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Turmeric Leaves Extraction (*Curcuma Longa L.*) as a Natural Preservative Using Ultrasound-Assisted Extraction (UAE) Method

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Abstract. Turmeric leaves (*Curcuma longa L.*) contain phytochemical compounds that can be utilized as natural food preservatives or bioformalin due to their antibacterial properties. The extraction method used in this study is Ultrasound-Assisted Extraction (UAE), to determine the effect of variable sample-solvent ratio, time, and particle size on total flavonoid, tannin, and alkaloid compounds, as well as to determine the optimal shelf life of fresh tuna. This study used sample-solvent ratio variables of 1:10, 1:15, and 1:20; time variables of 10, 20, and 30 minutes; and particle size variables of 60, 80, and 100 mesh. The highest total flavonoid compound was obtained in the variable sample-solvent ratio of 1:20, the particle size of 100 mesh, and the time of 20 minutes at 98.076 mg/L. The highest total tannin compound was obtained in the variable sample-solvent ratio of 1:15, the particle size of 60 mesh, and the time of 10 minutes at 41.697 mg/L. The highest total alkaloid compound was obtained in the variable sample-solvent ratio of 1:10, the particle size of 100 mesh, and the time of 20 minutes at 10.092 mg/L. The optimum curing time for tuna is 36 hours at room temperature with variable sample-solvent ratio, time, and particle size of 1:20 g/mL, 20 minutes, and 100 mesh with 20% concentration. The running has the highest flavonoid compounds, so it can be concluded that flavonoid compounds have a major effect on the preservation process of tuna.

Keywords: *Turmeric leaves, phytochemical compounds, ultrasound-assisted extraction, and natural food preservatives.*

1. Introduction

Tuna is a type of fish that has a relatively high protein content and water content as well as dense fish meat. Tuna is prone to damage because it has a high-water content, so microorganisms multiply easily. The chemical content contained in tuna is 71-76.7% water

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content; 21.6-26.3% protein; and 1.45-3.4% ash content. Efforts made to inhibit the decay of tuna are smoking with conventional methods and the use of liquid smoke. Conventional smoking can lead to uneven flavor and concentration in the smoked part of the tuna. The time and temperature that are used are not uniform, so the smoking of the tuna is not perfect. The second method, smoking tuna using liquid smoke, provides a distinctive flavor and extends the shelf life of tuna. However, the liquid smoke used requires a purification process first because most of the materials used are waste [1]. Along with the development of technology and science, there is a recent method that is safer and more effective in preserving tuna, which is bioformalin. Bioformalin is a natural preservative derived from nature as a substitute for formalin, so it is safe to use to preserve fresh fish. The materials used in the manufacture of formalin come from plants, so the cost of bio-formalin production is relatively affordable. Natural compounds that can be utilized as antibacterials are flavonoids, tannins, alkaloids, saponins, and triterpenoids found in plants [2].

Turmeric (*Curcuma longa L.*) is a spice plant that can be used as herbal medicine in handling circulatory problems, anti-inflammatory, antimicrobial, and antibacterial. Research on turmeric plants currently only focuses on turmeric rhizomes, even though turmeric leaves also contain high phenolic compounds. Turmeric leaves extracted in this experiment are shown in Figure 1. Turmeric leaves can be utilized as an alternative to bioformalin which is safe for consumption by the human body because it has high antibacterial compounds. The mechanism of antibacterial compounds is to damage the cytoplasmic wall so that it can inhibit bacterial metabolism and denature the proteins contained in bacterial cells, causing the leakage of nutrients from the cells. Thus, bacterial cells will be inhibited from growing and then die [3]. The chemical composition contained in turmeric plants is 69.4% carbohydrates consisting of flour; 6.3% protein; 5.1% fat; 3.5% minerals; 13.1% water; and 3-5% curcuminoids [4]. Curcumin is classified as a phytochemical compound consisting of phenolic compounds including flavonoids, tannins, and alkaloids [5]. The percentage of phytochemical compounds contained in turmeric plants is flavonoid content of 2.71%; tannin content of 2.58%; and alkaloid content of 1.47% [6]. Antibacterial compounds found in turmeric leaves with the highest percentage are flavonoids.

Figure 1. *Curcuma longa L* [7].

The antibacterial ability of total flavonoid compounds is greater than the antibacterial ability of total tannin compounds. The strength of the antibacterial ability of a compound is due to the relation between the structure of the compound and the antibacterial ability. The structure of the tannin compound is larger than the structure of the flavonoid compound, so the tannin compound will be more difficult to bind to the active group because there are steric hindrances in the structure of the tannin compound. Tannin compounds have a working mechanism of antibacterial activity that involves disrupting protein transport in bacterial cells [8]. Alkaloids are phytochemical compounds with the lowest concentration in turmeric leaves. The ability of alkaloids as antibacterial is due to the ability of alkaloids to infiltrate DNA so that the constituent components of peptidoglycan in bacterial cells are disrupted, this causes the wall layer in bacterial cells to form imperfectly and results in cell death. Flavonoid compounds are considered more effective as antibacterials because flavonoids can directly interfere with the work functions of microorganisms by breaking down bacterial cell membranes so that bacteria cannot grow and survive [9].

Various extraction methods of phytochemical compounds have been improved from conventional methods to modern methods, including Ultrasound Assisted Extraction (UAE). The advantages of the UAE method are that it can increase the penetration speed of the solvent through the cell wall, the mass can move quickly, increase the yield obtained, uses low operating temperatures, requires a small amount of solvent, and short extraction time [10]. The use of the UAE method in this study is because some phytochemical compounds will be damaged when extracted at high temperatures. The high temperature will cause structural changes in flavonoid compounds resulting in a small yield [11].

Factors that affect the UAE extraction process are time, particle size, temperature, type, and amount of solvent. The extraction time is too long until it passes the optimum limit, which will cause the degradation of bioactive compounds and reduce the yield obtained [12]. According to Andriani *et al*, the use of temperature in the extraction process must be adjusted

to the material to be extracted. Extraction temperature and time that exceeds the optimum limit of the extracted material can result in bioactive compounds experiencing changes in chemical structure due to the oxidation process so that the yield produced is reduced [13]. According to Fachri *et al*, particle size is a variable that affects the yield of extracts obtained. The larger the particle size, the more surface area of the material in contact with the solvent so that the diffusion process will run quickly. The larger particle size will also cause a decrease in yield due to aggregation in the extraction process [14]. The more amount of solvent, the higher the yield produced. The more volume of solvent used, the less bioactive compounds will be extracted because the volume of solvent can cause microwaves to be focused on the solvent [12]. Table 1 represents previous research on the extraction of phytochemical compounds.

Table 1. Research on Phytochemical Compound Extraction

Materials	Methods	Results	Ref
Turmeric Leaves	Microwave Assisted Extraction (MAE)	This study used aquabidest solvent with variable power of 10%, sample-solvent ratio of 1:20 and time of 10 minutes to obtain total flavonoid compounds of 4.025 µg/g. Bioformalin was applied to meatballs with an optimum preservation time of 48 hours at room temperature.	[12]
Black turmeric rhizomes	Maceration	This study used 80% ethanol solvent. Maceration was carried out for 48 hours. The total flavonoid content was 2,775.650 mg/100-gram, total tannin was 2,578.140 mg/100-gram, alkaloid was 1,466.290 mg/100 gram.	[15]
Ruta graveolens Linn	Maceration	This study used 96% ethanol solvent. Flavonoid levels were produced at 1.67% and tannins at 7.04% maceration time for 24 hours.	[16]
Papaya leaves	Maceration	This study used 96% ethanol solvent with a sample-solvent ratio of 1:15, maceration time for 3 x 24 hours. The total flavonoid content was 9.41% and alkaloid content were 16.56%.	[17]
Palm fruit peels	Maceration	This study used 96% ethanol solvent and maceration was carried out for 4 days. The tannin content was 1.16%bb and alkaloid content were 930.120 mg/g extract.	[18]
Muntingia calabura L.	Microwave Assisted Extraction (MAE) and Ultrasound Assisted Extraction (UAE)	This study used 70% ethanol solvent. The use of MAE at 50% power, sample-solvent ratio of 1:25 produced flavonoid concentration of 132.410 mg/mL and the most optimal time was 5 minutes to produce flavonoid concentration of 91.669 mg/mL. The use of UAE with a frequency of 40kHz with a variable sample-solvent ratio of 1:10 obtained flavonoid concentration of 47.589 mg/mL and the optimal time was 10 minutes to produce flavonoid concentration of 56.777 mg/mL.	[19]
Tectona grandis	Ultrasound Assisted Extraction (UAE)	This study used an ultrasonic bath with a frequency of 40 kHz with 70% ethanol solvent. The most optimal total flavonoid compound content was obtained when the solvent ratio was 1:5 and the extraction time was 30 minutes as much as 6.688%	[20]

Materials	Methods	Results	Ref
Muntingia calabura L.	Soxhletation	This study used ethanol solvent with a sample-solvent ratio of 1:5 and an extraction temperature of 70°C. The tannin compound produced was 13.715 mg/L.	[21]
Avocado leaves	Ultrasound Assisted Extraction (UAE)	This study used ethanol solvent with a sample-solvent ratio of 1:10 and an extraction time of 40 minutes. The resulting tannin compound was 4.8 mg/L.	[22]
Etlingera elatior	Maceration	This study used ethanol solvent with an extraction time of 4 days. The alkaloid compound produced was 0.536 mg/L.	[23]

Phytochemical compounds can be obtained through an extraction process. The effectiveness of the extraction process depends on the type of solvent used [24]. The use of conventional extraction methods, such as maceration, takes longer and requires more solvent volume [25]. In addition, the MAE extraction process uses high temperatures so it will cause problems with the phytochemical compounds contained in turmeric leaves because they have thermolabile properties [26]. Based on these problems, an extraction method is needed that can extract phytochemical compounds in turmeric leaves by maintaining good extract quality, short extraction time, and producing high yields. However, it still maintains the concept of energy efficiency by using a small amount of solvent.

The novelty of this research is the extraction method, namely Ultrasound-Assisted Extraction (UAE). This study aims to determine the effect of sample-solvent ratio, time, and particle size on total flavonoid, tannin, and alkaloid compounds, as well as to determine the optimal storage time of fresh tuna. Aquabidest is used as a solvent in the extraction process because aquabidest is the result of a multistage distillation process (through 2 distillation processes) so its content is purer and safer as a solvent for food bioformalin. Based on the research of Susanti *et al*., showed that aquabidest solvent can produce the highest percent yield when compared to ethanol and methanol solvents. This is because aquabidest solvent has a higher solubility than ethanol and methanol solvents. The solubility of the solvent used in the extraction process can affect the percentage yield obtained. The high percentage of extract yield is because the aquabidest solvent can dissolve organic compounds contained in the plant optimally [27]. This extraction method utilizes ultrasonic waves that cause the formation of bubbles in the solvent to accelerate the rupture of cell walls and the release of bioactive compounds into the solvent more easily and quickly [28].

2. Metode

2.1 Materials

The materials used are aluminum chloride ($AlCl_3$) 10% Merck; aluminum foil Klin Pak; aquabidest solvent Merck; BCG solution Merck; caffeine Sigma-Aldrich; chloroform grade Pa

Smart-Lab; Folin-Ciocalteu reagent Merck; phosphate buffer pH 4.7 Supelco; quercetin Sigma-Aldrich; saturated sodium carbonate (Na_2CO_3) Merck; sodium hydroxide (NaOH) 1 M Supelco; sodium nitrite ($NaNO_2$) Merck; tannic acid Merck; tuna; and turmeric leaves from Bangsalsari, Jember.

2.2 Equipment

This research requires equipment, namely 60, 80, and 100 mesh test sieves; basin; BK-1200 digital ultrasonic bath; cuvette; filter paper; IKA rotary evaporator; Ohaus analytical balance; Pyrex glassware; Pyrex pipette; sharp oven; spatula; spoon; thermo scientific cimarec hot plate; UV-VIS spectrophotometric apparatus model 752AP; and vial bottle.

2.3 Research Variables

The independent variables used are the sample-solvent ratio of 1:10, 1:15, and 1:20; time of 10, 20, and 30 minutes; particle size of 60, 80, and 100 mesh, the fixed variables used are UAE frequency of 40 kHz, ultrasound temperature of 45℃, and water content of 4.5%, and the dependent variables used are total flavonoid compounds, total tannin compounds, and total alkaloid compounds.

2.4 Methods

2.4.1 Preparation of Equipment and Materials

Turmeric leaves were cut into pieces and dried using sunlight for 3 days to reduce the water content to obtain a water content of 4.5%. The dried turmeric leaves were then pulverized and sieved with 60, 80, and 100 mesh sieves.

2.4.2 Extraction Process

Turmeric leaves simplicial will be extracted using the UAE equipment to obtain phytochemical compounds with the help of aquabidest solvent. The extraction process uses three different variables to obtain optimal results. The extraction process using the UAE method is that 5 grams of turmeric leave simplicial mass is weighed three times for each particle size variable and put into a beaker glass, then aquabidest solvent is added with a sample-solvent ratio of 1:10, 1:15, and 1:20. The extraction process was carried out with an ultrasonic bath at a frequency of 40 kHz and a temperature of 45℃ for 10, 20, and 30 minutes. The filtrate obtained was filtered using filter paper and evaporated using a rotary evaporator at 75℃. The filtrate obtained was heated using an oven at 70℃ until the mass was constant. The turmeric leaf extract obtained will be tested for phytochemical compounds using UV-Vis

spectrophotometry [20]. The experiment was conducted in accordance with the results of the Design Expert Version 13 analysis using the Box Behnken Design method. The methods contained in Design Expert Version 13 consist of Composite Central Design (CCD), Box Behnken Design (BBD), and full three-level factorial design. The advantage of Box Behnken Design over the full three-level factorial design and CCD is that when using the same number of variables BBD produces a smaller number of experiments so it is more efficient and can reduce the cost of the experiment. Experiments using CCD, have the potential for failure in the process of forming nanoemulsion systems because there are extreme points of the test [29]. The selection of the BBD method in this study is because BBD is suitable for further research, while the CCD method will reveal new variables whose values are very complicated.

2.4.3 Measurement of Total Flavonoid Compounds

2.4.3.1 Preparation of Quercetin Standard Solution

Quercetin weighed as much as 10 mg and dissolved into 100 mL of aquabidest to obtain 100 ppm standard quercetin solution. A standard quercetin solution was prepared using several variables, such as 0, 20, 40, 60, and 80 ppm. The quercetin standard solution was taken as much as 0.5 mL using a pipette and then added 0.1 mL $NaNO_2$ 5%; 0.1 mL aluminum (III) chloride 10%; and 0.2 mL NaOH 1 M at minutes 0, 5, and 6. In the next stage, aquabidest was added until the total volume of the solution was 2.5 mL. The absorbance values of all standard solution concentrations were measured using a Uv-Vis spectrophotometer with a wavelength of 435 nm [12].

2.4.3.2 Preparation of Quercetin Standard Curve

The standard curve was made by correlating the concentration of standard quercetin solution with the absorbance shown in the measurement by UV-Vis spectrophotometry using a wavelength of 435 nm [12]. After making a standard curve, a linear regression equation will be obtained as Equation 1 as follows:

$$y = mx + c \quad (1)$$

2.4.3.3 Analysis of Total Flavonoid Compounds

Turmeric leaf extract of 100 mg was dissolved in 50 mL aquabidest solvent to obtain a solution concentration of 2000 ppm. 0.5 mL of the sample to be tested and then added 0.1 mL $NaNO_2$ 5%; 0.1 mL aluminum (III) chloride 10%; and 0.2 mL NaOH 1 M at minutes 0, 5, and 6. The next step, add aquabidest to a total solution volume of 2.5 mL. The solution was then

rested for 30 minutes. The absorbance of the calibration of the quercetin standard solution was measured by UV-Vis spectrophotometry using a wavelength of 435 nm. The total flavonoid compound content can be calculated by substituting the average absorbance value of the sample in Equation 1. The linear regression equation is obtained from the calibration curve of the quercetin standard solution that has previously been measured so that the total flavonoid compound is obtained [30].

2.4.4 Measurement of Total Tannin Compounds

2.4.4.1 Preparation of Tannic Acid Standard Solution

Tannic acid with an amount of 0.1 g was dissolved into 50 mL of aquabidest in a beaker glass, then the solution was poured into a 100 mL volumetric flask and aquabidest solvent until the limit mark. The tannic acid standard solution was made dilution variations of 0, 10, 20, 30, and 40 ppm. Each dilution variation was taken as much as 1 mL and put into a 10 mL volumetric flask which already contained 7.5 mL of aquabidest. Folin-Ciocalteau reagent was added to the flask as much as 0.5 mL. The solution was rested for 3 minutes, then 1 mL saturated Na_2CO_3 solution was added and the solution was rested for 15 minutes [31].

2.4.4.2 Determination of Maximum Wavelength

The maximum absorption wavelength can be determined by measuring the absorption of one of the standard solutions at a wavelength of 400-800 nm. The maximum wavelength is obtained if the wavelength produces the highest absorbance value [31].

2.4.4.3 Preparation of Tannic Acid Standard Curve

Various dilutions of the standard solution were read for tannin absorbance with a UV-Vis Spectrophotometer at the optimum wavelength of 640 nm. A standard curve was made by correlating the concentration of the standard solution with the absorbance resulting from measurements using a UV-Vis Spectrophotometer [31].

2.4.4.4 Analysis of Total Tannin Compounds

Turmeric leaf extract was weighed as much as 0.5 g, then dissolved in aquabidest until it reached a volume of 10 mL. Folin-Ciocalteau reagent was added to the flask as much as 0.5 mL. The solution was rested for 3 minutes, then 1 mL saturated Na_2CO_3 solution was added and the solution was rested for 15 minutes. The absorbance of the solution was measured using a UV-Vis Spectrophotometer at the maximum wavelength [31].

2.4.5 Measurement of Total Alkaloid Compounds

2.4.5.1 Preparation of Caffeine Standard Solution

Caffeine was weighed as 250 mg and dissolved into hot aquabidest, then diluted into a 250 mL volumetric flask to obtain a solution concentration of 1000 ppm. The solution was pipetted 2.5 mL and dissolved into aquabidest in a 25 mL volumetric flask to obtain a solution concentration of 100 ppm [32].

2.4.5.2 Determination of Maximum Wavelength

The absorbance of the caffeine solution was measured with a UV-Vis Spectrophotometer at a wavelength of 200-400 nm to determine the maximum wavelength. The maximum wavelength is obtained if the wavelength produces the highest absorbance value [32].

2.4.5.3 Preparation of Caffeine Standard Curve

A standard solution of 100 ppm caffeine was taken as much as 0; 0,3; 0,6; 0,9; 1,2; and 1.5 mL then diluted until the volume of the solution reached 10 mL so that the concentration of the standard solution was 0, 3, 6, 9, and 12 ppm. The standard solution was pipetted as much as 1 mL then added 1 mL phosphate buffer pH 4.7 and added 1 mL BCG solution, then the solution was extracted using 1.5 mL chloroform three times using vortex and separated chloroform phase. The extracted solution was then collected in a 10 mL volumetric flask and added chloroform to the limit mark [33]. The absorbance was measured using a UV-Vis Spectrophotometer at the optimum wavelength of 273 nm. A standard curve was created by correlating the concentration of the standard solution with the absorbance resulting from measurements using a UV-Vis Spectrophotometer [32].

2.4.5.4 Analysis of Total Alkaloid Compounds

Turmeric leaf extract was weighed as much as 10 mg and dissolved in 10 mL aquabidest to produce a solution concentration of 1000 ppm. The sample solution was pipetted as much as 1 mL and then added 1 mL phosphate buffer pH 4.7 and 1 mL BCG solution, then the solution was extracted using 1.5 mL chloroform three times using a vortex and separated the chloroform phase. The extracted solution was then collected in a 10 mL volumetric flask and added chloroform to the limit mark and the absorbance was measured using a UV-Vis Spectrophotometer at the maximum wavelength [33]. The absorbance that has been obtained is entered into the regression equation of the caffeine standard solution so that the total alkaloid compounds in turmeric leaves are known.

2.4.6 Determination of Optimum Shelf Time Based on Organoleptic Test

The extracted phytochemical compounds were then made into concentrations of 10% and 20%. Apply to fresh tuna. Tuna that has been washed with running water, then soaked in each treatment concentration of turmeric leaves extract (10% and 20%) for 2 hours. Soaking aims to allow the turmeric leaf extract to be absorbed by the flesh of fresh tuna (*E. affinis*). Fish that had been soaked with turmeric leaf extract was left in the open room for the specified time limit of 12 hours, 24 hours, and 36 hours at room temperature (25-28℃) and compared with fresh tuna without natural preservatives. Determination of the optimum shelf life was done by organoleptic test including aroma, texture, and color [34]. Analysis of the quality of tuna meat was carried out based on (SNI 01-2729.1-2006) by referring to the fresh fish organoleptic score sheet. Assessment includes meat (color and appearance), aroma, and texture. Fresh tuna has characteristics, such as color or appearance of brightly colored meat with no redness, a very fresh smell typical of tuna, and a solid texture and not slimy. The following is the assessment standard based on SNI 01-2729.1-2006:

a. Fresh: organoleptic score 7-9
b. Slightly fresh: organoleptic score 5-6
c. Not fresh: organoleptic score 1-3

3. Result and Discussion

3.1 Effect of Variables on Total Flavonoids

Determination of total flavonoid compounds using a standard solution of quercetin. Curve preparation of quercetin standard solution with concentrations of 0, 20, 40, 60, and 80 ppm and measured using a wavelength of 435 nm resulted in a linear regression equation as follows:

$$y = 0.00013x + 0.0645 \quad (2)$$

with an R-value of 0.9753. The R-value that is close to 1 indicates that the regression equation has met the linearity requirement [35]. Table 2 shows the total analysis of phytochemical compounds contained in turmeric leaves.

Table 2. Total Phytochemical Compounds of Turmeric Leaves

Run	Sample-Solvent Ratio (g/mL)	Particle Size (mesh)	Time (minutes)	Total Flavonoids (mg/L)	Total Tannin (mg/L)	Total Alkaloids (mg/L)
1	5 g/ 50 mL	60	20	55	41.261	6.246
2	5 g/ 75 mL	80	20	81.923	40.329	9.015
3	5 g/ 100 mL	100	20	98.076	37.428	8.092
4	5 g/ 75 mL	80	20	80.385	40.442	9.477

Run	Sample-Solvent Ratio (g/mL)	Particle Size (mesh)	Time (minutes)	Total Flavonoids (mg/L)	Total Tannin (mg/L)	Total Alkaloids (mg/L)
5	5 g/ 75 mL	80	20	87.307	40.355	8.707
6	5 g/ 50 mL	100	20	71.153	39.693	10.092
7	5 g/ 75 mL	100	10	87.300	39.083	7.630
8	5 g/ 50 mL	80	10	57.500	41.017	6.400
9	5 g/ 75 mL	80	20	80.076	39.972	9.323
10	5 g/ 75 mL	60	10	65	41.697	7.323
11	5 g/ 100 mL	60	20	89.615	40.077	9.169
12	5 g/ 75 mL	80	20	89.610	40.146	9.631
13	5 g/ 100 mL	80	10	82.692	40.703	8.400
14	5 g/ 100 mL	80	30	93.461	37.463	6.707
15	5 g/ 75 mL	60	30	77.308	39.501	6.861
16	5 g/ 50 mL	80	30	75.769	39.606	7.476
17	5 g/ 75 mL	100	30	91.153	37.185	6.553

Table 2 shows that the lowest total flavonoid compound content was obtained when the research parameters were 1:10 sample-solvent ratio, 60 mesh turmeric leaves particle size, and 20 minutes extraction time, which amounted to 55 mg/L. The highest total flavonoid compound was obtained when the research parameters were 1:20 sample-solvent ratio, 100 mesh turmeric leaves particle size, and 20 minutes extraction time, which amounted to 98.076 mg/L. Based on Table 2, it can be concluded that the extraction variables greatly affect the extraction process.

3.1.1 Statistical Analysis

The optimum variables in this study were analyzed using Box-Bahnken Response Surface Methodology (RSM) with 17 running. The resulting data in the form of total flavonoid levels were then analyzed using analysis of variance (ANOVA) on design experts. This analysis was conducted to determine whether the variables used in the extraction process could have a significant effect on total flavonoids. ANOVA results of total flavonoid levels with a linear model can be seen in Table 3 as follows:

Table 3. ANOVA Analysis for Flavonoid Content

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	2158.81	3	719.60	39.79	< 0.0001	significant
A-Sample-Solvent Ratio	1441.98	1	1441.98	79.73	< 0.0001	
B-Particle Size	461.46	1	461.46	25.51	0.0002	
C-Time	255.36	1	255.36	14.12	0.0024	
Residual	235.12	13	18.09			
Lack of Fit	160.03	9	17.78	0.9472	0.5685	not significant
Pure Error	75.09	4	18.77			
Cor Total	2393.93	16				

In this study, the p-value of the feed-solvent ratio was <0.0001; particle size was <0.0001; and extraction time was 0.0002. Variables can be said to have a significant effect if

the probability value (p-value) of the analysis results is <0.05 or 5%, while the p-value results on the lack of fit show> 0.05. The P-value can be interpreted as the error value of the statistical calculation results [36]. Based on Table 3, the three variables show a p-value <0.05; so the model from the analysis in this study is said to significantly influence the flavonoid levels produced. This model produces a coefficient of determination (R^2) of 0.9018 or 90.18% which indicates that the linear model is appropriate and can be used to analyze the flavonoid content produced from the UAE extraction method. The R^2 value of >70% indicates that the experimental value is quite precise with the value predicted by the design expert by giving results that are close to the 100% value [37]. The R^2 value of 0.9018 indicates that sample-solvent ratio, particle size, and extraction time have an influence of 90.18% on flavonoid content. The adjusted R^2 value is 0.8791, while the predicted R^2 value is 0.8316 so it can be said to be appropriate because the difference is <0.2.

The result of ANOVA analysis is a regression equation that describes the relationship between the predicted response, total flavonoids (Y), and the variables tested. This can be expressed using a linear equation model as follows:

$$Y = 77.98 – 13.08A + 7.59B + 5.65C \quad (3)$$

Description: Y = Total flavonoids (mg/L)

A = Sample-solvent ratio (g/mL)

B = Particle size (mesh)

C = Time (minute)

The regression equation can be used to calculate the total flavonoid value if the variable values of solvent ratio, particle size, and time are different from the known values. The coefficients of sample-solvent ratio, particle size, and time show the amount of increase or decrease in the total flavonoid concentration value. Negative values of sample-solvent ratio, particle size, and time will decrease the value of total flavonoid concentration, while positive coefficients will increase the value of total flavonoids [38].

The relationship between the data generated from the experiment and the data from the design expert model can be seen in Figure 2 as follows:

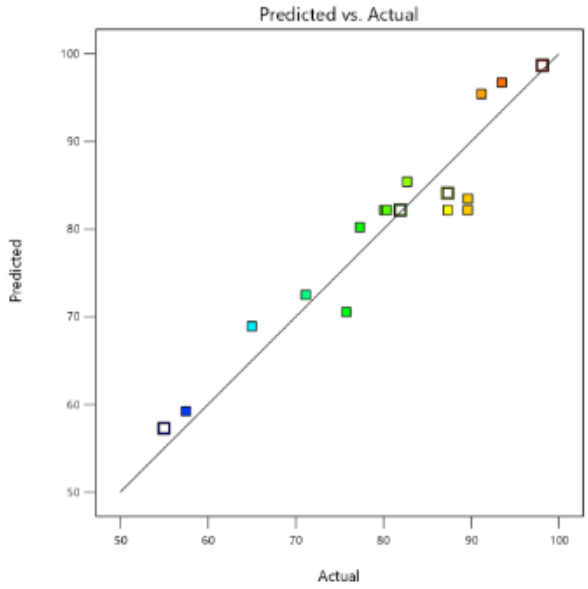
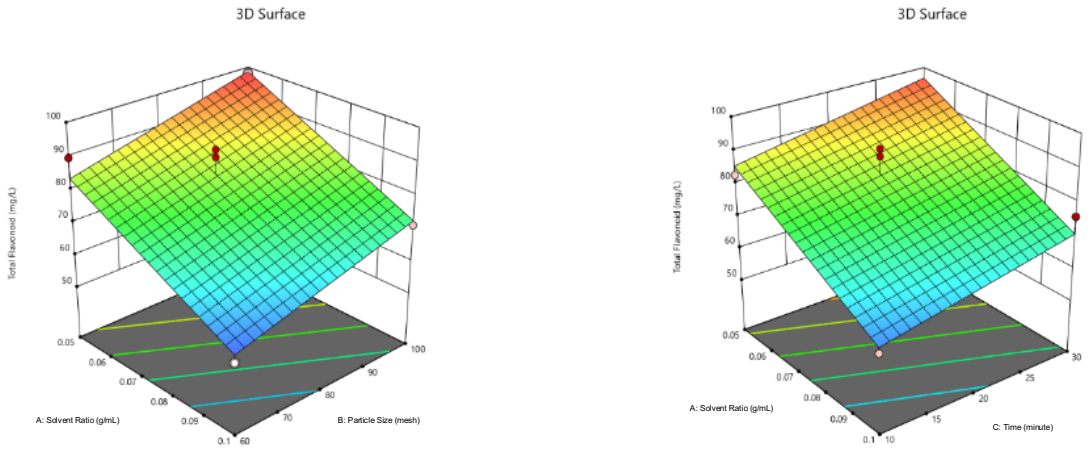


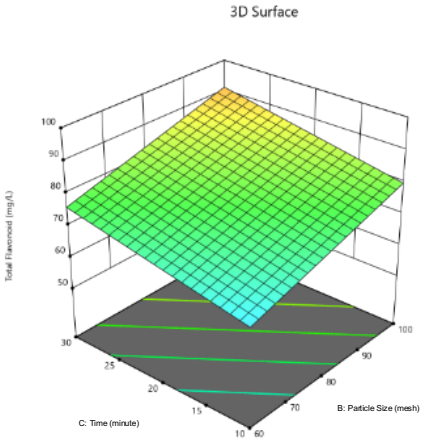
Figure 2. Relationship between model data and experimental data for flavonoid compound analysis

Figure 2 shows that the relationship between the experimental data that has been carried out and the predicted data is quite accurate, the layout distance between the experimental data and the tradeline shows fairly accurate data, this is characterized by the closer distance between the experimental data and the tradeline, the more accurate the data will be.

3.1.2 Effect of Extraction Parameters on Total Flavonoids



(a) (b)



(c)

Figure 3. Effect of extraction parameters on total flavonoid content (a) sample-solvent ratio with particle size, (b) sample-solvent ratio with time, (c) time with particle size

The effect of particle size on the extraction process is that the larger the mesh size or the smaller the particle diameter, the higher the flavonoid content produced, this is because the smaller the particle size in the sample, the solute will more easily reach the surface of the material to be extracted due to an increase in the contact surface area between the solid and the solvent. At smaller particle sizes, the solute diffusion path will be shorter on solid particles [39]. The next factor that can affect the flavonoid extraction process is the length of extraction time. The longer the extraction time, the higher the flavonoid content. This is because the longer the extraction time, the solvent can have a longer time to get into the particle cell wall and then remove the compounds in the particles so that the resulting flavonoid content will be higher. This is in line with research conducted by Isdiyanti *et al* [19]. The sample-solvent ratio can affect the flavonoid content produced, the greater the sample-solvent ratio used, the higher the flavonoid content produced. A higher sample-solvent ratio will increase the concentration gradient formed during the diffusion of solids into the solution, thereby increasing the extraction yield. This is in line with the research conducted by Mukti *et al* [12], that the optimum flavonoid content is produced at a sample-solvent ratio of 1:20.

Table 4. Comparison of Total Flavonoid Compound Results in Previous Studies

Raw material	Solvent	Method	Operating conditions	Total flavonoids (mg/L)	Reference
Turmeric Leaves	Methanol	Maceration	Extraction time 1x24 hours	48	[30]

Raw material	Solvent	Method	Operating conditions	Total flavonoids (mg/L)	Reference
Turmeric Leaves	Aquabidest	MAE	Sample-solvent ratio 1:20, power 10%, and extraction time 10 minutes	0.004	[12]
Turmeric Leaves	Aquabidest	UAE	Extraction time 20 minutes, particle size of turmeric leaves 100 mesh, and sample-solvent ratio 1:20	98.076	This research

Based on Table 4, the results of total flavonoid compounds of turmeric leaves from several literature are different. The total flavonoid compounds in this study showed higher results compared to the other two studies. This shows that the UAE extraction method is more optimal for extracting flavonoid compounds in turmeric leaves. The difference in results is due to differences in extraction conditions and methods used. In addition, extraction time and temperature also affect the total yield of flavonoid compounds obtained. The UAE method has the advantage of increasing the penetration speed of the solvent through the cell wall, the mass can move quickly, increasing the yield obtained, using low operating temperatures, requiring a small amount of solvent, and a short extraction time. Effendi's research [30] used the maceration method which was carried out at room temperature and did not use a benchmark for the amount of solvent used, so the solvent was added until the simplicial was submerged, while the research of Mukti *et al* [12], used the MAE method which was carried out with 199,5 watts of power. The use of a large wattage will also affect the total flavonoid compounds that can be extracted. This is because flavonoid compounds are susceptible to high temperatures.

3.2 Extraction of Total Tannin Content from Turmeric Leaves

Determination of total tannin compounds was carried out with a UV-Vis spectrophotometer using the Folin-Ciocalteu reagent. The standard solution used in the determination of total tannin compounds is tannic acid. The tannic acid standard solution curve was made with a solution concentration of 0, 10, 20, 30, and 40 and measured using an optimum wavelength of 640 nm to produce a linear regression equation as follows:

$$y = 0.0574x + 0.0946 \quad (4)$$

The equation is obtained by plotting the wavelength with the concentration of the solution. With the R-value of 0.9948. The R-value that is close to 1 indicates that the regression equation has met the linearity requirement [35].

Based on Table 2, the lowest total tannin content was obtained when the research parameters were 1:15 sample-solvent ratio, 100 mesh turmeric leaves particle size, and 30 minutes extraction time, which amounted to 37.1847 mg/L. The highest total tannin compound was obtained when the research parameters were 1:15 sample-solvent ratio, 60 mesh turmeric leaves particle size, and 10 minutes extraction time, which amounted to 41.6968 mg/L. The difference in the results of total tannin compounds in each experiment is due to the parameters used.

3.2.1 Statistical Analysis

Analysis of variance (ANOVA) with a quadratic model was used to analyze the data of total tannin compounds. In this study, the p-value of the sample-solvent ratio was <0.0001; particle size was <0.0001; and extraction time was <0.0001. Variables can be said to have a significant effect if the probability value (p-value) of the analysis results is <0.05 or 5%, while the p-value results in the lack of fit show >0.05. Based on Table 5, the three variables show a p-value <0.05; so, the model from the analysis in this study is said to significantly influence the tannin content produced. This model produces a coefficient of determination (R^2) of 0.9843 or 98.43% which indicates that the model is suitable and can be used to analyze the tannin content produced from the UAE extraction method. The R^2 value of >70% indicates that the experimental value is quite precise with the value predicted by the design expert by giving results that are close to 100%. The R^2 value of 0.9843 indicates that sample-solvent ratio, particle size, and extraction time have an influence of 98.43% on flavonoid content. The adjusted R^2 value is 0.9641, while the predicted R^2 value is 0.8079, so it can be said to be appropriate because the difference is <0.2.

Table 5. ANOVA analysis for tannin content

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	27.37	9	3.04	48.78	< 0.0001	significant
A-Sample-solvent ratio	4.36	1	4.36	69.93	< 0.0001	
B-Particle size	9.19	1	9.19	147.46	< 0.0001	
C-Time	8.04	1	8.04	129.03	< 0.0001	
AB	0.3393	1	0.3393	5.44	0.0524	
AC	0.7200	1	0.7200	11.55	0.0115	
BC	0.0221	1	0.0221	0.3548	0.5702	
A^2	0.6008	1	0.6008	9.64	0.0172	
B^2	0.9800	1	0.9800	15.72	0.0054	
C^2	0.6736	1	0.6736	10.80	0.0134	
Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Residual	0.4364	7	0.0623			
Lack of Fit	0.2942	3	0.0981	2.76	0.1759	not significant

Source	Sum of Squares	df	Mean Square	F-value	p-value
Pure Error	0.1422	4	0.0356		
Cor Total	27.81	16			

The amount of total tannin compounds (Y) as the response of the tested variables can be described in the equation model as follows:

$$Y = 40.53 + 0.7382A - 1.10B - 1.03C + 0.2841AB + 0.4138AC + 0.0744BC - 0.432A^2 - 0.4824B^2 - 0.4C^2 \quad (5)$$

Description: Y = Total tannin (mg/L)

A = Sample-solvent ratio (g/mL)

B = Particle size (mesh)

C = Time (minute)

The regression equation can be used to calculate the total tannin value if the variable values of the sample-solvent ratio, particle size, and time are different from the known values. The relationship between the data generated from the experiment and the data from the design expert model can be seen in Figure 4 as follows:

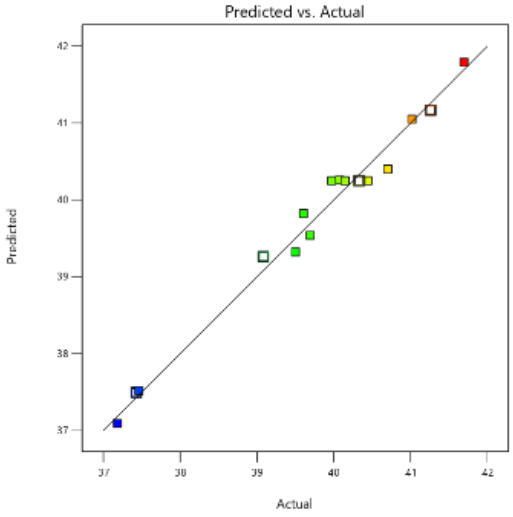


Figure 4. Relationship between model data and experimental data for tannin compound analysis

Figure 4 shows that the relationship between the experimental data that has been carried out and the predicted data is quite accurate, the layout distance between the experimental data and the tradeline shows fairly accurate data, this is characterized by the closer distance between the experimental data and the tradeline, the more accurate the data will be.

3.2.2 Effect of Extraction Parameters on Total Tannins

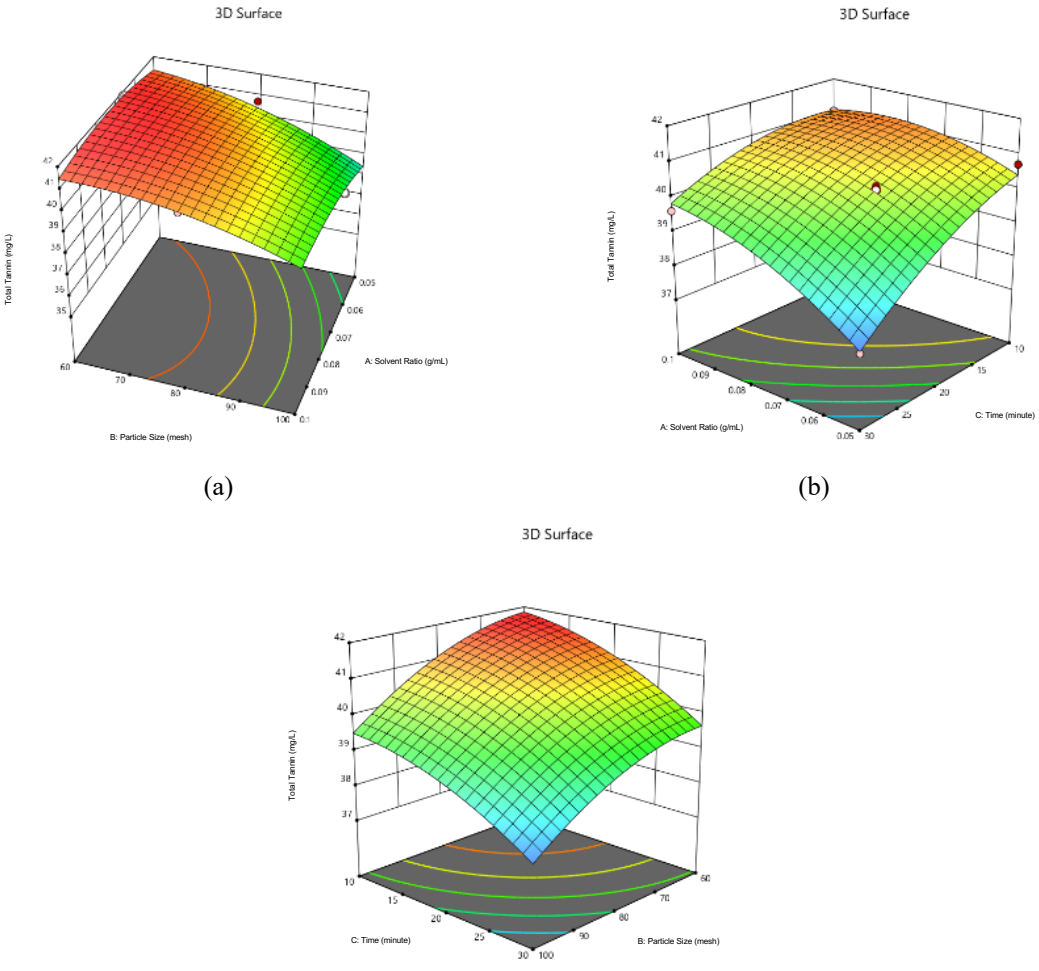


Figure 5. Effect of extraction parameters on total tannins (a) sample-solvent ratio with particle size, (b) sample-solvent ratio with time, (c) time with particle size

Based on Figure 5, it can be concluded that the total tannin content will decrease along with the longer the extraction time and the smaller the particle size (increasing mesh). This is in line with the research of Suharti, *et al*., that the longer time will cause tannin compounds to experience damage due to hydrolysis during the extraction process accompanied by a continuous heating process [40]. Based on the research of Rosalina, *et al*., the use of extraction time below 15 minutes aims to ensure that the media temperature during extraction does not exceed 45°C. The smaller the particle size, the greater the density between particles and the smaller the distance between particles. In this study, it can be seen that a particle size of 70 mesh produces tannin compounds that are less than optimal. This is because tannins are polar so in particle sizes below 70 mesh and short extraction times, tannin compounds will be extracted optimally. However, it is different from flavonoid compounds which require a particle

size of 100 mesh and a longer extraction time to extract flavonoid compounds optimally because flavonoids are semi-polar. The particle density can cause large obstacles to the ultrasonic waves during the propagation process towards the material. Thus, the higher the density of the material, the lower the propagation ability of the ultrasound waves [41]. The amount of tannin compounds will increase as the sample-solvent ratio increases, but the amount of tannin compounds will decrease when the volume of solvent used is excessive. This is in line with research conducted by Buanasari, that the more solvent used will inhibit the energy transfer process from ultrasonic waves to the solvent [42]. The solvent used in the extraction of tannin compounds is less than the extraction of flavonoids because the percentage of tannin compounds in turmeric leaves is lower at 2.58% compared to the percentage of flavonoids at 2.71%.

3.3 Extraction of Total Alkaloid Content from Turmeric Leaves

Determination of the total alkaloid compounds of turmeric leaves is done with two stages, including liquid extraction and UV-Vis spectrophotometer. The standard solution used in the determination of total alkaloid compounds is caffeine because caffeine is a xanthine group alkaloid compound with a crystalline form, easily soluble in water, and has a distinctive aroma and bitter taste [43]. Curve preparation of caffeine standard solution was carried out with solution concentrations of 0, 3, 6, 9, and 12 ppm and measured using an optimum wavelength of 273 nm to produce a linear regression equation as follows:

$$y = 0.0065x + 2.2984 \quad (6)$$

with an R-value of 0.9712. The R-value that is close to 1 indicates that the regression equation has met the linearity requirement [35].

Based on Table 2, the lowest total alkaloid compound content was obtained when the research parameters were 1:10 sample-solvent ratio, 60 mesh turmeric leaves particle size, and 20 minutes extraction time, which amounted to 6.246 mg/L. The highest total alkaloid compound was obtained when the research parameters were 1:10 sample-solvent ratio, 100 mesh turmeric leaves particle size, and 20 minutes extraction time, which amounted to 10.092 mg/L. Based on these data, it can be concluded that the extraction variables greatly affect the extraction results.

3.3.1 Statistical Analysis

Analysis of variance (ANOVA) with a quadratic model was used to analyze data on total alkaloid compounds. This study shows the resulting p-value is 0.0001; so the model of

analysis gives a real influence on the total alkaloid content. The variables of sample-solvent ratio, particle size, and extraction time have a p-value analysis result of 0.0507; 0.0022; and 0.2178 respectively. P-value <0.05 indicates that the model used is significant, while the insignificant model is characterized by a p-value greater than 0.1. From these data, it can be interpreted that the sample-solvent ratio and particle size variables have a real influence on the total alkaloids produced. While the time variable is insignificant it can be interpreted that the time variable has no real influence on the total alkaloids produced. This is expected because when alkaloid extraction uses ultrasonics, the time required is shorter, in contrast to the maceration extraction method. Ultrasonication produces mechanical agitation, cavitation, and thermal effects that can enhance the extraction process and the release of bioactive compounds. However, long ultrasonic times can cause degradation of the compounds present in the extract [44]. The results of the ANOVA analysis of tannin compounds are presented in Table 7 below:

Table 7. ANOVA analysis for alkaloid content

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	24.80	9	2.76	26.35	0.0001	significant
A-Sample-solvent ratio	0.5800	1	0.5800	5.55	0.0507	
B-Particle size	2.31	1	2.31	22.08	0.0022	
C-Time	0.1917	1	0.1917	1.83	0.2178	
AB	6.85	1	6.85	65.50	< 0.0001	
AC	2.02	1	2.02	19.35	0.0032	
BC	0.0946	1	0.0946	0.9043	0.3733	
A^2	0.2542	1	0.2542	2.43	0.1629	
B^2	1.02	1	1.02	9.76	0.0167	
C^2	11.41	1	11.41	109.16	< 0.0001	
Residual	0.7319	7	0.1046			
Lack of Fit	0.1817	3	0.0606	0.4404	0.7368	not significant
Pure Error	0.5502	4	0.1376			
Cor Total	25.53	16				

This model produces a coefficient of determination (R^2) of 0.9713 or 97.13% which indicates that the model is suitable and can be used to analyze the alkaloid content resulting from the UAE extraction method. The R^2 value of >70% indicates that the experimental value is quite precise with the value predicted by the design expert by providing results that are close to 100%. The adjusted R^2 value is 0.935, while the predicted R^2 value is 0.854 so it can be said to be appropriate because the difference is <0.2.

The total amount of alkaloid compounds (Y) as the response of the tested variables can be described in the Equation 7 model as follows:

$$Y = 9.17 - 0.2692A + 0.5502B - 0.1585C + 1.28AB + 0.6936AC - 0.1537BC - 0.2810A^2 - 0.4924B^2 - 1.65C^2 \quad (7)$$

Description: Y = Total alkaloids (mg/L)

A = Sample-solvent ratio (g/mL)

B = Particle size (mesh)

C = Time (minute)

The regression equation can be used to calculate the total alkaloid value if the variable values of the sample-solvent ratio, particle size, and time are different from the known values. The relationship between the data generated from the experiment and the data from the design expert model can be seen in Figure 6 as follows:

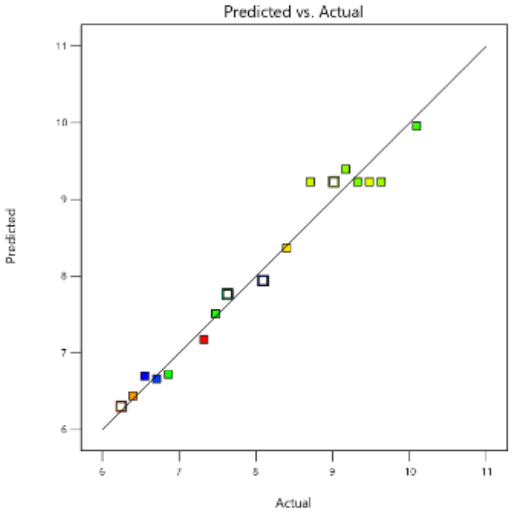


Figure 6. Relationship between model data and experimental data for analysis of alkaloid compounds

Figure 6 shows that the relationship between the experimental data that has been carried out and the predicted data is quite accurate, the layout distance between the experimental data and the tradeline shows fairly accurate data, this is characterized by the closer distance between the experimental data and the tradeline, the more accurate the data will be.

3.3.2 Effect of Extraction Parameters on Total Alkaloids

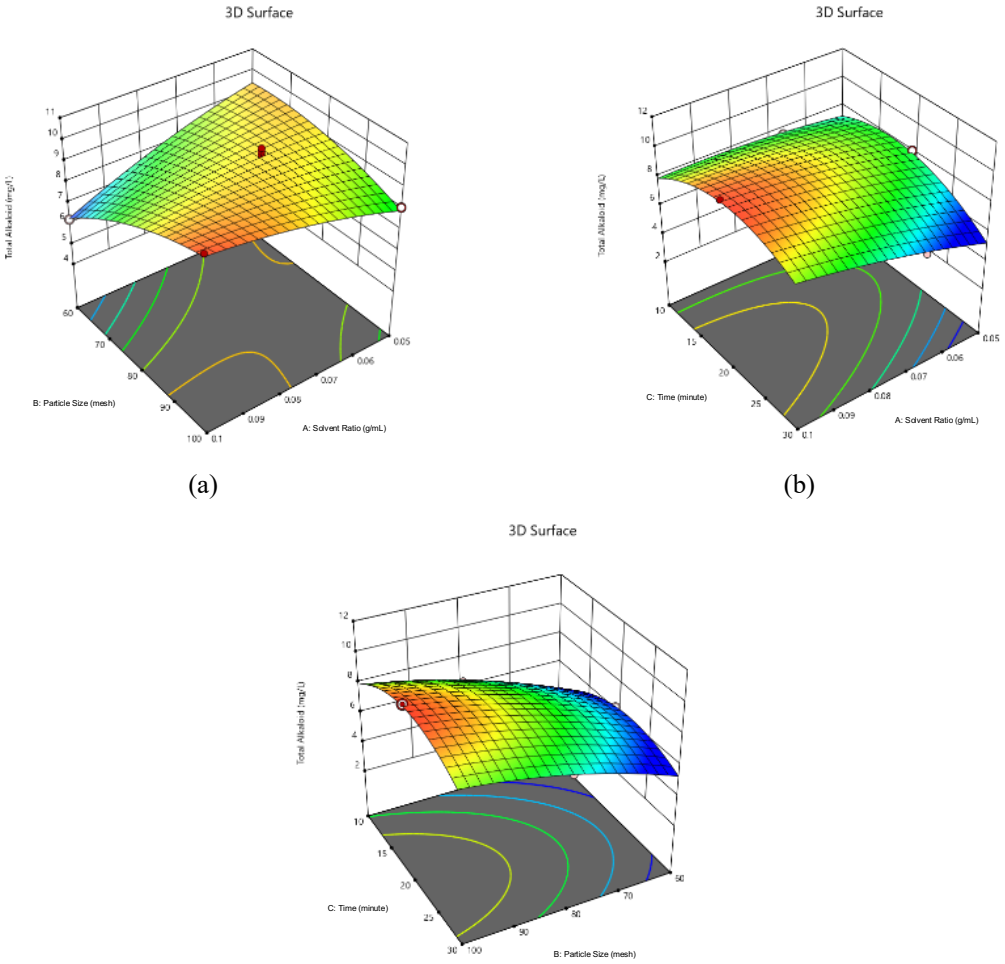


Figure 7. Effect of extraction parameters on total alkaloids (a) sample-solvent ratio with particle size, (b) sample-solvent ratio with time, (c) time with particle size

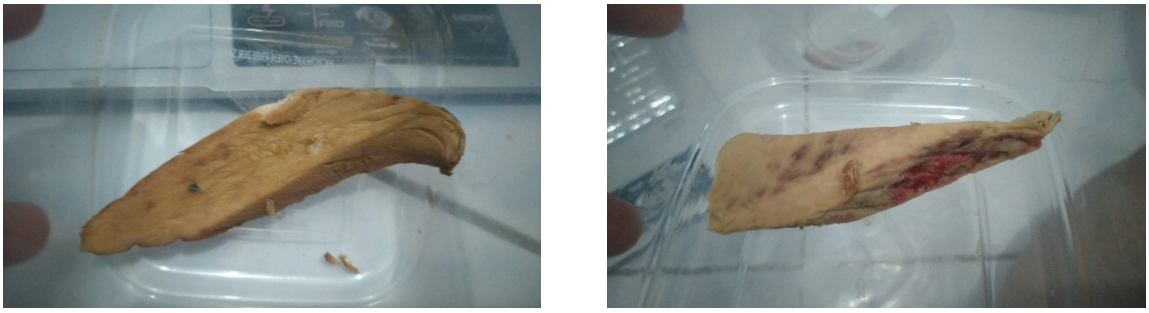
Based on Figure 7, the longer the extraction time, the more the obtained alkaloid compounds from a material. However, the longer extraction time will cause the extraction temperature to increase. Increased extraction time can provide an opportunity for the solvent to absorb ultrasonic waves for a longer time so that the heat generated from the process will increase the diffusion process of chemical compounds into the solvent. In this study, the total yield of alkaloid compounds increased from 10 minutes to 20 minutes of extraction time but decreased at 30 minutes of extraction time. This is because the percentage of alkaloids in turmeric leaves is small, so at an extraction time of 20 minutes the alkaloid compounds have been extracted perfectly. The use of a high sample-solvent ratio can increase the solvent's ability to absorb ultrasonic waves and convert them into heat energy so that it will easily extract

alkaloid compounds. However, the use of high solvent volumes can result in excessive swelling of the simplicial so that phytochemical compounds cannot be extracted optimally. The solvent used in the extraction of alkaloid compounds is less than the extraction of tannins because the percentage of alkaloid compounds in turmeric leaves is lower at 1.47% compared to the percentage of tannins which is 2.58%. Therefore, in this study the sample-solvent ratio of 1:10 produced the highest alkaloid compounds compared to the sample-solvent ratio of 1:15 and 1:20. This is consistent with the research of Mukhaimin *et al*. which states that the temperature of alkaloid degradation varies with each extraction method, if the extraction method used is direct heating of the material, then high temperatures can damage the alkaloid compounds contained in the material. In addition, modern extraction methods using high volumes of solvents can cause low yields of alkaloid compounds [45]. Extraction with the UAE method using a particle size of 100 mesh produces higher alkaloid levels of 10.092 mg/L compared to using a particle size of 60 mesh which is 6.246 mg/L. The opportunity to get high extraction results is by reducing the particle size, because the smaller the particle size (the larger the mesh), the surface area of a component increases so that the solvent can easily penetrate the wall of the material and bind the alkaloid compounds in it. This is following research conducted by Damanik *et al*., that the extraction process using a particle size of 100 mesh can produce a larger extract than with a particle size of 60 mesh. The smaller particle size will increase the number of pores formed in the sample powder so that the solubility of a substance can increase and the amount of solvent absorbed in the sample increases [46].

3.4 Analysis of Organoleptic Test

According to the results of smell, texture, and color analysis on tuna, it can be concluded that all treatments obtained the same results in the form of a decrease in quality value every 12 hours. Without the addition of bioformalin, tuna only lasts for 12 hours at room temperature. If more than 12 hours, tuna without bioformalin has characteristics such as slimy, moldy, rotten aroma, hard texture, and the meat turns red. Meanwhile, tuna with the addition of bioformalin can last for 36 hours at room temperature. If more than 36 hours, tuna with bioformalin starts to experience brownish discoloration, slimy texture, and slightly rotten aroma. The addition of bioformalin extract with a concentration of 20% is considered more optimal in the process of storing tuna compared to the addition of bioformalin with a concentration of 10%. The variables of sample-solvent ratio, time, and particle size respectively that produce optimum preservation time are 1:20 g/mL, 20 minutes, and 100 mesh. The highest rating was obtained for tuna added

with 20% turmeric leaf extract with variable ingredient ratios of 1:20, 100 mesh, and 20 minutes, namely a score of 9 for color, 8 for smell, and 8 for texture. The running has the highest flavonoid compounds, so it can be concluded that flavonoid compounds have a major effect on the preservation process of tuna. This is in line with research conducted by Rumayar *et al*. that the higher the concentration of extract used, the more optimal the storage time because it has good stability [47].



(a) (b)

Figure 8. Color difference in tuna with the addition of natural preservatives (a) and without the addition of natural preservatives (b)

4. Conclusions

Based on the results of this study, it can be concluded that the extraction variables sample-solvent ratio, time, and particle size greatly affect the total flavonoid, tannin, and alkaloid compounds. The highest total flavonoid compounds were obtained in the research variables of 1:20 sample-solvent ratio, 100 mesh turmeric leaves particle size, and 20 minutes extraction time, which amounted to 98.076 mg/L. The highest total tannin compound was obtained when the research variables were 1:15 sample-solvent ratio, 60 mesh turmeric leaves particle size, and 10 minutes extraction time, which amounted to 41.697 mg/L. The highest total alkaloid compound was obtained when the research variables were 1:10 sample-solvent ratio, 100 mesh turmeric leaves particle size, and 20 minutes extraction time, which amounted to 10.092 mg/L. The optimum preservation time was 36 hours at room temperature with variable sample-solvent ratio, time, and particle size of 1:20 g/mL, 20 minutes, and 100 mesh with 20% concentration. Running has the highest flavonoid compounds, so it can be concluded that flavonoid compounds have a major effect on the preservation process of tuna because flavonoids have the most important role in the preservation of tuna.

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