potravinárstvo®
Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 14, 2020, p. 351-357
https://doi.org/10.5219/1247
Received: 1 December 2019. Accepted: 2 February 2020.
Available online: 28 June 2020 at www.potravinarstvo.com

ISSN 1337-0960 (online)

SHELF LIFE OF TEMPEH PROCESSED WITH SUB-SUPERCRITICAL CARBON DIOXIDES

Maria Erna Kustyawati, Filli Pratama, Daniel Saputra, Agus Wijaya

ABSTRACT
Tempeh, a fermented soybean-based food originally from Indonesia, is a remarkably nutritious functional food with health benefits. Unfortunately, tempeh is highly perishable, with a shelf life of 24 – 48 hours. The goal of this research was to evaluate the possibility of a sub-supercritical CO_2 technique to increase the shelf life of tempeh by measuring the changes in the L^* (lightness) value and texture of tempeh via application of a kinetic approach and, based on the observations, to estimate its shelf life. Tempeh was processed with sub-supercritical CO_2 at 6.3 MPa for 10 min, then together with unprocessed tempeh (control), stored for 5 days at temperatures of 20, 30 and 40 °C. The Accelerated Self-Life Test (ASLT) with the Arrhenius model was used to measure the shelf life of processed and control tempeh. The calculated shelf life of processed tempeh using the ASLT by the Arrhenius method was 2.43 days at 20 °C, 3.7 days at 30 °C and 1.4 days at 40 °C, and the shelf life of unprocessed tempeh was 3.33 days at 20 °C, 2.90 days at 30 °C and 2.56 days at 40 °C. The conclusion was that the use of sub-supercritical CO_2 at 6.3 MPa for 10 min increased the shelf life of tempeh stored at 30 °C.

Keywords: sub-supercritical CO_2; kinetic change; shelf life; tempeh

INTRODUCTION
At present, consumers demand fresh food that is not only of high quality and safe but also has a long shelf life. High-pressure carbon dioxide (HPCD) technology has been developed as a food processing technology with the advantage of minimizing the loss of heat-sensitive nutrients. Carbon dioxide in the supercritical state has the dual properties of a gas with high diffusivity and a liquid with high solubility **(Ferrentino et al., 2010)**. These properties allow HPCD to diffuse easily through complex matrices, causing modification in either macromolecular or micromolecular substrates **(Garcia-Gonzales et al., 2007; Liao et al., 2010; Ferrentino, Balzan, and Spilimbergo, 2012; Guo et al., 2011)**. Many researchers have shown that HPCD could extend the shelf life of food by killing microbes and enzymes at a relatively low temperature whilst preserving the nutritional and sensory qualities of vegetables and food products. HPCD could inactivate *Salmonella*, *Listeria monocytogenes* and *Escherichia coli* **(Bourdoux et al., 2018; Liao et al., 2010)**, as well as the natural microbial flora **(Li et al., 2012; Cappelletti et al., 2015)**. HPCD has also been proven capable of inactivating the enzymes that cause food spoilage and lowering food quality, such as pectin methyl esterase, poly galacturonase, peroxidase, polyphenol oxidase and lipoxygenase **(Niu et al., 2019; Illera et al., 2018; Briongos et al., 2016; Liu et al., 2010; Hu et al., 2013)**.

Tempeh is an Indonesian fermented food made from soaked, hulled and cooked soybeans inoculated with the fungus *Rhizopus oligosporus*. Tempeh is a remarkably nutritious functional food with health benefits; however, it is highly perishable, with a short shelf life of 36 – 48 hours at room temperature **(Sparringa and Owens, 1999; Nout and Kiers 2005; Djunaidi et al., 2017)**. Several researchers have reported on methods of extending the shelf life of tempeh. Frozen storage (-18 °C) of tempeh resulted in loss of taste and texture which softened after thawing, while refrigerated storage caused discoloration and spoilage after 72 hours **(Witono et al., 2015)**. Meanwhile, tempeh kept under modified atmosphere packaging (15% O_2, 30% CO_2 and 55% N_2) spoiled in 24 hours **(Muslikhah, Anam and Andriani, 2014)**. A previous study performed by the author found that processing of tempeh with high pressure CO_2 at a pressure of 6.3 MPa for 10 min reduced the number of bacteria, yeasts and moulds in tempeh to 4.1, 5.1 and 4.3 log CFU g^{-1}, respectively. This discovery led us to study the effect of HPCD on the shelf life of tempeh. The objective of this research was to study the degradation kinetics of the quality parameters of tempeh processed with sub-supercritical CO_2 and to determine the shelf life of such tempeh.

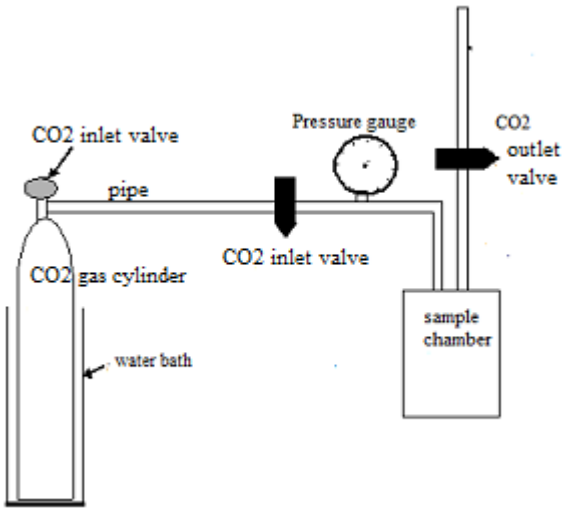

Figure 1 The set-up of the experimental apparatus **(Saputra, 2006)**.

Scientific hypothesis

The shelf life of tempeh stored at 20, 30, and 40 °C can be extended by treatment with CO_2 at sub-supercritical pressure.

MATERIAL AND METHODOLOGY

Treatment of tempeh with supercritical CO_2

Tempeh, in the form of a cylinder with a diameter of 35 mm and a length of 100 mm, fermented for 36 hours at 30 °C, was obtained from the Center of Home Industry Tempeh Making Palembang, Indonesia, placed in a cooler box and carried to the laboratory for direct processing **(Kustyawati et al., 2018)**.

The high-pressure CO_2 installation used for experimental treatments, consisting of a CO_2 gas cylinder, a cylindrical pressure chamber, pressure gauges, and a water bath at a constant temperature, is shown in Figure 1 **(Saputra, 2006).** Fresh tempeh was placed in a pressure chamber and then closed tightly. When the designated temperature in the water bath reached a constant 25 °C, and all pipe connections were secured, commercially available CO_2 was injected through the gas inlet valve from the gas cylinder into the pressure chamber, within 1 minute, until it reached the desired pressure of 6.3 MPa (sub supercritical CO_2 condition), which was indicated by the pressure gauge. After 10 min of treatment with sub-supercritical CO_2, the pressure was lowered to atmospheric pressure within 2 minutes by slowly opening the gas outlet valve. The tempeh was then collected aseptically from the pressure chamber using sterilized tongs, placed in a sterilized container, and stored in a refrigerator for further study. The processed and unprocessed tempeh (control) were analysed further for colour and texture, before and during the storage period.

Storage study

Tempeh processed with sub-supercritical CO_2 (6.3 MPa, 25 °C for 10 minutes) and unprocessed tempeh were employed as the treatment and control, respectively, in this experiment. All of the tempeh was stored for 5 days. Tempeh was stored as follows: tempeh samples were placed on a Styrofoam plate and covered with plastic film then stored at 20, 30 and 40 °C with the same relative humidity. Observations on quality parameter changes (*C*) were carried out by measuring the quality attributes represented by *L** and texture. Observations were made daily. A storage time of 5 days was chosen considering that the shelf life of fresh tempeh is normally around 24 – 48 hours at room temperature (28 – 30 °C).

The Accelerated Self Life Test (ASLT) with the Arrhenius model was used to determine the shelf life of tempeh, in which, if the food product deteriorates faster, then the shelf life is determined based on extrapolation to storage temperature. Changes in the quality factor were used to determine the degree of decrease in quality. Data were transformed into a kinetic plot, and an appropriate kinetic parameter model was obtained. The quality decrease in food is given by equation (1).

$$\frac{dQ}{dt} = k.Q^n \qquad (1)$$

Where: Q is the quality factor, t is time, k is a rate constant that depends on temperature, n is a degree factor or reaction order and $dQ\ dt^{-1}$ is the change in the Q factor per unit of time.

Most of all, a decrease in food quality includes zero-order (order 0) and first-order (order 1) reactions. The Arrhenius correlation chart was generated by evaluating the rate constant (k) at three different temperatures. The rate constant (k) was predicted by extrapolating the correlation between $ln\ k$ and 1/T at three temperatures. Shelf life is determined on the basis of the most influential factors on the product. One of the factors that can affect a product's shelf life is temperature. The Arrhenius kinetic approach was used to determine the shelf life and temperature limit factor. The equation for the Arrhenius model is shown in equation (2).

$$k_T = k_o e^{\frac{-E_a}{RT}} \qquad (2)$$

Where: k_T was the reaction rate constant of quality degradation, k_o was a constant (frequency factor, not dependent on temperature), E_a was activation energy, T was absolute temperature (K) and R was the gas constant (8.341 $J.mol^{-1}.K^{-1}$).

The zero-order and first-order quality reaction was measured by using equation (3) and equation (4), respectively **(Labuza and Szybist, 2001)**.

Zero order: $$t = \frac{(C_1 - C_o}{k} \quad (3)$$

First order : $$t = \frac{ln\frac{C_1}{C_o}}{k} \quad (4)$$

Where: C_o was the initial quality value of storage, C_t was the quality value at the storage time t, k was the reaction rate constant and t was the storage time (days). The determination of the order of the most suitable reaction was performed by selecting the equation with the highest R^2.

Colour and texture measurement

The surface colour analysis of tempeh was evaluated as the *CIE L*a*b** value and *LCH* colour scale using a colour difference meter (TC-1500, Tokyo, Japan). Results were expressed as L^* (Lightness), a^* (redness) and b^* (yellowness).

The total colour difference (ΔE^*) between the control and the processed tempeh was obtained using the following equation (5):

$$\Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \quad (5)$$

Where: ΔL^*, Δa^* and Δb^* were the differences between L^*, a^* and b^* after treatment and the L^*, a^* and b^* values of the standard colour. The standard colour used in this experiment was the L^*, a^* and b^* values of the unprocessed tempeh (control), which was L^*=76.6, a^*=3.1 and b^*=7.5.

Texture analysis

The texture analysed was tempeh hardness. The greater the value, the harder the sample being analysed. The LFRA Texture Analyzer (Brookfield AMETEK CT3-100-115), type A 7.1 was used to measure tempeh texture. The texture of the tempeh in this study was the quality of compactness of tempeh when sliced, because compact and dense soybean strands produce tempeh that is easily sliced. Tempeh has a non-homogeneous texture because it consists of woven soybean seeds arranged mycelia. This arrangement gives rise to varying angles/areas of penetration of the probe, for example, the possibility of probes piercing right into the soybean seeds or the soft areas between soybeans strands. Therefore, a Brooke-type probe blade was used in this study. The Brooke-type blade presses right in the centre of the sample. The peak load and final load numbers in units of gram force (gf) listed on the display were recorded. Measurements were performed in three replicates.

Statistical analysis

Statistical analysis was carried out using Microsoft Excel 2003 and Statistica 8.0 StatSoft software. Analysis of variance (ANOVA) was used to study differences between samples. A software program using Duncan's multiple range test was used to compare treatment means. A value of p <0.05 was considered statistically significant. All experiments were performed with at least three replicates.

RESULTS AND DISCUSSION

Kinetics of quality degradation

Processed and unprocessed (control) tempeh were used as the models in this experiment. Colour is very important to the sensory nature of tempeh because it is the first characteristic observed by the consumer. The colour of tempeh produced by the growth of mould was influenced by changes in the chemical composition of the tempeh and storage temperature. The lightness (L^*) value of the control was high, approximately 75.7 on the initial day, and then the lightness darkened to 57 at the third day of storage. The initial L^* value of the tempeh processed with sub-supercritical CO_2 glimmered to 74.4 and decreased to dark (69.3) by the fifth day (Table 1 and Table 2).

The data obtained from the experiments were plotted on a graph of the relationship between the degradation in the quality of L^* and texture, and the storage time at various temperatures. Based on the correlation coefficient (R^2) of texture and L^* (Table 3), the rate of change in the quality of tempeh followed the first-order reaction model. A higher correlation value indicates a faster decline in reaction product quality. This was in agreement with the findings of **Ahmed, Shivhare and Raghavan (2001)** that the degradation of betanin, a natural colour compound in beets induced by heat, followed a first-order reaction.

Figure 2 shows that the lightness value (L^*) weakened during storage at various temperatures, where it developed from light to dark. The colour of fresh tempeh is brownish yellow, due to the compounds furosine, hydroxymethylfurfural (HMF) and acrylamide, which are the products of the Maillard reaction in beans **(Zilic et al., 2014)**. Tempeh is made from cooked soybeans which have been heated to boiling temperature. In addition, during the depressurization process of the high-pressure CO_2 treatment, the mycelia of the mould are wiped from the surface of the tempeh, resulting in a brownish-yellow colour appearing on the beans. Moreover, a study performed by **Handoyo and Morita (2006)** found that over-fermentation of tempeh that occurred during storage could bring about protein depletion and produced a blackish-brown colour.

The hard texture of tempeh increased significantly during storage (p <0.05) (Table 1 and Table 2). The texture of tempeh is dense, compact and sliceable. It is formed by soybean cotyledons intertwined with the mycelia of moulds. As mould grows, it produces fluffy white mycelia which bind the beans, squeezing and penetrating the cell walls to create a cake texture and simultaneously producing enzymes that cause softening of the beans due to hydrolysis of various compounds during fermentation **(Duniaji et al., 2019; Jones et al., 2017; Wati et al., 2020)**.

Table 1 The lightness (*L**) and texture of processed tempeh during storage at 20, 30 and 40 °C.

20 °C			30 °C			40 °C		
Day	***L****	**Texture**	**Day**	***L****	**Texture**	**Day**	***L****	**Texture**
0	74.6 ±0.06 ^{a}	577 ±0.1^{a}	0	74.4 ±0.05^{a}	577 ±0.3^{a}	0	74.6 ±0.05^{a}	577 ±0.2^{a}
1	74.1 ±0.07^{a}	580 ±0.2^{ab}	1	73 ±0.050^{ab}	579 ±0.1^{ab}	1	69.3 ±0.06^{b}	701 ±0.2^{b}
2	73 ±0.02^{ab}	585 ±0.1^{b}	2	71.5 ±0.03^{b}	610 ±0.1^{b}	2	62.3 ±0.06^{bc}	789 ±0.3^{c}
3	71.4 ±0.03^{b}	589 ±0.1^{bc}	3	69.3 ±0.06^{bc}	625 ±0.3^{bc}	3	58.2 ±0.06^{bc}	860 ±0.23^{cd}
4	70 ±0.03^{bc}	592 ±0.3^{c}	4	68.4 ±0.06^{bc}	660 ±0.3^{c}	4	56 ±0.05^{c}	870 ±0.4^{cd}
5	69 ±0.10^{c}	593 ±0.3^{c}	5	66 ±0.07^{c}	690 ±0.2^{d}	5	55.5 ±0.07^{c}	885 ±0.1^{d}

Note: All values are the mean and standard deviation of three replicates. $^{a-d}$ Means within a column with different letters were significantly different (p <0.05).

Table 2 The lightness (*L*)* and texture of control (unprocessed tempeh) during storage at 20, 30 and 40 °C.

20 °C			30 °C			40 °C		
Day	***L****	**Texture**	**Day**	***L****	**Texture**	**Day**	***L****	**Texture**
0	75.3 ±0.11^{a}	505.7 ±1.0^{a}	0	75.3 ±0.09^{a}	506 ±0.09^{a}	0	75.3 ±0.9^{a}	505.5 ±0.1^{a}
1	70.8 ±0.58ab	346.5 ±1.0^{b}	1	74.2 ±0.08^{a}	396 ±0.05^{b}	1	69.5 ±1.0^{ab}	1233 ±0.09^{b}
2	66.7 ±0.11b	332 ±0.9c	2	69.5 ±0.1^{b}	286 ±0.1^{cd}	2	65.2 ±1.1^{b}	1321 ±0.04^{c}
3	63.2 ±0.12bc	338 ±0.9cd	3	65.7 ±0.12^{c}	305 ±0.1^{c}	3	60.7 ±1.2^{c}	1442 ±0.04^{cd}
4	59.5 ±0.09^{c}	340 ±0.7cd	4	60.4 ±0.11^{cd}	290 ±0.09^{cd}	4	55.3 ±1.1^{d}	1467 ±0.02^{cd}
5	58 ±0.09^{c}	300 ±1.0^{d}	5	57 ±0.8^{d}	277 ±0.1^{d}	5	54 ±0.9^{d}	1511 ±0.02^{d}

Note: All values are the mean and standard deviation of three replicates. $^{a-d}$ Means within a column with different letters were significantly different (p <0.05).

Table 3 Evaluation of the linear regression equation for the estimated shelf life of tempeh.

Quality parameters		**T, °C**	**Zero order**	***R²***	**First order**	***R²***
			Regression equation		*Regression equation*	
PT (Processed Tempeh)	*L**	20	y= -1.197x+75.01	0.982	y=0.0167x+4.318	0.981
		30	y= -1.657x+74.57	0.990	y=0.0236x+4.312	0.988
		40	y= -3.985x+72.61	0.919	y=0.0625x+4.287	0.933
	Texture	20	y=3.428x+577.4	0.970	y=0.0059x+6.359	0.969
		30	y=23.51x+564.7	0.959	y=0.0375x+6.339	0.965
		40	y=60.51x+629.0	0.877	y=0.0821x=6.443	0.847
CT (Control Tempeh)	*L**	20	y=-3.54x+74.43	0.983	y= -0.0537x+4.313	0.989
		30	y=-3.906x+76.78	0.980	y= -0.059x+4.347	0.975
		40	y=-4.388x+74.31	0.981	y= -0-069x+4.314	0.986
	Texture	20	y=-29.76x+434.7	0.581	y=0.0757x+6.061	0.615
		30	y=-41.25x+446	0.722	y= -0.111x+6.090	0.745
		40	y=167.1x+828.6	0.687	y= -0.174x+6.634	0.599

Shelf-life prediction

The influence of temperature on the reaction rate was described using the Arrhenius equation. The regression equation and value of the R^2 data for tempeh at 20, 30 and 40 °C are shown in Table 3. The lightness (*L**) decreased faster than the texture, which was indicated by the slope values. Plotting *ln k* against *1/T* produced a linear regression of the Arrhenius model in which the slope represents the E_a value (Table 4). The E_a values of *L** and texture were 12.27 and 25.59 kcal.mol^{-1}, respectively, indicating that lightness was more sensitive to temperature. The sensitivity of quality parameters to changes in temperature can also be evaluated based on the value of the correlation coefficient R^2, where the greater the value of R^2, the greater the relationship between changes in the rate constant (*k)* and temperature. The dates on which characteristic limits for processed tempeh were attained with respect to lightness characteristic criteria were 2.43, 4.88 and 9.36 days at storage temperatures of 20, 30 and 40 °C, respectively, while those with respect to texture characteristic criteria were 8.6, 3.7 and 1.4 days at storage temperatures of 20, 30 and 40 °C, respectively. The shelf life was defined as the earliest date of all the dates on which characteristic limits were attained when each characteristic criterion reached its limit. Therefore, the shelf life of processed tempeh was estimated to be 2.43 days at 20 °C, 3.7 days at 30 °C and 1.4 days at 40 °C. The dates on which characteristic limits were attained for the control (unprocessed tempeh), with respect to lightness characteristic criteria, were 3.33 days, 2.90 days, and 2.56 days at 20, 30 and 40 °C, respectively, while those with respect to texture characteristic criteria, were 6.89 days, 4.47 days and 2.99 days at 20, 30 and 40 °C, respectively. Therefore, the shelf life of unprocessed tempeh was estimated to be 3.33 days at 20°C, 2.90 days at 30 °C and 2.56 days at 40 °C. Summarizing the results, the shelf life processed tempeh was longer than that of unprocessed tempeh at 30 °C. However, the shelf life estimated in this study cannot be applied to all tempeh, because many factors including consumer palatability and consumer perspective, also play vital roles.

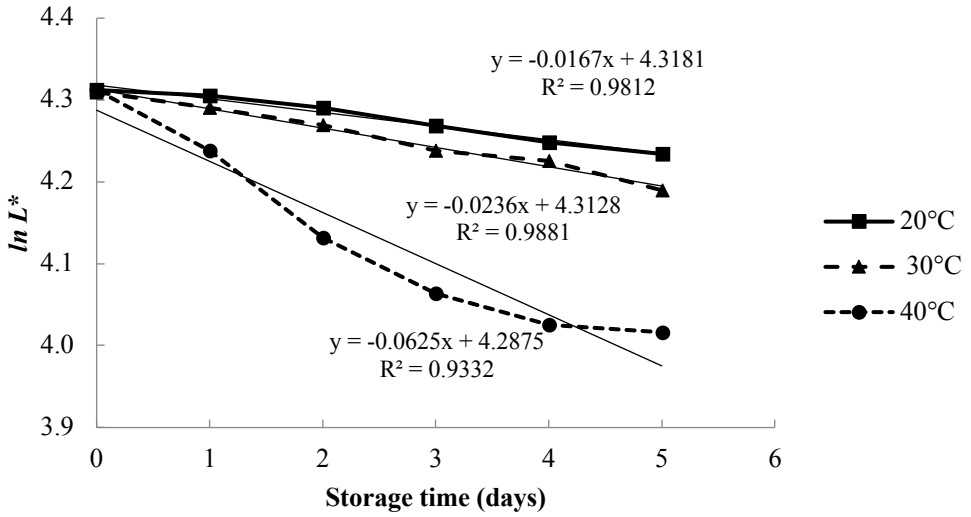

Figure 2 The correlation between lightness (*L**) of processed tempeh and time according to a first-order reaction.

Table 4 Regression equation of tempeh stored at 20, 30 and 40 °C.

	Quality parameter	T, °C	Regression equation	Reaction order	E_a, [1] kcal.mol-1	K,[2] day^{-1}	R^2	C_o[3] - C_t[4]	Shelf - life (days)
PT[5]	Lightness (*L**)	20	LnK= -6177.2x+16.839	First	12.27	0.292	0.923	5.1	2.43
		30				0.145			4.88
		40				0.758			9.36
	Texture	20	LnK= -12883x+38.85	First	25.59	0.938	0.950	48	8.60
		30				0.218			3.67
		40				0.559			1.42
CT[6]	Lightness (*L**)	20	LnK= -1206.8x+1.1719	First	2.40	0.053	0.984	9.6	3.33
		30				0.060			2.90
		40				0.068			2.56
	Texture	20	LnK= -3827.6x+10.458	First	7.60	0.073	0.995	201	6.89
		30				0.113			4.47
		40				0.169			2.99

Note: [1] Activation energy in kcal.mol^{-1}; [2] Rate constant; [3] Initial value of quality parameter; [4] Data of quality parameter as t time passes; [5] Processed tempeh; [6] Control (unprocessed tempeh).

All food expiration dates could be established as self-applied safety factors by each producer. For tempeh, expiration dates may not be mandatory because tempeh categorized as a fresh food product has a very short shelf life, and the spoilage of tempeh is easily detectable by looking at the colour, texture and aroma. Therefore, an expiration date is not necessary. From the point of view of microbial safety, tempeh, fermented soybean, is a reliably safe food because bacteria, yeasts and moulds that grow in tempeh have their own specific role. *R. oligosporus*, an important fungus in tempeh, is known to produce antibiotics against bacteria **(Kobayasi, Okazaki and Koseki, 1992; Wang et al., 1969)**. *Bacillus subtilis*, the most common bacteria in tempeh, contribute to the production of fatty acids and isoflavones **(Barus et al., 2017; Kanghae, Eungwanichayapant and Chukeatirote, 2017)**. The role of yeast in tempeh is not clear **(Nout and Kiers, 2005; Pleva et al., 2018)**; however, the authors' previous results show that co-culturing *Saccharomyces cerevisiae* with *R. oligosporus* in soybean fermentation produced tempeh with a pleasant yeast/tapai aroma that was liked by panellists **(Kustyawati, Nawansih and Nurdjanah, 2017)**. Further studies on the role of yeast in tempeh production are needed.

CONCLUSION

Sub-supercritical CO_2 processing at 6.3 MPa for 10 min increased the shelf life of tempeh at a storage temperature of 30 °C. The shelf life of processed tempeh was 2.43 days at 20 °C, 3.7 days at 30 °C and 1.4 days at 40 °C, and the shelf life of unprocessed tempeh was 3.33 days at 20 °C, 2.90 days at 30 °C and 2.56 days at 40 °C.

REFERENCES

Ahmed, J., Shivhare, U. S., Raghavan, G. S. V. 2001. Color degradation kinetics and rheological characteristics of onion puree. *Transactions of the American Society of Agricultural Engineers*, vol. 44, no. 1, p. 95-98. https://doi.org/10.13031/2013.2293

Barus, T., Wati, L., Melani, Suwanto, A., Yogiara. 2017. Diversity of protease-producing Bacillus spp. from fresh

Indonesian tempeh based on 16S rRNA gene sequence. *HAYATI Journal of Biosciences*, vol. 24, no. 1, p. 35-40. https://doi.org/10.1016/j.hjb.2017.05.001

Bourdoux, S., Rajkovic, A., Sutter, S. D., Vermeulen, A., Spilimbergo, S., Zambon, A., Hofland, G., Uyttendaele, M., Devlieghere, F. 2018. Inactivation of *Salmonella, Listeria monocytogenes* and *Escherichia coli* O157:H7 inoculated on coriander by freeze drying and supercritical CO_2 drying. *Innovative Food Science and Emerging Technologies*, vol. 47, p. 180-186. https://doi.org/10.1016/j.ifset.2018.02.007

Briongos, H., Illera, A. E., Sanz, M. T., Melgosa, R., Beltrán, S., Solaesa, A. G. 2016. Effect of high pressure carbon dioxide processing on pectin methylesterase activity and other orange juice properties. *LWT*, vol. 74, p. 411-419. https://doi.org/10.1016/j.lwt.2016.07.069

Cappelletti, M., Ferrentino, G., Endrizzi, I., Aprea, E., Betta, E., Corollaro, M. L., Charles, M., Gasperi, F., Spilimbergo, S. 2015. High Pressure Carbon Dioxide pasteurization of coconut water: A sport drink with high nutritional and sensory quality. *Journal of Food Engineering*, vol. 145, p. 73-81. https://doi.org/10.1016/j.jfoodeng.2014.08.012

Djunaidi, S., Puspitasari, M. D., Gunawan-Puteri, T., Wijaya, C. H., Prabawati, E. K. 2017. Physicochemical & microbial characterization of overripe tempeh. *INSIST*, vol. 2, no. 1, p. 48-51. https://doi.org/10.23960/ins.v2i1.33

Duniaji, A. S., Wisaniyasa, W., Puspawati, W., Indri, H. 2019. Isolation and identification of *R. oligosporus* local isolate derived from several inocolum resources. *International Journal of Current Microbiology and Applied Sciences*, vol. 8, no. 9, p. 2319-7706. https://doi.org/10.20546/ijcmas.2019.809.126

Ferrentino, G., Balzan, S., and Spilimbergo, S. 2012. Optimization of supercritical carbon dioxide treatment for the inactivation of the natural microbial flora in cubed cooked ham. *International Journal Food Microbiology*, vol. 161, no. 3, p. 189-196. https://doi.org/10.1016/j.ijfoodmicro.2012.12.004

Ferrentino, G., Balaban, M. O., Ferrari, G., Poletto, M. 2010. Food treatment with high pressure carbon dioxide: Saccharomyces cerevisiae inactivation kinetics expressed as a function of CO2 solubility. *The Journal of Supercritical Fluids*, vol. 52, no. 1, 151-160. https://doi.org/10.1016/j.supflu.2009.07.005

Garcia-Gonzales, L., Geeraerd, A. H., Spilimbergo, S., Eltst, K., VanGinneken, L., Debevere, J., VanImpe, J. F., Devlieghere, F. 2007. High pressure carbon dioxide inactivation of microorganisms in foods: The past, the present and the future. *International Journal of Food Microbiology*, vol. 17, no. 1, p. 1-28. https://doi.org/10.1016/j.ijfoodmicro.2007.02.018

Guo, J., Wu, Y., Xu, G., Xiao, M., Zhang, Y., Chen. 2011. Effects on microbial inactivation and quality attributes in frozen lychee juice treated by supercritical carbon dioxide. *European Food Research Technology*, vol. 232, p. 803-811.

Handoyo, T., Morita, N. 2006. Structural and functional properties of fermented soybean (tempeh) by using *R.oligosporus*. *International Journal of Food Properties*, vol. 9, no. 2, p. 347-355. https://doi.org/10.1080/10942910500224746

Hu, W., Zhou, L., Xu, Z., Zhang, Y., Liao, X. 2013. Enzyme inactivation in food processing using high pressure carbon dioxide technology. *Critical Reviews in Food Science and Nutrition*, vol. 53, no. 2, p. 145-161. https://doi.org/10.1080/10408398.2010.526258

Illera, A. E., Sanz, M. T., Trigueros, E., Beltrán, S., Melgosa, R. 2018. Effect of high pressure carbon dioxide on tomato juice: Inactivation kinetics of pectin methylesterase and polygalacturonase and determination of other quality parameters. *Journal of Food Engineering*, vol. 239, p. 64-71. https://doi.org/10.1016/j.jfoodeng.2018.06.027

Jones, M., Huynh, T., Dekiwadia, C., Daver, F., John, S. 2017. Mycelium composites: A review of engineering characteristics and growth kinetics. *Journal of Bionanoscience*, vol. 11, no. 4. p. 241-257. https://doi.org/10.1166/jbns.2017.1440

Kanghae, A., Eungwanichayapant, D. P., Chukeatirote, E. 2017. Fatty acid profiles of fermented soybean prepared by *Bacillus subtilis* and *Rhizopus oligosporus*. *Environmental and Experimental Biology*, vol. 15, p. 173-176. https://doi.org/10.22364/eeb.15.16

Kobayasi, S., Okazaki, N., Koseki, T. 1992. Purification and characterization of an antibiotic substance produced from *Rhizopus oligosporus* IFO 8631. *Bioscience Biotechnology and Biochemistry*, vol. 56, p. 94-98. https://doi.org/10.1271/bbb.56.94

Kustyawati, M. E., Pratama, F., Saputra, D., Wijaya, A. 2018. Viability of molds and bacteria in tempeh processed with supercritical carbon dioxides during storage. *International Journal of Food Science*, vol. 2018, p. 1-7. https://doi.org/10.1155/2018/8591015

Kustyawati, M. E., Nawansih, O., Nurdjanah, S. 2017. Profile of aroma compounds and acceptability of modified tempeh. *International Food Research Journal*, vol. 24, no. 2, p. 734-740.

Labuza, T. P., Szybist, L. M. 2001. *Open Dating of Foods*. Trumbull, Connecticut, USA : Food and Nutrition Press, Inc, 239 p. ISBN 0-91 7678-53-2.

Li, H., Zhao, L., Wu, J., Zhang, Y., Liao, X. 2012. Inactivation of natural microorganisms in litchi juice by high-pressure carbon dioxide combined with mild heat and nisin. *Food Microbiology*, vol. 30, no. 1, p. 139-145. https://doi.org/10.1016/j.fm.2011.10.007

Liao, H., Zhang, L., Hu, X., Liao, X. 2010. Effect of high pressure CO_2 and mild heat processing on natural microorganisms in apple juice. *International Journal of Food Microbiology*, vol. 137, no. 1, p. 81-87. https://doi.org/10.1016/j.ijfoodmicro.2009.10.004

Liu, X., Gao, Y., Xu, H., Hao, Q., Liu, G., Wang, Q. 2010. Inactivation of peroxidase and polyphenol oxidase in red beet (*Beta vulgaris* L.) extract with continuous high pressure carbon dioxide. *Food Chemistry*, vol. 119, no. 1, p. 108-113. https://doi.org/10.1016/j.foodchem.2009.06.002

Muslikhah, S., Anam, C., Andriani, M. A. M. 2014. Tempe storage by a method of modification atmosphere to maintaining quality and shelf life. *Jurnal Teknosains Pangan*, vol. 2, no. 3, p. 51-61. Avalaible at: https://jurnal.uns.ac.id/teknosains-pangan/article/view/4442/3788. (In Indonesian)

Niu, L., Li, D., Liu, C., Huang, W., Liao, X. 2019. Quality changes of orange juice after DPCD treatment. *Journal of Food Quality*, vol. 2019, p. 1-8. https://doi.org/10.1155/2019/6897583

Nout, M. J. R., Kiers, J. L. 2005. Tempe fermentation, innovation and functionality: Update into the third millenium. *Journal of Applied Microbiology*, vol. 98, no. 4, p. 789-805. https://doi.org/10.1111/j.1365-2672.2004.02471.x

Pleva, P., Cabáková, V., Butor, I., Pachlová, V., Buňková, L. 2018. Biogenic amines content in the fermented asian food in the Czech Republic. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, p. 292-298. https://doi.org/10.5219/896

Saputra, D. 2006. Puffing dehydrated vegetable with carbon dioxide. *Jurnal Keteknikan Pertanian*, vol. 20, no. 2, p. 157-

165. Available at: http://journal.ipb.ac.id/index.php/jtep/article/view/7609/5875. (In Indonesian)

Sparringa, R. A., Owens, J. D. 1999. Protein Utilization during soybean tempeh fermentation. *Journal of Agricultural and Food Chemistry*, vol. 47, no. 10, p. 4375-4378. https://doi.org/10.1021/jf981279u

Wang, H. L., Ruttle, D. I., Hesseltine, C. W. 1969. Antibacterial compound from a soybean product fermented by *Rhizopus oligosporus*. *Proceedings of the Society for Experimental Biology and Medicine*, vol. 131, no. 2, p. 579-583. https://doi.org/10.3181/00379727-131-33930

Wati, D. A., Nadia, F. S., Isnawati, M., Sulchan, M., Afifah, D. N. 2020. The effect of processed Tempeh gembus to high sensitivity c-reactive protein (hsCRP) and high-density lipoprotein (HDL) levels in women with obesity. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 14, no. 1, p. 8-16. https://doi.org/10.5219/1236

Witono, Y., Bambang W., Mujianto, M., Rachmawati, D. T. 2015. Amino acids identification of over fermented tempeh, the hydrolysate and the seasoning product hydrolysed by calotropin from crown flower (*Calotropis gigantea*). *International Journal on Advanced Science, Engineering and Information Technology*, vol. 5, no. 2, p. 103-106. https://doi.org/10.18517/ijaseit.5.2.494

Zilic, S., Mogol, B. A., Akillioglu, G., Serpen, A., Delic, N., Gokmen, V. 2014. Effect of extrusion, infrared and microwave, processing on Maillard reaction products and phenolic compounds in soybean. Journal of the Science of Food and Agriculture, vol. 94, no. 1, p. 45-51. https://doi.org/10.1002/jsfa.6210

Acknowledgements:

This article was presented in PATPI-SEAFAST International Conference: "SCIENCE-BASED INGREDIENTS: THE FUTURE FOR FOOD IN ASIA", °Ctober 3-5, 2018, Indonesia.

The manuscript was first edited and submitted during the Sabbatical Leave of the corresponding author at Mie University, Faculty of Bioresources in the laboratory of Bioinformatics and Food Engineering (BIFE) in 2019 funded by The Ministry of Research, Technology, and Higher Education of the Republic of Indonesia

Contact address:

Maria Erna Kustyawati, University of Lampung, Department of Agriculture Product Technology, Bandar Lampung, 35145, Indonesia. Tel. : +6281369994986,
E-mail: mariaernakustyawati@gmail.com
ORCID: https://orcid.org/0000-0001-7980-1844

Filli Pratama, University of Sriwijaya, Department of Agriculture Technology, Palembang, 30139, Indonesia, Tel.: +628153818913,
E-mail: fillipratama@gmail.com
ORCID: https://orcid.org/0000-0003-0282-9800

*Daniel Saputra, University of Sriwijaya, Department of Agriculture Technology, Palembang, 30139, Indonesia, Tel.: +6285279407485,
E-mail: drdsaputra@unsri.ac.id
ORCID: https://orcid.org/0000-0001-6264-8708

Agus Wijaya, University of Sriwijaya, Department of Agriculture Technology, Palembang, 30139, Indonesia, Tel.: +6281377484401,
E-mail: agus_wijaya@hotmail.com
ORCID: https://orcid.org/0000-0001-8280-2397

Corresponding author: *