

## Original article

# Green chili to reduce formaldehyde in tofu by spectrophotometric method using Schiff reagent

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### Abstract

Tofu is a processed soybean food ingredient that is widely consumed by the public. Tofu is not just very nutrient-dense but also reasonably priced and simple to find. Given that tofu has a limited shelf life and is easily harmed, makers frequently utilize the formaldehyde preservative, which is now banned for use. Formalin or formaldehyde is not included in the list of food additives because they are toxic and harmful to health. The aim of this study was to determine the reduction of formalin levels in white tofu by soaking green chilies. The study was carried out experimentally with the spectrophotometric method using the Schiff reagent. Absorbance measurements were carried out at a maximum wavelength of 580 nm. For 10 minutes of continuous immersion, 20 minutes, and 30 minutes, green chili solution concentrations of 0%, 2.5%, 5.0%, 7.5%, 10%, and 12.5% were added. Based on the results of the study, it was found that each addition of green chili solution concentration reduced the formalin level along with the soaking time. The largest reduction in formalin levels, or 69.82%, occurred after the addition of a 12.5% green chili solution for 30 minutes. These findings led to the conclusion that the addition of green chili solution could reduce formalin content in tofu.

## INTRODUCTION

Food is one of the basic human needs that must be processed properly in order to be beneficial for our bodies. Food products are materials derived from biological or water, processed or unprocessed products intended for food or beverages for human consumption (Saparinto and Hidayati, 2010). With the development of food science and technology, more human intervention in the process of forming or processing food. The food served must be available in an attractive shape and aroma, have acceptable taste, nice color, and consistency, and be durable. Food additives, often known as additives,

are frequently used to produce food that meets these requirements. (Widyaningsih and Murtini 2006).

Tofu as one of the processed soybean foods has been widely known and favored by the people of Indonesia (Supriatna, 2007). Tofu is a type of traditional food that is widely consumed because the price is relatively cheap and has a high nutritional content, particularly the protein content (10.9 grams per 100 grams of tofu). (Mahmud *et al.* 1990). Tofu has a high-water content of around 70-85%, so it is easy to decompose by bacteria (Harmayani *et al.* 2009). The average shelf life of tofu is 1-2 days at room temperature. If more than

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that the taste becomes sour, then gradually rots so that it is not suitable for consumption anymore (Koswara, 2009). Some manufacturers frequently include chemical compounds as preservatives to have a lengthy shelf life. (Cahyadi, 2023). The preservative that is often added is formalin because it functions as a disinfectant, able to inhibit the growth of microbes such as vegetative bacteria, fungi, or viruses (Reynold, 1982). The addition of formalin to tofu is typically done by soaking the tofu in water that has formalin added (Suparyanto, 2011).

Formalin, often known as formaldehyde, is a hazardous and harmful substance (Cahyadi *et al.* 2020) that should not be utilized as a food additive (Permenkes, 2012). Formalin is usually used for disinfecting laboratory equipment, preserving biological specimens, embalming corpses, and as raw materials for the chemical industry (WHO, 2001). The high formalin content in the body will react chemically with cells thereby suppressing their function, causing cell death and poisoning in the body (Azmiyawati, 2007). According to Alshendra (2013), formalin's toxic effects can harm the liver, create abnormalities of the central nervous system, lead to cancer and leukemia, influence the levels of blood hormones (Oldham *et al.* 1982), thyroid organs (Patel *et al.* 2003), and impair testicular function. (Zahra *et al.* 2007). WHO has established a safe level of formalin in the body at 1.0 milligrams per liter (Hastuti, 2010). There have been numerous studies done to lower formalin levels. Using white turmeric (Yazid and Putri 2017), tamarind (Juliadi *et al.* 2018), finger-meeting solution (Rakhmawati *et al.* 2020), aloe vera and turmeric (Al Hasyim *et al.* 2021), and lime juice (Mus *et al.* 2021) are a few among them.

Formalin analysis has been carried out with different methods. Some of them use titrimetric methods (Widayona, 2019), colorimetry (Purwanti *et al.* 2017; Thepchuay *et al.*, 2022), HPLC (Li *et al.* 2007; Bhowmik *et al.* 2017), GC-MS (Yeh *et al.* 2013), and spectrophotometry (Gigante *et al.* 2004; Yazid and Putri 2017; Cahyadi *et al.* 2020). Spectrophotometric methods are among the methods that are relatively inexpensive, simple, sensitive, and very popular (Gasparini *et al.* 2008; Yasri *et al.* 2015). This method is based on the reaction between formaldehyde and the type of reagent used, such as p-phenylenediamine, chromotropic acid, brilliant cresyl blue, and fluoral-P including Schiff's reagent (Gibson *et al.* 2008; Yasri *et al.* 2015).

The aim of this study was to use green chili solution and the spectrophotometric method with

Schiff's reagent to reduce the formalin levels in tofu. Green chili is a type of vegetable that is often used as a seasoning ingredient. According to Rosmainar *et al.* (2018), green chili contains various compounds that are beneficial to health such as flavonoids, capsaicin, vitamin C, and saponins. Besides being able to improve taste, the presence of vitamin C, and saponin bioactive compounds in green chilies is thought to reduce formalin compounds in tofu.

## MATERIALS AND METHODS

The materials needed in this study were 37% formalin solution, rosaniline hydrochloride, sodium bisulfite, 96% sulfuric acid, and 37% hydrochloric acid. All solutions were prepared using double distilled water (aquabidest). Big green chilies and white tofu are purchased directly from local sellers in the city of Gresik. The main equipment used was a UV-1600 PC spectrophotometer with a 1.0 cm quartz cell, an analytical balance, a suction filtration set, a magnetic stirrer, a blender, a volumetric flask, and Whatman filter paper (No. 42).

To determine the decrease of formalin levels in white tofu using green chili solution, an experimental research was designed with spectrophotometric methods using Schiff's reagent. Observations were made on six variables of green chili solution concentration, namely 0.0%; 2.5%; 5.0%, 7.5%; 10.0%, and 12.5% were soaked for 10 minutes, 20 minutes, and 30 minutes, respectively. Each treatment was repeated three times, then the absorbance was measured and the formalin content was determined.

### Standard Solution

Standard formalin solution (0.37%) was prepared by pipetting 10 ml of 37% formalin solution, put into a 1000 ml volumetric flask then diluted with aquabidest right up to the mark and shaken until homogeneous. A standard solution of 5.55% formalin was prepared by pipetting 15 ml of 37% formalin solution into a 100 ml volumetric flask diluted with aquabidest right up to the mark.

### Schiff's Reagent

Schiff's reagent was prepared according to the procedure from Auterhoff and Kovar (2002), by dissolving 100 mg of fuchsin (rosaniline hydrochloride) and heating with 50 ml of aquabidest until dissolved. After adding 1.25% sodium sulfite and 2.0 ml of 6 N HCl, the solution was diluted with aquabidest to 100 ml. After being left for 12 hours, the solution should be shaken with 0.5 g of activated carbon before filtering if it is still colored. Reagent shelf life is four weeks.

**Green Chili Solution**

The green chilies were washed and then cut into small pieces and mashed using a blender. After finely weighing each as much as 0.0 g; 2.5 g; 5.0 g; 7.5 g; 10 g; and 12.5 g then put into a 100 ml volumetric flask, diluted with aquabidest right up to the mark. The concentration of the chili solution obtained was 0.0%; 2.5%; 5.0%; 7.5%; 10%; and 12.5%. Furthermore, the solution was filtered using Whatman filter paper (No. 42), and the filtrate obtained was used for soaking treatment with white tofu.

**Sample Preparation**

The tofu was washed and then cut into cubes and soaked in 0.37% formalin solution for 2 hours. Next, the tofu was rinsed with aquabidest and weighed 5 grams. Tofu soaked with green chili solution with a concentration of 0.0%; 2.5%; 5.0%; 7.5%; 10.0%; and 12.5%, each left for 10 minutes, 20 minutes, and 30 minutes. After soaking, the tofu was rinsed with aquabidest and then pulverized using a mortar. The refined tofu was diluted with aquabidest, and filtered using a vacuum pump with the help of Whatman filter paper (No. 42). The resulting filtrate was then analyzed.

**Qualitative Analysis**

The qualitative test was carried out with the Schiff reagent color activity that adapted from the method used by Manoppo (2014). 1 mL of 96% H2SO4 (1:1) should be added through the tube wall to 1.0 mL of the filtered filtrate in a test tube. A purple red tint developed with the addition of 1.0 mL of Schiff's reagent indicated that the sample had been positively identified as formaldehyde.

**Maximum Wavelength**

The maximum wavelength was determined by testing a standard solution containing 0.444% formalin. 1 ml of 96% H2SO4 (1:1), 1 ml of Schiff's reagent, and 4.0 ml of the 5.55% formalin standard solution was mixed in the 50 ml volumetric flask. After being appropriately diluted in aquabidest, the solution was agitated until homogenous. After 5 minutes of standing time, the solution's absorbance was measured with a reagent blank using a UV-1600 PC spectrophotometer at a wavelength of 500–700 nm.

**Standard Curve**

Formalin standard solution was prepared with a concentration of 0.1-0.6%. Standard solution of 5.55% formalin 1.0 ml, 2.0 ml, 3.0 ml, 4.0 ml, and 5.0 ml were prepared in 50 ml volumetric flasks at

a time. Into each volumetric flask, 1.0 mL of 96% H2SO4 (1:1) and 1.0 ml of Schiff reagent was added, then diluted with aquabidest right up to the mark and shaken until homogeneous. The concentrations of standard formalin solutions obtained were 0.111%; 0.222%; 0.333%; 0.444%; and 0.550%. Then each solution was allowed to stand for 5 minutes, and the absorbance was measured at the maximum wavelength with a UV-1600 PC spectrophotometer using a reagent blank. The calibration curve was made by plotting the concentration of the standard formalin solution against absorbance

**Formalin Analysis**

In a 50 ml volumetric flask, 1.0 mL of 96% H2SO4 (1:1), 1.0 mL of Schiff's reagent, and 5.0 ml of the filtrate was added. After being diluted with aquabidest to the appropriate level, the solution was agitated until homogenous. The solution was left for 5 minutes and then the absorbance was measured with a UV-1600 PC spectrophotometer at the maximum wavelength using a reagent blank.

**RESULTS AND DISCUSSION**

**Qualitative Assay**

This research was conducted through several stages. In the first stage, the tofu samples were tested qualitatively to determine whether there was formalin content before the levels were determined. In the second stage, tofu is soaked in 0.37% formalin solution for 2 hours to ensure the formalin content before it is reduced. The findings of the observations indicated that the tofu's textural properties had changed. Before being soaked in formalin, tofu has a softer texture and a distinctive tofu smell. After soaking in formalin, tofu has a chewy texture, a rough surface, and a slight formalin odor.

**Table 1.** Qualitative test results of tofu with Schiff reagent

Treatment	Observation					Results
	1	2	3	4	5	
Before soaking	-	-	-	-	-	Negative
After soaking	+	+	+	+	+	Positive

The third stage, tofu that had been formalin-soaked was examined qualitatively. The test results using Schiff reagent before administration of formalin showed negative results. After soaking in

formalin, it showed a positive result with the formation of a purplish-red color. The results of the qualitative test of tofu before and after soaking in formalin are shown in Table 1. The formation of a purplish-red color is the result of a reaction between the aldehyde functional group on formalin (formaldehyde) with Schiff's reagent (Figure 1).

In the fourth stage, tofu in formalin is soaked in green chili solution with varying concentrations of 0.0% -12.5%. Green chilies are one of the most used seasonings in cooking and are rich in antioxidants that are beneficial to health, such as flavonoids and vitamin C. Antioxidants can improve the taste of tofu while also acting as a natural preservative that can replace dangerous chemical preservatives. The tofu was seen to have an intriguing texture, a little greenish tint, and a slight softness after being soaked in a green chili solution, and the distinctive smell of formalin had vanished, especially at a concentration of 12.5% green chili. After soaking the tofu in the green chili solution, a spectrophotometer was used to check for the presence of formalin.

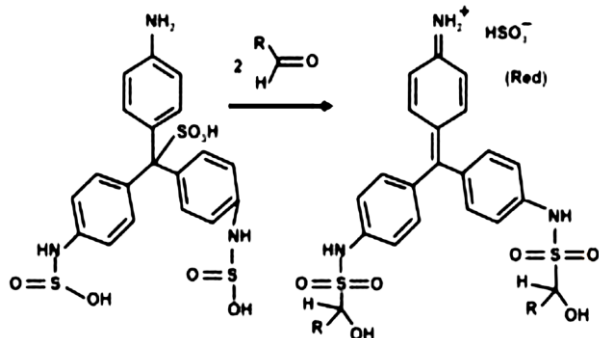


Figure 1. The chemical reaction of Schiff's reagent with formalin (Hubbe *et al.* 2019)

**Determination of maximum wavelength ( $\lambda_{maks}$ )**

Measurement of the maximum wavelength of formalin solution was carried out at 500-700 nm. The  $\lambda_{maks}$  measurement was carried out to obtain maximum sensitivity and absorption values (Yazid and Putri 2017). According to Fagnani *et al.* (2003), the  $\lambda_{maks}$  of formalin ranges from 570-580 nm, depending on the type and sensitivity of the tool used. The results of determining the  $\lambda_{maks}$  of formalin in this study were 580 nm. This wavelength is then used to measure standard solutions and formalin levels before and after treatment. The wavelength spectrum of the formalin solution is shown in Figure 2.

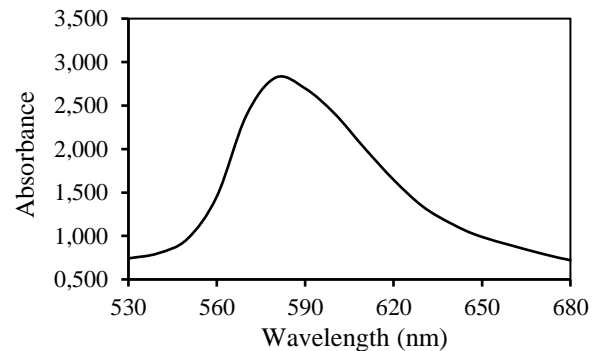


Figure 2. Wavelength spectra of formalin

**Determination of standard curve**

The formalin standard solution calibration curve obtained good linearity and complied with the Lambert-Beer law with a concentration range of 0.1-0.6%. Based on the standard curve, the regression equation is obtained  $y = 0.9809x + 0.2399$  with a correlation coefficient of  $r = 0.9948$ . The value of  $r$  is close to 1.0, illustrating that there is a linear relationship between formalin concentration and absorbance (Figure 3). Through the regression equation, the standard curve can be used to determine the concentration of formalin whose concentration is unknown after measuring the absorbance.

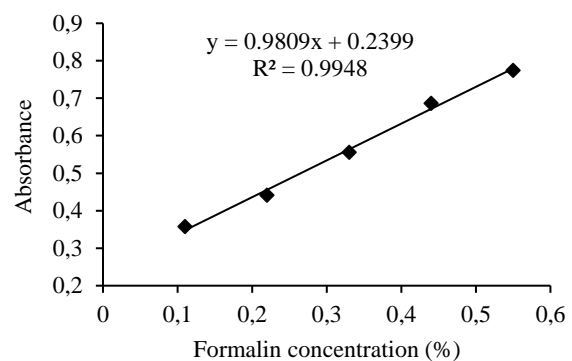


Figure 3. Calibration curve for formalin solution

**Determination of formalin levels**

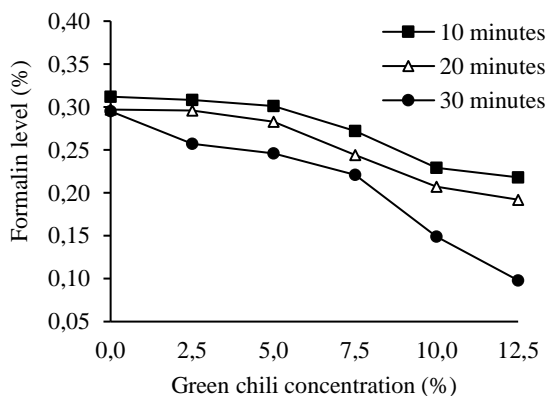
Analysis of formalin on the tofu was carried out before and after the addition of green chili solution. Before adding the chili solution, the tofu was first soaked in 0.37% formalin solution for 2 hours. The results of the analysis after soaking showed that the average formalin content in tofu was 0.319%. Furthermore, formalin tofu was added with green chili solution (filtrate) with varying concentrations of 0.0%; 2.5%; 5.0%; 7.5%; 10.0%, and 12.5% were soaked for 10 minutes, 20 minutes, and 30 minutes respectively. At a concentration of 0.0%, the tofu samples were soaked using double distilled water (aquadist) as a control.

**Table 2.** Formaldehyde content in tofu

No.	Green chili concentration (%)	Formalin level (%)		
		10 min*	20 min*	30 min*
1.	0,0	0.312	0.297	0.295
2.	2.5	0.308	0.296	0.257
3.	5,0	0.301	0.283	0.246
4.	7.5	0.272	0.244	0.221
5.	10,0	0.229	0.207	0.149
6.	12,5	0,218	0,192	0.098

The results of the formalin content in the tofu after treatment with the chili solution are listed in Table 2. Table 2 shows that the tofu that was soaked in water for 30 minutes or without the addition of chile (0%) had the greatest formalin concentration, which was 0.295%. In contrast, the tofu that had been soaked used chili solutions with concentrations of 2.5%, 5.0%, 7.5%, 10.0%, and 12.5% for 30 minutes, which led to reduced formalin levels of 0.257%, 0.246%, 0.221%, 0.149%, and 0.098%, respectively.

The results showed that the addition of green chili solution to tofu can reduce formalin levels, as well as immersion using water. However, the addition of water resulted in a smaller decrease compared to the chili solution. This is because the levels or residues of formalin in tofu are still more, so the reduction is smaller. The greater the decrease in formalin levels, the lower formalin levels will be obtained after soaking. Table 2 shows that as the concentration of the chili solution increases, the formalin level decreases with the soaking time. The graph of the decrease in formalin levels after adding chili solution by soaking for 10 minutes, 20 minutes, and 30 minutes is shown in Figure 4.



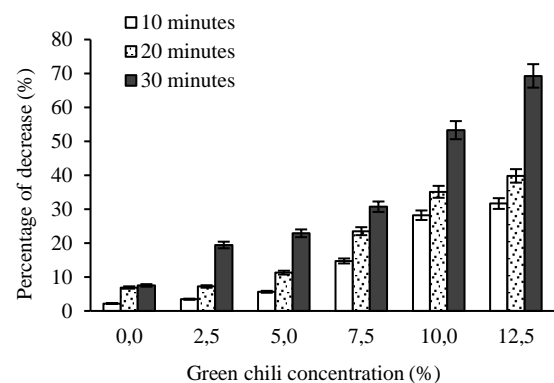
**Figure 4.** The reducing formalin levels

**Decreased Formaldehyde Levels**

Figure 5 illustrates that adding green chili solution at any concentration will lower the amount

of formalin in tofu. The concentration of the chili solution and the amount of soaking time both had an impact on the percentage of formalin reduction. The percentage decrease was 2.19, 6.90, and 7.52% after soaking for 10, 20, and 30 minutes at a concentration of 0.0% chili solution, respectively. Chili solution concentration 2.5% (3.45%; 7.21%; and 19.44%), chili solution concentration 5.0% (5.64%; 11.29%; and 22.88%), chili solution concentration 7.5% (14.73%; 23.51%; and 30.72%), chili solution concentration 10.0% (28.21%; 35.11%; and 53.29%), and 12.5% concentration of chili solution (31.66%; 39.81%; and 69.28%). The greatest percentage reduction was found in the administration of 12.5% chili solution concentration with 30 minutes of soaking time, namely 69.28%. A 30 minute immersion in water barely reduced the formalin by 7.52%.

Formalin is an active compound that can bind to food ingredients such as proteins, fats, and carbohydrates (Suntoro 1983). When formalin is added to tofu, a reaction will occur between the aldehyde groups in formalin and the protein in tofu to produce bonds that are difficult to break (irreversible). Formalin binds to proteins in foodstuffs in the form of reversible methyl-alcohol and protein cross-links, known as methylene bridges (-CH2-) which are irreversible to form methylene compounds (-NCHOH) (Kiernan 2000; Go *et al.* 2008).



**Figure 5.** The percentage reduction of formalin

One of the efforts to reduce formalin levels in foodstuffs such as tofu is to break the bond between formalin and protein. According to (Wilbraham and Matta 1992), this can be done using hydrolysis and the addition of acidic compounds. Meanwhile, according to Kierman (2000), it can be done by adding an acid catalyst, acting as a provider of H+ (polar) ions, and the presence of compounds that act as emulsifiers or surfactants.

In this study, water can reduce formalin in tofu presumably because of the hydrolysis reaction that

causes the release of formalin bonds with proteins. Formalin has a polar aldehyde functional group so it dissolves easily in water which is also polar. Water molecules will attract formalin which is tightly bound to protein (methylene bridge) so that formalin is released. This is to (Whitford 2013), that methylene compounds can decompose back into protein and formalin through hydrolysis reactions. This reaction takes place spontaneously because the H<sup>+</sup> ions from water molecules are not very reactive to methylene compounds, so only a small amount of formalin is released. It is proven that soaking using water can only reduce formalin by 7.52% (Table 3). The attraction of water molecules to formalin is affected by the duration of the interaction between water molecules and formalin in tofu.

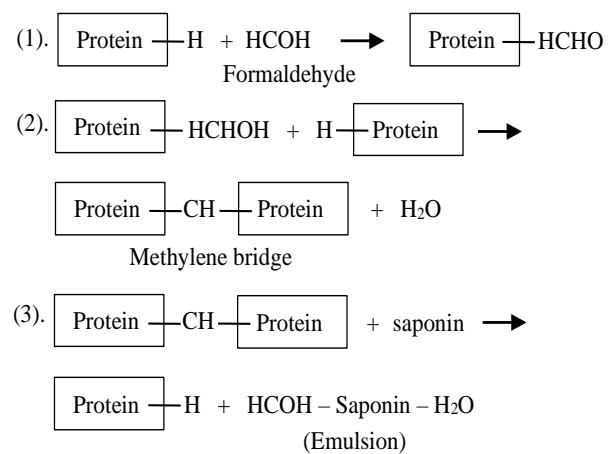
Green chili solution can reduce formalin because green chili contains vitamin C (84 mg/100g) (Depkes RI, 2013) and saponin compounds. It is believed that vitamin C (ascorbic acid), which is categorized as an organic compound and is acidic and polar so it can attract formaldehyde, which is also polar, and diminish formalin. (Kiernan 2000) asserts that the initial binding between formalin and protein results in methyl-alcohol, which is reversible and quickly degraded in the presence of an acidic chemical that serves as a source of H<sup>+</sup> ions.

The attraction of vitamin C to formalin is stronger than water molecules because it can act as a catalyst for providing H<sup>+</sup> ions which have a stronger reactivity to attract formalin so that the bonds are released. Acid compounds capable of reducing formalin are supported by research by (Juliadi *et al.* 2018), using tamarind can reduce formalin in sausages by up to 45%, (Burhan 2018), using acetic acid on anchovies reduces formalin by up to 97.62%, and (Mus, Sulfiani, and Musdalifah 2021), using lime juice on rebon shrimp reduces formaldehyde to 63.9%.

In addition to vitamin C, saponins contained in green chilies are also thought to reduce formaldehyde through saponification reactions or the formation of soaps belonging to the surfactant group (Gusviputri *et al.* 2013). Saponins are amphipathic glycoside compounds that have hydrophobic (non-polar) and hydrophilic (polar) groups, so they can act as emulsifiers or surfactants (Gunawan and Mulyani 2004; Damayanti, Ma'ruf, and Wijayanti 2014). In the formalin withdrawal mechanism, when formalin tofu is soaked in green chili solution, saponin compounds will emulsify the formalin bonds with proteins in tofu. Part of the polar group (hydrophilic) saponins will interact with polar water. Saponin compounds are adsorbed into the interphase region and bind to formaldehyde particles

until the emulsion stability of the polar groups occurs. The saponin compounds will disintegrate and produce micelles after being linked to formalin. Water and formalin interact through the micelles, distributing the formalin to create an emulsion with the water (Amran 2008; Damayanti, Ma'ruf, and Wijayanti 2014; Yazid and Putri 2017). Figure 6 depicts the purported withdrawal reaction of saponins to formalin.

The reduction of formalin in this study can be attributed to a catalytic and saponification reaction that took place in the presence of the acid compounds (ascorbic acid) and saponins present in green chilies. The methyl-alcohol bonds and cross-links known as methylene bridges were broken once formalin was reduced. (-CH<sub>2</sub>-). Following the release of formaldehyde from the protein, micelles are created by creating an emulsion system with water. Using the saponins found in white turmeric can lower the formalin content of tofu by 62.8%, while aloe vera can reduce the formalin content of tuna by up to 50%, according to study by Yazid and Putri (2017) and Rullyansyah, Azizah, and Kunsah (2020). Using saponins at Intersection keys reduced formalin in white tofu by 28.0% (Rakhmawati, Lestari, and Widyaningsih 2020).



**Figure 6.** Alleged withdrawal reaction of formalin by saponins

The reduction of formalin in this study can be said to have occurred through a process of catalysis and saponification reaction in the presence of acid compounds (ascorbic acid) and saponins found in green chilies. The reduction of formalin was followed by the breaking of the methyl-alcohol bonds and cross-links known as methylene bridges (-CH<sub>2</sub>-). Formaldehyde is then released from the protein to form an emulsion system with water to form micelles. Saponin compounds reduce formalin according to the research results of (Yazid and Putri 2017), utilizing saponins contained in white turmeric

can reduce formalin in tofu by 62.8%, (Rullyansyah, Azizah, and Kunsah 2020), using aloe vera can reduce formalin in tuna by up to 50%. (Rakhmawati, Lestari, and Widyaningsih 2020), using saponins at Intersection keys reduced formalin in white tofu by 28.0%.

## CONCLUSION

Based on the results of the study, it can be concluded that the tofu's formalin content can be decreased using the green chili solution. The higher the concentration and soaking time of the green chili solution, the greater the decrease. The addition of green chile solution at a concentration of 12.5% led to the largest reduction of formalin, which was reduced by 69.28%.

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