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Antioxidant activity and Total Phenolic Contents of Bread Enriched with Pumpkin Flour

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Abstract. Pumpkin is abundantly available in Indonesia and well known to contain high dietary fiber, β -carotene and phenolic compounds. The objective of this study was to evaluate the effect of pumpkin flour enrichment to the antioxidant activity and total phenolic content of bread. Breads were made by partially replacing of wheat flour with pumpkin flour at the level of 5% to 20%. The results showed that pumpkin flour significantly ($P < 0.05$) enhanced antioxidant activity of enriched bread measured by DPPH and ABTS. The highest antioxidant activity was observed in bread with enrichment of 20% of pumpkin flour. At highest values, antioxidant activity were 76,59% (DPPH) and 81,74% (ABTS), respectively. Total phenolic contents of enriched bread were also significantly affected by increased level of pumpkin flour. The highest total phenolic content (5,39 mg GAE/g) was observed in bread with enrichment of 20% pumpkin flour. Whereas, control bread contained total phenolic content of 1,38 mg GAE/g. Thus, we concluded that pumpkin flour can be employed to enhanced antioxidant activity and total phenolic content of bread.

Keywords: Antioxidant activity, bread, pumpkin flour, phenolic content

1. Introduction

Yellow pumpkin (*Cucurbita moschata*) is a local plant which is greatly abundant. In 2014, it was reported that Pumpkin production nationally was 523,063 tons. At present, the use of pumpkin as food is still limited to simple processing. Some processed products have been developed such as biscuits, bread, crackers, chips and several types of cakes. However, the availability of processed products in the market is still limited.

Pumpkin is an agricultural product that contains high pectin, bioactive substances, beta carotene, vitamin A, tocopherol, other vitamins including B6, K, C, thiamine, and riboflavin, as well as several types of minerals (K, P, Mg, Fe and Se). High dietary fiber content (pectin) can control the level of insulin serum, reduce blood sugar, enhanced glucose tolerance and provide protection against a number of diseases such as diabetes, cardiovascular disease, constipation and colon cancer [1;2]. Pumpkin contains carotenoid compounds, beta carotene compounds that can act as antioxidants in the



human body. Antioxidant compounds include phenolic acids, flavonoids, vitamin C, vitamin E, bilirubin, albumin and β -carotene.

Public attention to consume healthy bread rises along with the high prevalence of diseases such as obesity, diabetes and coronary heart disease. This strengthens the development of the bread industry for certain purposes such as highly nutritious bread and bread for diet [3]. The use of Pumpkin in the production of white bread did not only increase its organoleptic value but also improved its physical quality such as an increased in loaf volume [4].

Research on making white bread from pumpkin is still very limited in Indonesia. Therefore this study was conducted to determine the effect of pumpkin flour enrichment to the antioxidant activity and total phenolic content of bread.

2. Materials and Methods

2.1 Materials

The yellow pumpkin powder was made from good quality of pumpkins. The pumpkin cultivar was *bokor* or *cerme*. The characteristics of pumpkin used were flat rounded shape, having orange yellowish color of flesh and does not rotten, blemishes or bruises, evenly bright orange skin color. They were obtained from Pasar Tanjung, Jember. Wheat flour used was Cakra Kembar Premium (high protein contents). Other ingredients were yeast (Saf-Instant), sugar (Gulaku), salt (Kapal brand) and butter (Blue Band).

2.2 Methods

2.2.1 Pumpkin Powder Preparation Pumpkin powder was made following in several stages of processing, namely sorting, reducing of size, drying, and flouring. Pumpkin was sorted based on the condition of the pumpkin skin such as not rotten. Pumpkins were washed thoroughly and dried using a dehydrator at 65 °C for 7 hours. After drying, it was ground using a blender and sieved in 70 mesh like a standard flour.

2.2.2 Bread making Bread formulation is shown in Table 1. The bread making was done by replacing wheat flour with pumpkin flour (PF). PF replacement was done at a level of 0, 5, 10, 15 and 20%, based on the weight of wheat flour. Bread formulation is presented in Table 1. A bread maker (National SD-BT102, Panasonic, Osaka, Japan) was used to make breads using 4-h bread making setting. Baking was done three times (triplicate). After baking, the bread loaves were tempered at room temperature (28–30 °C).

Table 1. Formulation of bread enriched with pumpkin flour

Ingredient	Sample				
	A1 (0%)	A2 (5%)	A3 (10%)	A4 (15%)	A5 (20%)
Wheat flour (gr)	280	266	252	238	224
Yellow pumpkin flour (gr)	-	14	28	42	56
Xanthan gum (gm)	1,4	1,4	1,4	1,4	1,4
Sugar (gr)	16,8	16,8	16,8	16,8	16,8
Salt (gr)	5,6	5,6	5,6	5,6	5,6
Yeast (gr)	2,8	2,8	2,8	2,8	2,8
Butter (gr)	35	35	35	35	35
Water (ml)	200	200	200	200	200

2.2.3 Sample extraction Sample extraction was carried out based on the method of [5] with minor modification. Bread was cut about 1 cm of thickness and then dried in the oven at 40°C for 24 hours. Then the dry bread then mashed. The mashed bread was extracted with 80% methanol with a ratio of 5 grams of bread and 12.5 ml of methanol. Then bread extract was put in a shaker (100 rpm) for 2 hours at 37°C.

2.2.4 Antioxidant activity (DPPH) Antioxidant activity was measured based on the modified method of [6]. A sample of 0.1 ml was added with 3.9 ml of 0.2 mM DPPH. The mixture was allowed to stand for 30 minutes at room temperature. Absorbance of the mixture was then measured using a spectrophotometer (Shimadzu, UV-1601) at a wavelength of 510 nm. The antioxidant activity of the sample was determined based on the formula:

$$\% \text{ DPPH Scavenging} = \left[\frac{(A_{\text{Control}} - A_{\text{Sample}})}{A_{\text{Control}}} \right] \times 100\%$$

Where, A control is absorbance of DPPH solution without addition of sample and A sample is absorbance of DPPH solution by addition of sample.

2.2.5 Antioxidant activity (ABTS) Antioxidant activity based on ABTS scavenging was measured based on the method of [7] with slight modifications. ABTS 7.4 mM (0.203 g per 50 ml) and potassium persulfate 2.6 mM (0.035 g per 50 ml) were mixed in the same ratio. Mixture was put into a dark colored bottle and left in the dark room for 12 hours. From the ABTS solution, 1.5 ml was taken and diluted with ethanol 23.5 ml. A 0.01 ml sample was mixed with a 2.9 ml ABTS solution, then allowed to stand for 7 minutes and the absorbance was determined at a wavelength of 734 nm. The antioxidant activity of the sample was calculated based on the following formula:

$$\% \text{ ABTS Scavenging} = 100 - \left[\left(\frac{OD_{\text{sample}}}{OD_{\text{control}}} \right) \times 100 \right]$$

Where, OD sample is the absorbance value of ABTS mixture mixed with the sample and OD control is the absorbance value of ABTS solution without sample.

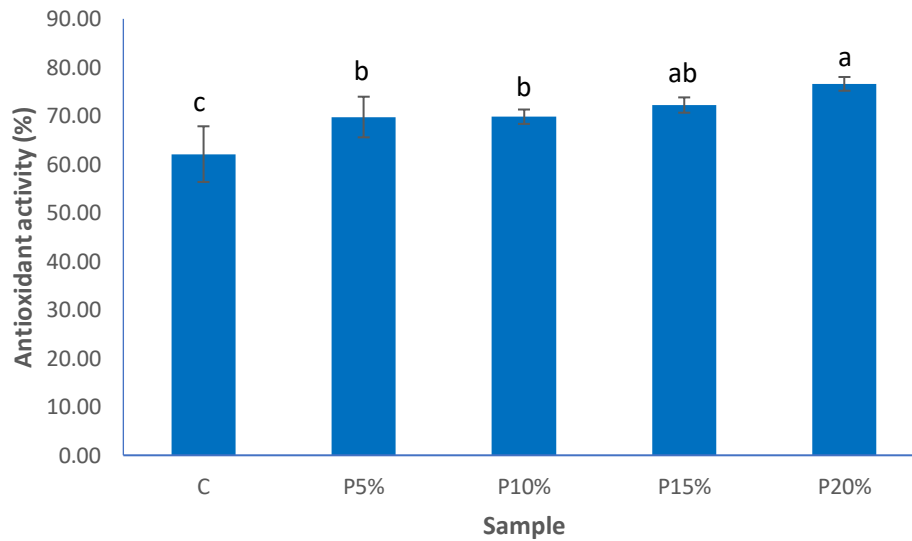
2.2.6 Total Phenolic Content Analysis of total phenolic content was carried out based on the [8]. A sample of 0.2 ml was put into a test tube. Furthermore, 1 ml of 10% Folin solution was added. After 10 minutes, 0.8 ml of sodium carbonate was added and then vortexed. The solution was incubated at room temperature for 2 hours. Afterward, the absorbance was determined at a wavelength of 765 nm. The phenolic content was determined from based on gallic acid standard curve. Total phenolic content are expressed as mg GAE (Gallic Acid Equivalent) /g dry matter.

2.2.7 Analysis Statistic Statistical analysis was done using SPSS for windows (ver. 19, SPSS, Inc., Chicago, IL, USA). The data were analyzed using one-way analysis of variance. Then, Duncan's multiple range test (DMRT) at $p < 0.05$ was implemented to compare means between samples.

3. Results and Discussion

3.1 Antioxidant Activity

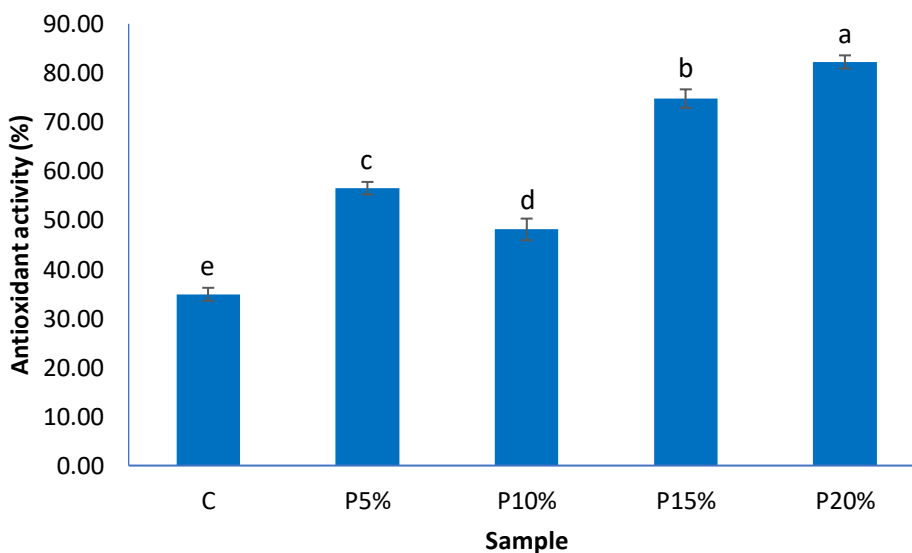
Antioxidant activity of bread enriched with pumpkin flour are shown in Figure 1 and 2. In figure 1, antioxidant activity of bread measured by the ability of the test sample to scavenge DPPH radical. Pumpkin flour addition significantly affected ($P < 0.05$) antioxidant activity (DPPH) of enriched bread.



Note : Control bread (C); 5% pumpkin flour (P5%); 10% pumpkin flour (P10%); 15% pumpkin flour (P15%); 20% pumpkin flour (P20%)

Different letter in the bar chart indicated a significantly different analyzed by Duncan Multiple Range Test (DMRT) at 5%.

Figure 1. Antioxidant activity of bread enriched with pumpkin flour measured by DPPH scavenging activity



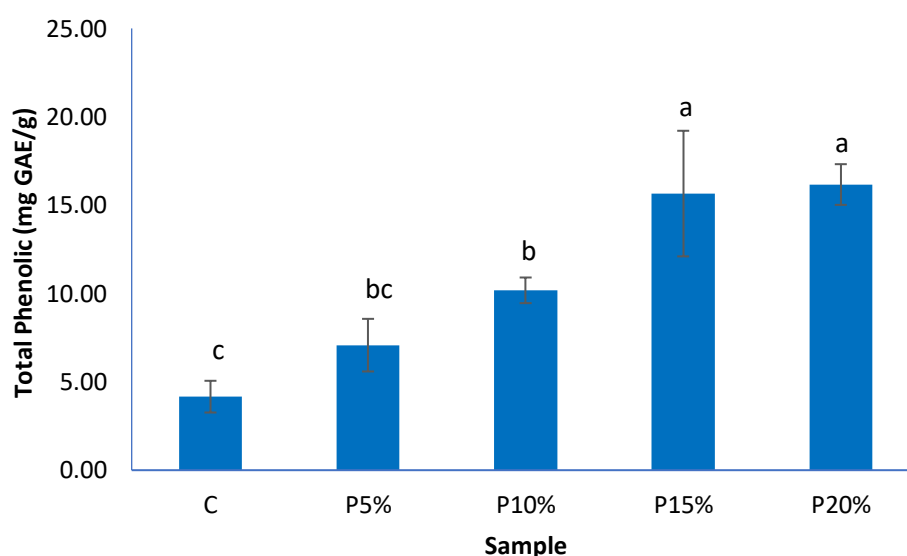
Note : Control bread (C); 5% pumpkin flour (P5%); 10% pumpkin flour (P10%); 15% pumpkin flour (P15%); 20% pumpkin flour (P20%)

Different letter in the bar chart indicated a significantly different analyzed by Duncan Multiple Range Test (DMRT) at 5%.

Figure 2. Antioxidant activity of bread enriched with pumpkin flour measured by ABTS scavenging activity

Figure 1 shown that increased pumpkin powder concurrently increased antioxidant activity of resulting bread. Addition of pumpkin powder at least 5% significantly increased antioxidant activity of bread compared to that of control bread as measured by DPPH scavenging activity. The highest antioxidant activity (76,59%) was presented by pumpkin enriched bread with 20% pumpkin powder. In the contrary, control bread presented the lowest (62,10%) antioxidant activity.

The similar finding was found when antioxidant activity of enriched bread was measured by ABTS scavenging activity (Figure 2). In general, addition of pumpkin flour significantly affected antioxidant activity of enriched bread. Increased pumpkin flour lead to increase antioxidant activity of enriched bread. The highest antioxidant activity exhibited in bread with 20% enrichment of pumpkin flour. The lowest antioxidant activity was found in control bread.



Note : Control bread (C); 5% pumpkin flour (P5%); 10% pumpkin flour (P10%); 15% pumpkin flour (P15%); 20% pumpkin flour (P20%)
Different letter in the bar chart indicated a significantly different analyzed by Duncan Multiple Range Test (DMRT) at 5%.

Figure 3. Total phenolic content of bread enriched with pumpkin flour

According to [9], the pumpkin peel and pulp are a good source of nutrition. The peel was found to be a good source for minerals such as phosphorus, iron and dietary fiber. On the other hand, the pulp was found to contain high β -carotene. In addition, the peels and pulp not only exhibited almost a comparable values polyphenols and antioxidants but also inhibition of DPPH activity. The hypocholesterolemic, antioxidant, hepatoprotective and prebiotic properties not only suggested for bakery enriched products but also for other food products.

Pongjanta [10] reported that the addition of pumpkin flour produced higher levels of β -carotene. The pumpkin flour has a higher β -carotene content (180 $\mu\text{g}/100\text{ g}$) than that of wheat flour which does not contain vitamin A. β -carotene has an ability to be an antioxidant that can play an important role in stabilizing carbon nucleated radicals. Ahzani [11] reported that the addition of pumpkin flour increased antioxidant activity due to an increase in β -carotene in pumpkin flour. β -carotene in pumpkin flour acted as a pro-vitamin A and also exhibited an antioxidant activity. Antioxidant activity

is also influenced by several components including phenol compounds which are the basic framework of compounds that have antioxidant activity.

c. Total Phenolic Content

The effect of pumpkin flour substitution on the total phenolic content of bread is presented in Figure 3. Based on statistical analysis showed that the addition of pumpkin flour has a significant effect ($P < 0.05$) on the total phenolic content of bread.

Figure 3 shows the total phenolic content of pumpkin enriched bread ranged from 4.16 mg GAE /g to 16.16 mg GAE /g. The bread enriched with 20% of pumpkin flour presented the highest total phenolic content (16.16 mg GAE/g). On the contrary, the control bread had the lowest total phenolic content (4.16 mg GAE/g).

Increased level of pumpkin flour produced a higher level of total phenolic content in enriched bread. Total phenolic content of pumpkin flour affected by drying of pumpkin chips during processing. Que [12] reported that high temperature used during drying lead to the formation of phenolic compounds. Additionally, [13] reported that drying has a positive effect on the total phenolic content of pumpkin flour.

Huang [14] reported that the antioxidant activity is directly proportional to the total phenol. The higher the phenolic content in a compound the higher its antioxidant activity. It is because the total phenolic content can be produced from a simple molecules namely phenolic compounds and a complex molecules such as tannins (hydrolyzed tannins and condensed tannins) [15]. Valenzuela [16] reported that the most available phenolic contents in pumpkin are flavonoids. Dragovic [17] also reported that there were chlorogenic acids in *Cucurbita pepo*, *Cucurbita maxima* and *Cucurbita moschata*.

4. Conclusions

Pumpkin flour enrichment significantly affect the antioxidant activity of enriched bread measured by DPPH and ABTS scavenging. The enrichment also significantly increased total phenolic content of enriched bread. Higher level of enrichment produced higher antioxidant activity and total phenolic content of enriched bread. Thus, producing a functional bread with an abundant antioxidant activity is feasible by using yellow pumpkin powder.

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