

# The Effect of Storage Conditions on Pasteurised Kenaf Seeds Milk Mh8234 Physicochemical Properties

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**Abstract:** Pasteurised kenaf seeds milk MH8234 (PKSM) has been produced from kenaf seeds which are considered waste in agricultural practices. Furthermore, studies on the effect of storage conditions on PKSM physicochemical properties have yet to be explored. Therefore, this study focused on identifying the effect of storage conditions at two different temperatures (ambient temperature (AT)  $27\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$  and chilled temperature (CT)  $4\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ ) for 30 days on the physicochemical properties (colour difference, emulsion stability index, viscosity, total soluble solids, turbidity and size index). This study showed that both storage temperatures and period significantly ( $p < 0.05$ ) affect all tested PKSM's physicochemical properties. This condition is due to the nature of the PKSM, which seeks to achieve thermodynamic stability and component hydrolysis reaction.

**Keywords:** Kenaf Seeds MH8234; Pasteurised; Storage Conditions; Physicochemical Properties

## 1. Introduction

Kenaf seed is scientifically known as *Hibiscus cannabinus* L. The kenaf seeds physical appearance is pointed oval, slate black, wedge shape and its average size is 6 mm long and 4 mm wide (Webber III et al., 2002). According to National Kenaf and Tobacco Board (NKTB) statistics, the total kenaf seeds produced in 2020 is 54 tons, based on a crop area of 127 hectares (National and Tobacco Board, 2020). Although the production of kenaf seeds is very high, most industry players are more interested in using kenaf fibre as a raw material. Therefore, kenaf seed is often considered an underutilised material and a waste material in the agricultural industry (Basri et al., 2014). Based on the author's knowledge, NKTB has diligently expanded its research on the commercialisation of kenaf seed-based products. This initiative aims to change public perception of this material and raise the standard of living of kenaf growers.

The development of pasteurised kenaf seed milk is one of the initiatives to diversify kenaf seed-based food products (Karim et al., 2020). Based on the literature review conducted by the authors, no studies have been published on the effect of storage on the PKSM physicochemical properties. Understanding the effect of storage conditions on PKSM physicochemical properties is crucial, as it mirrors the quality of the produced product. A good quality PKSM can be defined as having a high percentage of stability, low colour change value (Ferragut et al., 2015; Manso et al., 2001), a low turbidity value (Mirhosseini et al., 2008; Song et al., 2000)

and a low droplet size value (McClements, 2016). In general, manipulating the temperature and duration of storage will cause changes in the physicochemical properties of produced products. Among the causes of these changes are due to the product's internal reactions, which include droplet aggregation, coalescence, flocculation, and component hydrolysis reactions (McClements, 2016). Therefore, this study focuses on studying the effect of storage on two conditions: ambient temperature (AT:  $27 \pm 2$  °C) and chill temperature (CT:  $4 \pm 2$  °C) on the physicochemical properties of PKSM for 30 days. The results of this study can provide ideas to improve the quality of PKSM, either in terms of formulation or storage method procedures.

## 2. Materials and Methods

### 2.1 Source of Materials

30 kg of Kenaf Seeds MH8234 (KS) were bought from Zhanpu Zhonglong Kenaf Seed Co. Ltd, Fujian, China. The sample was delivered to the University of Technology Sarawak and stored at a chilled temperature ( $4 \pm 2$ °C).

### 2.2 Production of Pasteurised Kenaf Seeds MH8234 Milk (PKSM) and Storage Conditions

25 g of KS was soaked in 75 ml of filtered water in a 200 ml glass beaker. The KS was submerged in a water bath (Mettler D-91126, Germany) at 50 °C for 150 min. Then, the soaked KS were filtered using a plastic strainer and ground with 180 ml of 50 °C filtered water for 90 s using a laboratory blender (Waring, USA). The slurry was strained using double muslin cloths, and the KS ground hatch was discarded. The produced product was mixed with 0.1% (w/v) sodium carbonate, 0.3% (w/v) soy lecithin and 0.3% (w/v) Tween 80. The mixture was homogenised (Omnitech, United Kingdom) at 15,000 rpm for 3 min. Next, the homogenised mixture was pasteurised using the Armfield pasteuriser (PT175, United Kingdom) at 72 °C for 15 s. The released output (PKSM) was kept in a 200 ml measuring cylinder and a reagent bottle. Both containers were tightly sealed for further analysis. The sample was stored at two different conditions: ambient temperature (AT:  $27 \pm 2$  °C) and chilled temperature (CT:  $4 \pm 2$  °C) for 30 days. The PKSM processing summary is illustrated in Figure 1.

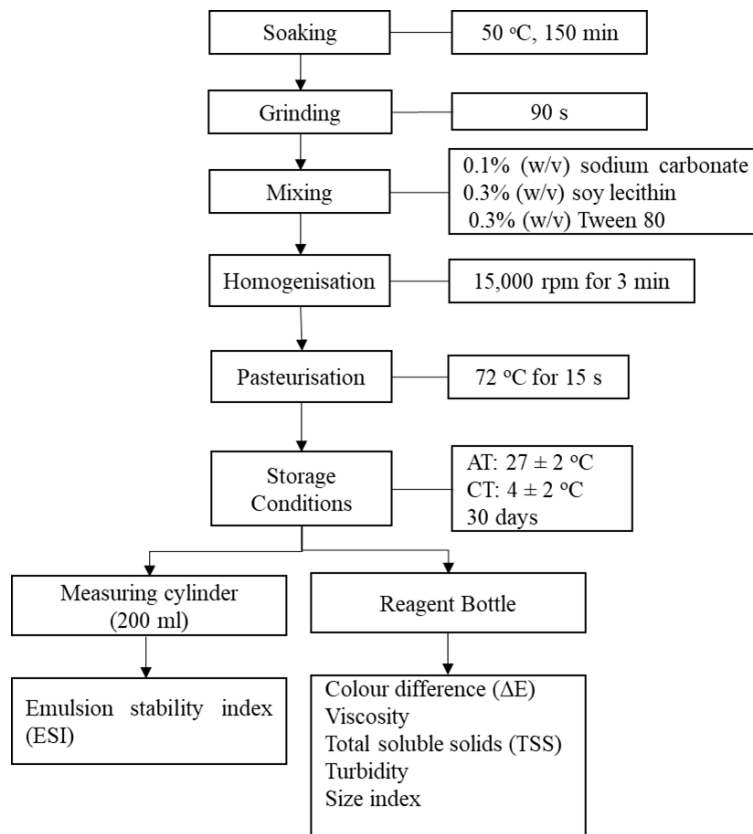


Figure 1: PKSM Processing Method Summary

### 2.3 Physicochemical Changes Analysis

The physicochemical changes of each sample were analysed for three days consecutively. There were five physicochemical analyses involved in this study: colour difference ( $\Delta E$ ), emulsion stability index (ESI), viscosity, total soluble solids (TSS), turbidity and size index. The sample's emulsion stability index was analysed using the sample stored in the 200 ml measuring cylinder. In contrast, other analyses utilised samples stored in the reagent bottle.

#### 2.3.1 Colour Difference ( $\Delta E$ )

The colour difference of each sample was measured according to (Calva-Estrada et al., 2018). A calibrated chromameter (Konica Minolta, Japan) with the parameter of L (lightness), a (redness) and b (yellowness) on the CIE Lab format was utilised to determine the colour reading. The terms  $L_1$ ,  $a_1$  and  $b_1$  are referred to fresh-made PKSM, while the  $L_2$ ,  $a_2$  and  $b_2$  indicate the colour reading was taken on the day it is supposed to be.

#### 2.3.2 ESI

The ESI of each sample was determined according to the method described by (Nasrabadi et al., 2016). The sample creaming and sedimentation height were measured using a calibrated vernier calliper (AKI, China) with a 0.01 mm range.

#### 2.3.3 Viscosity

The viscosity of each sample was determined using a calibrated Brookfield viscometer (Brookfield DVII, USA) equipped with a spindle No. 4. The reading was taken at 100 rpm with a constant volume of 20 ml.

#### 2.3.4 Total Soluble Solids

Each sample's total solids were analysed using a hand-held refractometer (Atago, Japan).

#### 2.3.5 Turbidity

The turbidity of each sample was analysed according to the method mentioned by (Gharibzahedi, Mousavi, Khodaiyan, et al., 2012; Nasrabadi et al., 2016; Taherian et al., 2006). Each sample was diluted with deionised water (1:1000) and measured its absorbance at 660 nm using a UV-VIS spectrophotometer (Agilent, USA). The distilled water was referred to as blank.

#### 2.3.6 Size Index

The size index value of each sample was evaluated using the method mentioned by (Harnsilawat et al., 2006). Each sample was diluted with deionised water (1:100), and the spectral absorptions was measured twice at 800 nm and 400 nm using a UV – VIS spectrophotometer (Agilent, USA). The deionised water was referred to as blank. The ratio readings between 800 nm over 400 nm are known as size index.

### 2.4 Statistical Analysis

Each experiment was conducted in triplicate. All results were analysed using SPSS version 23. The result is presented in the form of mean  $\pm$  standard deviation. The analysis of variance (ANOVA) and p-values ( $p < 0.05$ ) were applied for significance detection (Fattah et al., 2021).

## 3. Results and Discussion

### 3.1 Colour Difference

**Table 1.** shows the colour difference values for PKSM stored on AT and CT for 30 days. In this study, the reference colour values are  $L^*$  86.9,  $a^*$  -3.27 and  $c^*$  17.58, as they are the colour values for the newly produced PKSM. Referring to **Table 1.**, the AT and CT data have experienced a significant decrease from day 3 to 30. However, the AT sample decreased drastically compared to CT. Both samples share the condition of minimum and maximum colour difference values on the third and last day (AT: 4.31 and CT: 2.33; AT: 39.87 and CT: 4.63). In practice, high colour difference values indicate a significant difference between the fresh produced sample and the studied sample. This condition is caused by a non-enzymatic browning reaction between sugar and amino acid composition (Damodaran & Parkin, 2017; Ferragut et al., 2015; Manso et al., 2001). In the same study, samples stored at high temperatures recorded a drastic decrease. This condition is because the increase in temperature and storage time causes the promoting colour difference. Besides these factors, the colour

difference is also induced by duration, media and compounds responsible for colour (Peng et al., 2017).

**Table 1.** The Effect of Storage Conditions Towards Colour Difference.

Storage Days	Colour Difference	
	AT	CT
3	4.31 ± 0.06 <sup>a</sup>	2.33 ± 0.15 <sup>a</sup>
6	8.41 ± 0.06 <sup>b</sup>	2.64 ± 0.15 <sup>ab</sup>
9	13.31 ± 0.06 <sup>c</sup>	2.91 ± 0.15 <sup>c</sup>
12	16.41 ± 0.06 <sup>d</sup>	3.61 ± 0.15 <sup>d</sup>
15	19.35 ± 0.06 <sup>e</sup>	3.72 ± 0.15 <sup>c</sup>
18	23.51 ± 0.06 <sup>f</sup>	3.98 ± 0.15 <sup>cd</sup>
21	27.61 ± 0.06 <sup>g</sup>	4.13 ± 0.15 <sup>cde</sup>
24	32.63 ± 0.06 <sup>h</sup>	4.25 ± 0.15 <sup>de</sup>
27	35.67 ± 0.06 <sup>i</sup>	4.57 ± 0.15 <sup>e</sup>
30	39.87 ± 0.06 <sup>j</sup>	4.63 ± 0.15 <sup>e</sup>

\*Mean ± standard deviation with different superscripts within the same column are significantly different at P < 0.05.

### 3.2 ESI

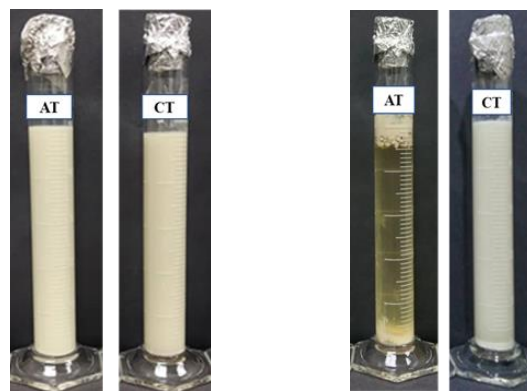
**Table 2.** shows the values of the ESI against PKSM stored at two different conditions for 30 days. **Figure 2.** shows the difference between the emulsion stability index values for the newly produced samples and those stored for 30 days. Based on observations, both samples have experienced a significant decrease. Furthermore, the maximum and minimum values of ESI for both samples were under the same conditions. The maximum and minimum ESI values for AT (94.80, 30.49) and CT (97.60, 72.97) were 3<sup>rd</sup> and 30<sup>th</sup>. Although both samples experienced a significant decrease in ESI values, the decrease values experienced by AT were more drastic than in CT. Theoretically, PKSM belonging to the oil in water emulsion group tends to undergo a separation phase. This condition is because the separation phase is driven by thermodynamic instability and surface tension experienced by each component in the PKSM such as fat and water (Nikolovski et al., 2016; Sapei et al., 2017; Sun et al., 2007). To achieve thermodynamic stability, a separation phase between PKSM components has occurred, which involves the process of droplet aggregation and gravitational separation (McClements, 2016). The high ambient temperature has also increased the rate of droplet aggregation between molecules, and

the result of the separated products is based on the creaming and sedimentation phases (Degner et al., 2014).

**Table 2.** The Effect of Storage Conditions on ESI.

Storage Days	ESI	
	AT	CT
3	94.80 ± 0.22 <sup>j</sup>	97.60 ± 0.01 <sup>j</sup>
6	88.54 ± 0.22 <sup>i</sup>	84.87 ± 0.01 <sup>i</sup>
9	77.77 ± 0.22 <sup>h</sup>	83.15 ± 0.01 <sup>h</sup>
12	67.82 ± 0.22 <sup>g</sup>	81.50 ± 0.01 <sup>g</sup>
15	56.50 ± 0.22 <sup>f</sup>	80.86 ± 0.01 <sup>f</sup>
18	44.78 ± 0.22 <sup>e</sup>	79.05 ± 0.01 <sup>e</sup>
21	41.78 ± 0.22 <sup>d</sup>	77.16 ± 0.01 <sup>d</sup>
24	38.52 ± 0.22 <sup>c</sup>	76.52 ± 0.01 <sup>c</sup>
27	34.67 ± 0.22 <sup>b</sup>	74.50 ± 0.01 <sup>b</sup>
30	30.49 ± 0.22 <sup>a</sup>	72.97 ± 0.01 <sup>a</sup>

\*Mean ± standard deviation with different superscripts within the same column are significantly different at P < 0.05.



Day 0

Day 30

**Figure 2.** Effect of Storage Conditions on the ESI of PKSM.

### 3.3 Viscosity

**Table 3.** shows the effect of different storage (AT & CT) on sample viscosity values over 30 days. Based on the observations showed both the data increased significantly. The minimum values recorded for both samples were on the third day (AT: 4.33 cp, CT: 3.15 cp). In contrast, the maximum value for both samples was recorded on the 30th day (AT: 9.68 cp, CT: 5.22 cp). Based on the data obtained, storage of PKSM at high temperatures produces a more viscous product. The results obtained are similar to the study's findings by Thakur et al., 2018, where the viscosity of wild aonla drink increased in line with the study period. Based on the results shows, the storage period is directly proportional to the sample viscosity value. Three phases cause this: droplet aggregation, particle size enlargement and creaming phase formation (McClements, 2016). In the first phase, the sample will undergo droplet aggregation to achieve thermodynamic stability. The second phase involved increased particle size. Lastly, a cream layer on the PKSM has been formed. The formation of the cream layer has increased the strain and shearing rate of PKSM and reduced the flow index of PKSM. PKSM with a low flow index value will form pseudo-plasticity. As a result, it will provide more resistance to the viscometer probe, and this causes an increase in viscosity (Suhaimi et al., 2016; Thakur et al., 2018).

**Table 3.** The Effect of Storage Conditions Towards Viscosity.

Storage Days	Viscosity (cp)	
	AT	CT
3	4.33 ± 0.08 <sup>a</sup>	3.15 ± 0.06 <sup>a</sup>
6	5.20 ± 0.08 <sup>b</sup>	3.52 ± 0.06 <sup>b</sup>
9	5.83 ± 0.08 <sup>c</sup>	3.95 ± 0.06 <sup>c</sup>
12	6.32 ± 0.08 <sup>d</sup>	4.36 ± 0.06 <sup>d</sup>
15	6.85 ± 0.08 <sup>e</sup>	4.62 ± 0.06 <sup>e</sup>
18	7.32 ± 0.08 <sup>f</sup>	4.74 ± 0.06 <sup>f</sup>
21	7.60 ± 0.08 <sup>g</sup>	4.85 ± 0.06 <sup>g</sup>
24	8.40 ± 0.08 <sup>h</sup>	5.01 ± 0.06 <sup>h</sup>
27	9.25 ± 0.08 <sup>i</sup>	5.07 ± 0.06 <sup>i</sup>
30	9.68 ± 0.08 <sup>j</sup>	5.22 ± 0.06 <sup>j</sup>

\*Mean ± standard deviation with different superscripts within the same column are significantly different at P < 0.05.

### 3.4 TSS

**Table 4.** displays the effect of storage at AT and CT temperatures on the TSS values of PKSM. Based on the observations, both samples recorded a significant decrease. The maximum and minimum values for the two samples were on: the third day (AT: 4.78, CT: 5.02) and the 30th day (AT: 2.70, CT: 3.90). According to (Magwaza & Opara, 2015), the value of TSS is more synonymous with the amount of sugar contained in a fluid-based product. In principle, the study on the sugar content in PKSM has not yet been implemented. The authors expect PKSM to have a low sugar value because the source of kenaf seeds rich in fat dominates the chemical changes of PKSM. Furthermore, the results recorded in this study contradict the study's findings by (Bhardwaj & Urvashi, 2014) and (Thakur et al., 2018). Hypothetically, the drastic decrease in TSS value at room temperature storage is due to the hydrolysis of sugars to acids occurring at a rapid rate. On the other hand, the low value of TSS change at cold temperature is because the temperature has inhibited the process of chemical change (Bhardwaj & Urvashi, 2014; O'Grady et al., 2014).

**Table 4.** The Effect of Storage Conditions Towards Total Soluble Solids.

Storage Days	Total Soluble Solids	
	AT	CT
3	4.78 ± 0.04 <sup>j</sup>	5.02 ± 0.03 <sup>j</sup>
6	4.58 ± 0.04 <sup>i</sup>	4.88 ± 0.03 <sup>i</sup>
9	4.35 ± 0.04 <sup>h</sup>	4.72 ± 0.03 <sup>h</sup>
12	4.03 ± 0.04 <sup>g</sup>	4.63 ± 0.03 <sup>g</sup>
15	3.80 ± 0.04 <sup>f</sup>	4.35 ± 0.03 <sup>f</sup>
18	3.52 ± 0.04 <sup>e</sup>	4.26 ± 0.03 <sup>de</sup>
21	3.35 ± 0.04 <sup>d</sup>	4.25 ± 0.03 <sup>d</sup>
24	3.22 ± 0.04 <sup>c</sup>	4.13 ± 0.03 <sup>c</sup>
27	3.05 ± 0.04 <sup>b</sup>	4.02 ± 0.03 <sup>b</sup>
30	2.70 ± 0.04 <sup>a</sup>	3.90 ± 0.03 <sup>a</sup>

\*Mean ± standard deviation with different superscripts within the same column are significantly different at  $P < 0.05$ .

### 3.5 Turbidity



**Table 5.** records the effect of storage at different temperatures on the turbidity value of PKSM. Based on the ANOVA evaluation, the turbidity values for both samples decreased significantly. The maximum and minimum turbidity values for each sample were day 3 (AT: 0.39, CT: 0.58) and day 30 (AT: 0.06, CT: 0.57). Storage at AT temperature has drastically lowered the turbidity value. Meanwhile, the sample stored at CT temperature experienced a slow increase in turbidity value. Theoretically, turbidity is one of the parameters closely related to the quality of a product (Mirhosseini et al., 2008; Song et al., 2000). This condition is because the value of turbidity is directly proportional to the value of the stability of a product. In this study, two factors influence the turbidity value of PKSM, namely storage temperature and storage period. The relatively high storage temperature and the long storage period lead to the AT sample's phase separation phenomenon, which occurs due to droplet aggregation, coalescence, flocculation, and creaming. In contrast, the decline in CT turbidity values can be attributed to previous studies on a mixture of orange juice and milk and grape juice. These conditions are due to sampling factors that undergo pectin degradation and the formation of suspended solids (Zulueta et al., 2013); the action of pectin methyl esterase, which has formed free radicals by catalysing the hydrolysis of methyl ester groups and has eliminated methoxy and carboxylic groups (Igual et al., 2014).

**Table 5.** The Effect of Storage Conditions Towards Turbidity.

Storage Days	Turbidity	
	AT	CT
3	0.39 ± 0.00 <sup>a</sup>	0.58 ± 0.00 <sup>a</sup>
6	0.33 ± 0.00 <sup>b</sup>	0.58 ± 0.00 <sup>b</sup>
9	0.29 ± 0.00 <sup>c</sup>	0.58 ± 0.00 <sup>c</sup>
12	0.25 ± 0.00 <sup>d</sup>	0.58 ± 0.00 <sup>d</sup>
15	0.21 ± 0.00 <sup>e</sup>	0.58 ± 0.00 <sup>e</sup>
18	0.19 ± 0.00 <sup>f</sup>	0.57 ± 0.00 <sup>f</sup>
21	0.15 ± 0.00 <sup>g</sup>	0.57 ± 0.00 <sup>g</sup>
24	0.10 ± 0.00 <sup>h</sup>	0.57 ± 0.00 <sup>h</sup>
27	0.07 ± 0.00 <sup>i</sup>	0.57 ± 0.00 <sup>i</sup>
30	0.06 ± 0.00 <sup>j</sup>	0.57 ± 0.00 <sup>j</sup>

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\*Mean  $\pm$  standard deviation with different superscripts within the same column are significantly different at  $P < 0.05$ .

### 3.6 Size Index

As shown in **Table 6.**, both samples experienced a significant increase in size index. In this study, the determination of the size index value is an adaptation of the turbidity assessment using the spectroscopy technique (Harnsilawat et al., 2006). The final evaluation showed that both samples' maximum size index value was on the 30th day: (AT: 1.02 and CT: 0.48) and the minimum on the 3rd day: (AT: 0.11 and CT: 0.09). Apart from determining the value of ESI and turbidity (Nasrabadi et al., 2016), the size index value also serves to interpret the stability state of the emulsion product. The determination of the size index is based on the rate of droplet aggregation that occurs in the emulsion product (Harnsilawat et al., 2006). In addition, high droplet aggregation values refer to large particle size levels (Nasrabadi et al., 2016). The large particle size indicates the occurrence of the separation phase as the data shown in **Tables 2.** and **5.** In comparison, the increase in the low CT size index value is due to the low-temperature storage that has caused the emulsifier to control the droplet aggregation rate (Dickinson et al., 1991; Gharibzahedi et al., 2012).

**Table 5.** The Effect of Storage Conditions Towards Size Index.

Storage Days	Size Index	
	AT	CT
3	0.11 $\pm$ 0.00 <sup>a</sup>	0.09 $\pm$ 0.00 <sup>a</sup>
6	0.26 $\pm$ 0.00 <sup>b</sup>	0.12 $\pm$ 0.00 <sup>b</sup>
9	0.39 $\pm$ 0.00 <sup>c</sup>	0.16 $\pm$ 0.00 <sup>c</sup>
12	0.49 $\pm$ 0.00 <sup>d</sup>	0.19 $\pm$ 0.00 <sup>d</sup>
15	0.59 $\pm$ 0.00 <sup>e</sup>	0.22 $\pm$ 0.00 <sup>e</sup>
18	0.68 $\pm$ 0.00 <sup>f</sup>	0.26 $\pm$ 0.00 <sup>f</sup>
21	0.79 $\pm$ 0.00 <sup>g</sup>	0.29 $\pm$ 0.00 <sup>g</sup>
24	0.88 $\pm$ 0.00 <sup>h</sup>	0.36 $\pm$ 0.00 <sup>h</sup>
27	0.99 $\pm$ 0.00 <sup>i</sup>	0.41 $\pm$ 0.00 <sup>i</sup>
30	1.02 $\pm$ 0.00 <sup>j</sup>	0.48 $\pm$ 0.00 <sup>j</sup>

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\*Mean  $\pm$  standard deviation with different superscripts within the same column are significantly different at  $P < 0.05$ .

## 5. Conclusions

This work was devoted to assessing the effect of storage conditions on PKSM physicochemical properties. This analysis was performed by storing PKSM at two different temperatures: ambient temperature (AT:  $27 \pm 2$  °C) and chilled temperature (CT:  $4 \pm 2$  °C). This study showed that the storage temperature and period had a significant ( $p < 0.05$ ) influence on all the tested physicochemical properties. The PKSM's physicochemical properties value reduction is lower by storing under CT. Therefore, PKSM should be kept under CT conditions. Future studies should focus on increasing the value of the emulsifying properties of PKSM by updating the formulation of PKSM. This improvement can be made by adding protein from animal and plant sources in the PKSM formulation.

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**Author's Contribution:** Abdul Fattah developed the product, conducted the analysis and drafted the article. Mohd Zahid and Mohammad Shahril are advisors on statistical analysis and reviewed the article. Mohd Hafsanjani and Mohd Syafiq designed the experiments and developed the product. Nurhayatie and Farah Syahirah are advisors in food chemistry and checked the article. Ashraf is an advisor in food physics and reviewed the article. All authors have read the article carefully and approved the final article.

**Conflicts of Interest:** The authors declare no conflict of interest.

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