Shelf Life Prediction of Tempeh Processed With Sub Supercritical Carbon Dioxides

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Abstract

Growth of *Rhizopus oligosporus* mycelium is an indication that tempeh is in good quality. Some of the *Rhizopus oligosporus* in tempeh processed with sub supercritical CO_2 do not die but recover during storage in favorable conditions, which may prolong the shelf life of the tempeh. Kinetic changes of quality parameters were used to predict shelf life of unprocessed and processed tempeh during storage at 20^o, 30^o, and 40^{o}C for 5 days. The result showed that the rate *(k)* degradation of *L** in processed tempeh was in the range of 0.007-0.027 and E_a was 12.20 kcal/mol K, whereas *k* of the Δ*E** decreased with the increase of storage temperature, and E_a was -1.96 kcal/mol K. Even though the E_a of unprocessed tempeh was smaller than processed tempeh, *k* value of *L** in either processed or unprocessed tempeh was sensitive to the temperature change. By analyzing *L** changes of tempeh during storage, it was found that the shelf life prediction of processed tempeh was 6.89±0.37, 10.28±1.48, and 2.70±0.12 days at 20, 30 and 40^{o}C respectively, while the unprocessed tempeh was 3.48, 2.21, and 2.67 days at 20, 30, and 40^{o}C respectively. The conclusion was that sub supercritical CO_2 processing can serve as an alternative method of cold pasteurization for tempeh which facilitates fungal growth during storage, thereby increasing shelf life.

Keywords: *subsupercritical* CO_2*, kinetic change, shelf life, tempeh*

Introduction

At present, consumers' needs for fresh food are not only in quality, safety and acceptance, but fresh food that has a long shelf life is also a consideration. Carbon dioxide in the states of supercritical is called as a supercritical CO_2, and it is sub supercritical CO_2 if it is slightly below near the supercritical region (at 25^{o}C and 6.3MPa). At this point, supercritical CO_2 has dual properties like a gas with high diffusivity and a liquid with high solubility which enable CO_2 to easily diffuse through complex matrices and cause the changes in either macromolecules or micro molecules substrates [1]. Since the molecules changes are happening at low temperature of processing, the supercritical CO_2 will be beneficial when applied for processing of food products. Many researchers have shown that high processing carbon dioxide (HPCD) can extend the shelf life of food in part because this technique can reduce the number of microorganisms in food and inactivate enzymes that cause food spoilage. However, some bacteria, especially those belonging to probiotic bacteria and certain types of molds such as *Rhizopus oligosporus* may need to be preserved during processing. *R. coligosporus* is an important mold in fermentation of soybeans, namely tempeh. When tempeh processed with supercritical CO_2 at 6.3MPa for 10min, the *R.oligosporus* in it was survived at the number of 10^4CFU/g [2]. The freshness quality of tempeh can be judged by its white color produced by *R. coligosporus* mycelium. When *R. coligosporus* growth declines, sporulation will appear and produce black spores which give result to change of white color of tempeh to whitish-grey and blackish-white. On the other hand, when either mycelia growth ceases or there is over fermentation, the color of the tempeh gradually changes to brown and dark brown due to protein breakdown. This is what is so called spoilage of the tempe. Fresh tempeh is characterized as having mushroom odor, white color, firm cake texture, and beany flavor. Freshly made raw tempeh can still be eaten for 2 days at room temperature [3]. The possibilities that the sub-supercritical pressure of CO_2 can maintain the quality of tempeh needed to be disclosed because the molds of tempeh processed with sub-supercritical of CO_2 is not completely dying. The objective of this research was to ascertain the kinetic process of degradation of the quality parameters of tempeh processed with sub-supercritical CO_2, including the rate reaction (k) and activation energy (E_a) and to predict shelf life.

2. Material and Methods.

2.1. Tempeh Preparation.

Tempeh, in the form of cylinder with 35 mm in diameter, and 100 mm length, was fermented for 36 h at 30^{o}C [2], was obtained from Center of Home Industry Tempeh Making Palembang, Indonesia, placed in the cool box and carried to the laboratory for direct processing. The high pressure CO_2 installation used for experimental treatments consists of a CO_2 gas cylinder, a cylindrical pressure chamber, pressure gauges, and a water bath at constant temperature showed in Fig. 1 [4]. Fresh tempeh was placed in a pressure chamber and then closed tightly. When the designated temperature in water bath was reached (27±2^{o}C) and all pipe connections were secured, commercially available CO_2 (Pertamina, Jakarta, Indonesia) was injected through the gas inlet valve from the gas cylinder into the pressure chamber until it reached the desired pressure of 6.3 MPa (sub supercritical CO_2 condition) which was showed in the pressure gauge within 1 min. After being treated with sub supercritical CO_2 for 10 min, the pressure was lowered to atmospheric pressure within 2 minutes by slowly opening the gas outlet valve. Then the tempeh was aseptically collected from the pressure chamber using a sterilized tong, placed in the sterilized container and stored in a refrigerator before running further experiment, storage study. Analysis of tempeh including color, textures, water content, mold numbers were conducted at both unprocessed tempeh and processed tempeh before and during storage time.

2.2. Color and Texture Measurement.

The surface color analysis of processed tempeh and the control was evaluated as *CIE* $L^*a^*b^*$ value and *LCH* color scale using color difference meter (TC-1500, Tokyo, Japan). Results were expressed as L^* (Lightness), a^* (redness), and b^* (yellowness). The total color difference (ΔE^*) between the control and the processed tempeh was obtained using following equation: $\Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}$ Where: the L^*, a^*, and b^* values meant the difference between the L^*, a^*, and b^* value after the treatment and the L^*, a^*, and b^* values of the standard color. The standard color used in this experiment was the L^*, a^*, and b^* values of the unprocessed tempeh (control) which was L^*=76.6; a^*=3.1; b^*=7.5. The color values of processed tempeh with sub supercritical CO_2 was L^*= 74.4, a^*=3.8, b^* =8.9, and ΔE^*= 1.58.

2.3. Storage Study.

The processed tempeh with sub-supercritical CO_2 (6.3 MPa, 25^{o}C for 10 minutes) were stored for 5 days. The storage of tempeh was carried out as follows: tempeh were placed in a steroform plate and covered with plastic film then stored at 20, 30, and 40^{o}C with the same relative humidity. Observations on quality factor changes (C) were carried out by measuring the quality attributes represented by L^*, ΔE^*, water content, mold numbers, and textures. Observations were made daily. A storage time of 5 days was chosen considering that the shelf-life of fresh tempeh was normally around 24-48 hours at room temperatures (28-30^oC). Fresh tempe that is not processed by supercritical CO_2 used as a control was also stored in the same condition as processed tempeh. The kinetics changes of quality factor (*C*) which was *L** and Δ*E** color, water content and textures in this experiment over the time (during storage) under isothermal conditions could be represented as in equation 1 [5]:

$$\frac{dC}{dT} = -k(C)^n \qquad (1)$$

Where *k* is the rate constant, *C* is the quantitative indicator of a quality attribute at time *t*, and *n* is the order of reaction. The *Arrhenius* equation is applied to describe the reaction rate constant temperature dependence represented in equation 2 [5]:

$$k = k_0 \exp(-Ea/RT) \qquad (2)$$

The equation 2 was applied to a reaction in consideration. Plot of the rate constant on semi-logarithmic scale as a function of reciprocal absolute temperature (1/T abs) should give a straight line, and the activation energy can be determined as the slope of the line multiplied by the gas constant *R*. Determination of reaction order is carried out by using an integrated rate law method that compares the coefficient correlation (R^2) value of each linear regression equation based on the results of the plot between the quality parameter data *[C]* and time (t). Firstly plot between the concentration of the quality parameter *[C]* to time (t), *ln* of the concentration of quality (ln *[C])* to time (t) and plot between 1/*[C]* to time (t), and then create a linear line equation and curve. Next, determine the most suitable line based on the coefficient of determination (R^2). The integrated form for first-order kinetic models is listed in equations 3 [6].

$$\ln(C/C_0) = -kt \qquad (3)$$

Result and Discussion

Quality Degradation Kinetics

Unprocessed tempeh and processed tempeh which was tempeh treated with sub supercritical CO_2 at 6.3MPa for 10 min were used as the models in this experiment. Fresh tempeh has a bright white color produced by the growth of *Rhizopus oligosporus* mycelium, and compact, sliceable textures. Following the processing under sub supercritical CO_2 for 10 min, tempeh was removed from the high pressure CO_2 system and analyzed its color, textures and water content. The value of *L**, *a**, *b**, *ΔE**, textures and water content of the processed tempeh was 74.4, 3.8, 8.9, 1.58, 577.2 gf, and 65.33%, respectively before storage and was 69.3, 4.1, 14.0, 5.32, 410.6 gf, and 49.49%, respectively after storage for 5 days at 30±2^oC. Low *L** value resulted in dark color indicates the color degradation of tempeh. The lowest rate degradation of *L** was -1,197 when tempeh was stored at 20^oC. Quality degradation rate was not correlated to the increase of storage temperatures, where the smallest Δ*E** was found in tempeh storage at 30^oC, and the high value of texture indicated a hard texture produced at 40^oC of storage. Meanwhile, tempeh was softer at 20 than at 30^oC of storage. The increase in texture was due to the evaporation of water from the surface of tempeh. The higher the temperature the greater the heat energy that is carried by air so that the mass of water on the surface of tempeh was more evaporated.

The quality parameter values were subjected to linear regression with respect to time as represented by equation 1. Correlation coefficients values were used to select the combination which best fitted to the first-order reaction for the range of temperature used. The correlation coefficient of color (L^*) degradations were at the range of 0.93 to 0.97, whereas that of the ΔE^* were at the range of 0.78 to 0.97. Table 1 showed that the low value of k demonstrated that L^* color change was significantly affected by heat than any other quality parameters evaluated. The degradation rate of L^* at 40^{o}C which was 0.027 was a threefold increase compared to that of at 30^{o}C (0.009). This meant that the white color of tempeh was turning to greyest dark because of the production of *R.oligosporus* spores. The growing mycelium of *R.oligosporus* produces the color of the tempeh that result in the high L^* value. Meanwhile, the change of mold number observed during storage may not be used as a quality indicator of shelf life prediction because there are several factors that affect the growth of *R. oligosporus* such as water content, temperature, humidity, and oxygen availability during storage of the tempeh. Mycelium of *R.oligosporus* in tempeh requires high energy in order to grow because their optimum growth temperature is at 27-30^{o}C and 63-65% water content (Kustyawati et al, 2018). Similar to the processed tempeh, L^* degradation rate of unprocessed tempeh increased at the higher temperature.

Shelf Life Prediction

Since color is an indicator of spoilage of tempeh, the presence of mycelium is very important in efforts to prolong their shelf life. Growth of Rhizopus mycelium is an indication that tempeh is in good quality. Tempeh has a bright white color and will turn pale to yellow-brown due to temperature changes. Mold growth reached the logarithmic phase on storage day 2 at 30^{o}C and growth began stationary thereafter until day 4 (Fig. 6). At the same time tempeh was white because the growth of mycelium was enveloping it. However, after day 4 of storage the tempeh is brownish due to Maillard reaction that occurs in soy protein. Meanwhile, at a storage temperature of 20^{o}C the mold growth remained steady until the 6th day, resulting in white color in tempeh that did not change color until then. Since Table 2 showed that differences in k values indicated sensitivity to quality deterioration over time which was influenced by temperature. If the value of k was small, the decrease in the quality of tempeh was slower and resulted in a longer shelf life at temperatures lower than 20^{o}C. Changes in the quality of tempeh were fastest when stored at a higher temperature.

Color is crucial in tempeh sensorial properties since it is the first characteristics the consumer observes. The color of tempeh changes during storage. Whitest color of tempeh decreased and the product turned brown to black with the time and this is reflected by decreased in L^* and ΔE^* value. Color of tempeh produced by the fungus mycelium *R. oligosporus* and was influenced by chemical changes in food constituents as well as temperature. During storage of tempeh, molds use chemical elements such as carbohydrate, lipids and proteins for growth so as to produce changes in texture, and color of tempeh (Fig. 6). The color of tempeh was gradually turned to dark brown of soybean color when the mycelium of *R.oligosporus* was died; whereas, that of was whitest black when spores of *R. oligosporus* started germinating. The rate degradation of color was slower at low temperature because the optimum growth of *R. oligosporus* is in the range of 25-32^{o}C at 80% humidity. Meanwhile, the color degradation rate was higher at 40^{o}C because *R. oligosporus* produced black spores which gave rise to dark color in tempeh so that the L^* value increased. This is why the change of tempeh color was in correlation to the growth of *R. oligosporus.* This may explain that change of tempeh color was in correlation to the growth of R. oligosporus and followed 1st order reaction. This agreed with color degradation of onion puree [7] and degradation of betanin in beets induced by heat which followed 1st order reaction since betanin is a natural color compound in beets. The activation energy (*Ea*) of total different color change (ΔE^*) was negative (-1,96 Kcal/mol) indicating that reaction change of ΔE^* may occur depending on the energy generated from the kinetic energy collisions between particles of molecules in tempeh. The increase of temperatures will increase the kinetic energy of the particles, and the frequency to produce effective collisions between the particles are smaller, and consequently it does not produce a reaction. Therefore, the value of the reaction rate constant declined with increasing temperature. Kinetic energy collisions between molecules will result in potentially higher energy than the energy required for the reaction (*Ea*), resulting in the release of energy. This was therefore, the enthalpy in tempeh reduced or negative (-) ΔH. Fermented food products in the storage condition will experience in energy release reaction due to the release of internal energy in the products which is relatively large as a result from degradation reactions by microorganisms or enzymes. Tempeh is fermented food product of soybeans by *R.oligosporus*. Sielberberg [6] stated that the reaction of a change would have negative activation energy if the value of the reaction rate constant declined with increasing temperature which may occur in exothermic reactions. The activation energy is negative means that the thermal energy available exceeds the required energy barriers [8]. The lightness of tempeh color degraded faster compared to texture. Even though the rate texture degradation was the lowest, this was less sensitive to temperature change. The coefficient correlation (R_2) kinetic equation of textures during storage which were 0.599 and 0.781 indicated that the temperature relatively less affected on the texture degradation. Comparing the unprocessed tempeh to the processed ones, it was drawn the attention that the E_a of L^* of unprocessed tempeh, 2.41 kcal/mol K, was lower than that of in processed tempeh (12.20 kcal/mol K), but k of L^* in both tempeh was sensitive to the temperature change. This indicated that non thermal sub-supercritical CO_2 processing may have contribution to the prolonged of the tempeh during storage at specific temperature.

The principle of extending the shelf life of tempeh is basically to reduce the bacterial activity which is the main cause of deterioration to tempeh, maintain fungal growth, and modify environmental factors. Prediction of tempeh shelf life showed that shelf life varies based on observed quality parameters. Tempeh had the shortest shelf life based on water content quality parameters. Water content decreased during storage at the experimental temperature and produced tempeh with high hardness. Decreasing in water content of tempe does not cause the death of mycelium because the water activity of mycelium is 0.6 to 0.8, [9]. Bacteria in tempeh are very likely not to grow optimally when the water content is low, and therefore the spoilage of tempeh can be prevented. On the other hand, processed tempeh was predicted to have 10 days longer storage than unprocessed tempeh which was 6 days at 20^oC of storage (Table 3). Temperature, water content and viability of *R.oligosporus* can be the main factors that affect the decrease in the quality of tempeh during storage. The result of this finding was in accordance with most of the findings stated that the decrease in the total number of bacteria, changes in product acidity, enzyme inactivation, and physical and chemical modification of the food environment may prolong the shelf life of the product processed with high pressure CO_2. Study of *Kimchi* processed with high pressure CO_2 resulted in a shelf life of 5 days longer than that of unprocessed *Kimchi* [10], where the increase in shelf life of *Kimchi* was caused by high pH value and low titratable acidity and low total number of lactic acid bacteria. Whereas, in tempeh it was caused more by the molds which were resistance to the high pressure CO_2 process and therefore they can grow back during storage at the appropriate temperature. Sub supercritical treatment of CO_2 to tempeh causes changes to the carbohydrate, protein, fat macromolecules and moisture content in tempeh, which makes it easier for molds to use it [11]. Therefore the high pressure CO_2 process facilitates mold growth in tempeh. Other studies have also shown that inactivation of enzymes such as pectin methyl esterase, and poly galacturonase by the high pressure CO_2 process which decreased viscosity slow down the product deterioration so that it could prolong the shelf life in vegetables food products [12,13]. The property of the supercritical CO_2 in term of acidification of media as a result of the interaction of CO_2 with water can reduce bacteria to 97.6% and therefore able to maintain a shelf life of 6 month longer as in oyster [14]. Removal of vital substances from cell membrane caused by supercritical CO_2 treatment was also killed bacteria in foods.

Conclusion

The conclusions of the research were that the rate (k) degradation of L^*in either processed tempeh with sub-supercritical CO_2 or unprocessed ones during storage was high. Yet, the E_a in L^* degradation of processed tempeh was higher than unprocessed tempeh. The rate degradation of textures of processed tempeh was relatively less affected by the temperature than that of unprocessed one. Shelf life prediction of tempeh was constructed based on the L^* parameter degtradation. Processing of tempeh with sub supercritical CO_2 prolong its shelf life.

Acknowledgements

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Tabel 1. Quality parameter value of unprocessed and processed tempeh at t_o and t_t day of storage.

Quality parameter	at t_o day *(C_0)*		At t_2 *day*	at t_5 day *(C_t)*
	Unprocessed tempeh	Processed tempeh	Unprocessed tempeh	Processed tempeh
Water content (% dw)	65.7	65.33	62	51.88
Textures (gf)	560.0	577.2	486.0	410.6
Color *(L*)*	75.3	74.4	57.0	69.3
Color *(a*),*		3.8,		4.1
Color *(b*),*		8.9, and		14.0 and
Color and (*ΔE**)		1.58		5.32

Table 2. Shelf life prediction of tempeh based on the *k* value of quality parameter evaluated at each storage temperature.

Quality parameters	Temperature (°C)	Reaction order	Shelf life prediction (days)
	20	1st	10.28±1.48
Color (*L**)	30	1st	6.89±0.37
	40	1st	2.70±0.12
	20	1st	2.47±0.13
Water content	30	1st	2.11±0.21
	40	1st	1.75±0.10
	20	1st	6.01±0.10
Textures	30	1st	4.02±0.10
	40	1st	3.45±0.10
	20	1st	27.17±0.10
Mold loads	30	1st	4.54±0.11
	40	1st	0.85±0.10

Table 3. Kinetic parameter of processed and unprocessed tempeh with sub-supercritical CO_2

Quality parameters		T, °C	*k*	Model of *T vs k*	E_a kcal/mol K
Processed tempe	*L**	20	0.007	Ln k = 15.93+6155T	12.20
		30	0.009	R^2= 0.921	
		40	0.027		
	*ΔE**	20	0.31	Ln k = 4.556-989.1T	-1.96
		30	0.27	R^2 = 0.97	
		40	0.25		
	Textures	20	0.027	Ln k = 0.473+1193T	2.37
		30	0.032	R^2 = 0.975	
		40	0.035		
	Water content	20	0.089	Ln k = 1585- 2.818T	3.15
		30	0.106	R^2 =0.999	
		40	0.002		
	Mold loads	20	0.011	Ln k = 15880(1/T)-49.70	31.53
		30	0.067		
		40	0.355	R = 0.994	
Unprocessed tempeh	*L**	20	0.025	Ln k = 4.556-989.1T	2.41
		30	0.03	R^2 = 0.97	
		40	0.12		

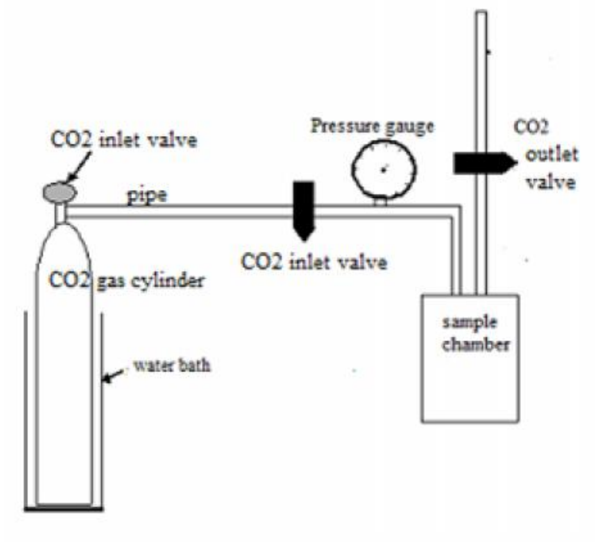

Fig. 1. The diagram of the experimental apparatus.

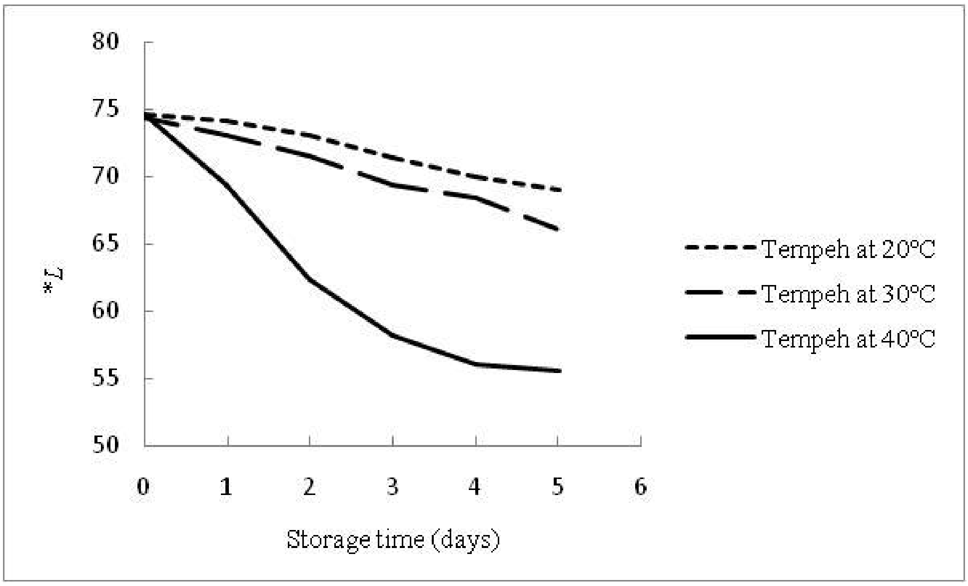

Fig. 2. Degradation of *L** value of tempeh during storage at 20, 30 and 40^{o}C.

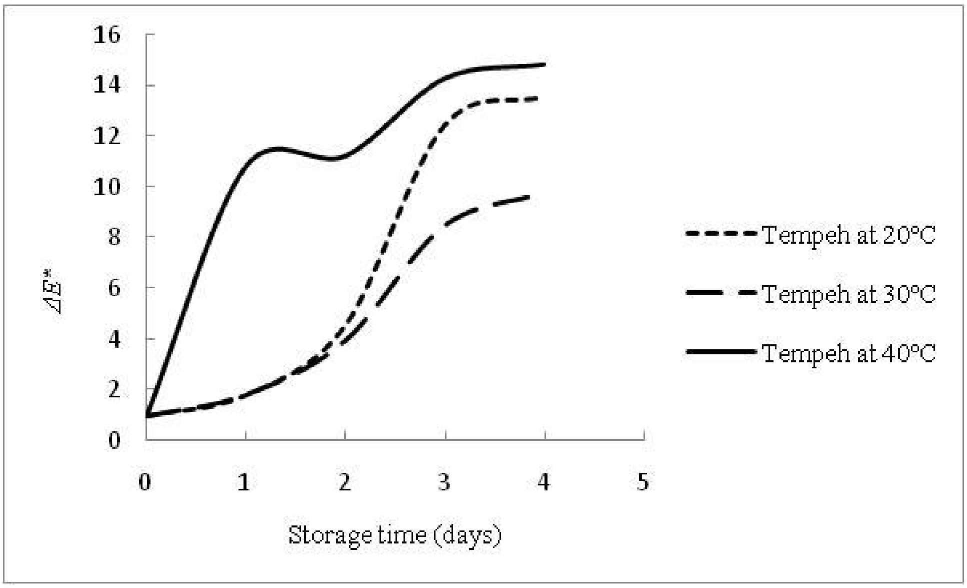

Figure 3. Degradation of Δ*E** value of tempeh during storage at 20, 30 and 40^{o}C.

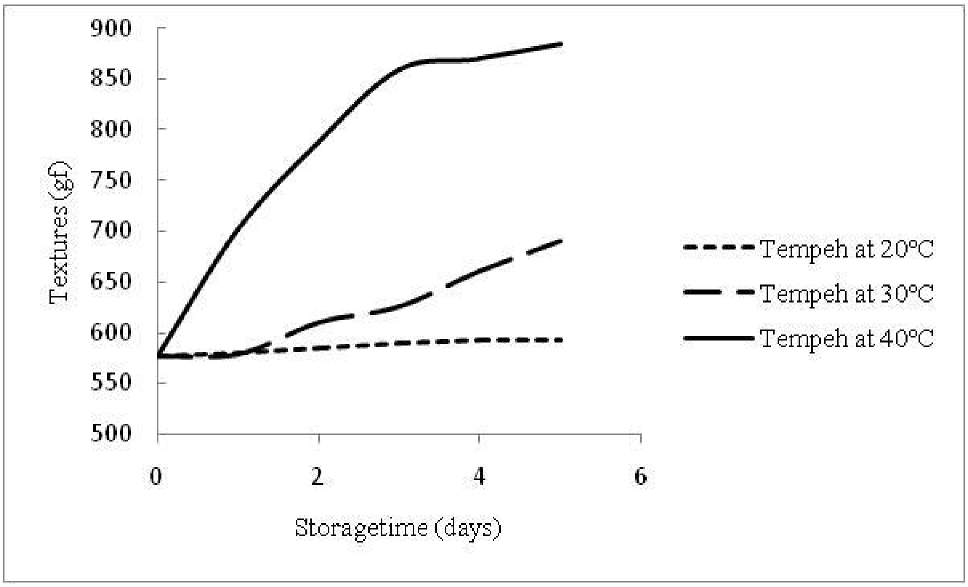

Figure 4. Degradation of textures of tempeh during storage at 20, 30 and 40^{o}C.

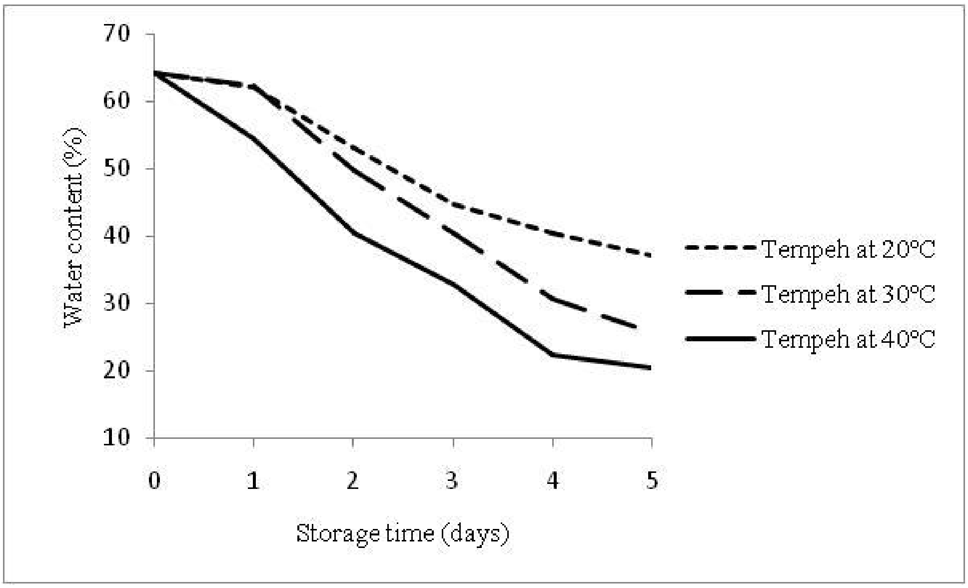

Figure 5. Change of water content of tempeh during storage at 20, 30 and 40^{o}C.

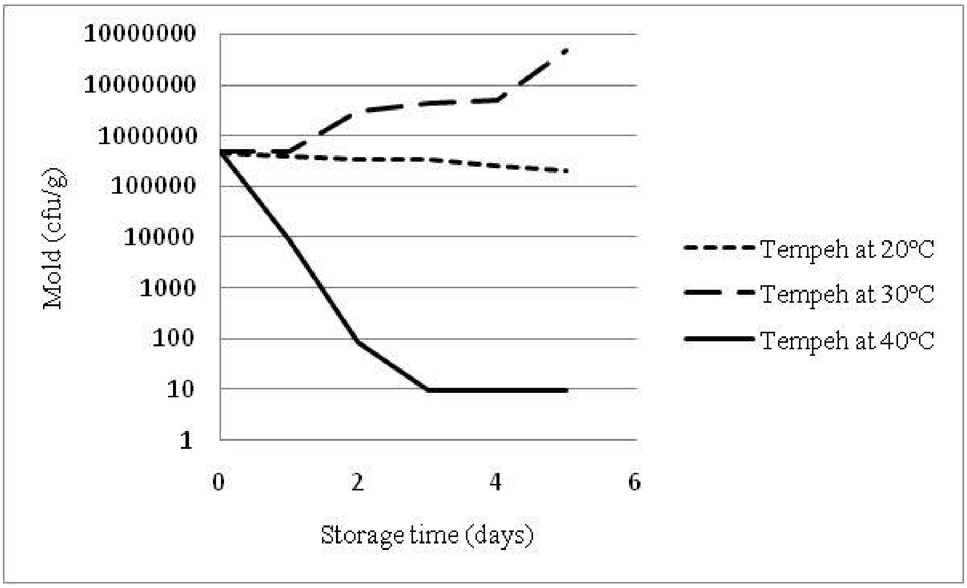

Figure 6. Growth *R. oligosporus* in tempeh during storage at 20, 30 and 40^{o}C.

The growth of molds began reached at stationary on day 2 to day 4 of storage at 30oC where the tempe