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Optimizing the sterilization process of canned yellowfin tuna through time and temperature combination

S H Anwar[1*], R W Hifdha[1], H Hasan[2], S Rohaya[1], Martunis[1]

[1]Agricultural Product Technology Department, Universityof Syiah Kuala, Banda Aceh 23111, Indonesia
[2]Electrical Engineering Department, Universityof Syiah Kuala, Banda Aceh 23111, Indonesia

[*]Corresponding author's e-mail: sri.haryani@unsyiah.ac.id

Abstract. Differences in can dimension, filled volume and type of sterilization equipment affect the heat penetration rate, temperature and time required for sterilization process in canned food. Thus, careful calculation and decision for those factors are critical to determine the final product quality. Therefore, this research aimed to investigate the quality of canned yellowfin tuna in a specific can dimension using a 24 L pressure canner by applying different time and temperature combination i.e., sterilization at 121°C for 20 minutes and at 115°C for 50 minutes. Two type of mediums used were palm oil and brine. Heat penetration study and calculation of sterilization time (Fo value) were conducted using thermocouple data logger for both methods. The results showed that the protein content decreased slightly from 24.6% (fresh tuna) to 23,57% (canned tuna sterilized at 121°C for 20 min) and to 13,31% for canned tuna sterilized at 115°C for 50 min. There was a sharp increment obtained for fat content of canned tuna in palm oil (0.54% to 10.6%). Heat penetration study indicated that the Fo for canned tuna in brine were longer (30.13 minutes) than tuna in oil (20.09 minutes). This research suggests that sterilization of canned tuna is recommended to be done at 121°C for 20 minutes with regards to the nutritional content of the final product, particularly the protein content.

1. Introduction

Tuna is a fishery commodity with the highest export potential and Indonesia has become the largest tuna exporting country in South East Asia. In 2011, the number of tuna exports were 142 tons which was equivalent to 5 trillion rupiah. Aceh Province is one of the largest fish producers in Indonesia and a great prospective type of fish in Aceh is Tuna (*Thunnus sp*.).

The term "tuna" in the world of Indonesian fisheries includes various types of fish, especially the group of *Thunnus sp*., (i.e. yellowfin, bigeye, SBT, and albacore) and tuna-like species such as marlin, sailfish and swordfish. The skipjack tuna is usually classified into a group of "cakalang" fish. The term "cob or *tongkol* in Bahasa Indonesia" is for small tuna, namely the eastern little tuna (*Euthynus sp*.), The frigate and bullet tunas (*Auxis sp*.), and long-tailed tuna (*Thunnus tonggol*) [1].

This research was designed based on the fact that there was no food industry In Aceh Province which processed fresh fish into canned fish such as canned tuna industry. However, unprocessed fish industries such as tuna fillet or whole tuna for export purpose are already present in Banda Aceh. Most of fish caught are shipped to North Sumatera Province or many countries worldwide. Canned tuna is one way to preserve and store tuna in cans which are hermetically sealed and sterilized so that the tuna will not turn spoiled physically and chemically, or contaminated biologically. In addition to extend the shelf life, canning also aims to increase the value added of tuna compared to fresh tuna before canning. Canned food must be commercially sterile which means sterile from pathogenic and microorganisms that could spoil food and if the microorganisms survive, the microbes will not grow and damage the canned food under storage conditions at room temperature.

The in-container technology begins with the process of selecting quality fresh tuna. The tuna is cleaned from gills, bones, fins and skin after the pre-cooking process. Tuna meat (fillets) are then filled in cans, packaged hermetically under vacuum condition after exhausting and sterilized [2]. The purpose of sterilization canned food is to kill as many as possible the pathogenic microorganisms that are harmful to health such as *Clostridium botulinum,* as well as heat-resistant microorganisms such as *Bacillus stearothermophilus (Geobacillus stearothermophilus), Bacillus sporothermodurans* and *C thermosaccharolyticum* which can cause spoilage in food products.

The main problem that often occurs in the process of food canning is safety and quality of food. The nutritional value of foods with high protein content such as fish (pH > 4.5) can be reduced after canning [3]. Changes in nutritional value after canning can be caused by improper temperature and time combination during sterilization. Sterilization using high temperature (± 121 ° C) for a long time in the canning process can cause protein denaturation[4]. In addition, canned fish can be contaminated with microbial heat-producing toxins. Other microbiological hazards are contamination by histamine (scromboid toxins) and algal toxins which are also heat-resistant [5].

The combination of temperature and time used greatly affects the final quality of canned tuna. A proper design of time and temperature combination for sterilization process is needed so that the product can meet the criteria for food safety and reducing quality as well as nutritional value that might occur can be minimized. In addition, the type of medium used can also affect the heat penetration process and the quality of canned fish produced. The guidelines regarding combination of temperature and time for canned tuna sterilization in literatures cannot directly be applied because of differences in several important factors such as type of tuna, can dimensions, can volumes, tuna weight, medium volume, sterilization equipment and the method used.

The medium added in the canning process serves as a conductor of heat, as a preservative and increases the degree of acidity. The medium also serves to give taste to the final product. [6] have examined the effect of added media to heat penetration in canned tuna. The media used was brine, tomato sauce, curry and groundnut oil. They concluded that the heat penetration is strongly influenced by the type of media used in the canning process. The fastest heat penetration is found in tuna with a medium of salt solution (brine) then followed by curry, tomato sauce and oil [6].

Researches with regard to canned fish have been done intensively however the existing literatures could not be applied directly as general guidelines if important parameters are different. These critical parameters including the can dimension, the amount of filling, the final volume of product, types of medium and types of sterilization equipment. All of these factors will determine sterilization value (Fo), the overall processing time and the profile of heat transfer in the product.

Therefore, this study aims to investigate the effect of temperature and time combination for the sterilization process of canned tuna. Different types of medium were also studied and both variables were examined toward the quality of canned tuna. The product quality was determined by chemical analysis for nutritional values. The nutritional changes were also compared between canned, pre-cooked and fresh tuna.

2. Materials and methods

Every step by step of canning in this research (handling, pre-cooking, exhausting, sterilization, cooling and ripening processes) followed the procedures written in the FAO Documents [7] with slight modification. The adjustments were done to fit the can dimension, the maximum filled volume, and the capacity of pressure canner (24 L only).

2.1. Handling and preparation of fresh tuna
The 48 kg yellowfin tuna (*Thunnus albacares*) was purchased from the Ocean Fishery Port Lampulo Baru, Banda Aceh. One of the medium, palm oil, and salt were bought from local supermarket in Banda Aceh. All chemicals for analysis were of analytical grade. Double distilled water was used in this study.

After careful visual examination for the freshness, the whole tuna was doused with running water and immediately cleaned of the head, gills, fins, bones, and skin. The tuna meat (fillet) was then cut into sizes of 1-1.5 kg and directly placed in a clean plastic bag without washing with water. The fresh tuna fillet was then stored in a freezer (-20°C) until the canning process began.

2.2. Pre-cooking, exhausting, and can-seaming processes
The frozen fillet was thawed until the ice melted and washed with running water. The fillet was cut into pieces then steamed (pre-cooked) at ±80 °C for 15 minutes. The pre-cooked tuna was cooled at room temperature. Tuna meat was cleaned from black meat and fine spines and further cut into chunks and flakes (± 2 cm). The chunks and flakes (± 80 g) were arranged carefully in each can. Beforehand, the cans were first washed using warm soapy water and rinse thoroughly. The mediums (80 ml each), i.e., palm oil and 5% salt solution (brine), were added into the cans that had been filled with fish meat. The medium was previously preheated at ± 70 °C. The total weight of tuna and the medium was ±160 g in each can.

Furthermore, the cans were exhausted. Exhausting was done by placing cans in a hot water bath (steamer). The time and temperature used for exhausting was ± 80°C for 10 minutes. This process aimed to remove as much oxygen as possible in the cans and create a vacuum condition inside the cans. Seaming process was done using semitro can seamer.

2.3. *Sterilization, cooling and ripening processes*
The canned tuna was sterilized using a pressure canner (All American 925, 24 L capacity). Combination of time and temperature were ± 121 °C for 20 minutes and ±115°C for 50 minutes. Upon sterilization completion, the cans were unloaded and cooled by soaking in the water bath. The cooling process must be done carefully and as quickly as possible.

Cooling was needed to reduce over cooking or processing which cause over cooked tuna meat and to prevent the re-contamination process after sterilization [7]. In addition, cooling also had an impact by giving stresses on bacteria that were still survive. Finally, the ripening evaluation was carried out to determine the perfection of the canning and sterilization processes. Curing was done by placing the cans in the upside-down position at room temperature, and monitored the cans' leak regularly. Ripening was done for at least 5-7 days.

2.4. Thermal recording process for the Fo calculation
For the measurement of thermal penetration in canned tuna, two temperatures were measured during sterilization, i.e., the pressure canner (retort) interior temperature and the core of the product temperature (inside a canned tuna). The measurements were conducted using Ellab thermocouples gland with built-in logger (TSP Micro, Ellab, Denmark).

To measure the retort temperature, a thermocouple was placed right above the water level in the lower part of the retort. For the core product temperature, measurement was done by placing a thermocouple inside a can before seaming. This thermocouple was positioning in the middle of the can pointing upward to the geometric center and was held by stuffing tuna meat (chunks and flakes). This was to measure the temperature at the slowest heating point (SHP) of the product. The thermocouple was inserted between tuna meat before the exhausting process was began. Therefore, the product dan retort temperatures had started to be recorded by the thermocouples right before the exhausting until the sterilization finish and the cooling process had accomplished. When the cans were cold enough, the thermocouples were removed. The recorded temperatures were converted into data using a docking system (Pro Multi Reader, Ellab, Denmark) and an application software (ValSuite Pro, Ellab). The data retrieve are the Lethality data which are automatically calculated into the Fo by the software.

2.5. Analysis of fresh, pre-cooked and canned tuna
Analysis of fresh and pre-cooked tuna included: analysis of water content (method of SNI 01-2891-1992, point 5.1), ash content (SNI 01-2891-1992, point 6.1), protein content (SNI 01-2891-1992, point 7.1), fat content (AOAC method 938.06-33.6.04.2005), carbohydrate content (by difference), and Vitamin A (Retinol) (AOAC method 2001.13- 45. ı .34.2005). Canned tuna, both in palm oil and brine, where undergone similar chemical analysis including water, ash, protein, fat and carbohydrate content. In addition, analysis of pH, and Vitamin A (Retinol) were also performed.

3. Results and Discussion
3.1. The Quality of Fresh and Pre-cooked Tuna: Proximate Analysis and Vitamin A (Retinol)
The proximate analysis of fresh and pre-cooked tuna can be seen in Table 1. The moisture content in fresh tuna was 69.4%. This result was lower than the research done by [8] which measured the water content of fresh yellowfin tuna which was 72.67%. The protein content in fresh tuna was 24.6% which was slightly higher (23.33%) than that obtained by Mohan et.al. [8]. However, this value was significantly higher than the result of Xavier et.al. [6] which was only 20.86%. Variations in protein content in fish can be affected by seasonal changes. The tuna species is said to be a source of high-quality protein for humans. Tuna contains a higher amount of protein when compared to other seafood that can be consumed [9].

Comparing the proximate analysis between fresh and pre-cooked tuna, two interesting results were noticed i.e., increasing the protein and fat content. Pre-cooked tuna analysed to have 30.5% protein than 24.6% in fresh tuna. The fat content was also higher which was 1.91% compared to only 0.54% in fresh tuna. These differences might be caused by decreasing the moisture / water content during pre-cooking process. The water had evaporated due to steam cooking at 80°C for 15 minutes. Therefore, the protein and fat content were increased while the carbohydrate content was decreased, accordingly.

Table 1. The Nutritional Value of Fresh and Pre-Cooked Tuna

Parameters	Unit	Fresh Tuna	Pre-cooked Tuna
Moisture	%	69.4	66.1
Ash	%	1.32	1.34
Protein (N x 6,25)	%	24.6	30.5
Fat	%	0.54	1.91
Carbohydrate	%	4.14	0.15
Vitamin A (Retinol)	IU/100g	<0.50	n.a.

The fish that contain more than 2% of fat are grouped into high-fat fish, one of which is tuna. However, the tuna fat content in this study was only 0.54%. Some studies also reported lower than 2% of fat content in fresh tuna which were 1.79% [8] and 1.93% [10]. The differences of tuna fat content can be influenced by the fish species, the size of the fish, the water condition in the area of fishing, eating habits, environment, season, food source, activity, growth phase and type of muscle, gender and age [11].

3.2. The Quality of Canned Tuna

3.2.1. The Water Content. The average moisture content of canned tuna was 66.27%. Figure 1 shows the statistical analysis of the data. It can be seen that the lowest water content was obtained from canned tuna sterilized at 121 °C for 20 minutes using palm oil as a medium. However, the water content of other treatments (S1M1, S2M1 and S2M2) were not statistically different from one to another.

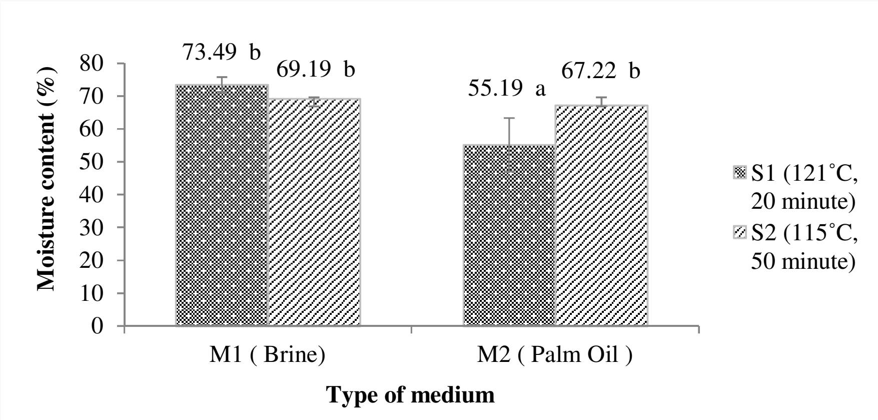

Figure 1. Effect of the sterilization conditions and type of medium on the moisture content of canned tuna

The water content in tuna generally decreases after the heating process is carried out, from 69,40% in fresh tuna (Table 1) to 66.27% (averagely) in canned tuna, except for S1M1. This might be caused by a heating process that occurs during pre-cooking, exhausting and sterilization. The heat treatment process causes fish to lose water content ranging from 5-13%. This is in accordance with the study of Mohan et.al. (2015) which underlined that during the pre-cooking process a decrease in water content was noticed of 5% and during the sterilization process was around 11-13%. Mohan et al. [8] also showed that the fresh tuna water content of 72.6% was reduced to 59-61% in the sterilization process in various oil mediums. In the pre-cooking process the water content can be reduced to 60-65%. This is caused by the release of water bound to muscle tissue. The amount of water content in tuna that is pre-cooked is related to the length of the cooking level.

The higher the temperature of sterilization the higher the decrease in water content [12]. A study also reported a decrease in water content caused by heating processes such as during frying and boiling. The greater and longer the heat given into food will result in reduced water content in sufficient quantities. The reduction is depending on the type of food, the temperature used and the length of the heating process [13].

Although most of the water content in canned tuna decreased, but at S1M1 treatment, the tuna water content was increased. However, the increments of water content among S1M1, S2M1 and S2M2 were statistically not significant. The increase in water content in canned tuna is thought to be caused by the addition of a medium in the form of a salt solution (brine), so that salt penetrates into the fish meat and absorbs the water. In general, food ingredients both before and after processing have hygroscopic properties, which have the ability to absorb water around it [14]. Salt is one of the hygroscopic substances.

3.2.2. Ash Content. Food contains organic and inorganic compounds called minerals or ash. The ash content is residual inorganic substances from the result of burning organic materials. Determination of the ash content is related to the levels of minerals contained in a food ingredient and the purity or cleanliness of a material produced. The more inorganic ingredients contain in a food product, the higher the value of the ash content. The results of ash content analysis in canned tuna ranged from 1.30% to 2.50% with an average value of 2.03%. The effect of sterilization conditions and the type of medium on the ash content of canned tuna can be seen in Figure 2.

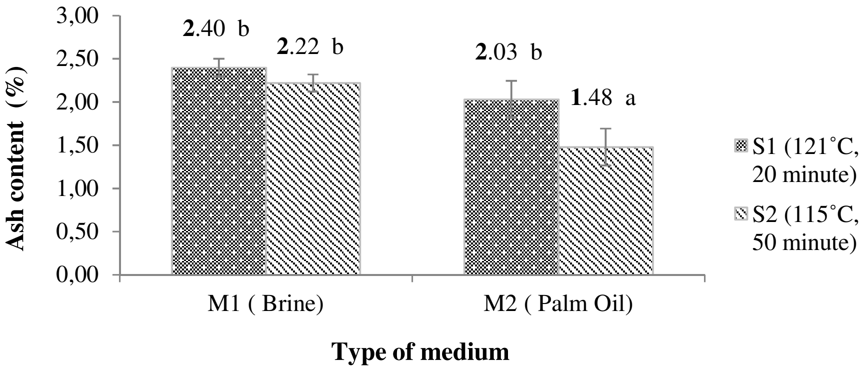

Figure 2.The effect of sterilization conditions and type of medium on the ash content of canned tuna

In this study the average ash content of canned tuna increased from the ash content of fresh tuna. This is in accordance with the study of Mohan et.al. [8] where the ash content of canned tuna (3.46 - 3.52%) has increased from fresh tuna (2.62%). It can be seen from Figure 2 that the tuna in brine had a higher amount of ash content than the tuna in palm oil. This increment can be attributed to the addition of salt as a medium in canned fish.

In a comparison study of canned fish products, the use of brine as a medium during pre-cooking by boiling the fish was evaluated with other pre-cooking method done by steaming. It is known that the fish muscle which is in contact with brine can increase the ash content in the final product. Canned fish, especially those using a salt solution as a filler media, cause an increase in the ash content resulting from the absorption of salt from brine[15].

Figure 2 also illustrated that although most of the ash content of canned tuna are higher than the fresh tuna, the ash contents of tuna in palm oil were actually lower than tuna in brine. The reducing in ash content is thought to be caused by an increase in fat content resulting from the absorption of palm oil by fish meat (chunks). This is in accordance with a study report which stating that an increase in fat levels can caused a decrease in ash content, particularly in meat [16].

3.2.3. Fat Content. The results of analysis of variance indicated that the sterilization methods did not significantly affect the fat content of canned tuna ($P \geq 0.05$). However, the type of medium has a significant effect ($P \leq 0.01$) on the fat content of canned tuna (Figure 3). As can be seen from Fig. 3, the fat content of tuna in palm oil was incredibly higher than tuna in brine.

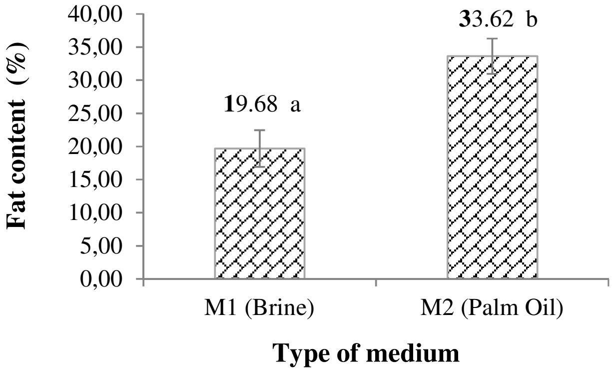

Figure 3. Effect of medium type on fat content of canned tuna

The high fat content in palm oil tuna is obviously due to the addition of palm oil as a medium during canning. Bahurmiz et.al. [15] who conducted research on nutritional and sensory characteristics of the local canned tuna in Hadhamout, Yemen also underlined similar finding. Canned tuna after adding the oil medium had a higher fat content than fresh tuna. Increased fat content can be associated with a decrease in moisture content and penetration of oil from the medium into the product (Mohan et.al. [8]).

3.2.4. Protein Content. Tuna is a fish species that contain a relatively high protein. The protein in tuna is much higher than other seafood. Results of analysis of variance showed that type of medium did not significantly affect the protein content but the sterilization conditions influenced the protein content of canned tuna, significantly ($P \leq 0.01$) (Figure 4).

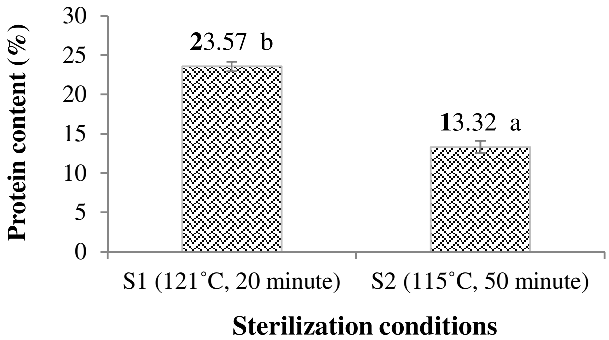

Figure 4. The effect of sterilization conditions on protein content in canned tuna

Figure 4 showed that the longer the sterilization time caused the lower protein content in canned tuna. Canning at 121°C for 20 minutes reduced the protein content only slightly (from 24,6% in fresh tuna to become 23,57%). The protein of canned tuna sterilized at 115°C for 50 minutes decreased significantly to become 13.32%. Maskur [12] summarized that the protein content of canned fish tended to decrease when the sterilization time was done longer. The decrease in protein content is thought to be caused by the occurrence of protein denaturation.

The heating process causes heat to penetrate to the fish chunks and reduce the functional properties of the protein. The resistance of protein to heat is strongly related to amino acids constituent. In canned products, the amino acids can be reduced by around 10-20%. Amino acids in processed foods can be decreased because of its sensitivity to heat. Heat treatment can cause protein to decrease with increasing temperature or the length of the processing time [2]. The loss of protein can be caused by three possible reasons, namely pre-cooking, diffusion into the liquid and heat destruction during thermal processing[17].

Another investigation related to the protein denaturation in freshwater fish (bighead carp) emphasized that the heating process can lead to reduction of myosin and actin content. In severe condition, myosin and actin might be decreased significantly or disappeared completely. After the cooking process at different temperatures, the intensity of the myosin chain bond reduces gradually and finally the fish protein changes [18]. They also stated that the bighead carp protein structure had not changed significantly during process at 90°C for 40 minutes. However, when the warm up time reached over 70 minutes, the chain link of myosin appeared thinner and tended to increase in line with the length of heating time. Increasing the heating temperature, the MHC (myosin heavy chain) bond also changed gradually. Moreover, increasing the heating time resulted severe protein degradation which lead to the damage offish muscle and fibres [18].

Reduction in protein content can also occur because of the large amount of water contained in a substance. The protein levels of skipjack fish on a wet base increased proportionally after frying process due to a decrease in water content. There is an inverse relationship between protein and moisture content. The concentration of protein in a material is measured higher when the water content is low [19].The protein content in a food is usually inversely proportional to its moisture content. Another study summarized that the protein content of canned fish increased during storage which was caused by a decrease in moisture content during thermal and storage processes [20].

3.2.5. Calculation of the Fo Value. According UN-FAO, the Fo value of a thermal process can be measured by means of physical or microbiological. In this research, the physical method was used where the temperature changes during thermal process at the slowest heating point (SHP)of the product were measured. The results are related to the thermal destruction rate at a reference temperature [21]. The F_0 value is the standard parameter used to indicate the success of a thermal treatment. The F_0 value of a thermal process in this research is determined by means of physical method which used the temperature changes during thermal process at the slowest heating point (SHP) of the product measured. The results are related to the thermal destruction rate at a reference temperature [21]. If the SHP of a product reached 121.1 °C then F_0 value was identical with time in minute.

The results of heat penetration study showed that the Fo for canned tuna in brine were longer (30.13 minutes) than tuna in oil (20.09 minutes). These mean the severity of heat penetration was higher in brine tuna than in palm oil tuna. This is in accordance with our previous findings [22]. Tuna in brine showed better heat penetration than tuna in oil because it was faster for salt solution to reach the holding temperature than the oil. As consequences, it is expected that tuna in brine will have lower number of spoilage bacterial spores (if they are still existed) compared to the cans with tuna in palm oil. In addition, these Fo values were higher than the requested standard written in FAO document. FAO requested that a botulinum cook must have an Fo value of at least 2.8 min, whereas freedom from thermophilic spoilage by *C thermosaccharolyticum* would necessitate an Fo value of at least 16 min.

Theoretically, all canners expect that all microorganisms dan their spores have been destroyed during canning. However, there is no guarantee for this to occur completely. That is why some outbreaks did happen in some countries and in Indonesia. If the spores are still existed in a small number, it is still acceptable as long as they are not able to replicate and deteriorate the canned product. Therefore, the storage test at room temperature is needed to prove if there is a potential threat, microbiologically and to make sure that the sterilization process is sufficient and successful.

4. Conclusion

The canning process done in this research is in accordance with the standards listed in the document of Food and Agriculture Organization for the United Nations (UN-FAO, 1988). The whole canning process takes approximately 3 hours starting from the process of pre-cooking, filling, exhausting, retorting and cooling. The whole sterilization process takes 2 hours. The sterilization conditions were 20 minutes at 121°C and 50 minutes at 115°C. Calculation of product lethality validates that the Fo value obtained is 30.13 minutes for tuna in brine and 20.09 minutes for tuna in oil.

Analysis of nutritional value showed a decrease in protein content of canned tuna when compared with fresh tuna. Canned tuna sterilized at 121 °C for 20 minutes had a higher protein content (on average > 22%) compared to sterilized tuna at 115 °C for 50 minutes (12-14%). Longer sterilization time can significantly reduce the protein content. There was a significant increment of fat content from 0.54% in fresh tuna to become 10.6% for canned tuna in oil, but decreased to 0.30 for canned tuna in brine. It should be underlined that canned tuna produced have meet the SNI standards for canned fish products.

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