OPTIMIZATION OF MAKING PROCESS OF API-API (*Avicennia marina*) MANGROVE LEAVES STEW WITH RESPONSE SURFACE METHODOLOGY ANALYSIS

Shofwatun Nida[*], Eko Nurcahya Dewi, Putut Har Riyadi
Department of Fisheries Processing Technology, Faculty of Fisheries and Marine Science, Diponegoro University
Jl. Prof. H. Soedarto S.H, Tembalang, Semarang, Central Jawa, Indonesia 50275
*Corresponding author: shofwatunnida01@gmail.com

ARTICLE INFO	ABSTRACT
	The leaves of the A. marina have the potential to be developed into functional foods such as tea. It contains alkaloids, saponins, tannins, phenolics, flavonoids, triterpenoids and glycosides, with antioxidants, antimicrobials, antifungals and antibiotics potential. This study want to examine the effect of oven duration and stewing duration on the stewing quality of A. marina leaves and to determine the optimum oven duration and stewing duration using analysis of Response Surface Methodology (RSM). The lower limit of the oven time used is 2 hours and the upper limit is 6 hours, while the lower limit for stewing is 5 minutes and the upper limit is 15 minutes. Analysis of the data carried out is a test for tannin levels, antioxidant activity test and hedonic test. Data of tannins and antioxidant activity processed using Software Design Expert 11. The progam suggests formulate with oven duration is 4 hours and stewing duration is10 inutes. The result of the tannin test are prdictd to produce 2.8676% and antioxidant activity 1.282 ppm. The desirability value is 0.961. that means the resulting formula is matchs.

INTRODUCTION

Avicenna marina or mangrove api-api is one of mangrove with abundant availability in Indonesia. Behbahani *et al.* (2018) stated that *A. marina* is a potential source of functional food because it has metabolite compounds such as alkaloids, flavonoids, steroids, terpenoids, tannins, and saponins. Its metabolites has potential as antioxidants, antimicrobials, antifungals and antibiotics (Rise *et al.,*2012). *A. marina* has a high potential as a component of functional food like herbal tea (Hardoko *et al.,* 2019).

Tea is a functional drink which is generally made from the shoots of *Camelia sinensis* (Noriko, 2008). The researchers stated that the secondary metabolites in tea

leaves include alkaloids, flavonoids, steroids, saponins, triterpenoids (Damayanti *et al.*, 2021). Over time, tea raw materials have developed. Many green plants with high levels of antioxidants are used as raw materials for tea making. Tea processing such as drying and stewing resulted in a decrease of tannin levels and antioxidant activity because these bioactive compounds are unstable in heat. A'yunin *et al.* (2019) stated that phenolic or polyphenolic compounds are relatively unstable to heat. The length of heating time on the tannin level of areca nut has an effect on the tannin level produced (Karina *et al.*, 2016).

High temperatures and longer drying reduce antioxidant activity (Sari, 2015). Different temperatures and stewing times have an effect on the level of metabolites dissolved in steeping water, one of which is antioxidant activity. Ajisaka (2012) stated that the stewing process aims to maintain the quality of the desired compounds, so that there is no degradation of the chemical content of tea. The short stewing time and the low temperature cause lower tannin compounds (Nindyasari, 2012). The length of time for oven and stewing affect the content of bioactive compounds (tannin content), antioxidant activity, and the level of preference such as color, aroma and taste. It is necessary to optimize using the *Response Surface Methodology* (RSM) with *Central Composite Design* (CCD) using two factors, namely oven time and stewing time.

The purpose of this study was to determine the effect of different drying method in *A. marina*'s antioxidant activity, tannin levels and hedonic tests. This study also want determine the optimum oven and steeping time on antioxidant activity and tannin levels using *Response Surface Methodology (RSM)* which is proven to be effective in optimizing and monitoring food processes CRiyadi *et al.,* 2019).

MATERIALS AND METHODS

Subjects

This study used *A. marina* leaves obtained from Mangrove Park, Kandang Panjang, Pekalongan, Central Java, Indonesia (**Figure 1**).

Place and time of research

The research was conducted at the Processing Laboratory in the Department of Fishery Processing Technology, Diponegoro University. Tannin levels were carried out by the Chem-Mix Pratama Laboratory, Yogyakarta from September to October 2021.

Picture 1. Mangrove Park, Kandang Panjang, Pekalongan

Tools and materials

The tools used are *beaker glass, blender*, oven, *thermometer* and scales. The main material used in this study was *A. marina* leaves with a leaf length of ± 6 cm.

Research procedures

The drying process was carried out using an oven at 50℃, referring to Aziz (2019). The stewing process is in accordance with SNI 01-1902-1995 at 100℃. The oven and stewing times were modified according to the *Central Composite Design* (CCD). The optimization stage was carried out with a CCD design with the *Response Surface Methodology* (RSM) method using 5 *center points* and producing 13 experimental treatment points, then each experiment was carried out by response analysis. Parameters tested for each response were tannin levels, antioxidant activity and hedonic tests (SNI 2346:2015).

Tannin Level Test (Adrianar et al., 2015)

A 80 ml of distilled water were added to 1 g of powdered sample. A 0.5 ml solution was then taken and 0.5 ml of *Follin Calcioteu* was added. The solution was incubated for 5 minutes. A 1 ml of sodium carbonate was added to the solution, allowed with 30 minutes incubation. The absorbance was read on a spectrophotometer 715 nm.

$$\text{Tanin} = \frac{10\,(A-B)\times N \times 0.00416}{Sample\,(g)} \times 100\%$$

Radical scavengin activity Test (Burda and Orzek, 2001)

Samples were taken as much as 2 ml with a concentration of 50 mg/L, 100 mg/L, 150 mg/L and 200 mg/L added each 1.5 ml of DPPH solution in ethanol and vortexed for 2 minutes. The purple color change into yellow indicates the efficiency of free radical scavengers. The absorbance was measured on a UV-Vis spectrophotometer on 517 nm after being incubated for 30 minutes. Then the comparison is observed. After the absorbance is obtained, the free radical scavenging activity (percent inhibition) is calculated as the percentage of DPPH color reduction.

$$\% \text{ Inhibisi} = \frac{Absorbansi\ blanko - absorbansi\ sample}{absorbsnsi\ blanko} \times 100\%$$

Optimization of RSM

Stages of process optimization with RSM using CCD. *software* used is Design Expert 11. The process of stewing *A.marina* carried out according to the variation of the combination of CCD determined by RSM with 2 factors are oven time and stewing time (**Table 1**).

Table 1. Model *design* CCD

Std	Run	Factor 1	Factor 2
		Oven Time (Hours)	**Stewing Time (Minutes)**
1	1	2	5
6	2	6	10
5	3	2	10
11	4	4	10
4	5	6	15
13	6	4	10
8	7	4	15
3	8	2	15
7	9	4	5
12	10	4	10
10	11	4	10
9	12	4	10
2	13	6	5

Data Analysis

Data analysis carried out on the steeping test of *A. marina* was a test for tannin levels and antioxidant activity. The test result data is then entered on the response surface

method and the centralized composite design or CCD. Data processing using *Software Design Expert* 11. The best result or response from the optimal solution is verified by making a steeping of *A. marina* in accordance with the optimal treatment of the predicted *response surface* and testing for tannin levels and antioxidant activity. Furthermore, the predicted value of the response surface is compared with the actual value.

RESULTS AND DISCUSSION

The content of tannins level in the stewing leaves *A. marina* has an attachment to antioxsidant activity. Data on the response of tannin levels and antioxidant activity are presented in (**Table 2**).

Table 2. Response Result Data

No.	Variables		Respon	
	Oven Time (Hours)	**Stew Time (Minutes)**	**Tannin Level (%)**	**Antioxidant Activity (ppm)**
1.	2	5	1,98	284,11
2.	6	10	1,81	309,18
3.	2	10	2,29	217,29
4.	4	10	2,92	145,22
5.	6	15	1,73	332,06
6.	4	10	2,87	148,71
7.	4	15	2,63	188,04
8.	2	15	2,17	255,37
9.	4	5	2,43	191,48
10.	4	10	2,83	167,81
11.	4	10	2,85	155,01
12	4	10	2,97	144,14
13.	6	5	1,62	357,72

Tannin level

The desired optimum response result is that the greater the tannin level produced, the greater the free radical scavenging. Tannin levels increased with 4 hours of oven time with 10 minutes of stewing (2,97%). Too long an oven will cause a decrease in tannin levels in the steeping of *A. marina*. The longer the heating, the tannin level tends to decrease (Karina *et al.*, 2016). Heating for 3 hours resulted in higher tannin levels than 2 and 4 hours. If the heating is too short, the tannin levels are less than optimal.

Tannin level is also affected by the stewing time. The longer the stewing, the more the compound is extracted. In the stewing process, an extraction process occurs, namely the withdrawal of soluble chemical compounds so that they are separated from the material with a liquid solvent. The longer the stewing time, the lower the tannin level due to a decrease in temperature in line with the stewing time (Mutmainnah *et al.*, 2018). Tannins are not extracted maximally at low temperatures. Sasmito *et al.* (2020) stated that the longer stewing at high temperatures resulted in a decrease in tannin levels, such as the breakdown of tannins into simpler phenols (Kim *et al.,* 2010).

Table 3. Results of ANOVA tanin level response

Source	Sum of Squares	df	Mean Squares	F-value	p-value	
Model	2,6	5	0,57	102,98	<0,0001	*significant*
A-Oven Time	0,27	1	0,27	49,08	0,0002	
B-Stew Time	0,04	1	0,04	7,49	0,02	
AB	0,0016	1	0,0016	0,28	0,60	
A²	1,55	1	1,55	279,00	<0,0001	
B²	0,20	1	0,20	36,10	0,0005	
Residual	0,03	7	0,0056			
Lack of Fit	0,02	3	0,0087	2,70	0,180	*not significant*
Pure Error	0,01	4	0,00322			
Cor Total	2,90	12				
Std. Dev.	0,07			R²		0,98
Mean	2,39			*Adjusted* R²		0,97
C.V. %	3,11			*Predicted* R²		0,92
				Adeq Precision		25,57

The model is declared significant and is called significant if a factor has a p*value* of less than 5% (<0.05). The criteria for these parameters are oven time (A) with a value of 0.0002 and stewing time (B) with a value of 0.02 (**Table 3**), which means that A and B have a significant (significant) effect on the response of tannin levels. The model has an *lack of fit* insignificant. This is because the model has a *lack of fit value* > 0.05, which is 0.180 which indicates that this value is not significant for pure error. The model is said to be appropriate to use.

The R² coefficient value is 0.98 which indicates that 98% of the *A. marina* leaf steeping is influenced by factors A and B **(Table 3)**. Only 2% of the factors that cannot be explained by the program. The resulting deviation value is 0.07. A low deviation value indicates the accuracy of the model. The relationship between the *predicted* R² 0.92 and

Adjusted R² 0.97 values is appropriate to conclude that they interact well. This is because the *predicted* R² and *Adjusted* R² have a difference of less than 0.2. Value *Adeq precision* must be more than 4, namely 25.57, indicating that the model can be applied in research design modeling. The model equation for the Y response (tannin level) which is influenced by the length of the oven (A) and the length of the stewinging time (B) (**Figure 2**).

$$Y = -0.187*A^2 - 0.010*B^2 - 0.002*AB + 1.412*A + 0.240*B - 1.034$$
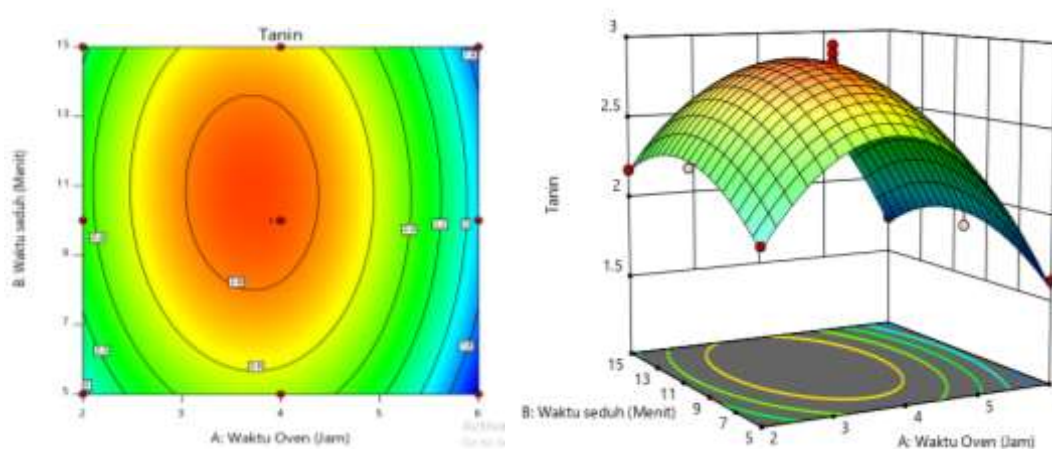

Figure 2. The 2D and 3D graphs of tannin level response

The effect of oven and stewing time is *quadratic* on the response of tannin levels. The influence of factors on the response of steeped tannin levels of *A. marina is* indicated by a horizontal line parallel to the X axis. The response to tannin levels will increase with increasing oven and stewing time. After reaching the optimal response, the tannin levels will decrease. This is because the longer the heating, the lower the tannin levels in the *A.marina*. Karina *et al.* (2016), also stated that the heating time on the tannin level of areca nut has an effect on the tannin level produced. The highest tannin level was found in the 3-hour heating period, which was 10.88%, compared to the 2-hour and 4-hour heating period of 10.79% and 10.87%, respectively.

The Radical Scavenging Activity

Antioxidant activity (Table 1) was found at 4 hours of oven time and 10 minutes of steeping time of 144.14 ppm and classified as moderate antioxidant activity. Molyneux, (2004), states that the classification of antioxidant activity is very strong ($IC_{50} < 50$ ppm),

strong (50 ppm $<$ IC$_{50}$ $<$ 100 ppm), moderate (100 ppm $<$ IC$_{50}$ $<$ 150 ppm), weak (150 ppm). $<$ IC$_{50}$ $<$ 200 ppm) and very weak (IC$_{50}$ $>$ 200 ppm). Oven time and stewing time affect the IC$_{50}$ steeping *A.marina leaves.*

The results of the antioxidant activity of steeping *A. marina* were positively correlated with the content of tannins. The more tannin level, the greater the antioxidant activity because tannins are composed of polyphenolic compounds that have free radical scavenging activity. The tannin contained in the leaves act as antioxidants by donating hydrogen. In the same treatment factor with 4 hours oven time and 10 minutes stewing time, the tannin levels and antioxidant activity produced were 2.97% and 144.14 ppm, respectively. According to Hidayah *et al.* (2018), there is a positive correlation between the increase in total phenolics, flavonoids, and total tannins with free radical scavenging activity. According to Rafsanjani and Putri (2015) stated that the more tannin level, the greater the antioxidant activity because tannins are composed of polyphenolic compounds that have free radical scavenging antioxidant activity. Thus, the best contributors to antioxidant activity were tannins, total phenolics, and total flavonoids, respectively.

Table 4. The ANOVA result of the radical scavenging activity

Source	Sum of Squares	df	Mean Squares	F-value	p-value	
Model	69268,56	5	13853.71	140.93	$<$ 0.0001	*significant*
A-Oven Time	9776,00	1	9776.00	99.45	$<$ 0.0001	
B-Stew Time	557,58	1	557,58	5,67	0,0488	
AB	2,37	1	2,37	0,0241	0,8810	
A²	36015,96	1	36015,96	366,37	$<$ 0,0001	
B²	4579,33	1	4579,33	46,58	0,0002	
Residual	688,14	7	98,31			
Lack of Fit	310,71	3	103,57	1,10	0,4470	*not significant*
Pure Error	377,43	4	94,36			
Cor Total	6995,70	12				

Std. Dev.	9,91	R²	0,99	
Mean	222,78	*Adjusted* R²	0,98	
C.V. %	4,45	*Predicted* R²	0,94	
		Adeq Precision	30,30	

Oven and stewing time affect the process of this model. The *lack of fit* is shown to be insignificant. The parameter that affects the discrepancy of the model is the p-*value*. The p-*value of lack of fi* is 0.447 which means greater than 0.05 and not significant (**Table 4)**. *Lack of fit* does not indicate that the model is good. In order to have a significant effect, the p-*value* must be less than 5% ($<$0.05) (Fathur *et al.,* 2018).

The R² coefficient value is 0.99 which indicates that 99% of the *A.marina* influenced by independent variables and only 1% of all variables that cannot be explained by the model. The relationship between the *predicted* R^2 0.98 and *Adjusted* R^2 0.94 values is appropriate and interacts well because it has a difference of less than 0.2. The *adeq precision* must have a ratio of more than 4, the *adeq precision* is 30.30 which means that it indicates an adequate signal **(Table 4)**. The equation for the Y response (antioxidant activity) which is influenced by the length of the oven (A) and the length of the stewing time (B) **(Figure 2).**

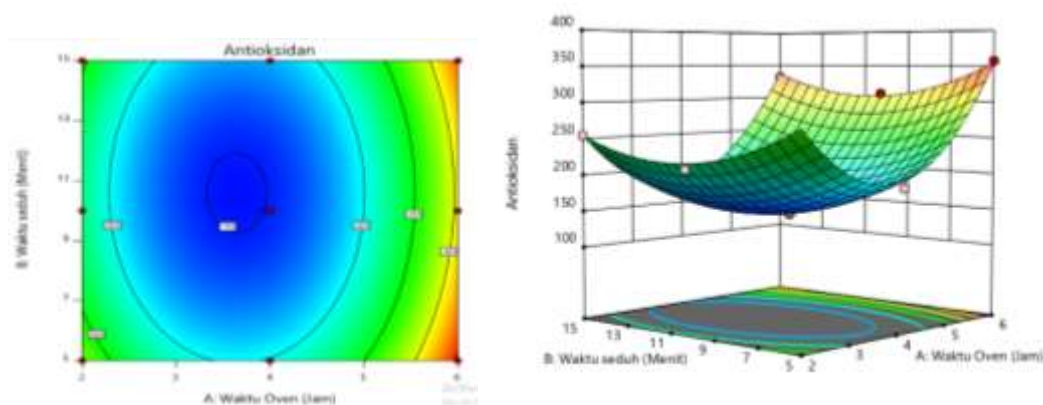$$Y = 28,548*A^2 + 1,628*B^2 + 0,077*AB - 208,975*A - 34.811*B + 712.563$$

Figure 3. The 2D and 3D graphs of antioxidant activity response

The influence of these two factor variables on the response is *quadratic.* The quadratic model is in the form of an open upward parabola in which the optimization shows minimal results. The effect of oven time and stewing time on the response of antioxidant activity can be seen by following a horizontal line parallel to the X axis. The antioxidant response (IC_{50}) will decrease with increasing oven and stewing time until the optimal point, then the antioxidant activity (IC_{50}) will increase return. This is due to its unstable antioxidant properties against heat. Minimal antioxidant activity was obtained at 4 hours of oven treatment and 10 minutes of stewing time with a value of 144.14 ppm Equator *et al.* (2020), stated that heating for a long time and using high temperatures can reduce antioxidant activity. Drying at low temperatures will result in long oven time, decay, and low antioxidant activity due to the inactivity of the polyphenol oxidase enzyme.

Optimizing steeping response of A. marina leaves

Oven and stewing time are optimized components with a range of upper and lower limits (**Table 5**). Both can affect the levels of tannins and antioxidant activity of the *A. marina* leaves. The response of tannin levels was chosen with a maximum target because the goal to be achieved was to get the maximum antioxidant activity and antioxidant activity with a minimal target tho obtain the effectiveness of counteracting free radicals. The *Design Expert* 11 program provides a solution that is close to the target.

Table 5. Optimization of response components, target, lowe limit, upper limit and levels of important in the formula optimization stage

Name	Goal	Lower Limit	Upper Limit	Importance
A:Oven Time	*is in range*	2	6	1
B:Stew Time	*is in range*	5	15	1
Tannin Level	*maximize*	1.62	2.97	1
Antioxidant Activity	*minimize*	144.14	357.72	1

The optimum results produced by the *software design expert* 11, with an oven time of 4 hours and a stewing time of 10 minutes, produced a tannin level of 2.885% and an antioxidant activity of 147.181 with a *desirability* 0.961 (**Table 6**). The formula solution is recommended as the optimal formula solution and then used as prediction validation because it has a *desirability* 0.961. According to Ramadhani *et al.* (2017), the optimal formula is a formula with a *desirability* value close to 1, indicating the ability of the program to produce the desired product is more perfect.

Table 6. Solutions produced in the optimization stage

Number	Oven Time	Stew Time	Tannin Levels	Antioxidant Activity	Desirability
1	4	10	2.885	147.181	0.961

Verification of optimum conditions for prediction results model

Verification is the process of examining the difference between the prediction results from the *software* and the analysis at the optimum point. The purpose of this verification is to confirm the actual optimal condition of the response of tannin levels and antioxidant activity. The optimal conditions for steeping *A. marina* were obtained at a long oven time of 3,680 hours and a stewing time of 10,702 minutes (**Table 7**).

Table 7. Comparison of actual validation results with program

Response	Prediction	Verification	95% CI low	95% CI high	95% PI low	95% PI high
Tannin Levels	2.862	2.71	2.78	2.93	2.67	3.05
Antioxidant Activity	151.28	170.497	141.54	161.01	125.89	176.66

The results of the response of tannin levels were lower and the value of antioxidant activity was higher than the predicted value **(Table 7)**. If the verification value is still in the range of 95% CI *low* and 95% CI *high*, then the solution recommended by *Design Expert* 11 software is considered good. According to Kusnandar *et al.* (2020), the comparison of the predicted and actual values can be done by verifying the optimization results. The model equation can be accepted that the actual verification results have conformity if it is within the CI and PI ranges.

Hedonic test

Color

The colour is dark red-brown to light yellowish brown. The drying process causes the green color of chlorophyll in the leaves to be oxidized to brown due to the browning event (Hermani, 2004). The highest average yield was sample A10 (6 hours of oven time, 10 minutes of brewing) with an average hedonic value of 8.80 (very favored by panelists) and light yellowish brown characteristics. This is influenced by the length of the oven and brewing time. This study is in accordance with Yamin *et al.* (2017), the fading of the color of the Chinese ketepeng leaf herbal tea is due to the degradation of the pigments in the Chinese ketepeng leaf, especially the chlorophyll pigment which is degraded to pheophytin causing the brown color to turn yellow.

Aroma

The highest aroma value was in sample A10 (6 hours oven, 10 minutes brewing) with an average hedonic value of 8.80 (very favored by panelists) and the characteristic aroma of *A. marina* faded. According to Sribudiani (2011), the longer the drying time, the less aroma of rosella herbal tea produced. This is due to the destruction of aromatic compounds in the drying process. According to Fellow (1988), the aroma in foodstuffs can be caused by volatile components, but these volatile components can be lost during the processing, especially heat.

Taste

The highest taste value in the A10 steeping sample (oven time 6 hours, brewing 10 minutes) was 7.93 (preferred by panelists) with a slightly bland characteristic. Irina and Mohamed (2012), stated that the bitter taste in food is usually caused by tannins. Tannins are one of the members of polyphenol compounds, namely compounds with a phenol group in their chemical structure found in plants, so they are often called plant polyphenols. Yang *et al.* (2007). The brewing process is the stage of the process of extracting aroma and flavor compounds by hot water.

CONCLUSION

The results of the analysis on *Design Expert* 11 software with CCD showed that the oven time and stewing time of *A.marina* had a significant effect on all responses. The validation results under optimal conditions for the stewing process of *A. marina* contained in the oven for 4 hours and a stewing time for 10 minutes, the tannin level of 2.86276% and antioxidant activity of 151.282 ppm with a *desirability* 0.961 were obtained.

REFERENCES

A'yunin, N. A. Q., Santoso, U., & Harmayani, E. (2019). Kajian kualitas dan aktivitas antioksidan bebagai formula minuman jamu kunyit asam. *Jurnal Teknoogi Pertanian Andalas*, *23*(1), 37-48. http://tpa.fateta.unand.ac.id/index.php/JTPA/article/view/184

Ajisaka. (2012). Teh Khasiatnya Dahsyat. Penerbit Stomata. Surabaya

Aziz, R. (2019). Kandungan antioksidan dan kadar air pada teh daun mangga quini (*Mangifera Indica*). *Journal of Agritech Science*, *3*(1), 1–9. https://doi.org/10.30869/jasc.v3i1.327

Behbahani, B. A., Yazdi, F. T., Shahidi, F., Noorbakhsh, H., Vasiee, A., & Alghooneh, A. (2018). Phytochemical analysis and antibacterial activities extracts of mangrove leaf against the growth of some pathogenic bacteria. *Microbial Pathogenesis,* 144, 225-232. https://doi.org/10.1016/j.micpath.2017.12.004

Damayanti, A. N., Riyadi, P. H., & Dewi, E. N. (2021). Characteristic and boactive potential of brewed *Sargassum* sp. with the additional bay leaf *(Syzygium polyanthum). IOP Conference Series: Earth and Environmental Science*, *890*(1), 012044.https://iopscience.iop.org/article/10.1088/1755-1315/890/1/012044/meta

Fellow, P. J. (1988). *Food Processing Technology Principle and Pactice*. Ellis Horwood. New York.

Hardoko., D., Nafiah., Sasmito, B. B., & Halim, Y. (2019). Antidiabetic activity of herbal green tea extract from white mangrove *(Avicennia marina)* leaves towards blood glucose level of diabetic wistar rats *(Rattus novergicus)*. *International Journal of Food Studies*, *8*, 43-52. https://iseki-food-ejournal.com/ojs/index.php/e-journal/article/view/553

Irina, I. & Mohamed, G. (2012). Biological activities and effects of food processing on flavonoids as phenolic antioxidants. *Advances in Applied Biotechlogy*. https://doi.org/10.5772/30690

Karina, Indrayani, Y., & Sirait, S. M. (2016). Kadar tanin biji pinang (*Areca Catechu* L.) berdasarkan lama pemanasan dan ukuran serbuk. *Jurnal Hutan Lestari*, *4*(1), 119-127. http://dx.doi.org/10.26418/jhl.v4i1.15083

Khatulistiwa, I. P. W. B., Permana, I. D. G. M., & Puspawati, I. G. A. K. D. (2020). Pengaruh suhu pengeringan oven terhadap aktivitas antioksidan bubuk daun cemcem (*Spondias Pinnata* (L.F) *Kurz*). *Jurnal Ilmu dan Teknologi Pangan*, *9* (3), 350-356. https://doi.org/10.24843/itepa.2020.v09.i03.p11

Kim, T. J., Silva, J. L., Kim, M. K., & Jung, Y. S. (2010). Enhanced antioxidant capacity and antimicrobial activity of tannic acid by thermal processing. Food Chemistry *118*(3), 740–746. https://doi.org/10.1016/j.foodchem.2009.05.060

Kusnandar, F., Mutmainah, M., & Muhandri, T. (2020). Optimasi proses pembuatan sohun dari pati ubi banggai (*Dioscorea alata*). *Jurnal Pangan dan Agroindustri*, *8*(3), 163-174. https://doi.org/10.21776/ub.jpa.2020.008.03.6

Mutmainnah, N., Chadijah, S., & Qaddafi, M. (2018). Penentuan suhu dan waktu optimum penyeduhan batang teh hijau (*Camelia Sinensis* L.) terhadap kandungan antioksidan kafein, tanin dan katekin. (Undergraduate Thesis, UIN Alauddin Makasar). Retrieved from http://repositori.uin-alauddin.ac.id/4118/

Molyneux, P. (2004). The use of the stable free radical diphenylpicrylhydrazyl (dpph) for estimating antioxidant activity. Songklanakarin Journal of Science and Technology, *26*(2), 211–219.

Nindyasari, S. (2012). Pengaruh suhu dan waktu penyeduhan teh hijau (*Camellia sinensis*) serta proses pencernaan *in vitro* terhadap aktivitas inhibisi lipase. (Undergraduate Thesis, IPB University).

Noriko, Nita. (2013). Potensi daun teh (*Camelia sinensiss*) dan daun anting-anting (*Acalypha indica* L.) dalam menghambat pertumbuhan *Salmonella typhi*. *Jurnal Al-Azhar Indonesia Seri Sains dan Teknologi, 2*(2), 104-110. http://dx.doi.org/10.36722/sst.v2i2.131

Rafsanjani, M. K. & Putri, W. D. R. (2015). Karakterisasi ekstrak jeruk bali menggunakan metode ultrasonik bath (kajian perbedaan pelarut dan lama ekstraksi). *Jurnal*

Pangan   dan   Agroindustri,   *3*(4),   1473-1480.
https://www.jpa.ub.ac.id/index.php/jpa/article/view/271

Rise, C. L. V., Prabhu, V. V., & Guruvayoorappan, C. (2012). Effect of marine mangrove *Avicennia marina* (Forssk.) Vierh against acetic acid–induced ulcerative colitis in experimental mice. *Journal of Environmental Pathology, Toxicology and Oncology*,   *31*(2),   179-192.
https://www.dl.begellhouse.com/journals/0ff459a57a4c08d0,27db4dfa659d34e6,
0620e99548a804fd.html

Ramadhani, R. A., Riyadi, D. H. S., Triwibowo, B., & Kusumaningtyas, R. D. (2017). Review pemanfaatan design expert untuk optimasi komposisi campuran minyak nabati sebagai bahan baku sintesis biodiesel. *Jurnal Teknik Kimia dan Lingkungan*, *1*(1), 11-16. http://dx.doi.org/10.33795/jtkl.v1i1.5

Riyadi, P. H., Suprayitno, E., & Sulistiati, T. D. (2019). Optimization of protein hydrolysate from visceral waste of nile tilapia *(Oreochromis niloticus)* by response surface methodology. *Aquaculture, Aquarium, Conservation & Legislation*, *12*(6),   2347-2358.
https://www.proquest.com/openview/900b72235157f5c1ede817ef8f13c361/1?p
q-origsite=gscholar&cbl=2046424

Sari, M. A. (2015). Aktivitas antioksidan teh daun alpukat (*Persea americana* Mill) dengan variasi teknik dan lama pengeringan. (Undergraduate Thesis, Universitas Muhammadiyah Surakarta).

Sasmito, B. B., Dwi, T., & Dearta, D. (2020). Pengaruh suhu dan waktu penyeduhan teh hijau *Sonneratia alba* terhadap aktivitas antioksidannya. *Journal of Fisheries and Marine   Research*,   *4*(1),   109-115.
https://doi.org/10.21776/ub.jfmr.2020.004.01.16

Sribudiani, E., Parlindungan, A. K., & Volliadi. (2011). Kajian suhu dan lama pengeringan terhadap kualitas organoleptik teh herbal rosella (*Hibiscus sabdariffa* Linn.). *Jurnal Sagu*, *10*(2), 9-15. http://dx.doi.org/10.31258/sagu.v10i02.1443

Yamin, M., Ayu, D. F., & Hamzah, F. (2017). Lama pengeringan terhadap aktivitas antioksidan dan mutu teh herbal daun ketepeng cina (*Cassia Alata* L.). *Jom Faperta*, *4*(2), 1-15. https://media.neliti.com/media/publications/201304-lama-pengeringan-terhadap-aktivitas-anti.pdf

Yang, D. J., Hwang, L. S., & Lin, J. T. (2007). Effects of different steeping methods and storage on caffeine, catechins and gallic acid in bag tea infusions. *Journal of Chromatograph*.   *1156*(1-2),   312-320.
https://doi.org/10.1016/j.chroma.2006.11.088