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SYNERGISM TEST OF DATE (PHOENIX DACTYLIFERA L.) AND RAW TEMPE ON ANTIOXIDANT ACTIVITY

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Abstract

Introduction: Dates and tempeh are foods that both contain antioxidant compounds. Antioxidant compounds such as flavonoids and isoflavones are known to be found in dates and tempeh. This study was conducted to see how much antioxidant activity contained in dates and tempeh and how the effect of dates and tempeh consumed simultaneously on the increase in total antioxidant activity.
Method: This study uses a direct experimental design with prospective data collection. The selected samples were dates with the type of sukkari and tempeh wrapped in plastic. Data processing was carried out using a simple linear regression statistical method.
Results: The DPPH test method with UV-Visible Spectrophotometry instrument showed the results of the % inhibition value of dates fruit of 39.99% and tempeh of 24.52%. Testing the synergistic effect using 7 treatments showed that the treatment with a ratio of 50:50 had a higher % inhibition value than the other treatments, which showed that consuming dates and tempeh in a ratio of 50:50 could provide a synergistic effect on antioxidant activity.
Conclusion The results obtained indicate that both samples, both dates and tempeh have high antioxidant activity, consuming both simultaneously can increase antioxidant activity. So it can be concluded that there is an effect on the synergistic effect of antioxidant activity of dates and tempeh which are consumed simultaneously. It is recommended to conduct research on the antioxidant activity of dates and tempeh using other test methods, and further research on the formulation of the preparation or clinical trials using mice.
Keywords: Dates, Tempeh, Antioxidant activity, Synergistic effect, DPPH.

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INTRODUCTION

Currently, there are many recipes from natural ingredients that can improve the quality of health, increasing the degree of public health is not only pursued in health services, but also with traditional health which is one of the various activities in improving public health status based on Law No. 2009 about health (Maulana, 2017). The use of herbs and traditional medicine to treat health problems has been around for a long time. One of the herbal recipes that is currently being discussed is "Raw Tempe and Date Fruit Juice" which is very good for the digestive system so that it can treat ulcer disease because of the probiotics contained in dates and prebiotics contained in raw tempeh.

It is known that tempeh is a fermented soybean product that has antioxidant activity because it contains three types of isoflavones, namely daidzein, glycitein, and genistein. Soybeans that have been fermented for 3 days have an inhibition value of 81.43% (Barus et al., 2019). Meanwhile, there is antioxidant activity in dates due to the presence of polyphenolic compounds, including flavanol, flavonol, flavone, and hydroxy cinnamate groups. Research has been carried out by (Nazilah, 2019) that the methanol extract of dates has a % inhibition of 43.10%.

It is known that the combination of the two types of antioxidants allows for a higher potential total antioxidant activity, known as a synergistic effect (Lingga, 2012). With the previous research, it is necessary to test the synergism of antioxidants in raw tempeh and dates consumed simultaneously which is the background of this research.

METHOD

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Research design
The research design is a direct experimental laboratory with prospective data collection

Research Location and Time
This research was carried out at the Mitra Keluarga STIKes Laboratory in March 2021 - April 2021.

Population and Research Sample
Population: The population used was sukkari dates purchased at Tanah Abang Market, Jakarta and soybean tempeh wrapped in plastic purchased at the Cibuntu Tempe Factory, Bekasi. Samples: The samples used were methanol extract of date palm pulp and methanol extract of tempe

Research variable
Independent Variables: The independent variables used in this study were date extracts and raw tempeh extracts and their combinations. Dependent Variable: The dependent variable in this study is the antioxidant activity of date and tempeh extracts and the synergistic effect of antioxidant activity
Confounding Variables: The confounding variable in this study was methanol

Research material
The research materials used were Date palm pulp (Phoenix Dactylifera L.), Raw soybean tempeh, Methanol pro-analyst (EMSURE®), Technical Methanol, DPPH (2,2-diphenyl-1-picrylhydrazyl) (SMART-LAB), FeCl3 (ferric chloride) and Vitamin C (L-Ascorbic Acid) (Sangon Biotech)

Research tools
The research tools used were knives, trays, test tubes, test tube racks, Plastic wrap, Aluminum foil, Tissue, Measuring cup, Beaker glass, Spatula, Porcelain cup, Glass funnel, Filter paper, Stirring rod, Dropper pipette, Vial bottle, Glass cuvette, Micropipette, Analytical balance, Microtip, Rotary evaporator (IKA), Oven, Vortex mixer, UV-Vis Spectrophotometer (Thermo)

Research Flow
Date fruit determination test
The dates used were tested for determination at the Herbarium Bogoriense LIPI, Bogor. The determination test was carried out to ensure the correct identity of the plants used, as well as to avoid research sampling errors (Puspitasari et al., 2019).

Preparation of date fruit extract
The dates used are dates with the type of sukkari purchased from the Tanah Abang market, Jakarta with a weight of 1 kg. The dates to be extracted are separated from the seeds and then chopped to reduce the surface of the dates so that it is easier during the drying process, the chopped dates are in the oven at 80℃ for 1x10 hours. The simplicia of dried dates were blended until smooth and then weighed as much as 250.06 grams. The extraction process uses the maceration extraction method by soaking 250.06 grams of date powder into 500 ml of technical methanol using a 500 ml glass beaker for 2x24 hours with occasional stirring. Dates powder that has been soaked for 2 days then the filtrate and residue are separated, then the filtrate is evaporated using a rotary evaporator to obtain a thick extract (Abdillah et al., 2017). Extract results obtained are then calculated extract yield. The viscous extract obtained was then subjected to organoleptic tests.

Preparation of raw tempeh extract
The tempe used was a type of tempe from soybean seeds with a fermentation time of 3 days which was purchased from the cibuntu tambun tempe factory, Bekasi. Tempe used as much as 1 kg then blended. The tempeh that has been blended is weighed as much as 500.17 grams then soaked using 1000 ml of methanol for 24 hours in a 1000 ml Erlenmeyer with occasional stirring, after 24 hours the filtrate is separated from the residue, then the residue is soaked again using 500 ml of methanol for 24 hours in Erlenmeyer 500 ml with occasional stirring, then the filtrate is separated from the residue and the filtrate from maceration of

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tempeh is evaporated using a rotary evaporator to obtain a thick extract.(Istiani, 2010). The viscous extract obtained was calculated for extract yield. The results of the viscous extract were then carried out by organoleptic tests.

Determination of extract water content

The water content of the extract was tested using the gravimetric method. The gravimetric method was carried out by weighing 10 grams of extract in a container that had been tared, then dried in an oven at 60℃ for 3 hours and then weighed. Continue drying and weighing at 1 hour intervals until the difference between 2 consecutive weighings is not more than 0.25% (Ministry of Health RI, 2017).

Phytochemical screening of raw dates and tempeh fruit extract

Qualitative tests on samples were carried out by phytochemical screening using the flavonoid test. The flavonoid test was carried out by adding a few drops of FeCl3 into the thick extract samples of dates and tempeh in a test tube, respectively. The concentration of FeCl3 reagent used is 5%. A positive result in the flavonoid test is indicated in the form of a change in the color of the sample to purple, black, blue, green or red (Setyowati et al., 2014).

Preparation of DPPH solution

In this study, the antioxidant activity was tested using the DPPH test method, 1 mg of DPPH compound was used then dissolved in 25 ml of pro-analytical methanol into a dark and airtight bottle to obtain a DPPH solution with a concentration of 40 ppm, then a 40 ppm DPPH solution was diluted to 5 ppm into 100 ml of methanol and stored in a dark bottle with an airtight lid which is useful for avoiding damage to the DPPH compound due to oxidation by air and light. (Santi & Sicily, 2019).

Preparation of standard curve stock solution for vitamin C

The standard curve in this study used vitamin C from the Sangon Biotech brand. A standard curve mother liquor was made by dissolving 2.5 mg of standard vitamin C into 50 ml of pro-analytical methanol into a 100 ml volumetric flask to obtain a concentration of 50 ppm, then the mother liquor 50 ppm vitamin C is diluted to 5 ppm into 50 ml methanol (Santi & Sicily, 2019).

Determination of the maximum wavelength of DPPH

Quantitative test using UV-Vis spectrophotometric instrument, so that the determination of the wavelength on the DPPH, by dissolving 4 ml of DPPH solution with a concentration of 5 ppm into 1 ml of methanol pro-analysis then measuring the absorbance at a maximum wavelength of 400-800 ppm in order to obtain the maximum wavelength of DPPH (Santi & Sicily, 2019).

Determination of DPPH operating time

The DPPH solution used was carried out operating time to see how many minutes the test compound could be stable in the test sample. The operating time was carried out by reacting 50 l of 5 ppm vitamin C into 4 ml of 5 ppm DPPH solution, then homogenized with a magnetic stirrer for 1 minute, the absorbance of the homogenized solution was measured using UV-Vis spectrophotometry at 0, 5, 10, 15 minutes. , 20, 25, 30, 35, 40, 45, 50, 55 and 60 with predefined DPPH wavelengths (Ulfah & Sumantri, 2014).

Preparation and determination of absorbance of standard curve series solutions

The mother liquor standard curve for vitamin C with a concentration of 50 ppm was diluted, so that a serial solution of 4 ppm concentration was obtained; 5 ppm; 6 ppm; 8.5 ppm and 9 ppm. Dilution is carried out by dissolving 0.4 ml; 0.5 ml; 0.6 ml; 0.85 ml and 0.9 ml of 50 ppm standard curve mother liquor into 5 ml technical methanol each in a 20 ml vial, then each series solution was reacted with 1 ml of 40 ppm DPPH and incubated for 30 minutes, then each the maximum wavelength of the serial solution was measured using UV-Vis spectrophotometry in order to obtain a linear regression equation (Santi & Sicily, 2019).

Making a solution of extracts of dates and raw tempeh

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The mother liquor of the test sample was made by dissolving 100 mg of the thick extract of dates and tempeh with 50 ml of pro-analytical methanol, so that the concentration of the mother liquor of the extract sample was 2000 ppm, then the mother solution of the 2000 ppm sample was diluted each to 50 ppm sample solution. The mother liquor sample of 50 ppm thick extract was each reacted with 1 ml of 5 ppm DPPH into a vial and incubated for 30 minutes. Each incubated sample solution was measured for absorbance on UV-Vis spectrophotometry using a predetermined DPPH wavelength, then repeated 3 times (Santi & Sicily, 2019).

Test the synergism of dates and raw tempeh

The synergism test was carried out with 7 stages of mixing date extract and tempeh, then each mixing stage was tested for antioxidant activity using the DPPH method. The results of the inhibition value obtained were then carried out statistical analysis tests using the simple linear regression method to see the effect of mixing dates and tempeh on increasing antioxidant activity. The formulation treatment can be seen in Table 4.1.

Table 1. Formulation of raw dates and tempeh pulp extract

Code	Treatment	
	Date Fruit Extract (%)	Soy Tempe Extract (%)
F1	100	0
F2	60	40
F3	55	45
F4	50	50
F5	45	55
F6	40	60
F7	0	100

RESULTS

Date fruit determination test

The test was carried out at the Indonesian Institute of Sciences (LIPI), the test results stated that the fruit used in this study was a date palm with the Latin name Phoenix Dactylifera L., Arecaceae tribe.

Date fruit extract

Figure 1. Date powder

Figure 2 Thick extract of dates

Table 2. Date extract organoleptic data

Organoleptic data	
yield	8.82%
Form	Thick extract
Color	Black brown
Smell	Special dates

Raw tempeh extract

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Figure 3. Tempe porridge


Figure 4. Tempeh thick extract

Table 3. Organoleptic data of tempeh extract

Organoleptic data	
yield	5.25%
Form	Thick
Color	Yellow
Smell	Tempeh

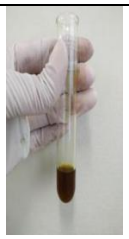
Determination of extract water content

The results obtained were that the water content of the thick extract of dates was 5.88% and the thick extract of tempeh was 43.32%.

Phytochemical screening of raw dates and tempeh fruit extract

Positive results obtained in the form of a change in the color of the sample to black. Positive results can be seen in Table 3.

Table 4. Phytochemical screening results

Date flesh extract		Tempeh Extract	
Before addition of FeCl3	**After the addition of FeCl3**	**Before addition of FeCl3**	**After the addition of FeCl3**
 Sorrel	 Black Positive Result	 Clear yellow color	 The positive result is blackish green color

Determination of the maximum wavelength of DPPH

Before testing the antioxidant activity using DPPH free radicals, the DPPH solution was determined to determine the maximum wavelength using a UV-Vis spectrophotometer. The maximum wavelength of DPPH is 515 nm with an absorbance of 0.586. The maximum wavelength spectra of DPPH can be seen in Figure 5.

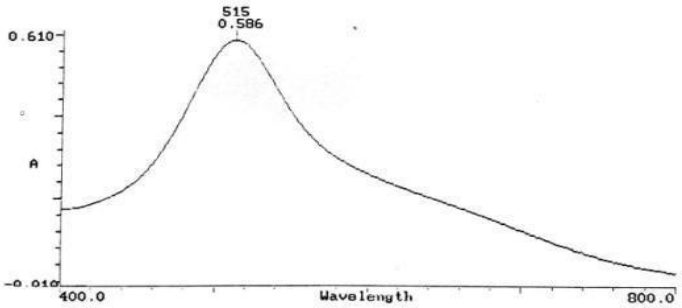


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Figure 5. Maximum wavelength spectrum of DPPH

Determination of DPPH operating time

After determining the maximum wavelength, the operating time is determined. The operating time graph can be seen in Figure 6.

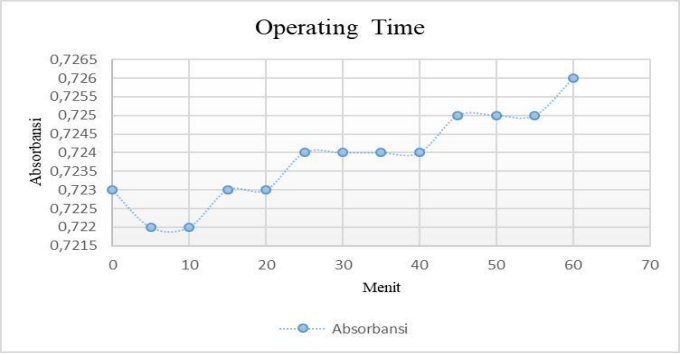

Figure 6. Chart DPPH operating time

Determination of the standard curve of vitamin C

Determination of the absorbance standard curve was carried out on a series solution with a concentration series of 4 ppm, 5 ppm, 6 ppm, 8.5 ppm and 9 ppm, the results of the regression equation Y = -0.0381x + 0.4273 with R2 = 0.99. From the absorbance data of vitamin C can be calculated the value of % inhibition and obtained data of % inhibition of vitamin C. The graph of the standard curve of vitamin C can be seen in Figure 7. The absorbance table of the standard curve concentration series can be seen in table 5.

Table 5. Absorbance and % inhibition data of vitamin C standard curve series

Concentration (ppm)	Absorbance	% Inhibition
4	0.39	41.08%
5	0.36	36.13%
6	0.31	27.15%
8.5	0.27	14.28%
9	0.24	5.78%

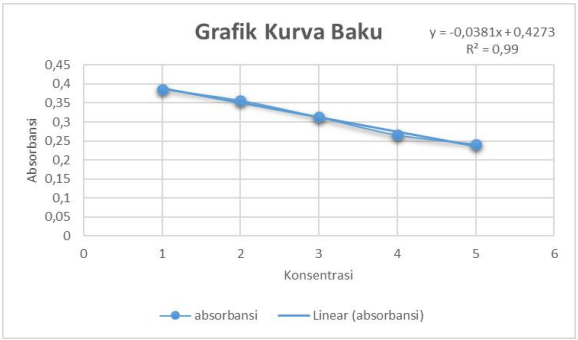

Figure 7. Vitamin C standard curve graph

Determination of antioxidant activity of dates and raw tempeh

The results of the absorbance of the sample and control obtained were calculated as % inhibition to see how much antioxidant activity contained in the extracts of dates and tempeh, with the following formula:

$$\%\text{Inhibition} = \frac{Absorbansi\ setelah\ reaksi - Absorbansi\ awal}{Absorbansi\ setelah\ reaksi}\ x\ 100\%$$

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The results of the inhibition of the date pulp extract were 24.52% and the tempeh extract was 39.99%.

Test the synergism of dates and raw tempeh

The absorbance measurement in the treated sample was repeated 3 times to obtain 21 data. The results of the formulation inhibition data can be seen in Table 6.

Table 6. Data % Inhibition of F1-F7 formulation

Formulation	Treatment		% Inhibition
	Dates	Tempe	
F1	100%	0%	24.52%
F2	60%	40%	31.31%
F3	55%	45%	27.79%
F4	**50%**	**50%**	**32.97%**
F5	45%	55%	29%
F6	40%	60%	31.28%
F7	0%	100%	39.99%

Mixing F4 with 50:50 treatment had the highest antioxidant activity compared to F2, F3, F5 and F6. To see the effect of treatment on increasing antioxidant activity, statistical analysis was carried out using a simple linear regression method. The normality test uses the Kolmogorov-Smirnov Test method with the Asymp value. Sig. 0.573 > 0.05 which means the data is normally distributed.

Table 7. Coefficients Tabel Table

Coefficients

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
(Constant)	24,186	2,923		8.275	.000
1 formulatio n	1,701	.654	.759	2,603	.048

a. Dependent Variable: inhibition

The coefficient value of the independent variable obtained can be seen how strong the influence of the independent variable on the dependent variable is through the regression coefficient interval in the following table:

Table 8. Regression coefficient interval

Interval	Information
80.00%	Very strong
60.00% – 79.99%	Strong
40.00% – 59.99%	Strong enough
20.00% – 39.99%	Weak

DISCUSSION

The initial stage of the determination test on dates was not carried out on tempe because the samples used were soybeans that had gone through a fermentation process. The results of the determination test showed that the plant used in this study was a date palm with the Latin name Phoenix dactylifera from the Arecaceae family. Sample preparation is an important step that must be done in this research. The initial stage of heating the pulp of the date palm which aims to remove the water content in the flesh so that it does not hinder the distribution of active compounds in the maceration process (Shahdadi et al., 2015). The maceration extraction method was chosen because the cold extraction technique is expected to attract more

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antioxidant compounds (polyphenols) where polyphenols are compounds that are not resistant to heat, so it is hoped that the extraction process will not damage the active compounds. (Sani et al., 2015).

The date palm extract sample was concentrated using a rotary evaporator instrument, the purpose of this concentration was to evaporate the solvent according to its boiling point with a temperature of 55°C, the temperature was chosen to prevent damage to antioxidant compounds due to heating. The yield of the viscous extract obtained after the solvent evaporation process using a rotary evaporator was 8.82%. Research conducted by (Nafisah, 2019) shows the yield of extracts on Ajwa dates by maceration method using 70% ethanol solvent of 49.48%.

Organoleptic tests were carried out on both samples by observing the shape, smell and color of each sample. The form of the date extract was obtained, which was thick, brownish black in color and had a distinctive smell of dates. These results are in accordance with research conducted by (Nafisa, 2019), he extracted Ajwa dates using 70% ethanol solvent with the maceration method and obtained a thick, blackish-colored extract and a distinctive smell of dates.

The viscous extract obtained was then tested for water content using the gravimetric method. The results of the water content obtained from the date fruit extract of 5.88% compared to previous research conducted by (Nafisa, 2019) showed that the water content of the date fruit extract was 13.15% which was carried out by testing the water content with the gravimetric method, if see from (Kementrian Kesehatan Republik indonesa, 2017)the water content that may be contained in the extract is not more than 0.25% so that the results obtained do not meet these rules. Judging from the purpose of testing the water content is to reduce the water content in the extract sample so that compound testing can be carried out optimally. From the results of the water content obtained, the sample can still be tested for optimal antioxidant activity.

Sample preparation on raw tempeh was carried out without heating because it was known that heating could reduce the antioxidant activity of soybean seeds by 28.68%-42.78% (Barus et al., 2019). The yield of tempeh extract was 5.25%. The results of the extract yields from the two samples obtained were in accordance with previous research conducted by (Savitri et al., 2017)he proved that by carrying out the maceration extraction process on samples containing polyphenolic compounds using methanol as a solvent, the yield of more than 4% was possible. This is because the methanol solvent is polar so that polyphenolic compounds that are polar will be very easily dissolved in polar solvents as well. followed by obtaining a thick extract and high yield value. The results of the organoleptic test on tempeh extract have a thick form with a yellow color and a distinctive tempeh smell, these results are in accordance with previous research conducted by (Mawaddah & Fakhrurrazi, 2018) that the obtained tempeh extract has a thick form with a yellowish color and has a distinctive tempeh odor.

The water content test carried out on the tempeh extract obtained a value of 43.32%. This does not meet the requirements, namely the water content contained in the thick extract is <0.25% (Kementrian Kesehatan Republik Indonesia, 2017), the high water content obtained in this study is due to the water content contained in tempeh and the addition of water in the process. making tempeh porridge, but the water content obtained in the tempeh extract can still be tested for optimal antioxidant activity.

The extracts of dates and raw tempeh were subjected to phytochemical screening which is a qualitative test to determine the content of active compounds in plants. The results of the phytochemical analysis of the extract obtained a blackish color which is in accordance with previous studies, this black color is caused by the phenolic compounds contained will form complex compounds with Fe3+ ions (Abdillah et al., 2017). The reaction of phenol compounds and FeCl3 can be seen in Figure 7.

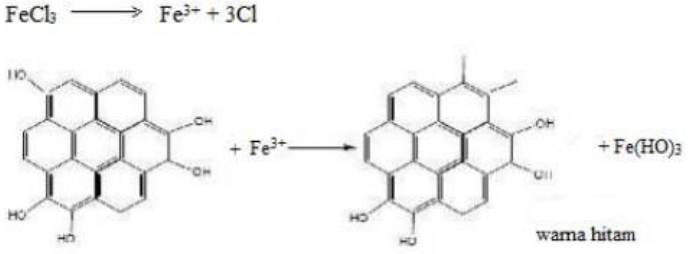

Figure &. Reaction of phenol compounds with FeCl3 (Setyowati et al., 2014)

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The DPPH method was chosen because the process is simple, fast, easy and sensitive and only requires a small sample of natural compounds. accurate and practical (Salim, 2018). The results of the DPPH wavelength test using the UV-Visible spectrophotometer instrument obtained a wavelength of 515 nm with an absorbance value of 0.586, this is in accordance with previous research conducted by (Salim, 2018) he determined the maximum wavelength on DPPH and obtained the wavelength results of 515nm with an absorbance of 0.750.

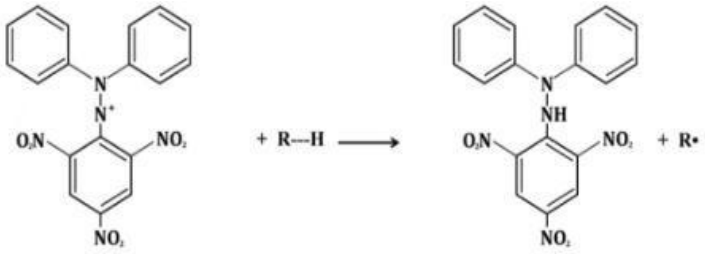

Figure 8. DPPH attenuation reaction (Molyneux, 2004)

The principle of the reaction of this method is the color change of DPPH from violet to yellow due to the hydrogen or electron donation process followed by a decrease in the absorbance of DPPH. Measurement of DPPH radical scavenging activity by methanol extract of date palm pulp and tempeh using a UV-Visible spectrophotometer will determine the value of free radical scavenging activity which is expressed by the value of % inhibition. The maximum wavelength provides optimal absorption from the test solution and provides great sensitivity so that it is hoped that the absorbance value can be obtainedoptimal on sample (Rizkayanti et al., 2017).

Antioxidant activity was observed by changing the color of the DPPH radical from purple to yellowish due to the donation of hydrogen atoms from antioxidant compounds followed by a decrease in DPPH absorbance, the higher the antioxidant concentration, the lower the DPPH absorbance. It is known that dates with the type of sukkari have a flavonoid content of1,983±0.104, The flavonoid content in sukkari dates is relatively smaller than the flavonoid content in Ajwa dates, which is equal to 2.787±0.138 (Nazilah, 2019)

In the test of the date extract sample, there was a change in the color of the DPPH radical to yellowish and the % inhibition value of the date sample was 24.52%. Comparing with previous research conducted by (Nazilah, 2019) the antioxidant activity of dates with the Ajwa type has an inhibition value of 43.10%. This shows that the research carried out is in accordance with previous research, that the sukkari type of date fruit has a relatively smaller antioxidant activity than the ajwa type of date fruit but both have the same strong antioxidant activity. The results of antioxidant activity showed the value of % inhibition for raw tempeh extract samples was 39.99%.

The % inhibition value obtained showed that the methanol extract of tempeh had a higher antioxidant activity than the methanol extract of the date palm pulp, this was due to the presence of factor-2 compounds which were only found in fermented tempeh. Soybean seeds contain isoflavone compounds, when soybean seeds are fermented, the isoflavone compounds in them undergo bioconversion from isoflavone glycosides to isoflavone aglucans with higher activity. new, namely factor-2 (Barus et al., 2019). This is shown in a previous study conducted by (Barus et al., 2019) that soybean seeds contain antioxidant activity of43.04% - 52.79, Soybean seeds subjected to heating and soaking decreased antioxidant activity by 28.68%-42.78%, while the fermented soybean seeds experienced an increase in antioxidant activity of 52.72%-67.61%.

The synergism test was carried out based on the mixing treatment which aims to see if there is an effect of increasing antioxidant activity on the flesh of dates and tempeh which are consumed together and then seen how many treatments are considered to have the highest increase in antioxidant activity. Treatments were taken by volume weight per volume (V/V) with a total formulation of 100%. The results showed that treatment F4 with a comparison of 50% date palm pulp extract samples and 50% tempeh extract samples had higher antioxidant activity with an inhibition value of 32.97% compared to F2, F3, F5 and F6 treatments. This is because the formulation with a ratio of 50% dates and 50% tempeh is balanced,

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To see the effect of the combination of date extract and tempeh extract on the increase in antioxidant activity, processing and statistical analysis were carried out using the linear regression test method. Simple Linear Regression Analysis was chosen because in this study only one independent variable was used. In simple linear regression analysis, only two main tables are needed, namely Model Summary and Coefficients(Wufron, 2020) Based on the Summary Model Table obtainedthe value of R Square which shows the percentage of the contribution of the independent variable in influencing the dependent variable while the rest is influenced by other variables. The R Square value of 0.575 means that the contribution of the independent variable in influencing the dependent variable is 57.5% while the remaining 42.5%.

Through the coefficients table, the independent variable value is 1.701, meaning that the effect of mixing treatment on increasing antioxidant activity is very strong because it has a percentage of 170%.Obtainedthe value of the coefficients of the independent variable is positive, which means that the independent variable has a positive effect on the dependent variable. It is observed from the regression coefficient table that the value of the independent variable is in the range of 80% so that the influence of the independent variable on the dependent variable is very strong. The significance value of the independent variable is 0.048, which is smaller than alpha 0.05, so H0 is rejected, meaning that there is a significant effect of the independent variable on the dependent variable.

CONCLUSION

Based on the results of the quantitative test using the DPPH method, the date palm pulp extract sample had antioxidant activity with an inhibition value of 24.52% and the tempe extract sample had antioxidant activity with an inhibition value of 39.99%. The results of statistical tests using the simple linear regression method showed that there was an effect of mixing date extracts and tempeh with a ratio of 50:50 on the increase in antioxidant activity.

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