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EFFECTS OF HIGH-PRESSURE PROCESS (HPP) TREATMENT OF TEMPOYAK ON MICROBIAL GROWTH

Tempoyak is a fermented durian paste made from an overripe durian under partial anaerobic conditions in a tight closed container. The anaerobic fermentation conducted by Lactic acid bacteria (LAB) can take place from 3 to 8 days in ambient temperature range from 24°C to 34°C and the final pH value is around 3.96 to 4.08. This study focus on the effectiveness of the High-Pressure Process (HPP) in Tempoyak and the relationship between pressure treatment with microbial activity in treated Tempoyak. A High-Pressure treatment of 600 MPa for 5 min on packed tempoyak was applied. Analysis of the pH, moisture content, total plate count (TPC), color and sensory were performed. Throughout four weeks of storage, the result of pH and moisture content showed no significant changes under chilled temperature (–4°C) for non-treated and HPP-Treated samples. The total plate count showed the microbial level of non-treated sample to be slightly higher when compared to HPP sample in both storage conditions. The L* values also showed significant change in the color analysis of the samples. From the analysis performed, HPP was proven to reduce the microbial activity in the tempoyak.

Keyword: Tempoyak; High Pressure Process; Enzyme; Microbes

1. Introduction

Tempoyak is a fermented durian paste created by mixing salt with overripe durian. It is a traditional cuisine that is frequently enjoyed by the Malays in the Southeast Asian region [1]. In Asia, Tempoyak is usually used as an acid-fermented sauce served with specific fish and vegetable meals. Tempoyak enables the preservation of durians that would otherwise be discarded [2]. Tempoyak is also known to have a creamy texture, distinctive durian flavor, and also yellow color for its apearance. Tempoyak is rarely to be consumed due to its raw smell and also sour taste [1].

Tempoyak can be fermented for months but typically requires around 7 days minimum for the development of flavour and acidity. Lactic acid bacteria (LAB) with an approximate 2.8 to 3.6% acidity provide a sour taste in Tempoyak [3]. Lactic acid bacteria (LAB) will carry out anaerobic fermentation at room temperature for 3-8 days (28-34°C) [1]. The pH of Tempoyak usually ranges from 3.96 to 4.08 [2].

Fermentation is a technology that uses of the development of metabolic process of microbes to preserve and transform food materials [4]. Fermented foods and beverages are quite popular because of their longer shelf life, safety, functionality, sensory, and nutritional qualities [5]. The shelf life of perishable produce is increased during food fermentation because the metabolites produced by the fermenting organisms inhibit the growth of spoilage and pathogenic organisms [4]. The thermal treatment is the most common method used in preserving fruit-based commercial products due to its capacity to inactivate microorganisms and spoilage enzymes. However, many of the natural phytochemicals are heat labile, easily destroyed, and lose their health benefits for consumers. The quality of fruit products can be influenced by thermal processing and have an impact on the acceptability of the product because the components responsible for colour, flavour, and taste are often heat-sensitive [6]. Thus, non-thermal treatment has been used as an emergence method to improve the microbiological safety and the shelf life of fruitbased products while having little impacts on the food sensory, physical and nutritional values [7]. High-Pressure Process treatment is known as a non-thermal technique of food preservation that will inactivate vegetative spoilage microorganisms and harmful pathogens by using pressure to induce the pasteurization effect without the need of overly intensive thermal processing

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or chemical preservatives [8]. HPP is effective not just for inactivating pathogens in raw materials, but also for heat-treated food products that become infected during slicing, packing, or cross-contamination with other food items [9].

Research on Tempoyak has indeed focused on its antimicrobial activity, largely attributed to the inhibitory activity of lactic acid bacteria (LAB). Several studies have investigated the antimicrobial potential of Tempoyak and its active compounds against various pathogens, including bacteria and fungi. These findings contribute to understanding its potential as a natural antimicrobial agent and its application in food preservation and health. LAB are frequently present in fermented products and frequently employed as starting cultures in the food sector [10]. LAB are known to produce organic acids, hydrogen peroxide, and bacteriocins, all of which contribute to their antimicrobial properties. For example, research by Mustapha et al. [11] investigated the antimicrobial activity of Tempoyak against Escherichia coli strains isolated from foodborne illness cases. The results demonstrated that Tempoyak extracts inhibited the growth of Escherichia coli strains in vitro, suggesting its potential as a natural preservative in food products. The results demonstrated that Tempoyak extracts inhibited the growth of Escherichia coli strains in vitro, suggesting its potential as a natural preservative in food products. Similarly, studies by Yusof et al. [12] explored the antimicrobial activity of LAB isolated from Tempoyak against Escherichia coli, highlighting the role of specific LAB strains, such as Lactobacillus plantarum and Lactobacillus fermentum, in inhibiting pathogenic bacteria. Furthermore, ongoing research is focusing on elucidating the mechanisms underlying Tempoyak's antimicrobial activity, including the production of antimicrobial compounds and the modulation of microbial communities in food matrices. Overall, current research indicates that Tempoyak possesses significant antimicrobial potential, attributed in part to the inhibitory activity of LAB, and further studies are warranted to explore its applications in food preservation and safety.

This study is focusing on the effectiveness of the High-Pressure Process (HPP) of non-thermal systems for Tempoyak and the relationship between pressure treatment with microbial activity in treated Tempoyak. The treated Tempoyak will undergo a microbiology test (total plate counts) and graph will be plotted based on the result obtained. The samples that will be analyzed were stored in two different conditions which were at room temperature (25°C) and chilled temperature (–4°C).

2. Experiment

2.1. Materials

1 g of Tempoyak sample is used from non-treated sample and HPP sample. Molten Agar ($400\,\text{mL}$) is used from the mixture of $400\,\text{mL}$ distilled water and potato dextrose agar ($15.6\,\text{g}$) and $63\,\text{mL}$ of Phosphate Buffer Solutions pH 7.40 is used in total plate count analysis.

2.2. Microbiology Analysis (Total Plate Count)

The analysis begins by labelling the dilution blanks as 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} , and 10^{-7} and 1 g of the durian paste is added to 9 ml of sterile phosphate buffer solutions labelled 10^{-1} . The original sample was proceeded to dilute for up to 10 times. The contents were then mixed by rolling the tube back and forth to obtain uniform distributions of the organism. 1 ml of suspension from the first dilution was transferred while in motion to the dilution blank of 10^{-2} with a sterile 1 ml pipette.

From the 10⁻² suspension, 1 ml of suspension was transferred to dilution blank, thus diluting the sample. This step was repeated till the original sample has been diluted 10^{-7} times. From the appropriate dilutions $(10^{-1} \text{ to } 10^{-7})$, 1 ml of suspension was transferred to sterile petri dishes. Agar plated was prepared by mixing the growth medium with molten agar (Potato dextrose agar) and then autoclaving to sterilize. Once the molten was agar cooled down to 50°C, it was poured about 15 ml into sterile petri dishes and left to set. The contents of each plate were mixed by rotating it gently to distribute the cells throughout the medium. The plates were let to solidify and were incubated the plates in an inverted position for 24 to 48 hours at 37°C. The appearance of the bacterial colonies was then observed for all the plates. The number of colonies counted were determined on the plate. The colonies in the range from 25 to 250 by placing each plate one by one on the platform of a Quebec colony counter.

$$Organism \ per \frac{ml}{g} of \ sample =$$

$$= Colonies \ number \times Average \ of \ replicates \div$$

$$Amount \ plated \times dilutions \tag{1}$$

2.3. pH Analysis

For pH quantification data, the Tempoyak or durian pulp were homogenized with distilled water (1:10 w/v) for 1 minute and subjected to pH measurement with a pH meter [13].

2.4. Determination of Moisture Content (M)

The moisture content for Tempoyak was determined by using the AOAC (1990) technique where 2 g of the samples were weighed on petri dishes and placed in the oven, uncovered up to 3 hours until the mass of the sample remained constant. The calculation below was used to determine the moisture content of the sample.

$$Moisture content = \frac{weight loss \times 100\%}{Weight of sample}$$
 (2)

TABLE 1

2.5. Color Analysis of Tempoyak Samples

The colour of the Tempoyak sample is determined using a Chromameter (CR-400, Konica Minolta Sensing, Japan). Chromameters work fundamentally by using a sensor that simulates how the human eye perceives colours and then quantifies colour by measuring the three basic components of colour known as primary colours (red, green, and blue).

The colour is measured by pressing the tip of the chromameter receptor (shaped like a flat surface) against the sample. The flat section of the equipment must be completely covered throughout the measurement. The CIELAB parameters of L* (lightness), $a^*(+a = red, -a = green)$ and (+b = yellow, -b = blue) were taken [13].

2.6. Sensory Evaluation

The sensory evaluation was done through individual testing. A triangle test was used to identify the samples among non-treated samples with HPP-Treated durian samples. Both samples were tested for three days each week. The samples were placed in two different conditions which were at room temperature condition and chilled temperature condition. For samples at room temperature condition, the sample was tested until the taste of non-treated and HPP-treated samples began to turn sour and changes in the texture.

2.7. Shelf-life Estimation

The Arrhenius model of Accelerated Shelf Testing (ASLT) is used to predict the shelf-life of Tempoyak sample. The data obtained from the observation of moisture content of HPP sample are plotted into a graph and the slop value was used to calculate the estimate shelf-life by using Eq. (3).

$$Shelf - life = \frac{A_e - A_o}{K} \tag{3}$$

Where A_e – Initial quality value; A_o – Value of product quality remaining after period of time; K – Constant.

3. Results and Discussion

3.1. Microbiology Analysis (Pour Plate Method)

The result for value of CFU/g for non-treated Tempoyak and HPP Tempoyak for room temperature and chilled temperature are tabulated in TABLE 2. The samples for non-treated samples and HPP samples was analysed under room and chilled temperature for 4 weeks of storage period. The pour plate method is used to analyse the microbiology analysis of both samples.

Based on Fig. 1, the microbial activities in HPP samples are slightly higher compared to the non-treated samples. This could happen due to cross contamination occurs during the process. Meanwhile, the HPP samples under chilled temperature is lowered compared to non-treated samples Fig. 2. Specifically, HPP reduced the total plate count (TPC) in tempoyak below

Total plate count of Tempoyak for non-treated and HPP samples under room and chilled temperature

	Total plate count (log ₁₀ CFU/g)									
Week	Non-treated Tempoyak (room temperature)	HPP Tempoyak (room temperature)	Non-treated Tempoyak (chilled temperature)	HPP Tempoyak (chilled temperature)						
1	6.81	6.81	8.79	8.97						
2	8.14	ND	7.18	7.18						
3	6.12	7.57	7.02	7.02						
4	6.00	7.43	9.00	6.77						

ND: not detected, > 25 CFU/g estimated

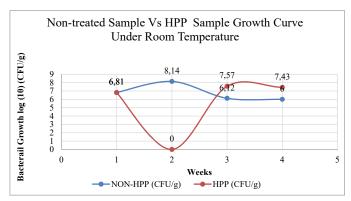


Fig. 1. Growth Curve of Non-treated vs HPP Sample Under Room Temperature

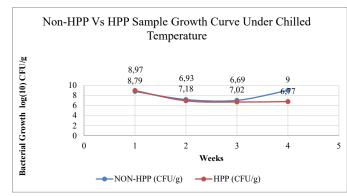


Fig. 2. Growth Curve of Non-treated sample Vs HPP Sample Under Chilled Temperature

The results for non-treated and HPP treates Tempoyak under room and chilled temperature

Week	Dilutions	Colony Count (non-treated Tempoyak under room temperature)	Colony Count (HPP Tempoyak under room temperature)	Colony Count (non-treated Tempoyak chilled temperature)	Colony Count (HPP Tempoyak under chilled temperature)	CFU/g (non-treated Tempoyak under room temperature)	CFU/g (HPP Tempoyak under room temperature)	CFU/g (non-treated Tempoyak under chilled temperature)	CFU/g (HPP Tempoyak under chilled temperature)
	10^{-1}	Uncountable	Uncountable	Uncountable	Uncountable				
	10-2	Uncountable	Uncountable	Uncountable	Uncountable		6.4×10^6		
	10^{-3}	Uncountable	Uncountable	Uncountable	Uncountable				
1	10 ⁻⁴	472 CFU	Uncountable	Uncountable	Uncountable	6.4×10^6		620×10 ⁶	930×10^6
	10^{-5}	64 CFU	64 CFU	Uncountable	Uncountable				
	10^{-6}	5 CFU	No changes	481 CFU	771 CFU				
	10^{-7}	2 CFU	No changes	62 CFU	93 CFU				
	10^{-1}	No changes	Uncountable	Uncountable	Uncountable		0 CFU	15.1×10 ⁶	8.5×10 ⁶
	10-2	No changes	Uncountable	Uncountable	Uncountable				
	10^{-3}	Uncountable	Uncountable	Uncountable	711 CFU				
2	10 ⁻⁴	Uncountable	Uncountable	Uncountable	776 CFU	137×10 ⁶			
	10^{-5}	Uncountable	No results	104 CFU	85 CFU				
	10 ⁻⁶	137 CFU	No results	62 CFU	12 CFU				
	10^{-7}	16 CFU	No results	20 CFU	3 CFU				
	10^{-1}	Uncountable	Uncountable	Uncountable	Uncountable		37×10 ⁶	10.5×10 ⁶	4.9×10 ⁶
	10-2	Uncountable	Uncountable	Uncountable	Uncountable				
	10^{-3}	650 CFU	Uncountable	Uncountable	Uncountable				
3	10 ⁻⁴	133 CFU	Uncountable	600 CFU	Uncountable	1.33×10^6			
	10 ⁻⁵	17 CFU	289 CFU	105 CFU	49 CFU				
	10 ⁻⁶	2 CFU	37 CFU	19 CFU	No changes				
	10^{-7}	No colony	No colony	6 CFU	15 CFU				
	10^{-1}	Uncountable	Uncountable	Uncountable	Uncountable		26.8×10 ⁶	1010×10 ⁶	
	10 ⁻²	Uncountable	Uncountable	Uncountable	Uncountable				
	10^{-3}	Uncountable	Uncountable	Uncountable	Uncountable				
4	10 ⁻⁴	Uncountable	Uncountable	Uncountable	Uncountable	1.01×10^6			5.9×10 ⁶
	10 ⁻⁵	64 CFU	149 CFU	310 CFU	415 CFU				
	10 ⁻⁶	No changes	146 CFU	362 CFU	59 CFU				
	10^{-7}	No changes	5 CFU	101 CFU	23 CFU				

detection levels throughout the storage periods. Meanwhile, the microbial growth for non-treated samples was relatively higher. This is possible due to the high risk of contamination occurring since the process required more handling and eventually results in higher initial microbial counts.

3.2. pH Analysis of Tempoyak

Based on Fig. 3, there was a significance different in the pH during storage. Generally, Tempoyak has a pH level ranging from 3.5 to 4.5, making it acidic. However, due to variations in production methods and regional preferences, the pH level may slightly differ. The results show the pH result obtain for both samples under room temperature, the pH of the Tempoyak decreases ranging from 4.79 to 3.64 and is more acidic with the increases in days and temperature. Meanwhile, for non-treated Tempoyak and HPP Tempoyak samples under chilled temperature, there is no big difference of the pH taken and the sample does not become more acidic with the increasing of time (days). Accord-

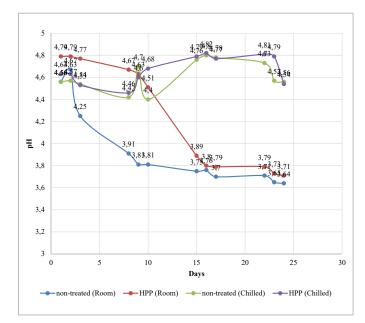


Fig. 3. Graph of pH against days for sample under room and chilled temperatures for 4 weeks storage

ing to [9], pH measurement is used to determine the quality and the freshness of durian. Durian with lower pH is not suitable for consumption since it might be too acidic whereas higher pH increases the possibility of microbial spoilage. A storage condition at chilled temperature (-4°C) seemed to be a better option since the pH remained stable after 4 weeks storage.

3.3. Moisture Content of Tempoyak

For everyday use or cooking, you can gauge the moisture content of Tempoyak based on its consistency and appearance. Tempoyak with a higher moisture content tends to be more creamy and spreadable, while Tempoyak with lower moisture content may be firmer and drier. The moisture content of both samples of Tempoyak are ranged from 63.18% to 67.32% under room temperature meanwhile, the samples under chilled temperature are range from 53.86 to 70.9% where the samples is analyzed at a temperature of 135°C. The analysis of non-treated and HPP sample under room temperature are done until the sensory evaluation of the samples changes which include the taste, texture, smell and color. The value of the moisture content is calculated using Eq. (2) and the value is shown in Fig. 4 for samples under both conditions. Moisture content can influence the flavour, texture, appearance and shelf-life of the fruit sample. The value of moisture content of the Tempoyak sample obtain is accordance with [14], where the durian fruits are within the range of 58.80-79.35%. The durian tends to have short shelf life due to the higher water content.

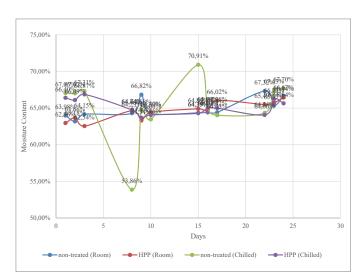


Fig. 4. Graph of moisture content against days for sample under room and chilled temperatures for 4 weeks storage

3.4. Chromameter Analysis

In the case of measuring the color of Tempoyak (fermented durian paste), certainty levels would depend on various factors including the texture and consistency of the Tempoyak, its color variations due to ripeness, fermentation process, and any added

ingredients. The colour analysis of the Tempoyak samples has been analysed using Chromamater (CR-400, Konica Minolta Sensing, Japan). TABLE 3 shows the L*, a* and b* value where L* (lightness), a*(+a = red, -a = green) and (+b = yellow, -b = blue). The L* values of L* of non-treated samples and HPP samples showed no significant differences throughout 4-week storage under both conditions. Based on the value obtain, the L* value is above 60 which it shows a yellowish colour based on the scale used in Fig. 5. The a* and b* colour are both positive

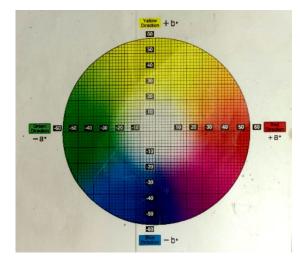


Fig. 5. Scale used for color analysis

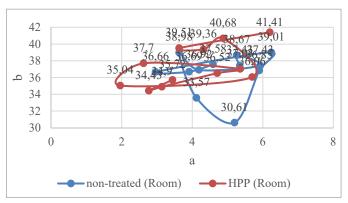


Fig. 6. Color analysis for non-treated and HPP samples under room temperature

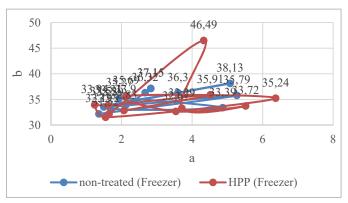


Fig. 7. Color analysis for non-treated and HPP samples under chilled temperature

L*, a* and b* value for non-treated	tampovals and HDD	Tampoval under room and	chilled temperature
L, a and b value for non-neared	i tempoyak and i ii i	Tempoyak under room and	cimiled temperature

Weeks	Days	Non-treated Tempoyak under room temperature			HPP Tempoyak under room temperature			Non-treated Tempoyak under chilled temperature			HPP Tempoyak under chilled temperature		
		L*	a*	b*	L*	a*	b*	L*	a*	b*	L*	a*	b*
	1	67.18	4.59	37.58	66.24	3.44	35.72	65.2	1.36	32.19	66.93	1.65	32.2
1	2	65.56	3.91	36.69	63.96	2.77	34.43	66.63	5.27	35.79	66.72	4.33	36.49
	3	66.24	2.96	36.66	66.76	3.14	34.90	65.87	1.5	33.53	63.54	3.71	33.29
	1	66.1	6.24	39.01	65.58	4.71	36.52	67.34	3.6	36.3	67.18	5.52	33.72
2	2	65.03	5.9	36.85	65.64	5.36	37.03	64.89	5.08	38.13	66.4	3.54	32.67
	3	61.28	5.2	30.61	66.07	2.62	37.70	63.43	1.68	33.39	65.51	2.13	35.69
	1	65.59	4.12	33.57	63.06	1.96	35.04	68.39	4.87	33.39	67.55	6.37	35.24
3	2	64.96	3.63	38.98	63.88	5.7	36.06	66.42	1.94	35.27	67.04	1.55	31.52
	3	63.29	4.19	36.92	65.98	4.88	40.68	66.18	2.12	33.95	66.16	1.24	33.94
	1	64.01	5.93	37.43	64.56	4.32	39.36	65.99	2.83	37.15	65.18	1.62	33.81
4	2	64.06	5.26	38.67	64.39	3.63	39.51	65.64	2.67	36.32	64.93	4.52	35.91
	3	63.02	5.35	37.43	64.57	6.2	41.41	65.56	2.83	37.15	64.47	2.07	32.83

thus the a* colour tend to be in the red part and the b* in the blue part of the scale.

Tan stated that the color property of durian would not be affected by HPP treatment [13]. The non-treated sample could display a change in color and it could be related to microbial and enzyme activites in the sample.

3.5. Sensory Evaluation

Based on the triangle test, the result for sensory analysis which includes the taste, smell, and texture for non-treated sample and HPP sample is shown in the TABLE 4. The first question used to evaluate the analysis is what the taste of the sample is where the answer listed is between sweet, slightly sweet and sour. Second question is what the smell of the sample is where the answer list is no odour, weak odour, and strong odour. For last question used is what the texture of the sample where the answer listed is between hard, semi-hard, semi-liquid and liquid. Based on the result, the taste of non-treated sample began to turn sour and give a strong odour when the samples have been stored for two weeks. The texture of the samples also starts to become liquids. Meanwhile, the taste, smell, and texture of HPP samples began to change during week 3. For the samples stored at chilled temperature, there is no significant difference of taste, smell, and texture for both samples until week 4 (24 days) storage. This is possibly due to lower temperatures that inhibit any enzymatic activities that occur in the Tempoyak samples.

Based on Fig. 8, the taste of Tempoyak for non-treated started to change to slightly sweet and sour at day 8 and 9 respectively. The non-treated Tempoyak turn sour after 9 days until 24 days of storage. The non-treated Tempoyak turn sour quickly compared to HPP Tempoyak where the HPP samples turn sour at 15 days of storage. Meanwhile, Figs. 9 and 10 shows the change of smell and texture of non-treated Tempoyak and HPP Tempoyak respectively. Based on Fig. 9, the non-treated Tempoyak start so have strong odour at 9 days of storage and HPP

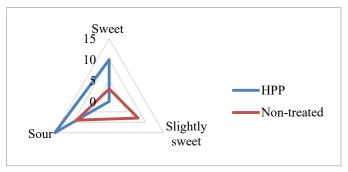


Fig. 8. Days taken for Tempoyak taste to change

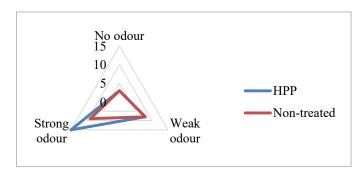


Fig. 9. Days taken for Tempoyak smell to change

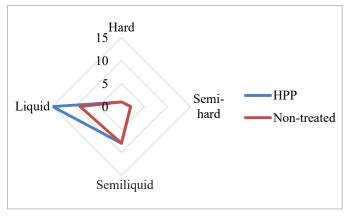


Fig. 10. Days taken for Tempoyak texture to change

Liquid

Weeks	Days	Taste (non-treated Tempoyak)	Taste (HPP Tempoyak)	Smell (non-treated Tempoyak)	Smell (HPP Tempoyak)	Texture (non- treated Tempoyak)	Texture (HPP Tempoyak)
	1	Sweet	Sweet	No odour	No odour	Hard	Hard
1	2	Sweet	Sweet	No odour	No odour	Semi-hard	Semi-hard
	3	Sweet	Sweet	No odour	No odour	Semi-hard	Semi-hard
	8	Slightly- sweet	Sweet	Weak odour	Weak odour	Semi-liquid	Semi-liquid
2	9	Sour	Sweet	Strong odour	Weak odour	Liquid	Semi-liquid
	10	Sour	Sweet	Strong odour	Weak odour	Liquid	Semi-liquid
	15	Sour	Sour	Strong odour	Strong odour	Liquid	Liquid
3	16	Sour	Sour	Strong odour	Strong odour	Liquid	Liquid
	17	Sour	Sour	Strong odour	Strong odour	Liquid	Liquid
	22	Sour	Sour	Strong odour	Strong odour	Liquid	Liquid
4	23	Sour	Sour	Strong odour	Strong odour	Liquid	Liquid

Strong odour

Sensory evaluation of non-treated sample under room temperature and chilled temperature

Tempoyak start to turn sour at 15 days of storage. The textures of non-treated Tempoyak start to change to liquid at 9 days of storage which is quicker compared to HPP Tempoyak where the HPP Tempoyak texture start to become liquid when it is already been stored for 15 days at room temperature.

Sour

Sour

24

3.6. Shelf-life Estimation

The value of the moisture content is used to determine the shelf life of the Tempoyak sample. The moisture content data of HPP Tempoyak under room temperature was used to plot the data in Fig. 8. The sample stored for 4 weeks under room temperature conditions where the graph plotted are between times in week and the average of the moisture content of each week. The graph below was plotted using the value of HPP sample under room condition.

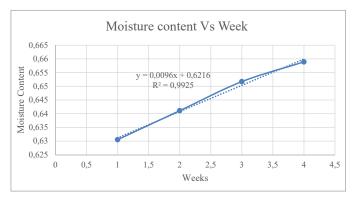


Fig. 11. Graph moisture content against week

Slope = 0.0096

Eq. (4) was used to calculate the shelf life of the Tempoyak sample.

Shelf life =
$$\frac{0.6680 - 0.6305}{0.0096} = 3.9 \text{ weeks}$$
 (4)

From Fig. 11, the value obtain of shelf life for HPP sample under room temperature is expected to be until four weeks based on the calculation used from the Arrhenius model of Accelerated Shelf Testing (ASLT)

Liquid

Strong odour

4. Conclusion

From the analysis performed, HPP was shown to be an ideal method for preserving Tempoyak samples. HPP was proven to inactivate the microorganism and reduce the enzymatic activities in the packed samples of the Tempoyak. In summary, HPP can be used to prolong the shelf life and maintain the quality of Tempoyak samples as HPP shows no significant difference on physical properties (pH, moisture content.

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