lemongrass

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THE EFFECTIVENESS OF IMMERSION TIME IN LEMONGRASS POWDER IN REDUCING FORMALIN LEVELS IN SALTED FISH

Abstract

Introduction: Salted fish is one of the foodstuffs that are widely consumed by the community. The rate of food poisoning due to chemicals in 2019 based on BPOM data was 7% and increased in 2020 to 16%. This increase was due to the Covid-19 pandemic so that traders experienced a decline in sales. Many traders try to extend the shelf life of their products by adding prohibited preservatives, one of which is formalin, which causes an increase in poisoning rates. Saponins are one of the bioactive compounds in plants that can reduce formaldehyde. Lemongrass is a plant that has been studied to contain saponins. The use of lemongrass is also processed in powder form to make it more practical and extend the shelf life of lemongrass. Lemongrass powder is used as an alternative ingredient in reducing formalin levels in foodstuffs, namely salted fish. The purpose of this study was to determine the effect of soaking salted fish with lemongrass powder in reducing formalin levels based on variations in soaking time.

Method: This research was conducted at the STIKes Mitra Keluarga laboratory. The type of research used is experimental. with 3 experimental groups. The control group (KK) is a negative and positive control. The treatment group (X1) was salted fish before the soaking treatment with lemongrass powder was carried out. The treatment group (X2) was salted fish after soaking treatment with lemongrass powder based on 6 time variations, namely, 5 minutes, 15 minutes, 25 minutes, 35 minutes, 45 minutes, and 55 minutes.

Result: Positive samples were determined using a UV-VIS spectrophotometer and Nash reagent. The lowest increase in formalin reduction was 9,299 ppm by soaking lemongrass powder for 5 minutes. The highest level was 0.553 ppm by soaking lemongrass powder for 55 minutes.

Keywords : Salted Fish, Formalin, Saponin, KMnO4, UV-Vis Spectrophotometry.

INTRODUCTION

Fish is a marine product that has a source of protein and is in great demand by the public. Marine products are easily damaged, so it is necessary to add additives. In the food processing business, preservatives are often applied to maintain product durability and freshness. Many producers still use dangerous food additives (BTP) such as formalin in food, one of which is salted (Widayanti & Laksmita W., 2017). Salted fish products are widely consumed because of their affordable price, longer shelf life, and easy availability (Lestari *et al*., 2022)

Formalin or formaldehyde (H_2CO) is a colorless solution with a pungent odor. Formalin sold in the market generally has a content of 37% in water with the addition of methanol up to 15% as a preservative stabilizer (Modeong *et al*., 2022)Formalin is added by some manufacturers to maintain product shape, extend shelf life, improve visualization of food products and manipulate various foods that are no longer fit for consumption. Economic motives are the reason traders add harmful BTP in their products to reduce production costs (Wijaya *et al*., 2012)

Foods containing formaldehyde have negative effects that will appear several years later. Short-term effects that will occur are irritation, stomach pain, and dizziness. Long-term effects that cause damage to the respiratory tract, liver, kidneys, pancreas, heart, brain, central nervous system and cancer. Formalin is prohibited as a food additive based on the Regulation of the Minister of Health No. 33 of 2012 (Fatma *et al*., 2021)

Food poisoning due to chemicals in 2018 reached 11% (BPOM, 2018). Food poisoning due to chemicals in 2019 reached 7.01% (BPOM, 2019). Food poisoning due to chemicals in 2020 reached 16(BPOM, 2020). The increase in the number of food poisoning due to chemicals in 2019-2020 was due to the Covid-19 pandemic. Merchant turnover experienced a decline in sales. Many traders work around so that their products last longer by adding preservatives. Preservatives used include those that are prohibited,

causing an increase in poisoning rates.

Saponins are one of the bioactive ingredients in plants studied that can reduce formalin levels. Saponins are widely contained in several tribes of flowering plants, namely Liliaceae, Amaryllidaceae, Poaceae and Dioscoreaceae (Darma & Marpaung, 2020). Lemongrass is one of the plants belonging to the Poaceae family which contains saponins and is easy to obtain and widely used in everyday life. The use of lemongrass is also widely processed in powder form to make it more practical and extend the shelf life of lemongrass. Based on the problems and impacts regarding the formalin contained in salted fish, it is necessary to conduct research on reducing formalin levels in salted fish using natural ingredients as an alternative solution, one of which is lemongrass powder.

Research conducted by Sugiarti (2020), showed a maximum decrease in the formalin content of salted squid by 72.11% from soaking in salt water for 90 minutes. The formalin level reduction test was also carried out by Sarwindah & Wardoyo (2019), which showed the level of formalin in white tofu with a solution of miana leaves (*coleus benth*) containing saponins, the highest decrease was 95.51% for 90 minutes. Cases of using formalin in salted fish still occur, one of which is in the city of Bandung. The results showed that from 25 samples sampled from 5 different traders, it was stated that 6 samples of salted fish in the Bandung City Simple Market were positive for formalin (Noorrela & Munggaran, 2021).

Based on the above case, there are still irresponsible traders who use formalin as a food preservative. The lack of research that proves the decrease in formalin levels with saponin compounds in lemongrass powder is the reason why researchers conduct research on the title "Effectiveness of Soaking Lemongrass Powder in Reducing Formalin Levels in Salted Fish". Salted fish samples were soaked in 4% formalin for 60 minutes and tested for levels using UV-Vis spectrophotometry, then soaked with lemongrass powder for 5 minutes, 15 minutes, 25 minutes, 35 minutes, 45 minutes, and 55 minutes again by UV-Vis spectrophotometry.

METHOD

This research was conducted from February to June 2022 at the Mitra Keluarga College of Health Sciences Laboratory. The type of research to be conducted is experimental with the pretest-posttest control group design. In this study, the treatment carried out was soaking salted fish containing formalin using lemongrass powder based on time variations of 5 minutes, 15 minutes, 25 minutes, 35 minutes, 45 minutes, and 55 minutes.

Qualitative test phytochemical test of saponins on lemongrass powder was carried out by dissolving 1 gram of lemongrass powder with 1 mL of distilled water (1:1). The solution is shaken for 1 minute, if there is foam then 0.1 N HCl is added. Stable foam for 10 minutes with a height of 1-3 cm indicates the presence of saponins (Selfiana *et al*., 2022). Qualitative test of $KMnO_4$ on salted fish was carried out by making positive control by soaking salted fish in 4% formalin for 60 minutes and negative control made from salted fish which was not soaked in 4% formalin. the negative control was soaked in distilled water for 60 minutes. A sample of 10 grams was ground with a mortar and pestle. The sample powder was dissolved in 30 ml of distilled water, then filtered. 2 ml of the sample filtrate was pipetted and 2 drops of 0.1N KMnO4 were added. The presence of formalin is indicated by the loss of the pink color of KMnO4 (Rambe *et al*., 2022)

Quantitative test of UV-VIS Spectrophotometry was carried out by making Nash reagent according to SNI ISO 14184-2:2015. Nash reagent was prepared by dissolving 37.5 g of ammonium acetate and dissolved in distilled water. The solution was added 0.75 mL of glacial acetate and 0.5 mL of acetyl acetone. The sea was transferred to a 250 mL volumetric flask and diluted using aquadest to the right mark. The reagents were stored in a dark bottle for 12 hours before use (Yulianti & Safira, 2020).

Preparation of 1000 ppm formalin mother liquor was carried out by dissolving 2.70 mL of 37% formalin (V/V) or equivalent to 370,000 ppm. The solution was put into a 1000 ml volumetric flask. The solution was added with distilled water up to the mark and homogenized. Standard solutions of 0 - 2 ppm were made with concentrations of 0 ppm, 0.05 ppm, 0.1 ppm, 0.5 ppm, 0.75 ppm, 1 ppm, 1.5 ppm and 2 ppm. The 1000 ppm mother standard solution was pipetted 0 ml, 0.005 ml, 0.01 ml, 0.05 ml, 0.075 ml, 0.1 ml, 0.15 ml and 0.2 ml, respectively. The solution was added with distilled water up to a volume of 100 ml at each concentration. The sea was pipetted as much as 2 ml of each concentration and added Nash reagent up to 3 ml into the volumetric flask. The solution was heated in a water bath at 40±2°C for 30 minutes and cooled for 10 minutes. The absorption was observed using a UV-Vis spectrophotometer (Suseno, 2021).

The maximum wavelength with nash reagent was determined by pipetting 2 mL of a standard solution of 0.05 ppm formalin and put into a closed test tube, then 3 mL of nash reagent was added. The test tube was then homogenized and the solution was heated in a water bath at a temperature of 40±2°C for 30 minutes after which it was cooled. The absorption was observed using a UV-Vis spectrophotometer (Wardani *et al*., 2020)

Quantitative testing of the sample was carried out by taking 2 ml of salted fish solution that had been soaked in 4% formalin. Then 3 ml of nash reagent was added to the solution and heated with a water bath at 40±2°C for 30 minutes and cooled for 10 minutes. The absorption was observed using a UV-Vis spectrophotometer. After that prepared measuring cups that have been labeled with names 5 minutes, 15 minutes, 25 minutes, 35 minutes, 45 minutes, and 55 minutes, adding 4 grams of lemongrass powder to each measuring cup. Add 100 ml of distilled water until dissolved. Furthermore, salted fish that has been soaked in 4% formalin is put into a measuring cup as much as 10 grams. The solution was allowed to stand for an appropriate period of time in each measuring cup, then the solution was filtered and tested for levels with a UV-Vis spectrophotometer.

RESULTS

The qualitative test of saponin compounds tested on lemongrass powder showed a stable foam for 10 minutes with a height of 1-3 cm indicating the presence of positive saponin compounds.

Figure 1. Saponin foam qualitative phytochemical test

Qualitative test with $KMnO_4$ was carried out on positive controls made by soaking salted fish in 4% formalin solution for 60 minutes, while negative controls used salted fish without formalin. The results of the examination showed that the positive sample contained formalin where the pink color of the $KMnO_4$ reagent was missing.

Figure 2. $KMnO_4$ test results positive samples contain formalin

Determination of the maximum wavelength using a 1000 ppm formalin solution whose absorption was observed at a wavelength of 400-600. Based on these measurements, the maximum wavelength was found at 535 nm.

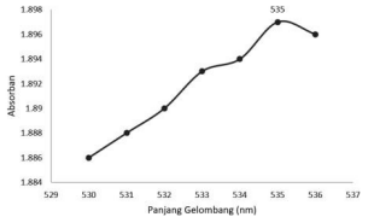

Figure 3. Maximum wavelength graph

Determination of the calibration curve using the concentration of standard solutions obtained absorption results at five concentrations, namely 0 ppm, 0.05 ppm, 0.1 ppm, 0.5 ppm, 0.75 ppm, 1 ppm, 1.5 ppm and 2 ppm showing the absorbance value. of 0, 0.219, 0.297, 0.356, 0.450, 0.577, 0.699 and 0.797. The calibration curve equation is used to show the relationship between the x and y axes. The linear regression equation obtained is y = 0.1061x - 0.0531 with a correlation coefficient of R2 = 0.9823.

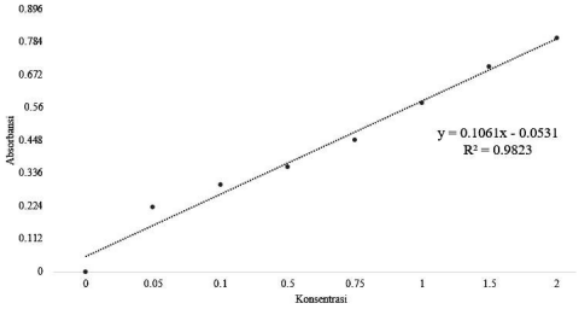

Figure 4. Linear regression curve of absorbance relationship with formalin concentration

Quantitative test using UV-Vis spectrophotometer at a wavelength of 535 nm to increase the reduction of formalin levels in salted fish with lemongrass powder is obtained in Table 1.

Table 1. Formalin Levels in Salted Fish Samples After Soaking in Lemongrass Powder Based on Time Variations

Repetition	Formalin Levels (ppm) After Soaking Lemongrass Powder Based on Time Variations (minutes)						
	0	5	15	25	35	45	55
1	12.932	9.388	7.447	5.637	2.470	1.273	0.576
2	12.781	9.219	7.437	4.959	2.433	1.377	0.557
3	13.262	9.313	7.334	5.109	2.414	1.217	0.538
4	12.781	9.275	7.305	4.911	2.414	1.330	0.538
Mean	12.939	9.299	7.381	5.154	2.433	1.299	0.553
Standard deviation	0.227	0.071	0.072	0.333	0.027	0.069	0.018
Reduced formalin	0%	28.13%	42.9%	60.7%	81.2%	89.9%	95.7%

The statistic test was carried out with the ANOVA test using SPSS version 26. The data obtained <α 0.05, the data were normal and homogeneous. The result of the ANOVA <α test is 0.05 which proves that there is an effect of the effectiveness of reducing formalin levels by soaking lemongrass powder.

Table 2. One way anova statistical test

	Soaking time (minutes)					
	0 and 5	0 and 15	0 and 25	0 and 35	0 and 45	0 and 55
	Significance					
Normality test	0.143	0.143	0.143	0.143	0.596	0.596
Homogeneity Test	0.154	0.173	0.500	0.070	0.106	0.105
One way anova test	0.000	0.000	0.000	0.000	0.022	0.022

DISCUSSION

The qualitative test of saponin compounds tested on lemongrass powder showed foam stability. Stable foam for 10 minutes with a height of 1-3 cm indicates the presence of positive saponin compounds (Selfiana *et al*., 2022). Foaming occurs due to the nature of saponins which can lower the surface tension of water when shaken. The decrease in surface tension caused by the presence of hydrophilic compounds that can damage hydrogen in the air.

The addition of HCl serves to increase the polarity so that the hydrophilic group will bind more stable and form foam (Ngginak *et al*., 2021). Research conducted by Purbowati (2021) where the foam is stable as high as 1-3 cm which indicates a positive result containing saponins in lemongrass. Another study on the saponin foam test conducted by Sapitri (2022) on lemongrass leaves produced a stable foam which indicated the presence of saponins.

Samples of salted fish that have been soaked in 4% formalin in a qualitative test with $KMnO_4$. Positive control was made by soaking salted fish in 4% formalin solution for 60 minutes, while negative control used salted fish without formalin. The results of the examination showed that the positive sample contained formalin where the pink color of the $KMnO_4$ reagent was missing. This is in accordance with Rosita's research (2022) where the identification of formalin in tofu samples reacted with 0.1 N KMnO4 will still produce a pink-purple color if the sample is negative for formalin. A positive sample containing formalin will not change color, so the sample will remain clear when $KMnO_4$ is added.

In Rambe's study (2022) a solution containing formalin compounds when $KMnO_4$ was dripped changed its color, namely the loss of the pink-purple color of the $KMnO_4$ reagent. The color change is due to the functional group containing an aldehyde, and a ketone is a carbonyl, which makes the presence of a carbonyl group more active than a ketone. The aldehyde group is rapidly oxidized to a carbonyl group or formic acid with an oxidizing agent such as KMnO4. Samples that does not contain formalin, it does not have the antioxidant KMnO4 which causes no color change because it does not have a substrate that can be oxidized by KMnO4.

The determination of the maximum wavelength is observed at wavelength 400-600 and the maximum wavelength is found at 535 nm. Based on the research of Suseno (2021) obtained a maximum wavelength of 412.5 nm, when compared with this study, there is a change in the maximum wavelength. According to Krisnawati (2018) formalin with Nash reagent has an optimum absorption at 400-600 nm. The wavelength of 535 nm in this study still entered the criteria for the optimum measurement of formalin levels. The difference in wavelength occurs because the scope of research is different from one researcher to another, causing a shift in wavelength by several factors such as equipment conditions, differences in reagents, differences in standard standard solutions and differences in tools used (Sari *et al*., 2021)

The calibration curve is used to show the relationship between analyte concentration (x-axis) and calibration (y-axis). To make calibration curve, the standard is prepared with the analyte concentration is known. The standard absorbance is determined at the wavelength and the results are depicted in a graph (Seidman et al., 2021). Determination of the calibration curve using the concentration of the standard solution, that is, the absorption results at the five concentrations are 0 ppm, 0.05 ppm, 0.1 ppm, 0.5 ppm, 0.75 ppm, 1 ppm, 1.5 ppm and 2 ppm showing the value absorbance of 0, 0.219, 0.297, 0.356, 0.450, 0.577, 0.699 and 0.797.

The calibration curve equation is used to show the relationship between the x and y axes. The linear regression equation obtained is y = 0.1061x - 0.0531 with a correlation coefficient of R2 = 0.9823. According to Yulianti & Safira (2020) the linearity of the calibration curve can be seen by calculating the value of the correlation coefficient (r), the curve can be said to be linear if the value of r 0.98. Thus, the

test curve satisfies the test requirements.

At a wavelength of 400-600 the ocular color in the solution is green-yellow and the absorbent color will be absorbed into purple (Seidman *et al*., 2021). Formalin is basically colorless. Nash reagent is used to add color to the test solution. Acetylacetone and ammonium in Nash reagent will react with formalin, where condensation will occur to form a compound 3,5-diacetyl-1,4-dihydro-2,6-lutidine (DDL). The heating treatment will produce a fixed yellow color, then the color on the instrument will produce a purple color (Hayun *et al*., 2017)

Reducing formalin levels in salted fish using lemongrass powder based on time variations, showing that all samples experienced decrease in any given time variation. Formalin salted fish without immersion (0%) the formalin content was calculated using visible spectrophotometer. Average levels of formalin in fish samples salty which is not treated using lemongrass powder as much as 12,939 ppm. Formalin levels in salted fish samples soaked with lemongrass powder experienced an increase in reduction and the highest increase was 95.7% at the longest immersion time variation of 55 minutes.

Formalin is reduced by saponin compounds in lemongrass powder. The decrease in levels was caused by the saponification reaction (soap formation process), where soap is included in the surfactant class. Surfactants in saponins are amphipathic, i.e. they contain hydrophobic (non-polar) and hydrophilic (polar) groups, where the surface active mechanism binds to formaldehyde molecules, reduce the surface tension to a very low level, making a soap solution (surfactant) this makes the ability of saponins to reduce formalin better than soaking with plain water. After formalin binds to saponins, saponins dissolve and form micelles. The round and oval parts of the micelles are heads that point and interact with water, formalin (polar) and formalin that react with micelles can dissolve in water (Mayasari *et al*., 2022).

According to the International Program on Chemical Safety (IPCS), the safe limit level of formalin that can be tolerated in the body is 1 mg/L (Donal Nababan *et al*., 2019). while according to the European Food Safety Authority (EFSA) in 2014 the limit for formalin exposure through food consumption is 100 milligrams per day (BPOM, 2019). So the results of reducing formalin levels in salted fish with lemongrass powder carried out in this study showed the most effective immersion for the longest time, namely 0.553 ppm at 55 minutes, where the level of formalin at that time was already below the recommended threshold.

The results of statistical tests carried out by normality test, data $<\alpha$ that is 0.05 which proves that the data is normally distributed. The homogeneity test of data $<\alpha$ is 0.05 which proves that the data is homogeneously distributed. One Way Anova statistical test data $<\alpha$ is 0.05 which proves that there is an effect of the effectiveness of reducing formalin levels by soaking lemongrass powder for 0 minutes and 55 minutes. If the probability (Sig) < then Ho is rejected and Ha is accepted which indicates a significant effect of variations in the soaking time of lemongrass in the form of formalin in salted fish.

CONCLUSION

The effectiveness of formalin reduction using lemongrass powder based on time variations with UV-VIS spectrophotometry obtained the longest time of 55 minutes with a concentration of 0.553 ppm and reducing formalin by 95.7%.

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