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Effect of Temperature and Time on the Sterilization Process of Soft-boned Milkfish

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Abstract
The objectives of this research are: 1) Analyzing the effect of sterilization temperature and time on protein content in soft spiny milkfish, 2) Analyzing the effect of sterilization temperature and time on the bacterial content in soft spiny milkfish, and 3) Knowing the ideal temperature and time in the milkfish sterilization process soft spines. There are various types of diversification of processed milkfish, including processing it into presto milkfish. The shelf life of pressure-cooked milkfish can be increased by sterilization using a high-pressure pressure cooker using a retort tool. In this study, the sterilization process used a combination of temperatures of 110°C, 121°C, 130°C, and times of 10, 20, and 30 minutes. The research method uses an experimental approach. The research location is CV Sekar Tekno, Semarang. The data collection technique uses observation, which is carried out by systematically observing and recording the symptoms being investigated. Data analysis techniques use descriptive statistics and analysis of variance/ANOVA. The results of the research showed that the effect of temperature and time on the sterilization process of soft spiny milkfish did not have a significant effect on the protein content of soft spiny milkfish. Although the protein content of soft spiny milkfish experienced changes up and down during the sterilization process, the protein content did not decrease significantly. The effect of high temperature in the sterilization process of soft spiny milkfish has a significant effect on the number of bacteria present in soft spiny milkfish after the sterilization process. This is indicated by the phenomenon of a constant decrease/value of bacterial content as shown in the following data: temperature 110°C is 148.44 CFU/g, temperature 121°C is 86.78 CFU/g, and temperature 130°C is 25.44 CFU/g. On the other hand, the effect of long sterilization time on the bacterial content of soft spiny milkfish tends to show an up-and-down phenomenon. This is indicated by an increasing trend in the number of bacteria at 30 minutes, which was 125 CFU/g. This phenomenon is caused by a leak in the retort pack used for soft spiny milkfish and other causes that are not yet known for certain in this process. Based on the results of the effect of temperature and time on protein levels and bacterial content, it shows that a temperature of 130°C has a high protein content of 16.6% with the lowest bacterial content, namely 45 CFU/g. At 20 minutes, it had a high protein content of 14.296% with the lowest bacterial content, namely 32.67 CFU/g. So the ideal temperature and time for the sterilization process of soft spiny milkfish is 130°C and 20 minutes.

1 Introduction

Indonesia is a maritime country because 2/3 of its territory is the ocean [1]. In percentage terms, 70% of Indonesia's territory consists of oceans, while the remaining 30% is land [2]. Potentially, Indonesia's

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maritime sector is the largest in the world, especially in the aspect of fisheries, both capture and aquaculture. One type of fishery with various potential, opportunities, and great benefits for health is milkfish [3][4]. Milkfish is a rich source of protein (20-24%), amino acids, fatty acids, minerals, and vitamins. The highest amino acid composition is glutamate at 1.386% (freshwater) and 1.268% (brackish water). The highest unsaturated fatty acid is oleic, 31-32%. The macro minerals in milkfish meat are: Ca, Mg, Na, and K. Its micro minerals consist of Fe, Zn, Cu, and Mn [5][6]. One of the milkfish producing areas in Indonesia is Central Java, precisely Semarang City, with production reaching 1,046.77 tons per year in 2020 [7].

Diversification of milkfish processed into various kinds of products to increase the economic value of milkfish. One of them is presto milkfish. Presto milkfish contains a variety of spices that cause the product to not last long in terms of its shelf life. Until now, milkfish preservation techniques to extend shelf life have varied, ranging from heating, salting, smoking, and so on, including sterilization, which has not been widely applied. Presto-cooked milkfish will make the spines soft and safer for consumption with a high-pressure and low-temperature cooking system. This heating system causes the risk of protein denaturation, fat oxidation, and vitamin loss[8][9]. One way to determine the protein content in soft-boned milkfish or presto milkfish is by laboratory tests on proximate levels (protein).

Processed milkfish, including presto milkfish, is a processed food product that is vulnerable to microbiological bacterial contamination. This can be caused by several things, such as less hygienic sanitation, less clear water, or the unfit condition of the milkfish itself. One of the parameters to determine whether or not the milkfish is suitable for consumption based on microbiological aspects is by analyzing the Total Plate Count (ALT) laboratory test. The ALT test refers to the Indonesian National Standard (SNI) 7388:2009 concerning the Maximum Limit of Microbial Contamination in Food.

The sterilization method is a food processing process to kill bacteria, microbes, or harmful microorganisms to make it healthier, safer for consumption, and last longer [10][11]. Sterilization is carried out if the food ingredients to be sterilized have been packaged using packaging that is resistant to high temperature and pressure, such as cans or plastic. This packaging aims to ensure the process or initial stages of food hygiene and quality [12][13]. The milkfish after being packaged will go through a sterilization process, which is a preservation method while maintaining quality, and is the basis for the destruction of microorganisms by heat.

The sterilization process is carried out by heating presto milkfish at a certain temperature with a predetermined length of time to destroy pathogenic spores and other spoilage bacteria. The working process is that the retort machine will heat up and evaporate water to form hot steam [14][15]. Temperature and time play an important role in influencing the nutritional content of processed food. Related to sterilization time analysis. The sterilization process using a retort machine must be done correctly so that the processed products are as desired. The control system over temperature and time is an important concern so that the sterilization process runs optimally. Based on this, the author intends to conduct research with the title "Analysis of the Effect of Temperature and Time on the Sterilization Process of Soft-Boned Milkfish".

2 Research Methods

This research method is experimental, the most reliable scientific research (most valid), because it is carried out by strictly controlling the variables, confounding variables outside the experiment. The focus of this research is to examine the effect of temperature and time on the sterilization process of soft milkfish.

In this study, researchers involved various parties in obtaining accurate, precise, and integrated information. Independent variables are temperature and time, dependent variables are protein content and bacterial content, and control variables. Control variables are variables that are controlled or made constant so that the effect of the independent variable on the dependent variable is not influenced by external factors that are not studied [16], such as milkfish, seasonings, retort tools, and so on.

The data sources for this research are primary and secondary. Primary data can be obtained directly from subjects related to the research. Primary data is data that is collected directly by data collectors [17].

Primary data can be obtained from questionnaires conducted by researchers. Secondary data is a data source that does not directly provide data to data collectors [17]. Examples of secondary data are obtained from other people and other related documents. In addition, secondary data can be obtained from reference books, teaching modules, government reports, journal articles, and so on. [18] added that in quantitative research, the data collection technique often used is a questionnaire. Meanwhile, in supporting accurate, factual, and comprehensive data, data collection is also carried out through interviews, observation, and documentation.

The analysis technique used in this research is descriptive statistics and analysis of variance/ANOVA. [16] argues that descriptive quantitative is a statistic used to analyze data by describing the data that has been collected as it is without intending to make general conclusions or generalizations. Included in descriptive quantitative, among others, are data presentation through tables, graphs, pie charts, pictograms, calculation of mode, median, mean (measurement of central tendency), calculation of deciles, percentiles, calculation of data distribution through calculation of mean and standard deviation, calculation of percentage. In this study, tests and measurements were carried out by repetition on each test sample. Each sample will be given a code as an identity.

3 Result and Discussion

3.1 Effect of Sterilization Temperature and Time on Protein Content of Soft Spine Milkfish

Table 1. Descriptive Statistics of Temperature and Time on Protein Content

Dependent Variable: Protein_Content

Duration	Temp	Mean	Std. Deviation	N
10 minutes	110°C	13.943	1.46739	3
	121°C	16.996	.99445	3
	130°C	22.253	2.83009	3
20 minutes	110°C	15.343	2.86015	3
	121°C	12.673	.57640	3
	130°C	14.873	2.89429	3
30 minutes	110°C	12.766	.82809	3
	121°C	20.710	9.48829	3
	130°C	12.673	.49541	3

Source: Processed Primary Data (2024)

Table 2. Test of Between-Subjects Effect of Temperature and Time on Protein Content

Dependent Variable: Protein_Content

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	301.359[a]	8	37.670	2.849	**.031**
Intercept	6743.440	1	6743.440	510.008	**.000**
Duration	55.465	2	27.732	2.097	**.152**
Temperature	43.227	2	21.613	1.635	**.223**
Duration * Temp	202.668	4	50.667	3.832	**.020**
Error	238.000	18	13.222		
Total	7282.800	27			
Corrected Total	539.360	26			

a. R Squared = .559 (Adjusted R Squared = .363)

Source: Processed Primary Data (2024)

From the corrected model, it can be seen how much influence the independent variable (independent variable) has on the dependent variable (dependent variable). In this case, the independent variables are factors that will be measured by researchers (length of time, temperature, and length of time * temperature) to determine the relationship between the dependent variable (the value to be observed). From the table above, based on the value (sig), if the sig value < 0.05, namely (0.031 < 0.05), it means that the model obtained is valid. The intercept value in this case is the value of Kadar_Protein in the value variable that

contributes to the value itself without being influenced by the independent variable, meaning that the intercept value is the value of the protein level itself without being influenced by the independent variable, meaning that changing the value of the dependent variable does not have the slightest influence by the independent variable. From the table above, based on the value (sig), if the sig value < 0.05, namely (0.000 < 0.05), it means that this intercept contributes significantly.

Whether or not the length of time affects the protein content is indicated by the significant value, from the table above the sig value is 0.152 or the value (0.152 > 0.05) in this case means that the length of time does not significantly affect the protein content. Whether or not the temperature affects the protein content is indicated by the significant value, from the table above the sig value is 0.223 or the value (0.223 > 0.05) in this case means that the temperature does not significantly affect the protein content. This test aims to determine whether there is a significant relationship between 2 factors. In this case, we will test whether or not there is an interaction between the length of time and the temperature group.

if F count < F table or sig value > 0.05, then H0 is accepted
if F count > F table or sig value < 0.05, then H0 is rejected, so accept H1.

From the table above, it can be seen that the significant value obtained from the table above is 0.020. So it can be concluded that there is an interaction between the length of time and the temperature group. Because there is an interaction between the length of time and the temperature group, we need a Post Hoc further test. Here's how the 2-way ANOVA post Hoc further test (Tukey).

Table 3. Multiple Comparisons of Temperature and Time on Protein Content

Dependent Variable: Protein_Content
Tukey HSD

(I) Temp	(J) Temp	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
110 C	121 C	-2.7756	1.71414	**.263**	-7.1503	1.5992
	130 C	-2.5822	1.71414	**.311**	-6.9570	1.7925
121 C	110 C	2.7756	1.71414	**.263**	-1.5992	7.1503
	130 C	.1933	1.71414	**.993**	-4.1814	4.5681
130 C	110 C	2.5822	1.71414	**.311**	-1.7925	6.9570
	121 C	-.1933	1.71414	**.993**	-4.5681	4.1814

Based on observed means.
The error term is Mean Square (Error) = 13.222.

Source: Processed Primary Data (2024)

3.2 Effect of Sterilization Temperature and Time on Bacterial Content in Soft Spined Milkfish

Table 4. Descriptive Statistics of Temperature and Time on Bacterial Content

Dependent Variable: Bacterial_Content

Temp	Duration	Mean	Std. Deviation	N
110 C	10 menit	196.67	262.694	3
	20 menit	48.67	49.602	3
	30 menit	200.00	100.000	3
121 C	10 menit	96.67	89.489	3
	20 menit	67.00	57.158	3
	30 menit	130.00	147.224	3
130 C	10 menit	15.67	25.403	3
	20 menit	15.67	25.403	3
	30 menit	45.00	.000	3

Source: Processed Primary Data (2024)

From the Descriptive Statistics image above, we can see the description of the results of the Bacterial Content value based on temperature. For example, a time of 10 minutes obtained an average value (mean) for a temperature of 110°C of 196.67, a standard deviation of 262.694. Furthermore, a temperature of 121°C

obtained an average value of 96.67, a standard deviation of 89.489. Meanwhile, a temperature of 130°C obtained an average value of 15.67 and a standard deviation of 25.403. Likewise, for the long time of 20 minutes and 30 minutes.

Table 5. Test of Between-Subjects Effect of Temperature and Time on Bacterial Content

Dependent Variable: Bacterial_Content

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	121297.185[a]	8	15162.148	1.179	.363
Intercept	221589.481	1	221589.481	17.235	.001
Temperature	68799.185	2	34399.593	2.676	.096
Duration	31764.963	2	15882.481	1.235	.314
Temp * Duration	20733.037	4	5183.259	.403	.804
Error	231419.333	18	12856.630		
Total	574306.000	27			
Corrected Total	352716.519	26			

a. R Squared = .344 (Adjusted R Squared = .052)

Source: Processed Primary Data (2024)

From this model correction, it can be seen how much influence the independent variable (free variable) has on the dependent variable (bound variable). In this case, the independent variable is the factors that will be measured by the researcher (length of time, temperature, and length of time * temperature) to determine the relationship between the dependent variable (the value to be observed). From the table above based on the value (sig), if the sig value > 0.05, namely (0.363 < 0.05) means that the model obtained is not valid. The intercept value in this case is the Bacteria_Content value on the value variable that contributes to the value itself without being influenced by the independent variable, meaning that changing the value of the dependent variable does not affect the independent variable. From the table above based on the value (sig), if the sig value < 0.05, namely (0.001 < 0.05) means that this intercept contributes significantly.

Whether or not temperature affects Bacterial Content is indicated by a significant value, from the table above the sig value is 0.096 or the value (0.096 > 0.05) in this case means that temperature does not have a significant effect on Bacterial Content. Whether or not time affects Bacterial Content is indicated by a significant value, from the table above the sig value is 0.314 or the value (0.314 > 0.05) in this case means that time does not have a significant effect on Bacterial Content. This test aims to determine whether there is a significant relationship between 2 factors, in this case we will test whether or not there is an interaction between time and temperature groups. If F count < F table or sig value > 0.05, then H0 is accepted. If F count > F table or sig value < 0.05, then H0 is rejected, so accept H1. From the table above, it can be seen that the significant value obtained from the table above is 0.804. So it can be concluded that there is no interaction between temperature groups and time. Because there is an interaction between temperature groups and time, we do not need further Post Hoc (Tukey) tests here.

3.3 Ideal Temperature and Time in the Sterilization Process of Soft Spined Milkfish

The sterilization process is carried out in 3 (three) repetitions. This aims to determine the ideal temperature and time in the sterilization process of soft-spined milkfish, especially related to the levels of proximate tests (protein) and bacterial content (ALT). Laboratory tests to determine protein levels were carried out at the Chemistry Laboratory of Universitas Negeri Semarang. Meanwhile, laboratory tests for bacterial content (ALT) were carried out at the UPTD Health Laboratory, Semarang City Government.

Visually, in the form of a graph, the effect of temperature and sterilization time on protein levels and bacterial content in soft-spined milkfish can be described as follows:

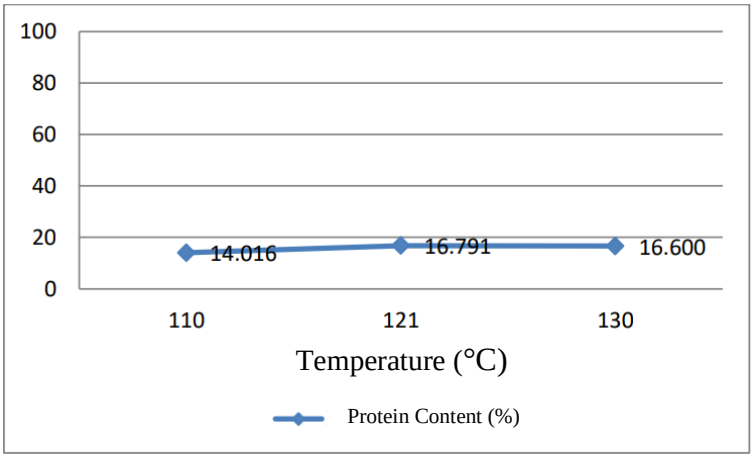

Figure 1. Effect of Temperature on Protein Content in the Sterilization Process of Soft Bone Milkfish

Source: Processed Primary Data (2024)

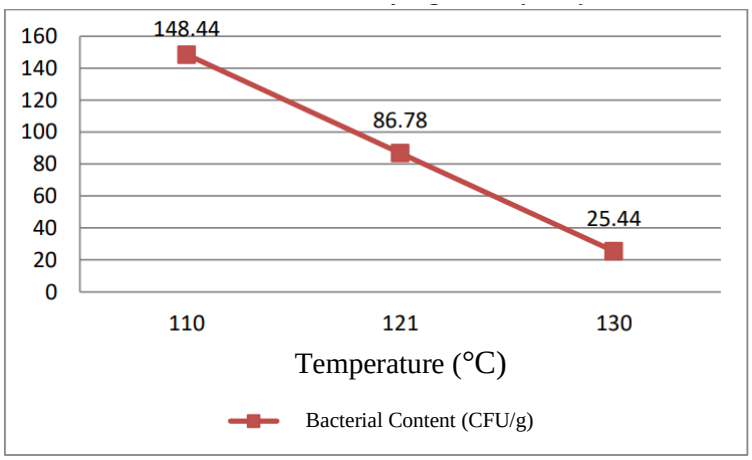

Figure 2. Effect of Temperature on Bacterial Content in the Sterilization Process of Soft Bone Milkfish

Source: Processed Primary Data (2024)

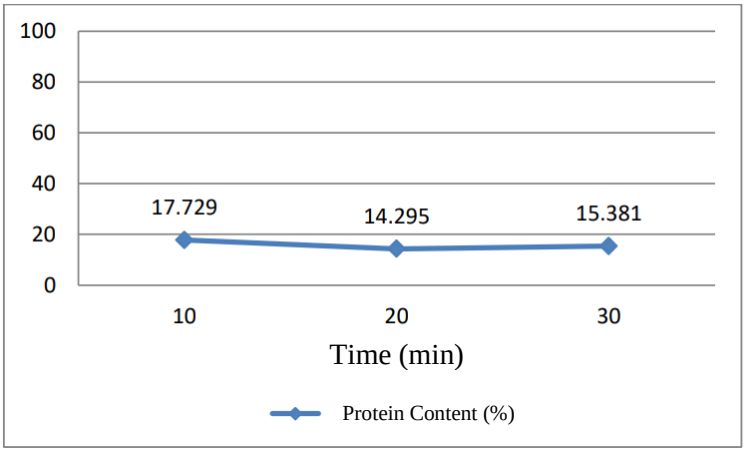

Figure 3. Effect of Time on Protein Content in the Sterilization Process of Soft Bone Milkfish

Source: Processed Primary Data (2024)

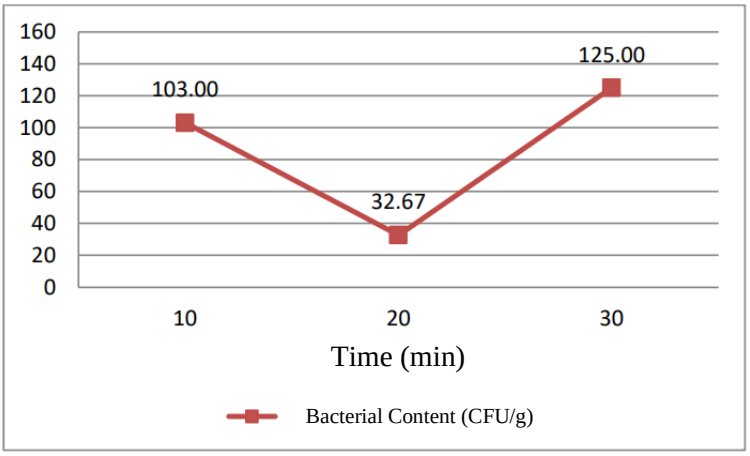

Figure 4. Effect of Time on Bacterial Content in the Sterilization Process of Soft Bone Milkfish

Source: Processed Primary Data (2024)

4 Conclusion

Based on the research conducted, the conclusions in this study are as follows:

1. The effect of temperature and time on the sterilization process of soft-boned milkfish does not significantly affect the protein content of soft-boned milkfish. Although the protein content of soft-boned milkfish experiences up and down changes during the sterilization process, the protein content does not decrease significantly.
2. The effect of high temperatures on the sterilization process of soft-boned milkfish has a significant impact on the number of bacteria in soft-boned milkfish after the sterilization process. This is indicated by the phenomenon of a decrease/value of bacterial content that decreases constantly, as in the following data: temperature 110°C of 148.44 CFU/g, temperature 121°C of 86.78 CFU/g, and temperature 130°C of 25.44 CFU/g. Conversely, the effect of the length of sterilization time on the bacterial content of soft-boned milkfish tends to show an up-and-down phenomenon. This is indicated by the increasing trend in the number of bacteria, at 30 minutes of 125 CFU/g. This phenomenon is caused by leakage of the retort pack packaging used in soft bone milkfish and other causes that are unknown in the process.
3. The effect of temperature and time on protein levels and bacterial content shows that at a temperature of 130 ° C, it has a high protein content of 16.6% with the lowest bacterial content of 45 CFU/g. At 20 minutes, it has a high protein content of 14.296% with the lowest bacterial content of 32.67 CFU/g. So, the ideal temperature and time for the sterilization process of soft bone milkfish are at a temperature of 130 ° C and 20 minutes.

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