Aloe vera and lemon juice capability in decreasing formaldehyde content in tofu sumedang with cold storing

C Daniela*, H Rusmarilin and H Sinaga

Department of Food Science, Universitas Sumatera Utara, Medan, North Sumatera, Indonesia

E-mail: *conniedaniela83@gmail.com or herla_surabaya@yahoo.com

Abstract. Formalin compounds are carcinogenic but are often used as preservatives for foods such tofu, which is harmful to health. This study aims to determine the concentration, soaking time of Aloe vera and lemon juice and the duration of cold storage to decrease formalin in tofu Sumedang. In this study, tofu was soaked in 5% formalin. Formalin tofu soaked with aloe and lemon juice in comparison (100%:0%, 80%:20%, 60%:40%, 40%:60%, 20%:80%, 0%:100%) and long immersion (30, 60, 90 minutes). Tofu with the best comparison and soaking is stored in cold storage (10°C) for 0, 3, 6 and 9 (days). The results showed that 100% Aloe vera with 90 minutes immersion reduced formalin levels by 65.82%, formalin residues by 28.777ppm, texture (5.592 (N/cm²), pH (5.273), brightness (84.287) and color index (83.519). Search results for cold storage for as long as 3, 6, 9 (days) respectively, formalin (26.261 ppm, 25.510 ppm, 23.440 ppm), texture (5.104, 4.325, 2.758), pH (5.043, 4.62, 3.92), mirror (79.916, 79.803, 79.410) and colour index (82.988, 83.514, 83.699). Aloe vera juice 100% containing saponin levels of 570µg/ml was more effective in removing formalin levels in Sumedang tofu because saponins as surfactants that bind formalin and air-soluble.

1. Introduction

Sumedang tofu is a favourite food for many people in addition to its delicious and savoury taste. Sumedang tofu has health benefits because it contains nutrients and vitamins. The weakness of the tofu product is low shelf life, because it contains water 82.94% and the nutritional content is quite complete so it is easily overgrown with microbes [1], this certainly causes losses to tofu producers. Avoiding this, producers often add preservatives such as formaldehyde. Formalin is widely used because the price is much cheaper. Formalin is a reactive compound that can bind compounds in food ingredients, such as proteins. Formalin acts as a preservative when reacting with proteins to form sequences between adjacent proteins, the result of this reaction, hardened and insoluble proteins [2]. About 6.136 people were exposed to food suspected to contain formaldehyde causing poisoning and 8 people died. One effort was made to reduce formalin compounds in food ingredients by using natural ingredients such as Aloe vera and lemon. Active compounds found in Aloe vera such as saponins have the potential to reduce levels of formalin. Based on previous research [3] stated that 80% Aloe vera juice can reduce formalin to milkfish fillets during cold storage by 66.48%. The acid content of lemon juice has catalysed the release of formaldehyde and protein, based on research shows [4] that aldehydes can be separated in a mixture using acidic compounds.
2. Materials and Methods

2.1. Materials

Soybeans are obtained from the traditional market Tj. Rejo, Medan North Sumatra, Aloe vera type Barbadensis Miller was obtained from the Medan Sunggal Aloe vera plantation and the lemon was obtained from the lemon Sempakata plantation, Medan Selayang. Chemicals used in this study is CH₃COOH (acetic acid technical), K₂SO₄ (Merck), CuSO₄ (Merck), H₂SO₄ (Merck) acid, NaOH (Merck) acid, boric acid, an indicator mengsel, alcohol, HCl (Merck) acid, formaldehyde 37% (Merck), 2.4-Dinitrophenylhydrazine (DNPH) 0.1% (Sigma Aldrich), CH₂Cl₂(dichloromethane) (Merck), methanol (Merck), plate count agar (PCA) (Oxoid) and anisaldehyde (Merck). The equipment used in this research ovens (Thermo Scientific, Precision Compact Oven), distillation devices, glassware, soxhlet tools, UV-VIS spectrophotometer, U-1800, Shimadzu type LC-20AT/SPD-20A HPLC, Cecil CE4201 HPLC, autoclave, colony counter and spatula.

2.2. Tofu making process

Soybeans are sorted by choosing good quality soybeans, soaked for 24 hours. Soybeans are crushed using blender with the addition of warm water with a ratio of soybeans to 1:2 water. Filtering is done, then filtrate is heated, stirred, acetic acid is added to form a lump. Put the tofu on the mold, let stand for 30 minutes and the tofu is ready for use.

2.3. The process of making Aloe vera and lemon juice

Aloe vera is sorted by sorting plants according to the quality and size of plants that meet healthy standards. Cleansing/washing is done, blended, crushed with a blender and filtered. Aloe vera juice is weighed according to the required percentage (100%, 80%, 60%, 40% and 20%). Lemon is sorted by choosing lemon yellowish green with a fruit shape that is not damaged or perforated. Cut a lemon in half, squeezed, and filtered resulting in a clear juice, lemon juice weighed in accordance with the required percentage (100%, 80%, 60%, 40% and 20%).

2.4. Making Aloe vera mixtures and lemon juice to reduce levels of formalin in formalin tofu

The formalin tofu was soaked into a mixture of Aloe vera and lemon juice with a ratio of 100%:0% (A₃), 80%:20% (A₂), 60%:40% (A₄), 40%:60% (A₅), 20%:80% (A₆) and 0%:100% (A₇) which are then soaked for 30 minutes (L₁), 60 minutes (L₂) and 90 minutes (L₃), each treatment was carried out an analysis of the levels of formalin, texture test, pH test, brightness (L), colour index, organoleptic of colour value, organoleptic of flavor value and organoleptic of texture value.

2.5. Determination of formalin using HPLC analysis

Formalin determination method added DNPH solution and extracted using dichloromethane [6] by HPLC instruments (Model SPD-M20 A).

2.5.1. Sample preparation. Weighed a sample of ± 5 g and added 5 ml of aqua demineralization, with a temperature of 96°C the sample was heated for 30 minutes. Filtered samples to obtain the filtrate contained formaldehyde. The filtrate was centrifuged at 5000 rpm for 15 minutes and supernatant was obtained, then put into a bottle and degassed using the Branson 5200 Ultrasonic Cleaner.

2.5.2. Determination of formaldehyde derivatization. Filtrates of samples and standard solutions respectively (20 ppm, 60 ppm, 80 ppm and 100 ppm). Each sample filtrate concentration and standard solution was taken 1 ml. Added 0.5 ml of 0.1% DNPH solution, extracted using dichloromethane. Dichloromethane is evaporated and the evaporation residue is dissolved with 1 ml of methanol, filtered using a 0.45 µm membrane filter.
2.5.3. Analytical conditions of HPLC. HPLC model used is Shimadzu type LC-20 AT/SPD-20A. Tool specifications consist of pumps with LC-20AT type, columns with VP-ODS type (250 x 4.6 mm).

2.6. Texture test (Brookfield texture analyser)
Tofu texture was measured using the Brookfield CT3 Texture Analysers [7]. The sample is placed under the TA47 probe 8 mm in diameter for the type of hard tofu. In the tofu product, hardness measurement is done by inserting a probe that is attached to the tool as deep as 10 mm into the sample.

\[
\text{Product hardness} = \frac{\text{the number that appears on the tool (kg)}}{\text{wide needle on the tool}} \times 9.8
\]

(1)

2.7. pH test
pH value was determined by the AOAC method [8]. The type of equipment used was pH meter (Fischer Accumat model 230 pH/ion meter). Total of 10 grams of sample were mashed, added little distilled water and stirred until evenly distributed.

2.8. Determination of colour values
Color testing is carried out by hunter method [9] using Minolta Chroma meter (CR 200 type, Japan). Measurement produces values of L, a, b and \( \theta \text{H} \) (\(^{\circ}\)Hue).

\[\theta \text{H} = \tan^{-1} \left( \frac{b}{a} \right)\]

(2)

2.9. Selection of the best treatment
The best treatment of Sumedang tofu was chosen based on the weighting technique with a variable weight value of formalin 1, texture test 0.9, brightness 0.8, colour index 0.7 and pH value 0.6.

2.10. Saponin content test
The saponin content test was determined by Quillaja Bark [10]. The sample used is Aloe vera juice. Taken as much as 0.5 ml of aloe vera sample. Added 5 ml of water then extracted, 50 \( \mu \text{L} \) of anisaldehyde was added, 2 ml of 50\% sulfuric acid added heated at temperature of 60\( ^\circ \text{C} \). The sample was read with UV-VIS spectrophotometer (UV-VIS spectrophotometer, U-1800, High Technology Corporation, Japan) at a wavelength of 435 nm.

2.11. Organoleptic test of colour, flavour and texture
Organoleptic tests of colour, flavour and texture were determined using a hedonic value scale and randomly tested by 20 panellists. Testing was done on a sensory basis based on the hedonic scale.

2.12. Statistical analysis
Data were analysed using SPSS software (22.0). Differences in mean values were tested by analysis of variance and significance levels were obtained with Duncan’s test. A significance level of \( P < 0.05 \) was used.

3. Results and discussions
The results of the analysis of chemical properties and physical properties of tofu without formalin treatment and tofu. Table 1 shows the results of the analysis of tofu without treatment with formalin. Based on the texture, brightness and colour tofu index, formalin tofu has a higher value than tofu without treatment, but based on the tofu pH test without treatment has a lower value than tofu formalin, this shows that the pH of tofu without treatment is more acidic than tofu formalin. Tofu with
the addition of formalin causes the texture to be more compact, because formalin reacts with protein, hardens proteins and cannot dissolve [3].

### Table 1. Results of analysis of tofu without treatment and tofu formalin

| Parameter                        | Tofu without treatment | Tofu formalin         |
|----------------------------------|------------------------|-----------------------|
| Formalin content (ppm)           | -                      | 84.190                |
| Texture test (N/cm²)             | 5.045                  | 10.039                |
| pH test                          | 4.023                  | 5.388                 |
| Brightness (L)                   | 84.490                 | 84.520                |
| Index colour                     | 84.390                 | 84.619                |
| Organoleptic of colour value     | 4.140                  | 4.130                 |
| (numeric)                        |                        |                       |
| Organoleptic of flavour value    | 4.330                  | 2.340                 |
| (numeric)                        |                        |                       |
| Organoleptic of texture value    | 3.160                  | 4.730                 |
| (numeric)                        |                        |                       |

*Data is the means of three replicates*

Based on the organoleptic of colour value that tofu without formalin and formalin tofu have the same assessment by panellists, that is, at the preferred level. The results obtained it is shown that the assessment of the texture of tofu formalin is not preferred by panellists due to the high levels of formalin added. This is related to the mechanism of formalin as a preservative was states that aldehyde groups which are easy to react with proteins form methylene compounds. Thus, when protein foods soaked with formalin solution, then the aldehyde group from formaldehyde will bind the protein element as the cause of the texture to be compact and hard [13].

### 3.1. Effect of the concentration ratio of Aloe vera and lemon juice on quality parameters of Sumedang tofu

Table 2 shows that table 2 shows that A₁ treatment (100%:0%) with the remaining formalin was 34.823 ppm, the formalin level was reduced by 58.64%. The active compounds that play a role in reducing formalin are saponins. The mechanism of saponin in reducing formalin through saponification reaction which is associated with work methods such as surfactant that is to be able to form water emulsion and formalin [14]. The treatment with the highest texture was obtained at A₆ (0%:100%) with a value of 7.794 N/cm². The higher the texture value of tofu, the resulting tofu has hard and solid, research result [7] shows that formalin tofu has a chewy and stiff texture. The treatments that have the highest pH, brightness (L) and colour index (*Hue*) values were obtained in A₁ treatment (100%:0%). Decreasing the brightness level of tofu with soaking on lemon juice is caused by the absorption of the colour of lemon juice in tofu. Treatment colour index A₁ (100%:0%) which shows a reddish yellow colour, this is because components contained in *Aloe vera* such as anthraquinone aloin and aloe-emodin [15]. Based on organoleptic of colour and flavour value A₁ treatment (100%:0%) had the highest, but organoleptic of texture value, treatment A₆ (0%:100%) had the highest value.

### 3.2. Effect of soaking duration of Aloe vera and lemon juice mixture on Sumedang tofu parameters

The results showed that the immersion time had an effect on the levels of formalin (ppm), texture test (N/cm²), pH test, brightness value (L) and colour index (*Hue*). Influence comparison *Aloe vera* and lemon juice to the observed quality parameters are presented in table 3. Table 3 shows the effect of *Aloe vera* soaking time and lemon juice on the parameters (formalin content, texture test, pH test, value brightness (L), colour index, organoleptic of colour value, organoleptic of flavour value, organoleptic of texture value. From the results obtained that the longer the immersion, the formalin content decreases. Soaking 30 minutes, formalin content in tofu was 56.572 ppm while at 90 minutes
soaking, formalin tofu content was 40.629 ppm or decreased by 15.943 ppm (28.20%). This decrease occurs because the longer the immersion time, the more the amount of methylene bond that is hydrolysed and causes formalin to no longer bind to the protein to tofu. The results of the study for the texture showed longer soaking, the smaller the number of textures obtained, this shows that the texture of the tofu was soft. This is due to a decrease in formaldehyde in the tofu because formalin is soluble in water [16].

Table 2. Effect of Aloe vera and lemon juice comparison on Sumedang tofu parameter

| Parameter                  | Treatment                  |
|----------------------------|----------------------------|
|                            | A1 (100%:0%)              | A2 (80%:20%)              | A3 (60%:40%)              | A4 (40%:60%)              | A5 (20%:80%)              | A6 (0%:100%)              |
| Formalin content (ppm)     | 34.823± 5.787F            | 39.747± 8.485E            | 44.244± 10.529D          | 52.273± 9.393C            | 56.572± 6.953B            | 65.143± 5.167A            |
| Texture test (N/cm²)       | 5.687± 0.068F             | 5.904± 0.085E             | 6.233± 0.075D            | 6.485± 0.075C             | 6.988± 0.086B             | 7.794± 0.117A             |
| pH test                    | 5.270± 0.012A             | 4.846± 0.010B             | 4.417± 0.031C            | 4.192± 0.037D             | 3.781± 0.027E             | 3.453± 0.019F             |
| Kecerahan value (L)        | 84.349± 0.063A            | 83.469± 0.077B            | 82.328± 0.026C           | 81.534± 0.060D            | 80.801± 0.080E            | 79.058± 0.089F            |
| Color index (<Hue)         | 83.131± 0.191A            | 81.119± 0.170B            | 80.204± 0.084C           | 80.138± 0.093C            | 80.097± 0.173E            | 79.870± 0.122C            |
| Organoleptic of color value (numeric) | 3.806± 0.0025A        | 3.588± 0.014B             | 3.424± 0.020C            | 3.286± 0.013D             | 3.090± 0.013E             | 2.820± 0.030F             |
| Organoleptic of flavor value (numeric) | 4.273± 0.059B       | 4.652± 0.047A             | 4.023± 0.089B            | 3.590± 0.059C             | 3.261± 0.076D             | 2.756± 0.116E             |
| Organoleptic of texture value (numeric) | 3.104± 0.100c        | 3.313± 0.145D             | 3.631± 0.162C            | 3.722± 0.134C             | 4.411± 0.121B             | 4.586± 0.039A             |

*Data is the means of three replicates

3.3. Selection of the best treatment

The best treatment was determined based on the levels of formalin, texture test, pH test, brightness (L), colour index, organoleptic of colour value, organoleptic of flavour value and organoleptic of texture value [5] then the best sample was obtained from the largest decrease in formalin content which was then continued for storage treatment. A1L3 treatment (soaking with 100% Aloe vera juice for 90 minutes) was chosen as the best treatment.

3.4. Effect of storage time on the quality of Sumedang tofu

Table 4 shows the effect of storage time on parameters (formalin content, texture test, brightness value (L), colour index (<Hue) and pH test). Difference in formalin levels between formalin tofu at storage 3, 6, 9 days and tofu with the best treatment at storage 3, 6, 9 days is not much different and the value tofu tends to decrease but the decline occurs slow due to cooling temperature. This is because the formalin tofu is difficult to release quickly and is affected by cold storage so that the evaporation
process is slow [6]. The biggest texture decrease was obtained in T0 treatment (tofu without treatment), which was 55.17%. Decrease in texture value is caused by storage that is too long causing the decay process to tofu by microbial activity that causes an increase in water content so that the texture will become soft and slimy [11]. The brightness value (L) of tofu will decrease with the longer storage time, this decrease can be caused by the decay process that occurs during storage. Decomposition of sugar in the product which causes colour changes due to the presence of colour precursors (eg 5-(hydroxymethyl)-2-furaldehyde [17]. The colour index value during storage in each sample is between 79-84 °Hue.

**Table 3.** Effect of immersion time for *Aloe vera* and lemon juice on Sumedang tofu parameters

| Parameter                              | Treatment                        | L1 (30 minutes) | L2 (60 minutes) | L3 (60 minutes) |
|----------------------------------------|----------------------------------|-----------------|-----------------|-----------------|
| Formalin content (ppm)                 |                                  | 56.57±11.149A   | 49.20±10.964Bb | 40.62±12.547C   |
| Texture test (N/cm²)                   |                                  | 6.61±0.030Aa    | 6.51±0.030AB   | 6.41±0.030Bb    |
| pH test                                |                                  | 4.32±0.659Aa    | 4.32±0.675Aa   | 4.32±0.678Aa    |
| Value brightness (L)                   |                                  | 81.99±1.888Aa   | 81.90±1.911Aa  | 81.87±1.902Aa   |
| Colour index (*°Hue*)                  |                                  | 80.93±1.479Aa   | 80.72±1.428Bb  | 80.61±1.429Aa   |
| Organoleptic of colour value           |                                  | 3.36±0.013Aa    | 3.32±0.013Aa   | 3.32±0.013Aa    |
| Organoleptic of flavour value          |                                  | 3.71±0.755Aa    | 3.75±0.655Aa   | 3.81±0.677Aa    |
| Organoleptic of texture value          |                                  | 3.91±0.582Aa    | 3.79±0.586Bb   | 3.67±0.606C     |

Data is the means of three replicates

**Table 4.** Effect of long cold storage on the quality of Sumedang tofu

| Treatment (cold storage) | Formalin content (ppm) | Texture test (N/cm²) | Brightness value (L) | Color index (*°Hue*) | pH test |
|--------------------------|------------------------|----------------------|----------------------|----------------------|---------|
| T0                       | 0.00±0.000C            | 4.72±0.09C           | 80.85±0.07Aa         | 84.06±0.02Bb         | 5.02±0.18a |
| T1 (3 days)              | 77.33±0.166Aa          | 9.71±0.10Aa          | 80.43±0.06AB         | 84.15±0.01Aa         | 5.097±0.16a |
| A1L3                     | 26.26±0.106Bb          | 5.10±0.07B          | 79.92±0.29Bb         | 83.15±0.06Bb         | 5.043±0.13a |
| T0                       | 0.00±0.000C            | 3.70±0.16Bb         | 80.45±0.19Aa         | 82.77±0.04Ab         | 4.40±0.13b |
| T1 (6 days)              | 72.64±0.231Bb          | 8.28±0.34Ab         | 80.58±0.25Ab         | 83.85±0.03Ab         | 4.91±0.11a |
| A1L3                     | 25.51±0.064Bb          | 4.32±0.23Ab        | 79.80±0.21B          | 82.27±0.03Ab         | 4.62±0.01b |
| T0                       | 0.00±0.000C            | 2.26±0.28Bb         | 80.06±0.23B          | 81.66±0.02Bb         | 3.38±0.21c |
| T1 (9 days)              | 69.55±0.337Ab          | 7.00±0.23Ab         | 80.20±0.25Ab         | 83.58±0.17Bb         | 4.70±0.15a |
| A1L3                     | 23.44±0.006Bb          | 2.75±0.20Ab        | 79.41±0.18B          | 82.23±0.19Bb         | 3.92±0.11b |

Data is the means of three replicates

### 4. Conclusion

The treatment of 100% Aloe Vera juice was able to reduce the levels of formalin in Sumedang tofu with a percentage of decrease reaching 58.64% and based on this treatment weighting technique became the best treatment. The longer the cold storage of Sumedang tofu, the lower the formaldehyde level, the cold storage formalin level to 9th day was 23.44 ppm in A1L3 treatment, while the safe threshold of formalin in the body ranged from 1.5-14 mg/L/day, this means the rest formalin is still too large. Therefore, further treatment is needed, such as more extended soaking time.
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