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Determination of Antioxidant Activity and Shelf-Life Test of Turmeric (*Curcuma longa)* Fortified Bread

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Abstract

The increasing public awareness for healthy living is parallel to community demands for healthy food that contains good nutritional compounds to support the physiological function of the body but also has good and attractive taste. In Indonesia, bread, as a substitute for rice, has now become part of the community. With some fortification, the bread will become healthier. In this research, turmeric fortified bread is evaluated for its antioxidant activity and shelf-life test. Turmeric is one of the most popular spices in Indonesia because of its health benefits as a strong antioxidant and antiinflammation. Curcumin, the active ingredient of turmeric, exhibits therapeutic potential for the treatment of diabetes, cardiovascular disorders, various types of cancers and functions as hepatoprotective. Antioxidant activity of ethanolic extract of turmeric-fortified bread was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, using Rigol U3600 UV/Vis spectrophotometer. The result showed that antioxidant activity in terms of inhibitory concentration (IC_{50}) value of plain bread has 227.59 ppm (weak), of 4% turmeric bread has 114.8 ppm (medium), 6% turmeric bread has 80.37 ppm (strong), and 8% turmeric bread has 63.09 ppm (strong). The shelf-life test (according to flavor, appearance, and texture) of bread, packaged using PP-plastic, was conducted for 15 days. The result showed that plain bread has 4.11 days of shelf-life, 4% turmeric bread has 10.11 days, 6% turmeric bread has 11.55 days, and 8% turmeric bread has 12.11 days. The research concluded that turmeric-fortified bread enhances the antioxidant value of bread and acts as a powerful natural preservative.

Keywords: bread, turmeric, antioxidant, DPPH, shelf life.

INTRODUCTION

Free radicals are unpaired molecules and are very active, which can cause damage to surrounding molecules. Free radicals that take electrons from the human body can cause changes in the structure of DNA (deoxynucleic acid) so that mutated cells arise. Exposure to free radicals for the human body is accumulative, which causes various diseases to appear when the human immune system can no longer tolerate the presence of free radical compounds (Fakrhia et al., 2019). On the other side, antioxidants stabilize free radicals by complementing the electron deficiency of free radicals and inhibiting the chain reaction of free radical formation. Many antioxidants are sourced from plants, one of which is a spice, namely turmeric (Malangngi, et al., 2012).

Turmeric has several types, including black turmeric, yellow turmeric, white turmeric, and red turmeric (Retnaningsih, 2015). Turmeric is a plant that has the potential as an antipyretic, fever therapy, antihepatotoxic, anti-inflammatory, bacteriostatic, hypocholesterolemia, choleretic and

spasmolytic because of the active compound curcumin (Azis., 2019). Studies have shown that the curcumin in turmeric has powerful antioxidants (Fanniakusuma et al., 2019). Turmeric also functions as a food preservative since its curcumin and essential oils content can inhibit bacterial growth (Khalil O.A.K. et al., 2012).

Bread is a flour-based substitute for rice. Indonesian people have made bread as a snack, even making bread as a breakfast menu for some people (Hidayah., 2015). Fortification is the addition of a type of nutrient into food to prevent deficiency and improve health and aims to increase the level of consumption and nutritional status of the population from added nutrients (Setyaningrum et al., 2017). This Turmeric Fortified Bread is expected to increase the living standard of the Indonesian people.

The research attended to test the antioxidant activity of turmeric bread extract with the DPPH method and continued with the shelf-life measurement as standard testing of food.

METHODOLOGY

The research is divided into four parts, which are 1) preparation of simplicia, 2) making of fortified bread, 3) antioxidant activity test, and 4) shelf-life test.

Turmeric (*Curcuma longa*) and bread ingredient was purchased from the traditional market nearby. Analytical chemicals, including ethanol solvent and DPPH, were using Merck. Equipment used were slicer/cutter (VC 60 mp Ellane), pollinator blender (Vitamax MPS 1394), bamboo winch, black cloth, soft brush, sieve, digital analytical scale (METTLER TOLEDO), shaker, vacuum rotary evaporator (B-ONE), spectrophotometer (Rigol U 3600), micropipette and other general glassware.

Preparation of turmeric powder and Bread

Turmeric was sorted from dirt, washed, and dried. The sliced turmeric was then dried using a dehydrator (Wirastar FDH-10) at 55 $^{\circ}$C for 5 hours. Dried turmeric was powdered using a grinder (Universal mill) and sieved at 200 mesh.

Bread is prepared with ingredients: margarine, butter, sugar, egg, salt, milk, yeast, water, high protein flour, and turmeric powder. Variation is made by the concentration of turmeric powder used in bread dough, which is 4%, 6%, and 8% of turmeric powder compared to flour, e.g., 8% equal to 920 gr of flour with 80 gr of turmeric powder. Plain bread is made with 1000 g of flour.

The dough was developed at room temperature for 45 minutes and baked in a Hock Oven at 190 $^{\circ}$C for 45 minutes. Loaves of bread were allowed to cool at room temperature, and each loaf was packed in one PP (Polypropylene) plastic bag. The bread was evaluated for antioxidant activity and shelf life.

Antioxidant Activity Test

This study uses UV-Vis Rigol 3600 spectrophotometry to obtain absorbance data converted into percent inhibition values using the following formula:

$$Inhibitory\ Activity\ (\%) = \frac{Abs.\ Blanko - Abs.\ Sample}{Abs.\ Blanko} x\ 100\%$$

The blank is a homogeneous DPPH solution that is used as a comparison solution used to measure the percent antioxidant activity.

Shelf-life Test

The shelf-life test was carried out for 15 days, and observations were made on 4%, 6%, and 8% turmeric bread (3 loaves of bread per percent turmeric bread) which were packaged in PP plastic and stored at room temperature. The results of this test will be written into a table using a scale of values from 1-9, with 9 being the best, the worst being 1, and 6 being the most critical value scale for the product. The types of testing from shelf life are 1) odor/smell, 2) appearance, and 3) texture.

RESULTS AND DISCUSSION

Simplicia Extraction Results

Extraction of turmeric fortified bread simplicia and turmeric powder was carried out by maceration method using 96% ethanol as solvent. The weight of the simplicia used was fortified turmeric bread (4%, 6%, and 8%) 50 grams with a ratio of 1:5, and turmeric as much as 30 grams with a ratio of 1:3. Each simplicia was macerated for 2x24 hours on a rotator shaker. The extract was then filtered and extracted using a rotary evaporator to separate from the solvent. The thickened extract was then poured into a petri dish and put in an oven at 50 $^{\circ}$C until a thick extract was obtained. The data calculation formula used is as follows:

$$Yield = \frac{Extract\ weight}{Sample\ Weight} \times 100\ \%$$

Table 1: Yield of Extraction

Sample	Rendemen (%)
Plain Bread	15,88
Turmeric Bread 4%	13,45
Turmeric Bread 6 %	13,57
Turmeric Bread 8%	17,52

From Table 1 above, it can be explained that the highest yield was on 8% turmeric bread with a weight of 17.52%, then plain bread with a weight of 15.88, then 6% turmeric bread with a weight of 13.57, and 4% turmeric bread with a weight of 13,45. Based on these results, the difference in yield results was caused by differences in the percentage of content in the bread.

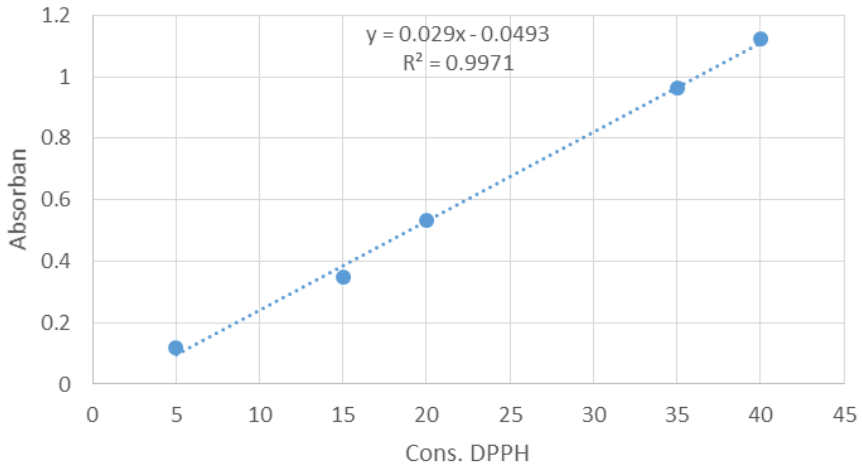
Antioxidant Activity Test

The purpose of testing the antioxidant activity is to get the results of antioxidant activity and calculate the concentration of the inhibitor of antioxidant activity (IC_{50}) in samples of plain bread and turmeric bread.

Linearity Test of Spectrophotometric Method

A linearity test was conducted to determine the ability of the method used to obtain results in accordance with the measured concentration. The results of the linearity test spectrum can be seen in Figure 1.

Figure 1: Linearity Test of DPPH Spectrum



Antioxidant Activity Test of Bread

The antioxidant activity test was obtained based on the test data of 1,1-diphenyl-2-picrylhydrazil (DPPH) so that the percent antioxidant activity was obtained as presented in Table 2.

Table 2: Average Antioxidant Activity of Bread Samples

Sample	**Concentration (ppm)**				
	12,5	25	50	100	200
Plain Bread	40,06	40,51	41,97	44,82	49,64
Turmeric Bread 4%	38,68	39,12	39,95	40,71	41,86
Turmeric Bread 6%	40,03	40,38	40,95	45,22	47,04
Turmeric Bread 8%	39,94	40,91	42,40	46,65	47,58

The result of the interpretation of the antioxidant activity testing method with DPPH is IC50. The strength of antioxidant activity is grouped with values ranging from IC50 < 50 ppm very strong, 50-100 ppm strong, 101-250 ppm moderate, 250-500 ppm weak, and more than 500 ppm

inactive (Mustarichie et al., 2017). The results of the calculation of the concentration of the inhibitory antioxidant activity of IC50 from the extract can be seen in Table 3.

Table 3: Inhibitory Concentrations (IC_{50}) of Bread Samples

Sample	IC_{50} (ppm)	Category Antioxidant Power
Plain Bread	227.59	Moderate
Turmeric Bread 4%	114.79	Moderate
Turmeric Bread 6%	80.37	Strong
Turmeric Bread 8%	63.09	Strong

Test Results of the Shelf life of Turmeric Bread

The results of the analysis of the shelf life of bread based on smell, texture, and appearance were investigated using the Extended Storage Studies method based on the points, namely 1) odor/smell, with a value of 6, the specific type of bread/flour almost disappeared, no disturbing odor, 2) appearance, with a value of 6, almost not moldy, quite interesting, the type specificity is almost lost, and 3) the texture, with a value of 6, is slightly soft, typical of plain bread is reduced. The results of shelf life (days) based on odor, appearance, and texture are in Table 4.

Table 4: Shelf-Life Data at Critical Point (value 6)

Specification	Plain Bread	Turmeric Bread 4%	Turmeric Bread 6%	Turmeric Bread 8%
Odor	4.33	9.67	11.33	13.33
Appearance	4.00	9.67	11.33	13.33
Texture	4.00	9.67	11	12
Average	4.11	10.11	11.55	12.11

Bread packaged using polypropylene plastic at room temperature, plain bread without adding turmeric flour reached a critical point (value 6), reaching a shelf life of 4.11 days, namely the bread began to grow mold. While bread with the addition of 4% turmeric powder reached a critical point (value 6) reached a shelf life of 10.11 days, bread with added 6% turmeric reached a critical point (value 6) reached a shelf life of 11.55 days, and bread with added turmeric 8% reached a critical point (value 6) reached a shelf life of 12.11 days. This shows that the addition of turmeric powder has an effect on the shelf life of bread. Therefore, turmeric powder is proposed to be used as an additive to improve shelf life (Khalil O.A.K. et al., 2012).

CONCLUSION

Based on the results of research that has been done, namely regarding antioxidant activity and shelf life of bread fortified with turmeric powder, it can be concluded several things:

1. The addition of 4% turmeric powder in bread extended the shelf life by an average of 10.11 days, the addition of 6% turmeric powder to bread extended the shelf life by an average of 11.55 days, and the addition of 8% turmeric powder to bread extended the shelf life by an average of 12,11 days, compared with plain bread with a mean of 4.11 days.
2. Antioxidant activity in 4% turmeric bread with IC_{50} = 114.79 (moderate), 6% turmeric bread with IC50 = 80.37 (strong) and 8% turmeric bread with IC_{50} = 63.09 (strong), compared to plain bread with IC_{50} = 227.59 (moderate).
3. The addition of turmeric powder affects the antioxidant activity and extends the shelf life. The more powder is added, the higher the antioxidant activity and the longer the shelf life.

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