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Kinetic changes of antioxidant capacity and physical quality of tempe during heating

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Abstract

Tempe is a traditional fermented soyfood from Indonesia which has a short shelf-life. Thermal process destroying microorganisms might be applied to extend the shelf-life of tempe. The study on evaluation of quality changes of tempe during heating is very limited. The main objective of this research was to study the kinetics of antioxidant capacity and physical changes during isothermal heating of tempe at different temperature (75°C, 85°C and 95°C). Tempe was cut and placed in vial tubes with 2% of aqueous salt (NaCl) solution as heating medium. Changes of antioxidant capacity (2,2-diphenyl-1picrylhydrazyl (DPPH) scavenging capacity and total phenolic content (TPC)) and physical quality (texture and color) of tempe during heating were analyzed and modeled as first order kinetic reaction. Thermal process affected on antioxidant capacity and physical quality of tempe. During heating the rate constant of antioxidant capacity and physical quality in samples increased with increase of time and temperature process. The changes of DPPH scavenging capacity had similar trend to TPC indicating that TPC strongly influenced on the antioxidant capacity of tempe. Based on Arrhenius equation, the most heat sensitive parameter was color properties, followed by hardness, TPC and lastly antioxidant capacity.

Introduction

Tempe is an Indonesian traditional food made by fermentation of soybean with *Rhizopus* spp. and some other moulds, such as *Mucor* spp. Some enzymes produced during fermentation such as proteases, lipases, carbohydrases and phytases degrade macromolecules into micromolecules (Nout and Rombouts, 1990) contributing to the appearance, aroma, texture, flavor and nutritional quality of tempe (Wiesel *et al.*, 1997). Fungal biomass growth binds the bean cotyledons together tightly, thus tempe resulted has a firm texture and unique appearance.

After soybean fermentation, the hardness and lightness of tempe decreased (De Reu *et al.*, 1997; Handoyo and Morita, 2006), but the antioxidative capacity became higher than the raw soybeans (Chang *et al.*, 2009). Some subtances contributed to improve antioxidant capacity of tempe, such as free amino acids, peptides, isoflavonoid aglycones, 3-hydroxyanthranilic acid (HAA) and gamma-aminobutyric acid (GABA) (Esaki *et al.*, 1996; Watanabe *et al.*, 2007). Sheih *et al.* (2000) reported that a thirds of antioxidant capacity in tempe were contributed by aglycone isoflavones and the other two-thirds were contributed by peptides liberated during fermentation.

Furthermore, as compared to soybeans tempe had improved bioactive compounds, such as glucosamine, GABA, folate, vitamin B_{12}, vitamin B_6, ergosterol, aglycone isoflavones and some minerals (Wiesel *et al.*, 1997; Eklund-Jonsson *et al.*, 2006; Feng *et al.*, 2007; Koh *et al.*, 2012; Mo *et al.*, 2013). However, the great healthy benefits of tempe were not balanced with the short shelf-life of tempe. For this reason, thermal process might be applied as an alternative methods to extend the shelf-life because it destroyed microorganisms effectively. On the other hand, thermal treatment induced some changes to the physical, nutritional and sensory properties of food.

Some researchers stated that thermal treatments affected on quality attributes of soybean products. Boiling yellow soybean flours for 120 min could decrease the lightness and increase the chroma intensity (Xu and Chang, 2008). Moreover, thermal processing significantly affected on the antioxidant capacity, total phenolic components, total flavonoid content and total isoflavones in soymilk and yellow soybeans (Huang *et al.*, 2006; Xu and Chang, 2008; Xu and Chang, 2009).

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Thus, kinetic models are required to predict quality changes at different processes to provide a safe product and maximize the quality retention. But, a study of quality evaluation of tempe subjected to the thermal processing conditions is very limited. This study was conducted in model systems because it would be easier to explore some changes of tempe during thermal process. The aim of this research was to develop a kinetic model of antioxidant capacity and physical quality changes of tempe as affected by various heating treatments. Therefore, the changes that occur during thermal processing of tempe can be predicted for optimal product quality.

Materials and Methods

Tempe preparation

Yellow-seeded soybeans (*Glycine max* L.) were obtained from Organization of Tempe and Tofu Producers (KOPTI) Bogor, Indonesia. Clean soybean grains (300 g) were soaked with an acetic acid solution pH 3.5 (650 mL) for 22 hours at 27°C. The soybeans were then dehulled manually, and the separated cotyledons were boiled (95°C) in tap water (2 L) for 40 min. After cooling down to room temperature, solid state fermentation was performed by inoculating 0.03% mixed culture (Raprima brand, Bandung, Indonesia) into cooked soybeans in perforated polypropylene bags (25 cm × 12 cm) and then incubated for 40 hours at 30°C.

Thermal treatments

Fresh tempe was cut into cylindrical shape with diameter of 12 mm and height of 20 mm. Three tempe cuts (± 6 g) were placed in vial tubes (d: 20 mm, h: 75 mm) considering to minimize lag time. Before closing the tube, 2% aqueous salt (NaCl) solution (± 20 mL) was added, which was usually used as heating medium in thermal process. Samples were heated in water bath (Gesellschaft für Labortechnik, Germany) set at three different temperature (75°C, 85°C and 95°C) for 0-120 min. Process time was calculated after lag time of 6 min at 75°C and 7 min at 85°C and 95°C. Following the heat treatments, samples were immediately cooled in ice water to minimize quality deteration during cooling. For chemical analysis tempe and salt solution were separated and freeze-dried, whereas for physical analysis only tempe samples were determined.

Extraction of samples

The freeze-dried samples were accurately weighed into centrifuge tubes and extracted with 50% acetone (Merck, Germany) and 80% acetone (1:5, w/v) for TPC and DPPH scavenging capacity respectively. Solvents were selected according to a solvent comparison study by Xu and Chang (2007), which found 50% acetone extracts exhibited the highest TPC values and 80% acetone the highest DPPH scavenging capacity values for yellow soybean. The sample flour-solvent mixtures were vortexed 16 times during 4 h of extraction according to Ferreira *et al.* (2011). The tubes were then centrifuged by 5810R Centrifuge (Hamburg, Germany) at 3000 rpm for 10 min and the extracts were stored at 5°C in the dark for use.

Determination of DPPH scavenging capacity

The 2,2-diphenyl-1picrylhydrazyl (DPPH) scavenging capacity of samples were evaluated according to the method of Xu and Chang (2007). The sample extract (0.2 mL) was added to 3.8 mL ethanol (Merck) solution of 0.1 mM DPPH radical (Sigma, USA). The mixture was vortexed vigorously for 1 min and then kept at room temperature in the dark for 30 min. The absorbance of samples was measured using a UV-Visible Spectrophotometer (U-2900, Hitachi, Tokyo, Japan) at 517 nm against ethanol as a blank. A negative control was mixture of DPPH solution and extraction solvent. The inhibitory percentage of DPPH was calculated according to the following equation:

$$\text{Inhibition (\%)} = [1 - (A_{sample}/A_{control})] \times 100$$

The DPPH free radical scavenging activity was expressed as millimoles of ascorbic acid equivalent per gram of freeze-dried sample (mmol of AAE/g). The standard calibration curve range was 10 to 1000 μM ($R^2 = 0.996$).

Determination of total phenolic content (TPC)

Total soluble phenolics in the extracts were determined using a Folin-Ciocalteu assay as described by Xu and Chang (2007). The sample extract (50 μL), distilled water (3 mL), 250 μL Folin-Ciocalteu's reagents solution (Merck) and 750 μL of 7% Na_2CO_3 (Merck) were vortexed and left to stand for 8 min at room temperature. Thereafter, distilled water (950 μL) was added to the mixture and the absorbance was read using the U-2900 Spectrophotometer at 765 nm after 2 h of incubation against distilled water blank. The TPC was expressed as milligrams of gallic acid equivalents per gram of freeze-dried sample (mg of GAE/g). The standard calibration curve range was 10 to 1000 μM ($R^2 = 0.998$).

Texture analysis

The texture measurement was accomplished by using TA-XT2i Texture Analyzer (Stable Micro Systems Ltd., Godalming, Surrey, UK) with cylindrical probe (d: 30 mm, h: 50 mm), by performing texture profile analysis (TPA). Tempe were compressed to 50% of their original height by two compressions. Texture profile was generated by keeping pre-test, test and post-test speeds of 2, 1 and 2 mm/s respectively. The waiting time between the two-cycles of the TPA test was 5 s. The peak force of the first compression cycle of the sample was marked the maximum force and recorded as the indicator of hardness. Various texture parameters were obtained from texture profiles by using Texture Expert 1.22 software.

Color analysis

Analysis of visual color was performed by using CR310 Chromameter (Konica, Minolta, Tokyo, Japan). Hunter's color parameters (L, a and b values) for the surface of treated tempe samples were recorded. The L value indicated lightness, a, the red (+) or green (−) coordinate and b, the yellow (+) or blue (−) coordinate.

Kinetic modeling

In this study the changes of quality attributes were modeled as first order reaction characterized by logarithmic relationship between concentration of food quality and time (Villota and Hawkes, 2007):

$$-\ln\left(\frac{C_t}{C_0}\right)=k_1 t \quad (1)$$

where C_t is the concentration at time (t), C_0 is the initial concentration and k_1 is rate constant.

The temperature dependence of a reaction rate constant can be expressed by the Arrhenius equation:

$$k=k_0 \exp\left(-E_a/RT\right) \quad (2)$$

where k_o is the frequency or collision factor, E_a is the activation energy, R is the gas constant (8.314 J/K mol) and T is the absolute temperature (K).

Results and Discussion

Changes of antioxidant capacity

The DPPH assay measures against the 2,2-diphenyl-1-picryhydrazyl radical which is reduced to the yellow colored in alcoholic solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH-H (Gülçin, 2012). Kinetic modeling in radical DPPH scavenging capacity of tempe during heating is shown in Figure 1. The results of spectrophotometric analysis indicated an increase in antioxidant capacity of salt solution and total of tempe and salt solution due to thermal treatments. In contrast, antioxidant capacity of tempe decreased during heating period of 120 min.

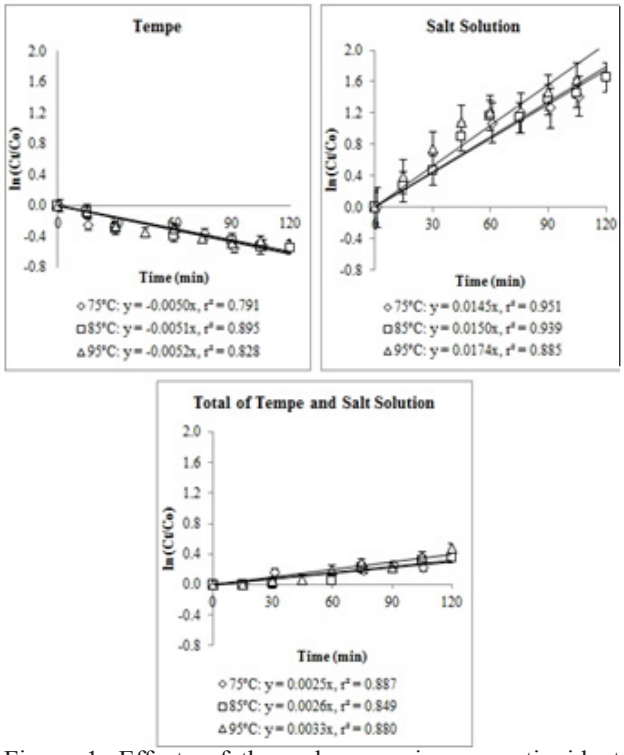

Figure 1. Effects of thermal processing on antioxidant capacity of tempe at 75°C, 85°C and 95°C.

The rate of decreasing antioxidant capacity in tempe increased very slightly with increase of temperature (k_{75}=0.0050, k_{85}=0.0051 and k_{95}=0.0052 min^{-1}). Heating tempe at 75°C, 85°C and 95°C for 120 min gave almost same effects on antioxidant capacity. In addition, the rate of increasing antioxidant capacity in salt solution (k_{75}=0.0145, k_{85}=0.0150 and k_{95}=0.0174 min^{-1}) was greater than in total of tempe and salt solution (k_{75}=0.0025, k_{85}=0.0026 and k_{95}=0.0033 min^{-1}). Totally the thermal treatments of sample at 75°C and 85°C gave almost same effects on total of tempe-salt solution for 120 min. However, heating at both of temperature was significantly different as compared to at 95°C. The rate constant of antioxidant capacity changed drastically at temperature over 85°C.

A similar pattern had been reported for ultra high temperature (UHT) process of soymilk (Xu and Chang, 2009), pasteurization of tea extracts (Manzocco *et al*., 1998), and steaming of several vegetables, such as carrots, spinach, mushrooms, asparagus, broccoli, cabbage, red cabbage, green and red peppers, potatoes and soybeans (Halvorsen *et al.*,

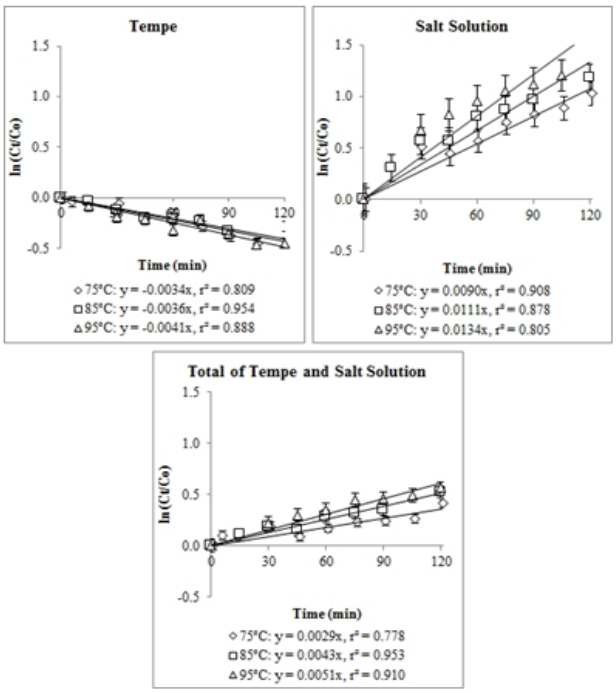

Figure 2. Effects of thermal processing on total phenolic content (TPC) of tempe at 75°C, 85°C and 95°C.

2006), which showed an increase in the antioxidant capacity. The steaming process significantly increased about 75-140% DPPH values of antioxidant capacity in yellow soybeans as compared to the raw soybeans (Xu and Chang, 2008).

According to Sheih *et al*. (2000) that two-thirds of antioxidant capacity in tempe was contributed by peptides. Tempe containted 17 amino acids (Kwon *et al*., 2010), which tyrosine, phenylalanine, cysteine, lysine, arginine, aspartate, glutamate, histidine, glycine and proline were suggested playing important role in antioxidant power of soybean (Riison *et al*., 1980; Chen *et al.,* 1996; Fang *et al*., 2002; Hu *et al*., 2003; Saiga *et al*., 2003; Je *et al*., 2004; Rajapakse *et al*., 2005). Soybean protein isolate was reported of conducting denaturation at 76.5°C with high water content (Kitabatake *et al.*, 1990). However, antioxidant capacity of protein and amino acids was relatively constant by thermal treatments (Arcan and Yemeniciog˘lu, 2007).

Isoflavones in soybean have been considered to be the source of antioxidant. Genistein, daidzein and their glycosides had a radical DPPH scavenging capacity, ferric reducing-antioxidant power (FRAP) and suppression of low-density lipoprotein (LDL) oxidation (Lee *et al*., 2005). Heating soybean product at 70-90°C caused degradation of glucoside isoflavones to aglycone form (Eisen *et al*., 2003) which the aglycones had greater antioxidant capacity than glucoside forms (Pratt and Stafforini, 1979). As compared to raw soybean, boiled yellow soybean had significant increases of β-glucoside (daidzin, glycitin and genistin) and aglycone (daidzein, glycitein and genistein) isoflavones, but significantly decreased the content of the malonylglucoside forms (malonyldaidzin, malonylglycitin and malonylgenistin) (Xu and Chang, 2008). Therefore, this research indicated that the isoflavones from phenolic group were the responsible components affecting the changes of antioxidant capacity of tempe during heating.

On the other hand, the decline of antioxidant compounds in solid phase was due to the increase of water soluble antioxidant subtances to 2% of salt solution. Thermal process might decrease firmness and adhesion of cell walls (Van Buren, 1979), thus it induced releasing of bound phenolic compounds, such as flavonoids, accumulated in the vacuoles (Brecht *et al.*, 2008). Briefly, as longer heating time and higher temperature, the bound antioxidant components were more liberated from cell and leached into liquid phase.

Changes of total phenolic content (TPC)

The total phenolic content in samples was measured using the Folin-Ciocalteu assay which was based on the transfer of electrons from phenolic compounds to the Folin-Ciocalteu reagent in alkaline medium forming blue complexes that can be detected spectrophotometrically at 750–765 nm (Singleton *et al.*, 1999). The changes of total phenolic contents during heating expressed as mg of galic acid equivalent/g of sample are shown in Figure 2.

When samples were subjected to the thermal processing, the TPC changed with the same trend as antioxidant capacity. The rate of decreasing TPC in tempe increased with increase of temperature (k_{75}=0.0034, k_{85}=0.0036 and k_{95}=0.0041 min^{-1}). Moreover, the TPC of salt solution elevated more quickly (k_{75}=0.0090, k_{85}=0.0111 and k_{95}=0.0134 min^{-1}) than in total of tempe and salt solution (k75=0.0029, k_{85}=0.0043 and k_{95}=0.0051 min^{-1}).

The increase of TPC occured in salt solution, whereas TPC of tempe decreased during heating period of 120 min indicating that heat treatments caused water-soluble phenolic compounds leaching into heating medium. Totally heating of samples increased the total amount of TPC in tempe and salt solution. Heating of tempe might cause the degradation of polyphenols and release of bound phenolic compositions from the vacuoles (Brecht *et al*., 2008). According to correlation analysis (data not shown), there were significant correlations between TPC and DPPH scavenging capacity ($p < 0.05$) at 75°C, 85°C and 95°C in all components of samples.

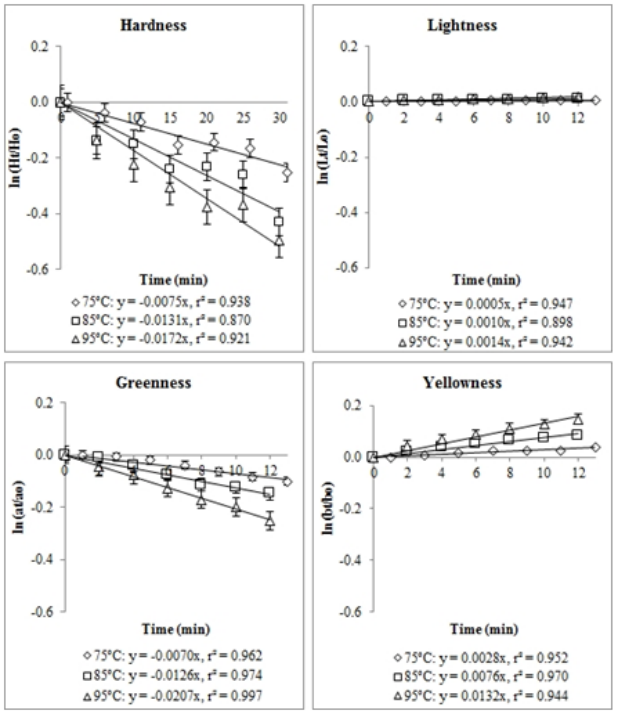

Figure 3. Effects of thermal processing on physical quality of tempe at 75°C, 85°C and 95°C.

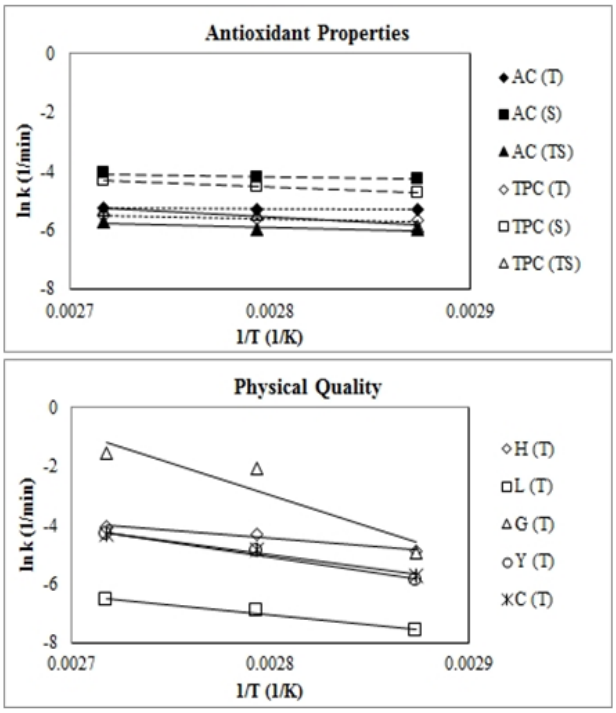

Figure 4. Arrhenius plot for antioxidant properties and physical changes of tempe at 75oC, 85oC and 95oC. (Key: T=tempe, S=salt solution, TS= total of tempe and salt solution, AC=antioxidant capacity, TPC=total phenolic content, H=hardness, L=lightness, G=greenness, Y=yellowness).

It can be assumed that phenolic compounds strongly influenced the antioxidant capacity of tempe.

The changes of phenolic content in tempe was affected by composition of phenolic acids. Raw and boiled yellow soybean contained free phenolic acids both benzoic type, such as gallic acid, 2,3,4-trihydroxybenzoic acid, vanillic acid and protocatechualdehyde, and cinnamic type, such as chlorogenic, sinapic and trans-cinnamic acid. Moreover, conjugated phenolic acids were also detected in both raw and boiled yellow soybean, such as benzoic type (gallic, protocatechuic, 2,3,4-rihydroxybenzoic, p-hydroxybenzoic, gentistic, syringic, vanillic acid, protocatechualdehyde and vanillin) and cinnamic type (caffeic, p-coumaric, m-coumaric, o-coumaric, sinapic and trans-cinnamic acid) (Xu and Chang, 2008).

Thermal processing caused complex variations in phenolic acid profiles of soy products. For instance, boiling soymilk caused significant increases in free gallic, protocatechuic, 2,3,4-trihydroxybenzoic, sinapic acid and subtotal benzoic acids (Xu and Chang, 2009), whereas boiling treatment of yellow soybean significantly increased 2,3,4-trihydroxybenzoic acid (Xu and Chang, 2008). In addition, pressure steaming of yellow soybeans caused the increase of benzoic acid and also the TPC value. Beside phenolic acids, the changes of TPC during heating were affected by isoflavonoids as explained before.

Changes of hardness

Generally thermal softening in fruits and vegetables was expressed as the first order reaction rate with the hardness as the primary textural attribute (Bourne, 1987). The maximum force (kg) parameter of the samples during thermal treatments at three different temperatures is given in Figure 3. As temperature elevated, the rate of softening tempe increased (k_{75}=0.0075, k_{85}=0.0126 and k_{95}=0.0165 min^{-1}) which at 95°C textural degradation occured most rapidly.

The firmness of various bean was strongly correlated with soluble pectin content increasing due to thermal treatment (Huang and Bourne, 1983). Intercellular adhesive material, such as pectin, held the firm texture of cell wall structure. When broken by heat, the pectin was depolymerized, as a result the plant tissue lost its resistance to fracturability (Loh and Breene, 1982). Thermal process affected on turgor cell and changes in wall-pectin composition leading to a decrease in firmness (Van Buren, 1986; Revilla and Vivar-Quintana, 2008). In addition, the presence of salt (NaCl) in the medium accelerated the solubility of pectin (Van Buren, 1986). Thus, the more salt dissolved in solution, the texture of tempe became softer.

Table 1. Activation energy for antioxidant properties and physical quality of tempe during thermal treatments.

Parameters	Component	Equations	r^2	Ea (kJ/mol)
Antioxidant capacity	Tempe	y = -251.1x – 4.5769	0.999	2.09
	Salt solution	y = -1160.3x – 0.9188	0.873	9.65
	Total	y = -1765.4x – 0.952	0.842	14.68
Total phenolic content	Tempe	y = -1194.1x – 2.2653	0.945	9.93
	Salt solution	y = -2549.3x + 2.6167	0.999	21.19
	Total	y = -3627.2x + 4.6135	0.957	30.16
Hardness	Solid	y = -5330.3x + 10.467	0.968	44.32
Lightness	Solid	y = -6612.4x + 11.453	0.968	54.98
Greenness	Solid	y = -21823x + 58.122	0.868	181.44
Yellowness	Solid	y = -9952.8x + 22.787	0.978	82.75

Color changes during heating

Figure 3 also shows typical results on the changes of color parameters of tempe surface at 75°C, 85°C and 95°C. The lightness (L) and yellowness (b) values increased, but the greenness (a) values tended to decline during thermal processing. It can be seen from the graphs that the rate of changes in color attribute at 95°C occured most rapidly, followed by heating at 85°C and 75°C. The increase rate of yellowness (k_{75}=0.0028, k_{85}=0.0076 and k_{95}=0.0132 min^{-1}) occured more rapidly as compared to lightness (k_{75}=0.0005, k_{85}=0.0010 and k_{95}=0.0014 min^{-1}) for 12 min of heating. On the other hand, the rate of decreasing in greenness was the greatest (k_{75}=0.0070, k_{85}=0.0126 and k_{95}=0.0207 min^{-1}) among other color parameters.

According to Clydesdale and Ahmed (1978), object-light interactions might affect on color measurement of samples, such as reflection from the surface. Heating tempe in salt solution caused the increase of water content entering into the tissue. When the source light from chromameter came on the tempe surface, it would be resulted increasing intensity of object-light interactions. In this way, increasing water content of tempe produced the more reflected light from the tempe surface. This might increase the lightness of tempe and change the other color propeties.

A food system which contain a carbonyl group of reducing sugar and an amine group of free amino acids subjected to high temperature treatment should experience the Maillard reaction involving the formation of brown pigmen (Kim and Lee, 2009). It is well known that tempe contains high protein (23-55%) and also some sugars (Kwon *et al.*, 2010). When tempe was heated for certain time, the carbonyl group and amine group might interact to form brown color of Maillard reaction. But, the presence of salt in heating medium could decrease the rate of Maillard Reaction due to the decrease of water activity value (BeMiller and Huber, 2008). Therefore, it can be assumed that the use of 2% salt solution as heating medium might be effectively retain the visual appearance of tempe.

Activation energy

The Arrhenius plot (ln k vs. 1/T, the reciprocal absolute temperature) of antioxidant activity and physical changes of tempe is given in Figure 4. The Arrhenius equation described the effects of typical temperature ranges on tempe attribute changes during thermal process. It can be seen from the curves that the slope for degradation of physical properties was sharper than antioxidant capacity indicating that the rate of physical change was more heat sensitive than other parameter.

The activation energy (E_a) (Table 1), which represents the least amount of energy needed for a chemical reaction to take place, was calculated by the slope of curves (E_a/R). The activation energy also indicated the parameter sensitivity to temperature changes. Determination of activation energy from physical attributes showed that activation energies for color changes were greater than textural changes meaning that color properties were more heat sensitive than textural attributes. The activation energy for antioxidant capacity was smaller than total phenolic content meaning that TPC was more heat sensitive than antioxidant capacity of tempe. A positive value of E_a means that the reaction rate increases with increasing temperature. The information from Arrhenius parameters can be used to optimize thermal process and maximize quality retention of tempe by choosing an appropriate time-temperature combination.

Conclusion

Thermal process affected on antioxidant capacity and physical quality of tempe. During heating the rate of change in antioxidant capacity and physical quality of tempe increased with increase of time and temperature process. The changes of DPPH scavenging capacity had similar trend to TPC indicating that TPC strongly influenced on

the antioxidant capacity of tempe ($p < 0.05$). The presence of salt (NaCl) in heating medium improved solubility of pectin accelerating textural degradation and also decreased water activity value inhibiting the Maillard reaction, thus the appearance of fresh tempe could be relatively maintained. Based on Arrhenius equation, the most heat sensitive parameter was color parameters, followed by hardness, TPC and lastly antioxidant capacity.

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