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Application of vacuum packaging to extend the shelf life of fresh-seasoned tempe

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Abstract

Tempe is an indigenous fermented food from Indonesia, prepared by the action of molds, Rhizopus spp., on cooked soybeans. In 2012, up to 60% of soybean production went to tempe industry with consumption of tempe reaching 8.5 kg per person per year in Indonesia. However, the export of tempe seems still impossible due to its perishable characteristic whose shelf life is very short. Pre-blanching and vacuum packaging were expected to extend the shelf life of tempe. This research was aimed to study the application of blanching and vacuum packaging on the shelf life of fresh-seasoned tempe. Steam blanching at 80°C for three minutes was selected from pre-investigation for pre-treatment of the tempe without inducing cooked-tempe characteristic. The results of this research study revealed that steam blanching and vacuum packaging were able to extend the shelf life of fresh-seasoned tempe for two days in room temperature (23-24°C), 32 days in refrigerated temperature (4-6°C), and 49 days in chilling temperature (0-4°C). The pH of tempe was found to be increasing during storage with more tenderized texture during storage.

Introduction

Tempe is an indigenous fermented food from Indonesia, prepared by the action of molds, Rhizopus spp., on cooked soybeans through controlled fermentation process. Tempe is well known for its nutritional value and health benefit (Astuti *et al.*, 2000; Astawan, 2008; Babu *et al.*, 2009; Messina *et al.*, 2009). Indonesia is acknowledged as world's biggest producer of tempe and centre of soybean market in Asia. In 2012, up to 60% of soybean stocks went to tempe industry with average population consumption of tempe in Indonesia reached 8.5 kg per person per year (BPS, 2012).

Tempe becomes one of meat alternative for people living as vegetarian or veganism due to its high protein content. Sarwono (2012) reported that 100 g of fresh tempe contained around 18.3 g protein while 100 g of beef contained 12.8 g protein. Following this fact, potential market for tempe is not only limited to domestic scale but also at international level where countries like India, China, Japan, Australia, European countries (Vegetarian Victoria, 2011), as well as United States are listed as top countries with high number of vegans (Stahler, 2009). In 2013, there were up to 540 million people were vegetarian with the top consumers spread in India up to 500 million people (Vegetarians, 2013) and the rest 40 million were European and American (Raw Food Health, 2008). The prediction, which said that up to nine billion people around the world would follow vegetarian trend in 2050, has even strengthened the prospect of tempe in the future (Huffpost Living, 2014).

Government of Indonesia has genuinely regulated the standard quality of tempe by establishing Indonesian National Standard of Tempe – SNI 01-3144-1998 that has been updated to SNI 3144-2009. In the 36th Codex Allimentarius Commission (CAC) Assembly in Rome (2013), tempe standard was adopted as regional CODEX standard and soon will become worldwide tempe standard (CODEX Indonesia, 2013). It showed how world has also put highest concern upon tempe, the indigenous food from Indonesia to an international level.

Despite the above facts, the export of tempe seems still impossible due to its perishable characteristic whose shelf life is very short. Many researches have been done in studying the methods of extending the shelf life of tempe. This include dried-flavoured tempe (Mutiara, 1985), drying and sterilization of tempe (Kemala, 2006), and also combination of vacuum packaging and sterilization (Indriani, 2006). Previous studies were mostly applying high temperature-thermal process that had been proven to extend the shelf life of food product. However,

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thermal process application changes the natural appearance and physical properties of fresh tempe which unable to meet consumers preference for fresh tempe. Therefore, the aim of this research was to apply blanching treatment and vacuum packaging to extend the shelf life or fresh seasoned tempe. Seasoning was projected as addition process to increase added value of the tempe and also to fulfil people nowadays' trend upon ready-to-cook food.

Materials and Methods

Materials

Local soybean *(Glycine max)* Grobogan from Central Java, Indonesia was used as raw material in tempe production. A 10 gram of salt was mixed with 30 gram of garlic and 200 ml water. All of these ingredients were blended homogenously prior to seasoning the tempe. Polyetylene (PE) plastic with 8 mm thickness was used for vacuum packaging.

Production of fresh-seasoned tempe

Production of tempe was based on procedures that has been applied in Indonesia House of Tempe, Bogor-Indonesia which included the practice of Good Manufacturing Practices (GMP). Sortation and washing of Grobogan soybean was carried out prior to soaking for three hours. Soaked-soybean was boiled, soaked again for 15 hours, and then dehulled. Soybean was then washed with hot water and cooled before it was inoculated with 0.1% R. oligosporus. This was followed by packing the soybean with perforated oval-shaped plastic (d= 2 cm) before incubation process (T=31^{o}C; RH=78-85%; 36-48 hours). Tempe was then marinated with seasoning prior to vacuum packaging. At the end of the production, tempe was stored at three different temperatures which were room temperature (23-24^{o}C), refrigeration temperature (4-6^{o}C) and chilling temperature (0-4^{o}C).

Determination of optimum blanching time

Steam blanching (80^{o}C) with 10 variations of blanching time (1-10 minutes) was applied to the tempe at the beginning of processing prior to treatments (seasoning and vacuum packaging). Optimum blanching time was determined by comparing the texture of blanched tempe and control (non-blanched tempe) using penetrometer. Selected tempe was the tempe that had no significance difference with control in term of texture ($p > 0.05$) using T-Test.

Determination of shelf-life

Shelf-life of tempe was determined using extended storage study (ESS) method through partially staggered design by drawing a simple regression of subjective data or organoleptic assessments result (Arpah, 2001). Ten trained panelists were asked to assess its acceptance based on attributes like color, texture, aroma, and overall acceptability during storage for fresh-seasoned tempe. Taste attribute was added for fried tempe sample for both control and treated tempe to allow panellist conducting comparison. Hedonic 7-scale test with scale point from 1 represents dislike extremely to point 7 which represent like extremely. Sensory evaluations were held every day for tempe that were stored in room temperature, every three days for tempe that were stored in refrigeration temperature, and every seven days for tempe that were stored in chilling temperature. Sensory analysis with average score below four was considered as deteriorated where score of that particular sensory aspect was then used to draw simple regression with x = day and y = score of sensory evaluation.

Measurement of pH

The change of pH of tempe during storage were measured using pH-meter (Eutech, Singapore).

Analysis of texture

The change in texture of tempe during storage was measured using penetrometer (Precision, USA).

Proximate analysis

Proximate analysis was carried out on control or fresh tempe sample (Day 0) and also at the end of storage which consisted of moisture content using oven drying method (AOAC, 2012), protein content using Kjeldahl method (AOAC, 2012), fat content using Soxhlet method (AOAC, 2012), ash content (AOAC, 2012), and carbohydrate content (by difference).

Microbial contamination analysis

Contamination of bacteria were analyzed following standard methodologies described in the Bacteriological Analytical Manual of *Escherichia coli* (Feng *et al*.,2002) and *Salmonella* spp. (Andrews and Hammack, 2007)

Heavy metals contamination analysis

Heavy metals that were analyzed involving lead (Pb), cadmium (Cd), tin (Sn), arsenic (As), and mercury (Hg). Analysis was conducted using atomic absorption spectrophotometer (AOAC, 2012).

Statistical test

The results were expressed as a mean ± SD. The statistical analysis of chemical composition of fresh seasoned tempe was carried out using one-way ANOVA followed by DMRT post-hoc test. The significance in the changes of the texture was based on T-Test SPSS.

Results and Discussion

Determination of blanching time

Blanching is a unit operation that is commonly carried out to inactivate enzymes, modify texture, preserve colour, flavour, and nutritional value, and remove trapped air in food. Blanching can be carried out through water blanching, steam blanching, microwave blanching, or gas blanching (Corcuera, 2004). Gokmen (2005) reported that exposing food with hot air at 80°C through blanching method could inactivate up to 90% of enzyme activities. Compared to hot water blanching, steam blanching was found to be more effective and beneficial in preventing excess of nutrients loss (Martinez *et al.*,2012; Xiao *et al*.,2014). Steam blanching at 80°C was applied in this research on tempe product as pre-treatment to suppress metabolic activity of molds (prevent continued fermentation) and also proteolytic and lipolytic enzymes which were the major reason of fresh tempe deterioration.

In this study, blanching time was pre-determined to ensure that shelf life of tempe can be extended and still have the fresh-like texture. The texture of blanched tempe from every variants of the blanching was assessed using penetrometer test and compared with non-blanched tempe as control. Duration of three minutes steam blanching was chosen as the best time after assessing the texture changing of blanched tempe when compared with the control. The result of penetrometer test (Table 1) showed that application of steam blanching 80°C with the duration longer than three minutes had caused significant ($p<0.05$) undesirable texture changing of fresh tempe, consequently tempe was assumed possessing cooked-tempe texture.

Shelf life determination

Shelf life of food product can be defined as a period between production time and retail purchase of a food product during which the product still serves satisfactory quality or retain desired sensory, chemical, physical, and microbiological characteristics (Ellis and Man, 2000). Shelf life determination was commonly conducted through Extended Storage Study (ESS) for perishable product, which only had shelf life less than three months and Accelerated Shelf life Testing (ASLT) for food products having longer shelf life (Herawati, 2008).

Tempe was categorized as highly perishable food product, which had shelf life less than two days in ambient temperature. In this study, ESS or conventional method was applied to determine the shelf life of the tempe. ESS was done through partially staggered design by drawing a simple regression of subjective data or organoleptic assessments result (Hough *et al.*, 2003). The type of plastic used in the research was polyethyene (PE). PE plastic is commonly used for vacuum packaging due to its characteristic, which are translucent to opaque, robust enough to be virtually unbreakable and quite flexible at the same time (Dirim *et al.*, 2004). Vacuum packaging was applied to this research in expectation to extend the shelf life of the tempe.

Sensory evaluation for ESS of tempe reported that panelists were more sensitive to attribute of aroma of raw tempe (control sample) than the other attributes. This sensitivity influences their acceptance upon raw tempe. During storage, sensory score for attribute of aroma got lower and the reducing rate was faster than the other attributes.

Off odor that was spread by the raw tempe was contributed by ammonia as the impact of continuous fermentation and also influenced of seasoning that was added. Pungent off odor and sour aroma of raw tempe were not detected by panelists on fried tempe but it came up as bitter taste in several samples. Consequently, these two attributes were mostly referred as detrimental attributes in determining shelf life of tempe. The summary result of extended storage study of the tempe could be seen in Table 2 and 3.

The result of shelf life determination revealed that vacuum packaging had successfully extended the shelf life of tempe. In the room temperature, normally tempe could only remain in a good and acceptable condition up to 24 hours or one day. Vacuum packaging was found to extend the shelf life of tempe up to three days for sample of non-seasoned tempe in room temperature and up to two days for seasoned tempe stored in room temperature. In low temperature storage, both refrigeration or chilling temperature, vacuum packaging worked better than in the room temperature. Tempe could eventually has longer shelf life in low temperature storage compared to ambient temperature.

Fresh seasoned tempe had shelf life up to 32 days while non-seasoned tempe had shelf life up to 35 days in refrigeration. Lower temperature, which was chilling temperature, could even extend the shelf life of seasoned tempe up to 49 days and up to 55 days for

Table 1. Effect of blanching time on texture of tempe

Time (minutes)	Texture (mm/ 5 seconds)	Time (minutes)	Texture (mm/ 5 seconds)
0 (control)	6.46 ± 049		
1	6.61±0.68	6	10.13±0.59*
2	6.87±0.55	7	11.49±0.92*
3	6.93±0.57	8	12.31±1.03*
4	8.86±0.95*	9	14.41±0.87*
5	9.40±0.76*	10	15.94±1.13*

·Results were expressed as mean ± SD
·Symbol * showed significant difference compared to control ($p<0.05$) using T-test

Table 2. Extended storage study for fresh seasoned tempe

	Vacuum	Non Vacuum
	Room temperature (23-24°C)	
Y	y = -0.6186x + 5.67	y = -0.7051x + 5.444
R^2	0.9545	0.9889
Shelf life (days)	2	1
	Refrigeration temperature (4-6°C)	
Y	y = -0.0514x + 5.8405	y = -0.1437x + 5.8633
R^2	0.9003	0.9756
Shelf life (days)	32	14
	Chilling temperature (0-4°C)	
Y	y = -0.046x + 6.38	y = -0.1236x + 6.31
R^2	0.9551	0.9991
Shelf life (days)	49	21

·x axis represented day(s)
·y axis represented sensory attribute

non-seasoned tempe. Vacuum packaging worked by expelling all air inside the packaging without altering with other gas (Jay, 2000), thus, eventually vacuum packaging is known to be a good barrier for oxygen and water (Masniyom *et al*., 2013) and effective to extend shelf life of food product (McMillin, 2008; Brenesselova *et al*., 2015).

Normally, tempe without pre-blanching treatment and vacuum packaging could only remain in a good condition up to two weeks in low temperature storage. Tempe would undergo texture changing and undesirable odor for the time of storage longer than that. The absence of oxygen in the packaging could retard the growth of aerobic spoilage bacteria such as Pseudomonas spp., Enterobacteriaceae, Shewanella putrefaciens (Arashisar *et al*., 2004; Stamatis and Arkoudelos, 2007; Mastromatteo *et al*., 2010). The application of hurdle concept by involving low temperature storage could also delay deterioration due to microbial or chemical activities.

Shelf life of seasoned-tempe was shorter than non-seasoned one. Theoretically, garlic that was added as spices seasoning of tempe possess antimicrobial substance,allicin (diallythiosulphinate), which is able to suppress microbial growth (Suryati *et al*., 2014). However, odor or unpleasant smell produced by combination of salt and garlic which owns highly reactive sulfur molecules (Gitin *et al*., 2014; Kimbaris *et al*., 2006) that were stored in vacuum for few days contributed to the production undesirable attribute or odor for panelists, remembering the shelf life determination was done through sensory evaluation. Nevertheless, seasoned tempe owned the strength in term of its simple practicality due to its pre-seasoning step compared by existing product. Thus, consumers could directly cook the tempe without seasoning at first.

Observation of texture and pH changes during storage

Analysis of texture changing of tempe during storage was done using penetrometer as a form of objective observation upon shelf life determination of tempe. Texture of tempe can be pointed as one of objective characterization upon shelf life and quality of tempe since consumers often assess the texture of tempe before purchasing.

In this research, penetrometer was chosen to analyse the texture of tempe during storage. Needle of penetrometer was injected in several areas of tempe and applied for five seconds. Score displayed indicating the depth of needle that can dig the tempe. The higher score of penetrometer indicated the more tender or overstuffed the tempe. Tempe were found gradually getting overstuffed or tender during storage that was reported by the increasing score of penetrometer test each tempe. Phenomenon above could be caused by continued fermentation that still running during storage.

The tempe had been blanched and packed in vacuum that could suppress metabolic activities of microorganism, such as molds. However, due to storage condition (room temperature), continued fermentation by *Rhizopus* spp. still running in lower rate that can destroy the inter-matrix of soybeans. Consequently, tempe were getting softer and mushy

Table 3. Extended storage study for fresh non-seasoned tempe

	Vacuum	Non vacuum
	Room temperature (23-24°C)	
Y	y = -0.384x + 5.252	y = -0.3051x + 4.624
R^2	0.9869	0.9371
Shelf life (days)	3	2
	Refrigeration temperature (4-6°C)	
Y	y = -0.0285x + 5.1716	y = -0.0867x + 4.8183
R^2	0.8917	0.9578
Shelf life (days)	35	11
	Chilling temperature (0-4°C)	
Y	y = -0.0386x + 5.8634	y = -0.1143x + 6.1333
R^2	0.9324	0.9999
Shelf life (days)	55	21

*x axis represented day(s)
*y axis represented sensory attribute

when fermentation process was still occurring and additional water existed due to hydrolysis of carbohydrate' (Ferreira *et al.*, 2011). Other than that, tempe which has high amount of protein could undergo coagulation and lose its water binding capacity which caused texture changing of the tempe (Fellow, 2000).

Stability of food product could be correlated with its easiness to undergo chemical changes. One of the manifestations of chemical changes on food is the change of pH during storage. Measurement of pH change of tempe was carried out as an observation to the shelf life of tempe. Measurement of pH was conducted periodically following subjective determination of shelf life of tempe using ESS method. It is known to be important to measure the change of pH of food as an intrinsic factor that work as an indicator of quality and acceptance of food. Previous study done by Pambudi (2013) reported that the pH of tempe tend to increase due to continued-fermentation process.

Decipherment of protein happens during storage of tempe. Protein would be degraded into base compound such as ammonia, which contributes in increasing the pH. The pH of food change due to degradation of protein that was caused by the acts of proteolytic enzyme into carboxylic acid, sulfide acid, and ammonia (Chamidah, 2000).

Initial pH of fresh tempe is influenced by its fermentation process. As other chemical changes, the pH change during storage was also manifested in sensory attribute of tempe. This chemical reaction's result influenced consumers' sensory acceptance on tempe through its contribution upon odor or unacceptable aroma and also taste. Normal tempe actually has unique aroma as manifestation of linoleic acid's recast which is dominant lipid acid in soybean into 1-octen-3-ol by lipoxygenase enzyme and hydroperoxide lyase enzyme (Feng *et al.*, 2006). However, as the pH kept increasing during storage, tempe produced pungent off odor and also bitter taste for fried tempe that could decrease consumers acceptance.

Chemical composition

Water is one of detrimental component in food affecting its quality, such as appearance, texture, and palatability. Water content in food also determines acceptability and shelf life of food product (Li *et al.*, 2011). Indonesian National Standard (SNI) for tempe 3144-2009 regulates the maximum water content of tempe is 65% and this had been complied by final product of fresh-seasoned tempe in this research with water content of 63.62%. Result of sequence water content analysis during storages through the analysis of variance revealed that there was no significant difference ($p>0.05$) found for water content during end storage in room and refrigeration. However, a highly significance difference ($p<0.01$) was found between water content in Day 0 tempe and at the end of storage of chilling temperature (65.86%). Water transfer for environment or cold storage to inside the packaging could cause this increment of water content. The vacuum condition of the packaging could possibly be broken after long storage in lower temperature, which were up to 49 days in this research. However, this water content increment that was close to maximum water content regulated by SNI happened in the end of storage when the tempe was nearly rejected.

Tempe is valued mainly as a rich source of high plant protein. As the result of fermentation process, bioavailability of protein of fermented soybean was known to be higher than unfermented one (Astawan *et al.*, 1994; Pawiroharsono, 2007; Susi, 2012; Bavia *et al.*, 2012). Molds which grown covering the tempe contain nucleic acid that can contribute to addition of nitrogen protein (Pasaribu, 2007). Soybean which was widely processed as raw material of tempe was also known to be rich in essential and non-essential amino acid. Astawan *et al.* (2015) reported that besides high in protein, the soybean is also rich in both essential and non-essential amino acid. Cysteine, isoleucine, leucine, lysine, methionine,

Table 4. Chemical composition of fresh seasoned tempe

Content (%)	Period of analysis				Req (SNI)
	Day 0	End of storage (Rejected tempe)			
		Room	Refrigeration	Chilling	
Water** (wb)	63.62 ± 0.08[a]	63.46 ± 0.44[a]	64.24 ± 0.27[a]	65.87 ± 0.18[b]	Max 65
Protein* (db)	48.98 ± 0.11[a]	50.72 ± 0.62[b]	49.44 ± 0.41[a]	49.86 ± 0.25[ab]	Min 45
Crude Fat* (db)	43.63 ± 0.49[a]	43.57 ± 0.67[a]	44.41 ± 0.55[a]	44.38 ± 0.06[a]	Min 28
Ash** (db)	2.19 ± 0.01[b]	2.15 ± 0.01[b]	1.86 ± 0.02[a]	1.80 ± 0.05[a]	Max 4
Carbohydrate* (db	5.20 ± 0.37[a]	3.58 ± 0.06[a]	4.29 ± 0.94[a]	3.96 ± 0.26[a]	-

·Results were expressed as mean ± SD
[*] Different words following the number in a row showed that result was significantly different ($p < 0.05$) using DMRT.
[**]Different words following the number in a row showed that result was highly significance different ($p < 0.01$) using DMRT.

phenylalanine, threonine, tryptophan and valine are among the group of essential amino acid while alanine, glycine, arginine, histidine, proline, tyrosine, aspartic acid and glutamic acid are among the group of non-essential amino acid owned by soybean. Crude protein analysis reported that protein content in the tempe was 48.98% db that was in the range of SNI requirement upon tempe, minimally 45% db. The result of analysis of variance revealed that protein content of fresh seasoned tempe increased significantly ($p<0.05$) between day 0 (controll) and end of storage in ambient temperature which was 48.982% to 50.72% protein, respectively.

The result of crude fat analysis showed no significant difference ($p>0.05$) between tempe day 0 (controll) and end of storage in room, refrigeration, and chilling temperature. The range of crude fat content was 43.63% to 44.38% dry basis that had been complied with requirement addressed by SNI 3144-2009 which is 28% in least content. Carbohydrate content that was obtained from by difference calculation reported that there was no significant increment ($p<0.05$) of its content from Day 0 (controll) to the end of every storage condition. The range for carbohydrate content were 3.58-5.20% db.

Ash content analysis is closely related to mineral content in food. The result of ash content though analysis of variance reported that there was no significant difference ($p>0.05$) of ash content of tempe (day 0) and tempe at the end of ambient storage. In the other hand, highly significant difference ($p<0.01$) was reported between day 0 tempe and end of refrigeration and chilling temperature storage which were 2.2 to 1.84 and 1.83% respectively. The trend of reduction was found during the change after storage in cold temperature. Fennema (1996) reported that mineral content in food can decrease during washing or physical separation. This mineral reduction was the manifestation of leaching of minerals into water (Helmy, 2003; Wang, 2010). It was also supported by Santoso *et al.* (2006) and Salamah *et al.* (2012), reporting that mineral content in food can decrease after boiling or other processing involving water since mineral is a water soluble. In this research, ash content of tempe stored in cold temperature was analyzed after thawing process, consequently mineral was presumably dissolved and went wasted. However, ash content of the tempe still fulfilled SNI 3144-2009 requirement, which is 4% by maximum. Full version of chemical composition can be seen in Table 4.

Microbiological analysis

Microbiological analysis is important to be carried out as one of quality assurance aspects to certify quality and safety of food, also to decrease prevalence upon food borne pathogens (McMeekin and Ross, 2002). Tempe, which is recognized as nutritious food that is high in protein, can be a suitable media for microbial growth. The major causes of microbial presence on food products are non-observance of relevant technological application, incompliance with recommended process standard and lacks of personal hygiene. Previous studies found that tempe has an antibacterial substances that works especially against Gram positive bacteria, such as *Bacillus subtilis, Staphylococcus aureus, Streptococcus,* and *Clostridium* spp. However, the same activities combating *Escherichia coli* and *Salmonella* spp. was not found (Roubos-van den Hil and Nout, 2011).

Escherichia coli, abbreviated as *E. coli,* is a Gram negative and facultative anaerobic bacteria which is a member of the normal intestinal microflora of humans and other mammals. Its cell ability to grow well outside in a limited time resulted this

Table 5. Heavy metal contamination analysis of fresh seasoned tempe

Metal	Result (mg/kg)	Max standard in SNI (mg/kg)
Lead (Pb)	0.016	0.250
Cadmium (Cd)	0.017	0.200
Tin (Sn)	<0.002	40
Arsenic (As)	<0.002	0.250
Mercury (Hg)	0.003	0.030

bacteria often being used as indicator organism for fecal contamination or hygiene practices in food manufacturing (Melliawati, 2009). *Salmonella* spp. belongs to rod-shaped Gram-negative bacteria. Similar with *E. coli, Salmonella* spp. is characterized as pathogenic bacteria which responsible with some diseases caused by its infection or known as salmonellosis (Narumi *et al*., 2009). The result of both bacteria (*Escherichia coli* and *Salmonella* spp.) which were negative indicated that standard quality upon microbial safety of Indonesian National Standard of Tempe or SNI 3144-2009 had been complied. Pre-blanching (steaming 80°C) applied in this research was indicated to be effective to suppress microbial growth in food (Gokmen, 2005).

Water activity is also known to be essential factor for microbial growth. Reducing water activity below which optimum condition of microbial can grow create a stress condition for microbes. Additional of salt as the seasoning which is very hygroscopic could decrease the water activity by binding the free water (Astawan *et al*., 1994). Other than that, in common, *Salmonella* spp. and *Escherichia coli*, which belong the family of Enterobacteriaceae, are sensitive to salt (Abdulkarim *et al*., 2009) whereas salt was used as seasoning in this research to marinate the tempe. Addition of garlic (*Allium sativum* Linn) as seasoning was presumed to have an active compound namely allicin which could work as antimicrobial agent (Syifa *et al.*, 2013) which effectively suppress both Gram-positive and Gram-negative bacteria such as *Staphylococcus aureus, Salmonella typhii, Pseudomonas aeruginosa*, *Escherichia coli* and *Proteus* spp. (Durairaj *et al.*, 2009; Syifa et al., 2013).

Heavy metal contamination analysis

Heavy metal contamination is one of the chemical aspects to watch out because it can threaten the health and safety of consumers. Heavy metal is a term used for the transition elements having atomic mass density greater than 5 g / cm^3 (Jarup, 2003). According to Astawan (2008), contamination of heavy metals in consumed food would be accumulated in the human body and in a long term can lead to nervous system disorders, paralysis, and premature death as well as a decrease in the level of intelligence of children. Heavy metals which are found frequently contaminate tempe in Indonesia are lead (Pb), cadmium (Cd), tin (Sn), arsenic (As), and mercury (Hg). The result of heavy metals analysis (Table 5) revealed that fresh-seasoned tempe had afforded safety regulation of tempe established through Indonesian National Standard. Producing method, which had applied principle of Good Manufacturing Practices, contributed in prevention of heavy metals contamination.

Conclusion

Steam blanching 80°C for three minutes can be applied to tempe to supress microbial growth without inducing cooked-like texture. Combination of vacuum packaging and low temperature storage was reported to be effective to extend the shelf–life of fresh-seasoned tempe up to 49 days (without vacuum was only 21 days) in chilling, while storage in refrigeration and ambient temperature with vacuum packaging were reported to have shorter shelf life, which were 32 and 2 days respectively. Increment of pH and penetrometer score were found during storage due to continued fermentation. Fresh-seasoned tempe (day 0) had complied regulation of tempe quality established by Indonesian National of Standard (SNI 3144-2009) in term of proximate, microbial safety, and heavy metals contamination.

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