DEGRADATION KINETICS OF RED BEET (*Beta vulgaris*) POWDER DURING THERMAL PROCESSING AND CHANGES OF pH

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ABSTRACT

Red beet (*Beta vulgaris*) is a source of antioxidants and often used as a natural colorant in the food processing. Antioxidant content in beets is called betalain, which are classified into betacyanin which is purplish red pigment and betaxhantin which is the yellow orange pigment. Antioxidants are compounds that can delay or prevent the oxidation of free radicals such as rancidity and discoloration but are very susceptible to degradation. Free radicals are highly reactive molecules because it has unpaired electrons in the outer orbital of the molecule so that it can react with the cells of the body that can cause disease. Antioxidant activity degradation is determined by calculating the activation energy. The activation energy is the minimum energy required to perform a reaction. The purpose of this study was to determine the effect of pH, temperature and heating duration on antioxidant degradation of red beet powder and to determine the degradation kinetics by calculating the value of the activation energy based on the decrease in antioxidant activity with Arrhenius model. Preparation of red beet powder is conducted by drying red beet using cabinet dryer. Methods of research conducted with three levels, i.e. pH 6, 7 and 8; with four different temperatures, i.e. 40^{o}C, 60^{o}C, 80^{o}C and 90^{o}C; and at five heating rate, i.e. 0, 5, 10, 15 and, 20 minutes. The analysis performed in this study is the analysis of antioxidant activity in red beet powder using DPPH method. The results were calculated using the Arrhenius equation. The results showed that lowest decreasing antioxidant activity is at pH 6, temperature 40^{o}C and 0 minute; is equal to 25.40% and the highest decreasing antioxidant activity is at pH 8, temperature 80^{o}C and 20 minutes is equal to 49.76%. The highest activation energy (Ea) is pH 6, at 0 minute that equal to 7303.85 J/mol. While the lowest Ea is pH 8 at 20 minutes that equal to 4230.16 J/mol.

Keywords: *red beet powder, antioxidants, activation energy, degradation kinetics*

1.INTRODUCTION

Red beet (*Beta vulgaris*) is purplish red tuber and often used as natural food colorant so the product can improve consumer attraction. Nowadays, synthetic food colorant already widely circulated in the community. However, it becomes dangerous if we consumed it for a long period because it will cause carcinogenic effects. To reduce the use of synthetic food colorant, we can use natural food colorant, i. e. red beet. The benefits of this colorant are safe use and also contain high of antioxidants. Antioxidants found in red beet called betalain. Betalain classified into two pigment, betaxhantin which has orange and yellow color and betacyanin which has purplish red color (Pitalua *et al*., 2010).

Besides betalain, antioxidants contained in red beet namely polyphenols, vitamins, flavonoids and folic acid. Antioxidants work to prevent the oxidation reactions of free radicals which can cause rancidity and damage to food products and can inhibit cancer cells that are good for health (Windono *et al*., 2001).

Red beet powder can be used as a natural colorant for beverage products. In red beet beverage processing using heat treatment, antioxidants found in red beets will be degraded. Stability of betalain influenced by light, oxygen, water activity, pH and temperature (Gaztonyi *et al*., 2001).

Temperature affects the rate of reaction as a factor affecting the decline of the food product quality. Higher temperature will caused reaction rate become faster so the antioxidant activity became decrease. Betalain stable at temperatures below 40^{o}C and in acidic conditions with a pH range of 4-6 (Fennema, 1997)

This research use three different pH condition; pH 6 (acidic), 7 (neutral), and 8 (alkaline). Selection of the pH to determine antioxidant degradation of red beet, because not all products are produced under conditions of acidic beverages. Based on that statement, the research is conducted to determine the degradation kinetics of the antioxidant activity in red beet powder with a combination of pH, temperature and heating duration. Besides that, the Arrhenius approach is used to calculate the changes of activation energy.

2.MATERIALS AND METHODS

2.1.Chemicals and Tools.

The materials used in this study are red beet powder, methanol, distilled water, DPPH solution, 10% HCl solution and 10% NaOH solution. The tools used in this study are the cabinet dryer, blender, slicer, pH meter, a closed reaction tube, water bath, thermocouple and spectrophotometer. The data analyses are conducted with Microsoft Excel 2010 and MatLab 2010a.

2.2.Sample Preparation.

Red beet is dried with dryer cabinet to form a powder blend. The powder is dissolved, and then treated with 3 levels of pH (pH 6, pH 7, and pH 8). NaOH solution is added to make alkaline condition; meanwhile HCl solution is added to make acidic condition of sample. Next, the sample are placed in a reaction tube and heated in a water bath. Each pH level is heated with four level of temperature (40^{o}C, 60^{o}C, 80^{o}C, and 90^{o}C) for 0, 5, 10, 15 and, 20 minutes. Heating temperature of 40^{o}C betalain pigments to represent optimum degree of stabilization of pigment; and the temperature of 60^{o}C, 80^{o}C and 90^{o}C are represent pasteurization temperature. Combination treatment performed three replications. Determination of antioxidant activity is conducted with DPPH (2, 2-diphenyl-1-picrylhydrazyl) method.

2.3.Antioxidant Activity of the Red Beet Powder (DPPH method).

0.5 gram sample was weighed and extracted with 5 ml of methanol for 2 hours in the dark room. 0,1 ml of extract were taken and treated with 3.9 ml of DPPH solution made by dissolving 2.4 mg of DPPH in 100 ml of methanol. Samples were incubated in the dark for 30 minutes and absorbance was measured at a wavelength of 515 nm. As a control, use 0, 1 ml of methanol were reacted with 3.9 DPPH solution and methanol used as a blank solution.

Antioxidant activity is expressed as % inhibition with the following equation:

$$\%inhibition = \{(A_B - A_A)/A_B\} \times 100\%$$

Description:

A_A = sample absorbance at 30 minutes

A_B= control absorbance (methanol+DPPH)

(Williams *et al*., 1995)

2.3.Logistic Equation.

The mathematics equation model (logistic equation) is used to determine degree of antioxidant activity degradation with equation:

$$A(t) = \frac{\quad}{1 + \ -}$$

Description:

A: antioxidant activity degradation

A_0: antioxidant activity degradation at t=0

A_f: antioxidant activity degradation at t time

r : constant

t: heating duration (min)

(Wolf & Venus, 1992 in Garnier *et al*., 1999; modification).

2.4.Antioxidant Degradation Kinetics of Red Beet Powder.

The determination of the Ea value using Arrhenius equation, the data obtained by analysis of linear equations where Ea is the activation energy, whose value is assumed to be constant at a certain temperature range, R is the gas constant (8.314 J/mol K), T is the temperature expressed in Kelvin (K). Arrhenius equation can be seen as follows:

$$\ln k = \ln k_0 - \frac{Ea/R}{T}$$

Where the rate constant k showed a decrease in quality, k_0 is a constant (independent of temperature), Ea is the activation energy, T is absolute temperature (K), R is the gas constant (8.314 J/mol.K) (Arpah, 2003).

3.RESULTS AND DISCUSSION

3.1.Antioxidant Activity Degradation of Red Beet Powder

Table 1. *Antioxidant Activity Degradation in Red Beet Powder with Variation of pH, Temperature, and the Heating Duration*

pH	Temperature (^oC)	Antioxidant Activity Degradation (%)				
		Heating Duration				
		0 minute	5 minutes	10 minutes	15 minutes	20 minutes
6	40	25.40±0.64	28.32±1,55	32.53±1.00	35.53±0.96	36.11±0,59
	60	27.77±0.29	29.53±0.99	34.30±2.72	35.85±2.62	36.79±2.35
	80	34.28±0.90	36.02±0.35	39.32±1.50	39.40±1.13	41.42±2.54
	90	37.76±0.21	42.11±0.75	43.98±1.70	45.02±1.82	45.52±0,47
7	40	29.05±0.20	32.86±0.76	32.95±1.18	36.03±1.20	37.58±3.38
	60	30.30±0.47	33.04±0.45	35.63±1.61	37.64±1.62	40.19±2.38
	80	34.20±0.88	37.45±0.36	39.21±1.94	41.77±1,49	42.72±1.03
	90	39.86±0.61	43.90±1.52	44.46±0.76	45.13±0.79	46.51±0.94
8	40	29.71±0.98	32.59±1.56	35.38±2.06	37.89±3.29	39.71±2.61
	60	31.13±0.41	35.09±1.96	39.11±2.84	41.20±0.46	42.50±1.43
	80	36.44±0.59	36.53±0.66	43.00±1.21	45.79±0.15	47.59±0.38
	90	41.05±0.89	44.98±1.79	46.01±0.15	47.33± 0.99	49.76±2.77

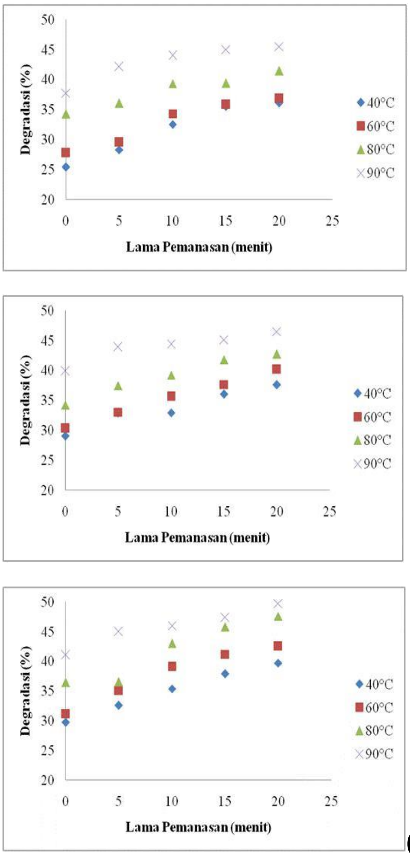
Description:

All values are mean ± SD (Standard Deviation)

From Table 1. there was gradual increase antioxidant activity degradation from red beet powder at pH 6 in temperatures ranging from 40^{o}C to 90^{o}C also in the heating duration is started from minute 0 to minute 20. At pH 7 and pH 8 also showed similar results, Antioxidant activity degradation will increase with higher heating temperature and longer heating duration. The higher degradation is at pH 8 with a temperature of 90^{o}C in the 20 minutes. Fennema (1997) said that red beet has a pH optimum between pH 4 and pH 6.

Therefore the antioxidant activity degradation of red beet at pH 6 is lower than at pH 7 and 8 because in pH 6 the sample is at stable pH conditions. Acidic conditions can increase antioxidant activity stability because it can prevent oxidation so as to minimize the damage of betalain pigments (Ravichandran et al., 2013). Temperature of 40^{o}C is the optimal temperature for the pigment stability so the damage or the degradation is still relatively lower than the higher temperature (Roy et al., 2004).

Heating causes degradation in the form of betalain pigment degradation. This pigment is converted into betalamic acid and cycloDOPA 5-O-glycoside. The longer the heating process, the pigment betalain breakdown process will be greater (Delgado-Vargas et al., 2000).

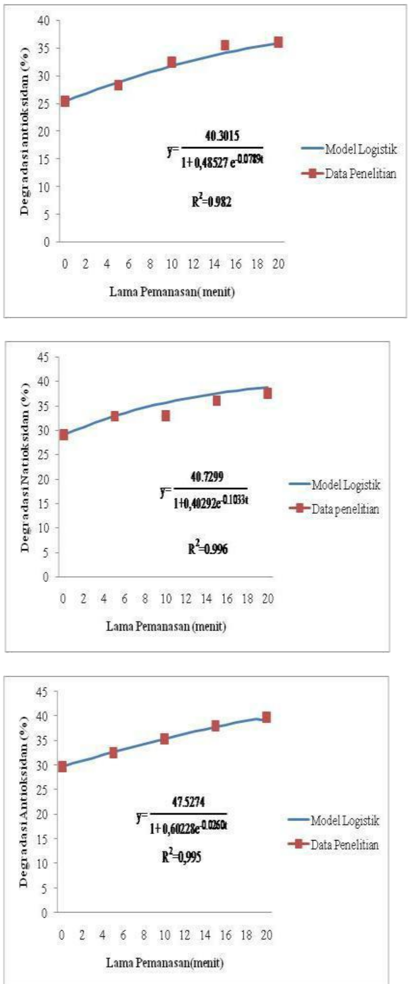


(a) (b) (c)

Figure 1. Decrease in antioxidant activity of red beet powder on (a) pH 6, (b) pH 7, (c) pH 8.

In Figure 1. shows that the higher pH will caused higher degradation of antioxidant activity of red beet powder. On the research from Stintzing and Carle (2004), it said that pH is a factor determines the stability of antioxidant activity. In addition to pH, temperature and heating duration can also cause antioxidant activity of red beet become unstable. Temperature affects the stability of the antioxidants in red beets. In theory of Stintzing and Carle (2004), betalain stability is affected by temperature, pH, moisture, light and oxygen. At high temperature, antioxidants in red beet powder will be damaged.

Logistic model



(a) (b) (c)

Figure 2. Decrease antioxidant activity of red beet powder to the logistic equation on (a) pH 6, (b) pH 7, (c) pH 8

In Figure 2. we can be see that the antioxidant activity degradation will increased with the longer heating duration. Highest antioxidant activity degradation is on 90^{o}C at 20 minutes and the lowest antioxidant activity degradation is on 40^{o}C at

5 minutes.

Table 2. *Correlation Coefficient of Linear Equations in Red Beet Powder*

Temperature	pH 6	pH 7	pH 8
40^{0}C	0.967	0.991	0,993
60^{0}C	0.935	0.997	0,954
80^{0}C	0.942	0.971	0.958
90^{0}C	0.953	0.846	0,946

Table 3. *Correlation Coefficient of Logistic Equations in Red Beet Powder*.

Temperature	pH 6	pH 7	pH 8
40^{0}C	0.982	0.996	0.995
60^{0}C	0.960	0.998	0.996
80^{0}C	0.957	0.986	0.979
90^{0}C	0.960	0.973	0.961

In the Table 2 and Table 3 it can be seen that the higher correlation coefficient, the relationship between x and y will be higher too (Labuza, 1978). Based on these results it can be concluded that the antioxidant activity degradation of red beet powder tends to use the principle of logistic equation, where the initial heating of antioxidant degradation begins to rise higher and then increases the longer the increase be fixed at some point. Timuneno (2008) states that the logistic equation is showed that at a certain point decrease in antioxidant activity reaches equilibrium, so that the graph will be a constant.

According to D'Amico *et al.* (2006), the temperature and time of pasteurization for red beet powder is 60^{o}C for 18 minutes; while according Klewicka & Czyzowska (2011) using temperature 80^{o}C for 10 minutes and 90^{o}C for 5 min. When the pasteurization temperature and time applied in the logistic equation for each pH, the value of % antioxidant activity degradation of red beet powder can be seen in Table 4. From Table 4 it can be seen that the pasteurization time and temperature are inversely related. The higher the heating temperature, the shorter the time required.

Tabel 4. *% Antioxidant Activity Degradation of Red Beet Powder Based on Temperature and Time Pasteurization*

Temperature/duration of pasteurization	% degradation		
	pH 6	pH 7	pH 8
60^{o}C/18 minutes	36.79%	40.19%	42.50%
80^{o}C/10 minutes	39.32%	39.21%	43.00%
90^{o}C/5 minutes	42.11%	43.90%	44.98%

3.2.Antioxidant Degradation Kinetics of Red Beet Powder

Degradation kinetics of the antioxidant of red beet powder was analyzed using the Arrhenius equation. The trick is plotting ln k (%antioxidants degradation) with 1/T (temperature heating) for each heating time and pH conditions in order to obtain a linear equation. From this linear equation obtained value gradient/slope equal to the activation energy that divided with the gas constant values (Table 5).

Table 5. *Activation Energy Values at Each pH*

pH	t (min)	*Slope* (k)	R (J/mol.K)	Ea (J/mol)
6	0	878,5	8.314	7303,85
	5	827,7	8.314	6881,50
	10	635,4	8.314	5282,71
	15	603,2	8.314	5015,00
	20	580.4	8.314	4825,44
7	0	720,7	8.314	5991,80
	5	718,1	8.314	5970,28
	10	618,8	8.314	5144,70
	15	599,6	8.314	4985,07
	20	529,6	8.314	4403,09
8	0	681,5	8.314	5665,99
	5	626,1	8.314	5205,40
	10	579,2	8.314	4815,46
	15	511,0	8.314	4248,45
	20	508,8	8.314	4230,16

Description

T = heating duration (menit)

Slope = gradient (K)

Ea = activation energy (J/mol)

R = gas constant (J/mol.K)

From the Table 5 it can be seen that the activation energy of each sample is proportional to the value of degradation quality constant. The highest activation energy is red beet powder at pH 6 were heated for 0 minutes; while the lowest activation energy is red beet extract at pH 8 were heated for 20 minutes. According Azeredo (2009), degradation of the pigment betalain will caused the value of the activation energy decreases. This means that the greater the degree of degradation of betalain pigments, the value of the activation energy decreases.

The activation energy the minimum amount of energy supplied to a reaction (Hariyadi, 2004). From the theory of Robertson (1993), states that the smaller the activation energy, the faster the product will be severely degrades. To calculate the activation energy, make a graph of ln k with 1/T, can be seen in Figure 3. it can be seen that the obtained linear equation y=mx+b. The slope of the equation above represents the value (-Ea/R) with constant gas of 8,314 J/mol.K. In the linear equation y=-878,5x+1.433 (R^2=0.995) obtained Ea value of 7303.85 J/mol.

CONCLUSION

- Decrease in the antioxidant activity of extracts of red beet will increase when the temperature and heating time increased. Decrease in antioxidant activity in red beet powder will increase when conditioned in neutral and alkaline pH.
- The best temperature of pasteurization is at a temperature of 60^{o}C while the pasteurization temperature which causes a decrease in antioxidant activity was highest in the temperature of 90^{o}C.
- The higher the temperature of

pasteurization, the heating duration will be lower, stable antioxidant activity carried out at a temperature of 60^{o}C at 18 minutes pasteurization, pasteurization is done at a temperature of 80^{o}C at 10 minutes and at a temperature of 90^{o}C at 5 minutes.

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