Life Science 15 (1) (2026)

Life Science

https://journal.unnes.ac.id/journals/UnnesJLifeSci

Effect of Retort Sterilization Temperature and Time on the Quality of Vacuum Packed Soft-Boned Milkfish

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Article Info

Submitted : 17 June 2025
Revised : 26 May 2026
Accepted : 31 May 2026

Keywords:
Soft-boned Milkfish;
Retort Sterilization;
Total Protein Content;
Hedonic Quality

Abstract

Soft-boned milkfish is a popular processed product of the milkfish (Chanos chanos), but it has a limited shelf life. Retort sterilization technology with vacuum retort pouch packaging can extend shelf life, but it is necessary to study the right combination of temperature and time to maintain product quality. This study aims to analyze the effect of a combination of temperature (110 °C, 121 °C, and 130 °C) and sterilization time (10, 20, and 30 min) on Total Plate Count (TPC), total protein content, and hedonic quality of the product. The parameters analyzed in this study were TPC with two replications (duplicates), total protein content measured by the Biuret method, and hedonic quality assessed through the hedonic test. The results showed that all treatments produced TPC below the SNI 7388:2009 limit of 5 × 10^5 CFU/g. The 121 °C treatment for 30 min maintained a total protein content of 26.17% and TPC value of 1.00 × 10^2 CFU/g, while the 130 °C treatment for 10 min produced a total protein content of 20.85% and TPC value of 1.00 × 10^0 CFU/g, and produced the most favorable hedonic quality. Therefore, 130 °C for 10 min is recommended as the optimal sterilization temperature and time, because it is able to produce products that are microbiologically safe and preferred by panelists. Moisture content plays a role in influencing TPC value, total protein content, and hedonic quality. It is recommended to research the effect of temperature and sterilization time of soft-boned milkfish retort on the water content of soft-boned milkfish.

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p-ISSN 2252-6277
e-ISSN 2528-5009

INTRODUCTION

Indonesia, as an archipelagic country with vast territorial waters, has enormous potential for marine and fisheries resources and can be used as a driving force for the national economy (Mubarok, 2023). One of the brackish fishery commodities that has high economic value and is widely favored by the public is milkfish (*Chanos chanos*) (Mukhlis et al., 2020). Indonesia is one of the countries that produces the most milkfish in the world, followed by the Philippines (Dharma et al., 2020; FAO, 2012), with a total production of 779,705.9 tons in 2022 (Kementerian Kelautan dan Perikanan Republik Indonesia, 2025b). In Central Java, Semarang City is a coastal area with huge potential for brackish water aquaculture. One of the main commodities cultivated in this region is milkfish (Kusuma & Anwar, 2017; Mubarok, 2023), with the total milkfish production in Semarang City in 2022 at 45.62 tons. (Kementerian Kelautan dan Perikanan Republik Indonesia, 2025a).

The high production of milkfish can be a business opportunity for the community, but if not processed properly, the fish is easily spoiled. One of the most famous preparations is presto milkfish. (Sulandjari et al., 2022). Processed milkfish is very popular with the public because milkfish has a delicious and savory taste. In addition, the price is affordable for everyone (Fitri *et al*., 2016). Milkfish have a protein content of 20-24% or 20 grams per 100 grams (Akbar et al., 2021; Hafiludin, 2015).

One of the main constraints in the production of presto milkfish is the short shelf life. Common methods such as vacuum packaging and freezer storage can only maintain quality for 2 days at room temperature and 2 months at cold temperatures (freezer) (Muflihati et al., 2020). As a solution, retort technology can be applied to extend the shelf life of Rogan josh/beef curry retort pouch products up to 1 year at room temperature, 28 ± 2 °C (Shah et al., 2017), in contrast to Sunyoto et al. (2024), who revealed that retort can extend the shelf life of soft milkfish products in retort pouch packaging up to 1 year. This method not only ensures microbiological safety but also helps maintain the quality and nutritional content of the product (Yang et al., 2022), This method not only ensures microbiological safety but also helps maintain the quality and nutritional content of the product (Yang et al., 2022), with heating standards generally 121°C for 15 min (Paramesti, 2022).

Retort sterilization has been applied by SMEs in Semarang, such as the milkfish SME in Tembalang Subdistrict. The production process is carried out in two stages: presto cooking to soften the spines, followed by retort sterilization after the product is vacuum-packed in retort pouches. This process results in a softened milkfish product that is safe for consumption, durable, and preferred by consumers. High-temperature sterilization is effective in reducing the number of microorganisms, but may reduce quality parameters, such as protein content and organoleptic quality (Abraha et al., 2018; Patmawati et al., 2022). Therefore, temperature and time optimization is essential to maintain product quality. Vacuum packaging can extend shelf life by creating conditions with minimal oxygen, thereby suppressing microbial growth (Purnamayati et al., 2018; Triyannanto et al., 2019). Retort pouches are an efficient packaging option because they absorb heat faster than cans, reducing sterilization time and energy consumption (Majumdar et al., 2015). The principle is similar to canning, but more practical and economical in maintaining product sterility (Ningrum et al., 2019).

The effect of sterilization temperature and time on food quality can be evaluated by conducting microbiological tests in the form of Total Plate Count (TPC), proximate tests in the form of total protein content tests, and hedonic tests. Therefore, based on the description above, it is necessary to research the effect of temperature and sterilization time of soft retort milkfish on microbial contamination, total protein content, and hedonic quality.

METHODS

This research is an experimental study using a factorial Completely Randomized Design (CRD) with 2 factors of temperature treatment (110 °C, 121 °C, and 130 °C) and sterilization time (10 min, 20 min, and 30 min), each with 2 replications. Materials used were milkfish, KH2PO4, and Plate Count Agar (PCA). The tools used in this research are a retort machine (Engineering with top height 60 cm, bottom height 15 cm, and diameter 55 cm), an incubator (Capp Incubator), smasher (Biomerieux), counter plate (Interscience scan 300), analytical balance (Ohaus), a magnetic hotplate stirrer (BioCote UC152D), and an autoclave (Hirayama HV HVE 50 L).

Presto and Sterilization of Milkfish

The seasoned milkfish is then restored at 115°C for 3.5 hours. After that, the milkfish is packaged using a retort pouch and vacuumed with a vacuum sealer. The milkfish is then sterilized using a retort at 110°C, 121°C, and 130°C for 10, 20, and 30 min. After the sterilization process was complete, the milkfish was stored in a container box for 14 days at room temperature (31-32 °C).

Total Plate Count Test (TPC)

The TPC test was carried out by the spread plate method using Plate Count Agar (PCA) media. A total of 11 g of homogeneous samples that have been mixed with 99 ml of KH_2PO_4 solution are crushed using a smasher, then inoculated with as much as 0.1 ml into two petri dishes (duplo) and leveled with a spreader. The dishes were incubated at 35 ± 0.5 °C for 48 hours in an inverted position (Salfinger, 2015). The number of colonies was counted using a digital colony counter. The results of the calculation were compared with the microbial contamination standard based on SNI 7388:2009, with a maximum TPC limit of 5×10^5 CFU/g with the food category of fish and fishery products, including molluscs, crustaceans, and echinoderms steamed or boiled and or fried (Badan Standarisasi Nasional, 2009). Then, data processing of colony count results was carried out by laboratory staff. TPC value data were analyzed using the Kruskal-Wallis test at a significance value of <0.05.

Total Protein Contents Test

Total protein content testing was conducted at the Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Negeri Semarang. The method used to test the total protein content is the biuret method. The biuret method works on the principle of forming a purple complex due to the interaction between two or more peptide bonds in the protein molecule with Cu salts in an alkaline solution (Purnama et al., 2019). Data on total protein content were analyzed using the Kruskal-Wallis test at a significance value of <0.05.

Hedonic Test

Analysis of color, appearance, texture, aroma, and taste was carried out sensorially using the scoring test method, which was determined based on the hedonic test (Arziyah *et al.*, 2022). A hedonic test assessment was conducted by 25 untrained panelists, namely university students. The assessment was done using the hedonic test form. Hedonic quality data were analyzed using the Kruskal-Wallis test at a significance value of <0.05. Furthermore, a further test was conducted using the Mann-Whitney test.

Table 1
Hedonic test rating scale

No.	Hedonic scale	Numerical scale
1.	Very like	5
2.	Like	4
3.	Ordinary	3
4.	Dislike	2
5.	Strongly dislike	1

Source: (Arziyah et al., 2022)

RESULT AND DISCUSSION

Total Plate Count (TPC)

The effect of temperature and time variations during the retort sterilization process on soft duri milkfish on TPC, referring to the Indonesian National Standard (SNI) 7388: 2009 concerning Maximum Limits of Microbial Contamination in Food, is described in Table 2.

Table 2
TPC calculation results of soft milkfish based on the combination of sterilization temperature and time

No.	Sample Code	Mean Total Plate Count CFU/g)	Maximum Limit of Microbial Contamination (SNI 7388:2009)
1.	AA	2,18 x 10^5	5 x 10^5
2.	A1	4,50 x 10^1	
3.	A3	4,50 x 10^1	
4.	A5	1,00 x 10^0	
5.	B1	7,25 x 10^1	
6.	B3	1,00 x 10^2	
7.	B5	1,00 x 10^0	
8.	C1	1,50 x 10^2	
9.	C3	1,00 x 10^2	
10.	C5	4,50 x 10^1	

Description: AA (control, presto at 115 °C for 3.5 hours), A1 (110 °C, 10 min), A3 (121 °C, 10 min), A5 (130 °C, 10 min), B1 (110 °C, 20 min), B3 (121 °C, 20 min), B5 (130 °C, 20 min), C1 (110 °C, 30 min), C3 (121 °C, 30 min), C5 (130 °C, 30 min).

TPC testing aims to determine the effectiveness of sterilization treatment in reducing the number of bacteria. Data in Table 2 shows that the average TPC value of the sterilized soft milkfish with various temperature and time combination treatments is in the range of 1.0 x 10^0 to 1.50 x 10^2 CFU/g, which is very much reduced compared to the control TPC results, which reached 2.18 x 10^5 CFU/g. In addition, all samples after the retort sterilization process have TPC values that are far below the maximum limit of microbial contamination set, which is 5 x 10^5 CFU/g, so the retort sterilization treatment applied is generally effective in reducing the number of bacteria in the sample.

The results of the TPC calculation of soft milkfish were statistically analyzed using the Kruskal- Wallis test. The Kruskal-Wallis test results gave a significance of p-value = 0.033, which indicates that p-value <0.05. Based on the results of the Kruskal-Wallis test, the combination of temperature and sterilization time influences the TPC value of soft-boned milkfish.

Retort sterilization treatment with different combinations of temperature and time provided variations in TPC reduction. Sterilization for 10 min and 30 min with a combination of temperatures of 110 °C, 121 °C, and 130 °C significantly reduced the TPC value because the sterilization process can kill microorganisms down to their spores (Hendrawati & Utomo, 2017). It is known that the higher the sterilization temperature, the lower the TPC value. Meanwhile, sterilization for 20 min at 110 °C and 121 °C increased the TPC value from 7.25 x 10^1 CFU/g to 1.00 x 10^2 CFU/g. According to Wahyuni *et al*. (2021), the increase in TPC value is caused by several factors, namely an increase in the number of spoilage bacteria due to pH and environmental conditions during storage. In addition, the availability of oxygen in food can also cause microbial growth in food. The improper packaging process will cause the entry of bacteria into the air (Danarsi & Noer, 2016). Microbial growth in food products can cause physical and chemical damage, making them unfit for consumption (Suci et al., 2024). Some of the factors that influence the growth rate and activity of bacteria in foodstuffs include pH, temperature, humidity, and oxygen (Fitria & Zulaika, 2019).

Total Protein Content

The total protein content of soft-boned milkfish processed using different sterilization temperatures and times is shown in Figure 1.

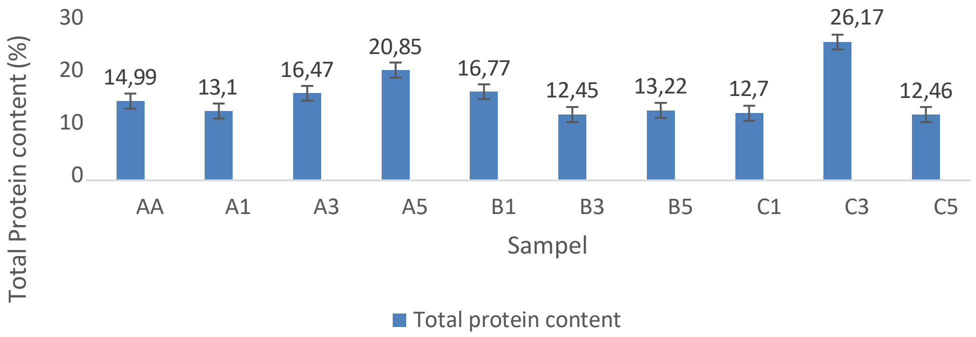

Figure 1
The results of measuring total protein content

One of the most important indicators to measure the texture and nutritional quality of fish is protein (Yu et al., 2022). The data in Figure 1 shows that the control soft-boned milkfish has a total protein content of 14.99%. Soft-boned milkfish with sterilization treatment at 121 °C for 30 min can maintain a total protein content of 26.17%, while sterilization at the same temperature for 20 min shows the lowest total protein content, which is 12.45%. The combined treatment of temperature and sterilization time gave varying results in total protein content. Sterilization for 10 min at 110 °C resulted in a total protein content of 13.10%, while the total protein content increased at 121 °C (16.47%) and 130 °C (20.85%).

The data obtained from the study showed that the total protein content of soft-boned milkfish was different in each treatment. This shows that there is an influence between the combination of temperature and sterilization time on the total protein content of soft-boned milkfish. Since the data were not normally distributed based on the normality test, the measurement data of total protein content was analyzed using the Kruskal-Wallis statistical test with a significance value of p-value <0.05 and obtained a significance value of 0.047, which means p-value <0.05, indicating variation in protein content between groups. Post hoc analysis did not reveal significant differences between treatment pairs. The protein content exhibited a non-linear pattern, where sterilization for 20 minutes tended to result in lower values, while the 10-minute treatment showed relatively higher values, and the 30-minute treatment showed fluctuating values. Based on the results of the Kruskal-Wallis test, the treatment of a combination of temperature and sterilization time has an influence on the total protein content of soft-boned milkfish.

Since the data were not normally distributed based on the normality test, the total protein content was analyzed using the Kruskal–Wallis test. The results showed a significant difference among treatments (p = 0.047), indicating variation in protein content between groups. Post hoc analysis did not reveal significant differences between treatment pairs. The protein content exhibited a non-linear pattern, where sterilization for 20 min tended to result in lower values, while the 10 min treatment showed relatively higher values, and the 30 min treatment showed fluctuating values.

This variation may be associated with the effects of thermal processing on protein structure and stability. Sundari *et al.* (2015) stated that the heating process using high temperatures causes protein denaturation and degradation or reduces the nutritional value of protein. This is supported by the statement of Irawati et al. (2016) that total protein contents decrease due to the heating process and cause protein hydrolysis. Hydrolyzed proteins become more soluble and may be carried into the heating medium during the retort sterilization process, which can contribute to changes in measured total protein content (Ciptawati et al., 2021). In addition, heating at high temperatures can cause evaporation of water from milkfish. The higher the temperature used, the more water molecules come out of the surface and become gas (Sundari et al., 2015). Although the water content in this study was not measured directly, such changes may affect the concentration of measured protein (Wulandari et al., 2021). The increase in measured protein content at higher temperatures may be associated with moisture loss during sterilization, resulting in a higher relative concentration of protein.

Hedonic Quality

The results of the hedonic test measurement of soft-boned milkfish after sterilization with the treatment of temperature and time variations are given in Table 3.

Table 3
Hedonic test results of soft-boned milkfish

Sample	Hedonic Test Mean Value				
	Color	Appearance	Textures	Flavor	Scent
AA	3.64[ab]	3.60[ac]	3.80[a]	3.72[a]	3.48[a]
A1	3.60[abc]	3.96[ab]	3.60[a]	3.72[a]	3.64[a]
A3	3.68[ab]	3.72[abcd]	3.68[a]	3.56[a]	3.40[a]
A5	4.00[a]	**4.12[b]**	3.88[a]	3.96[a]	3.88[a]
B1	3.88[a]	3.80[abc]	3.80[a]	3.76[a]	3.84[a]
B3	3.76[ab]	3.92[ab]	**4.00[a]**	**4.20[a]**	**3.96[a]**
B5	3.28[bc]	3.36[acd]	3.56[a]	3.56[a]	3.52[a]
C1	3.16[c]	3.20[ad]	3.88[a]	3.88[a]	3.76[a]
C3	**4.04[a]**	3.88[ab]	3.76[a]	3.72[a]	3.92[a]
C5	3.76[ab]	3.80[abc]	3.52[a]	3.36[a]	3.60[a]

Description:

a. *AA (control, presto at 115 °C for 3.5 hours), A1 (110 °C, 10 min), A3 (121 °C, 10 min), A5 (130 °C, 10 min), B1 (110 °C, 20 min), B3 (121 °C, 20 min), B5 (130 °C, 20 min), C1 (110 °C, 30 min), C3 (121 °C, 30 min), C5 (130 °C, 30 min),*

b. *Different letter notations indicate significant differences between treatments at a 5% confidence level.*

The data in Table 3 show that the treatment of sterilization temperature and time has an influence on the level of panelists' liking for soft milkfish. Sterilization treatment at 121 °C for 30 min resulted in the highest hedonic mean score for the color parameter, which was 4.04. Meanwhile, the highest score on the appearance parameter was obtained from the 130 °C temperature treatment for 10 min, with a score of 4.12. The highest hedonic mean scores for texture (4.00), flavor (4.20), and aroma (3.96) parameters were obtained at 121 °C for 20 min.

The results of a hedonic test on soft-boned milkfish were analyzed by Kruskal-Wallis non-parametric analysis. The results of the analysis showed that the treatment of temperature and sterilization time had a significant effect p-value <0.05 on the color and appearance of soft-boned milkfish but had no significant effect p-value >0.05 on texture, taste, and aroma. Then the Mann-Whitney test was conducted at the 5% level for color and appearance parameters. Based on the results of the Mann-Whitney test, and showed that there was a significant effect between treatments.

The color of foodstuffs plays an important role in determining their acceptability as well as the quality of the food product itself (Jannah et al., 2018). Sterilization at high temperatures and longer processing times can affect physicochemical properties, including changes in color that become darker in food products (Ariyana et al., 2023; Maherawati et al., 2022). Discoloration of soft-boned milkfish may be caused by fat oxidation and protein denaturation, which can lead to the formation of dark brown complexes contributing to browning in the product (Nguyen et al., 2023).

The first factor that customers assess when buying something is its appearance. Factors that affect appearance are the shape and color of the product produced (Derwin et al., 2022). High-temperature heating will cause evaporation of water from the fish meat. High water content on the outer surface of the meat can cause the fish to look pale and less attractive appearance, resulting in a less favorable appearance to the panelists (Riandi et al., 2023). Otherwise, sterilization at high temperatures and for a long time can make the milkfish spines soft, because the collagen in the fish spines becomes soluble, which eventually turns into gelatin. This can improve the appearance of the product (Susilo et al., 2014).

In general, the sterilization process can affect the texture characteristics of fish by breaking down proteins into simpler molecules, resulting in a softer texture (Ako et al., 2016). Heating at high temperatures can also cause damage to the fish's tissue structure, resulting in a less firm texture (Azhari et al., 2023). These conditions can affect the texture characteristics of soft-boned milkfish products. High microbial growth can cause the ability to break down materials into simple compounds to increase, and the density of fish samples to decrease (Jannah et al., 2018).

In addition to texture, the sterilization process can also affect the flavor of soft-boned milkfish. Flavor is one of the key factors in the acceptance of food products (Kuncoro et al., 2019). Changes in flavor can be influenced by the texture and consistency of the ingredients, as changes in texture can affect the stimulation of olfactory receptor cells and salivary glands (Arziyah et al., 2022). Additionally, hydrolysis, oxidation, and enzymatic activity during processing can also cause changes in the product's flavor (Jannah et al., 2018).

Food aroma is caused by volatile compounds produced through enzymatic and non-enzymatic reactions (Arziyah et al., 2022). Changes in aroma may occur due to lipid oxidation during heating, thereby affecting the product's aroma (Ako et al., 2016; Riandi et al., 2023). Additionally, protein degradation in the fish flesh can produce volatile compounds, such as ammonia (NH3) and H2S, which cause changes in the product's aroma (Dotulong & Montolalu, 2018).

Most of the panelists mentioned that the aroma, taste, appearance, and color of soft-boned milkfish were almost the same. This depends on the sensitivity of each individual in assessing the product, so the results are subjective (Triandini & Wangiyana, 2022). Overall, panelists preferred the soft-boned milkfish with 130 °C temperature treatment for 10 min, due to the less fish-like odor, flavor, and texture. This is supported by observations at one of the soft-boned milkfish SMEs in the Tembalang District, which indicate that customers prefer the taste of milkfish processed with a retort sterilization process compared to presto milkfish.

CONCLUSION

The combination of sterilization temperature and time significantly reduced the number of microorganisms in soft-boned milkfish, with all treatments showing TPC values below the SNI 7388:2009 limit (5×10^5 CFU/g). The 121 °C treatment for 30 min maintained the highest total protein content of 26.17%, whereas the 130 °C treatment for 10 min yielded a total protein content of 20.85%. However, the difference in total protein content between treatments was not significant. The 130 °C sterilization treatment for 10 min produced a hedonic quality that was preferred by panelists and gave a TPC value of 1.00×10^0 CFU/g. Therefore, the combination of 130°C for 10 min is recommended because it can produce products with the best microbiologically safe, preferred sensorially, nutritionally acceptable, shorter processing time. hedonic quality, good nutritional value, and microbiological safety.

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