, giving sensory scores not significantly different to those for 0:100 samples, is the most suitable.

Odor, mouth-feel texture, flavor and overall acceptability of cooked FFS were affected by the fermentation period ($p < 0.05$) (Table 4). However, meat homogeneity and color scores were not affected by the period ($p > 0.05$). Odor scores improved significantly on days 2–4 and deteriorated significantly on day 6 ($p < 0.05$) (Table 4). The results imply that FFS may ripen on day 2. However, the good odor in ripened FFS may last only 2 days from day 2 to day 4. After that, an unpleasant odor of overripe FFS was exhibited on day 6. This unpleasant odor may be caused by the accumulation of hydrocarbons, trimethylamine and acetic acid as previously described. The results also show that the mouth-feel texture score reduced on day 6. Proteolytic enzymes breaking down muscle structures as previously described may cause an undesirable soft texture which reduced the mouth-feel texture score in cooked FFS at the end of the fermentation period. Flavor and overall acceptability scores increased significantly on days 2–4 ($p < 0.05$). On day 6, overripe characteristics such as a very sour taste and pungent unpleasant odors were perceived during chewing of the product. These characteristics reduced the flavor score to 5.37 ± 0.55, which is close to the cut-off score of 5 points. The results indicate that FFS is suitable for consumption on days 2–4 according to overall acceptability scores of raw and cooked FFS. To extend the shelf life of FFS, the use of low temperature for storing ripened FFS may be required. The low temperature may suppress the growth of LAB and activity of proteolytic enzymes in FFS, thus delaying quality changes in the product after day 2. We found that storing ripened FFS at 4 $^{o}$C in a refrigerator could extend shelf life of the sausage from 6 days to 14 days (unpublished data). We, therefore, recommend that FFS should be fermented for 2 days. After that, the product should be kept in a refrigerator.

## 4 Conclusions

The ratio of TTM to tilapia fillet meat had no effect on YM, b* value, pH and titratable acidity. The 0:100 ratio produced lower LAB, TPC and L* values compared to 100:0, 80:20 and 60:40. The ratio also yielded a higher a* value than 100:0 and 80:20. The 0:100 ratio generated the best TPA values followed by 20:80. However, sensory acceptability of these two ratios were not significantly different. Conclusively, the 20:80 ratio, giving similar sensory quality to 0:100, is recommend for the production of FFS.

The highest LAB, TPC, hardness, springiness, cohesiveness, chewiness and resilience values were found on day 2. After that, the values declined. Adhesiveness reduced to the lowest value on day 4. After that, the value increased on day 6. YM, titratable acidity and color values (L*, a* and b*) increased as the fermentation period increased. However, pH decreased as the fermentation period increased. Most of the sensory qualities of raw and cooked FFS improved on days 2-4 and declined on day 6. The results reveal that FFS may ripen on day 2. The best quality of FFS was found on day 4.

Using TTM in combination with tilapia fillet meat is possible and recommended to the tilapia filleting industry in Thailand and other locations where TTM is available and FFS is popular. This research provides an example of the utilization of an edible fish meat by-product which may inspire researchers in other countries to explore the uses of local underutilized by-product meat. Future studies focusing on using transglutaminase to increase textural quality of FFS containing TTM may be explored.

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