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THE INFLUENCE OF PROXIMATE COMPOSITION AND AMINO ACID PROFILE ON THE SEPARATION PHASES OF PASTEURIZED KENAF SEEDS MH8234 MILK (KSM)

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Abstract:

The production of kenaf seed milk (KSM) is one of the initiatives to develop kenaf seed MH8234, which is considered byproduct in agriculture practise. However, the effect of pasteurisation at 72 ^{o}C for 15 s has caused it to separate into three parts, namely creaming, continuous and sedimentation phases. Therefore, this study was conducted to determine the cause of the separation phase through chemical characterisation that involved proximate composition and amino acid profile. The results of the study show that the formation of creaming phases is deduced from the high value crude fat (2.68 ± 0.2 %) Meanwhile, the formation of sedimentation is also promoted by the presence

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of hydrophobic amino acids such as phenylalanine (3.95 ± 0.21 g/100 g), tyrosine (3.31 ± 0.09 g/100 g) and alanine (2.87 ± 0.09 g/100 g). The results of the both studies revealed that the two factors were the dominant cause of the formation of the separation phase due to the effect of pasteurisation at 72ºC for 15 s. The data obtained has clearly deciphered the chemical properties of KSM and is a guide for the formulation of it that is stable upon pasteurisation.

Keywords:

Chemical Characterisation, Kenaf Seed MH8234 Milk, Proximate Composition, Amino Acid Profile.

Introduction

Pasteurisation is a thermal process utilized for the eradication of pathogenic microorganisms. The utilization of this method is appealing due to its capacity to maintain the nutritional properties of the product, coupled with the application of moderate heat for a short duration (Grundy et al., 2016; Wibowo et al., 2015). Additionally, this technique has been utilized to generate plant-based milk (PBM) variants, including soy (Ringgenberg, 2011), almond (Dhakal et al., 2016), pistachio (Shakerardekani et al., 2013), and walnut milk (Liu et al., 2016). Given the absence of any published pasteurisation research on KSM, it is logical to conduct a study on the pasteurisation of this substance. It is noteworthy to discuss the impact of pasteurisation on the substance in question, given that this is an initial investigation into the consequences of heating on KSM. Although pasteurisation is crucial for ensuring the safety of products, it has been found to result in the physical instability of plant-based milks (PBM) such as almond and hazelnut milk, as noted by(Bernat et al., 2015). One of the factors contributing to instability is the disproportionate ratio of fat to protein, which can have an adverse impact on the emulsification process that occurs during pasteurisation.

The present investigation involved the implementation of the pasteurisation method on KSM through the utilization of a pasteuriser (PT175, United Kingdom) and subjecting it to a temperature of 72 ^{o}C for 15 s. The examination of the pasteurised sample revealed that the process of pasteurisation resulted in the formation of three distinct phases of KSM, namely creaming (top part), continuous (middle part), and sedimentation (bottom part). The diagram depicted in Fig. 1 illustrates the partitioning of pasteurised KSM into discrete constituents. It seems like the separation that occurred had a significant impact on the stability of KSM products after pasteurization. To determine the cause of this separation, it is crucial to understand the chemical nature of each isolated component. Thus the objective of this study is to examine each of separate component via proximate analysis and amino acid profiling. By investigating these properties, it may be possible to identify the reasons behind the material instability and work towards solutions for more stable KSM product production.

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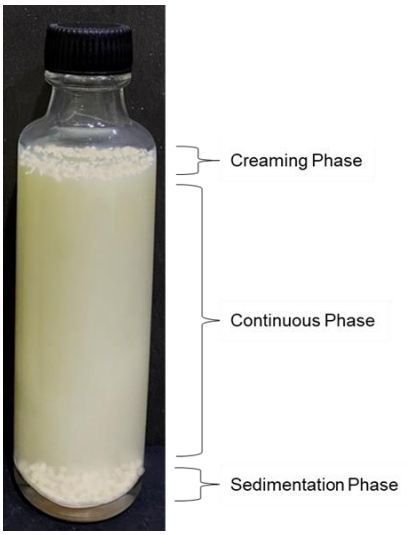

Figure. 1 The Separated Components of KSM Under Pasteurisation Treatment

Material

A total of 1 kg of MH8234 kenaf seeds were procured from Zhanpu Zhonglong Kenaf Seed Co. Ltd., located in Fujian, China. Upon arrival at the University of Technology Sarawak, the material was promptly stored in a designated chiller room at a temperature of (4 ± 2 ^{o}C).

Methods

Preparation Of KSM Separated Components

One litre of raw KSM was produced according to the method mentioned by (Ab Razak et al., 2022). Next, the material was pasturised using a pasteurizer (Armfield PT175, United Kingdom). The pumping speed of the equipment was established at 8.5 ml/s, while the heating parameters were maintained at a fixed temperature of 72 ^{o}C for 15 s. The resultant product was stored in reagent bottles. The pasteurised sample was subjected to additional centrifugation using a centrifuge (Thermofisher, Germany), operating at a speed of 4,000 rpm for 90 min. The centrifugation process has yielded three distinct components, namely the creaming, continuous, and sedimentation phases. Each distinct phase was extracted using either a pipette or spatula and subsequently preserved in separate amber containers. The specimens were stored at a refrigerated temperature of (4 ± 2 ^{o}C) in preparation for subsequent examination.

Proximate Analysis

The proximate analysis consist of six different analyses, namely: crude fat, crude protein, ash content, crude fibre, moisture content and carbohydrate. All the analysis were conducted accroding to the method mentioned in (AOAC, 2016).

Amino Acid Profile

High-Efficiency Liquid Chromatography (Agilent 1220, Agilent Technologies) was utilised to analyse the amino acid profile of each constituent. The apparatus utilised in the study was equipped with a UV 338 nm detector, and a mobile phase consisting of a ratio of 1:2:2 of 100 mM sodium sulphate ($Na_2SO_{4)}$ with a pH of 7.2, acetonitrile (CH_3CN), and methanol (CH_3OH)

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(v/v/v) at a flow rate of 0.45 ml/min. The experiment was conducted at an operating temperature of 40 °C, as reported by (Ab Razak et al., 2022). Standard solutions were prepared for experimentation using an amino acid standard hydrolysate provided by Waters®. The hydrolysate contained 2.5 mM of each amino acid, including lysine, aspartic acid, serine, glutamic acid, cysteine, histidine, arginine, threonine, glycine, hydroxyproline, alanine, proline, tyrosine, valine, isoleucine, phenylalanine, tryptophan, and methionine. The analytical technique employed was chromatography, which was carried out using a Waters Acquity UPLC system in accordance with the manufacturer's application system guide note (Waters Corporation, 2006). The quantification of the 18 amino acid constituents was performed in accordance with the methodology outlined by (Mota et al., 2016).

Result and Discussion

Proximate Analysis

The proximate composition values for each component, namely the creaming, continuous, and sedimentation phases, are presented in Table. 1. According to the data presented in Fig. 1 and Table. 1, it can be observed that the creaming phase exhibits the lowest density and the highest crude fat content in comparison to the other constituents. The elevated level of crude fat can be attributed to the presence of membranous lipid droplets that are suspended on the surface of the sample. The creaming crude fat value obtained from the experiment exhibits a resemblance to the kenaf oil in water emulsion across various cultivars, as reported by (Bouterfas et al., 2014) and (Saidin, 2019). The factor of seed-to-water ratio and the volume of cream components separated have been found to exert an influence on the highest crude fat value in this section, as reported by (Munir et al., 2017). The sedimentation phase exhibited the highest crude protein value, owing to its comparatively higher density in relation to the other two constituents. The elevated levels of crude protein can be attributed to the resemblance of the phase separation procedure to the manufacturing techniques employed in the production of isolated soy protein (Wellard et al., 2014) and pumpkin protein (Quanhong & Caili, 2005). Furthermore, the amino acid and fatty acid profiles of KSM are responsible for the majority of the value of crude fat and crude protein present in the creaming and sedimentation phases.

Table 1. Proximate Composition of Creaming, Continuous, and Sedimentation Phases

Proximate composition	Creaming phase	Continuous phase	Sedimentation phase
Crude fat	2.68 ± 0.20^{a}	0.83 ± 0.18^{c}	0.15 ± 0.14^{b}
Crude protein	0.15 ± 0.05^{c}	0.53 ± 0.05^{a}	2.06 ± 0.05^{b}
Ash content	0.25 ± 0.01^{b}	0.50 ± 0.02^{c}	1.06 ± 0.03^{a}
Crude fibre	0.06 ± 0.05^{a}	0.09 ± 0.04^{b}	0.11 ± 0.03^{c}
Moisture content	91.07 ± 0.03^{a}	91.85 ± 0.08^{b}	90.27 ± 0.01^{c}
Carbohydrate	5.79 ± 0.01^{c}	6.20 ± 0.02^{a}	6.35 ± 0.03^{b}

Note: Mean with different superscript within the same column are significantly different at ($p<0.05$).

Furthermore, the sedimentation phase exhibited the highest levels of ash content and crude fibre values, in addition to a high crude protein value. The elevated ash content can be attributed to the minerals that amassed within the partially solidified component. The significance of crude fibre remains uncertain due to its reliance on processing variables, material diversity, and soil characteristics, as noted by (Ugochi et al., 2015). The moisture content component is predominantly governed by the continuous phase. Based on the empirical findings, it can be

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inferred that the continuous phase constitutes a liquid state, which serves as the primary constituent in the KSM processing. The sedimentation phase exhibited the highest value in comparison to the other components in the latter part of the carbohydrate aspect. This value denotes that the sedimentation phase may yield a greater amount of energy compared to the other constituents. The determination of proximate composition values for each component is a fundamental aspect of comprehending the analysed data, including the amino acid profile and fatty acid profile.

Amino Acid Profile

The amino acid composition of the creaming, continuous, and sedimentation components is presented in Table. 2. The investigation of the separation phase experienced by KSM upon pasteurisation at 72 ^{o}C for 15 s involves the quantification of the amino acid profile at each composition. The classification of the nineteen amino acids presented in Table. 2 can be dichotomized into two distinct groups based on their chemical properties, namely hydrophilic and hydrophobic. Table. 2 exhibits multiple constituents that are denoted as N.D. The study's findings indicate that the amino acid component was not detected.

Table. 2 Amino Acid Composition of Creaming, Continuous, and Sedimentation Phases

Amino Acid	Amount (g/100 g)		
	Creaming	Continuous	Sedimentation
Lysine	0.91 ± 0.03^{a}	1.14 ± 0.02^{c}	0.12 ± 0.01^{c}
Aspartic acid	N.D	2.19 ± 0.03^{a}	0.04 ± 0.02^{b}
Serine	0.03 ± 0.03^{c}	1.81 ± 0.12^{a}	0.11 ± 0.09^{b}
Glutamic acid	N.D	2.32 ± 0.21^{b}	0.16 ± 0.04^{a}
Cysteine	N.D	0.04 ± 0.04^{a}	N.D
Histidine	0.01 ± 0.01^{a}	0.93 ± 0.02^{c}	0.05 ± 0.01^{b}
Arginine	0.14 ± 0.04^{b}	2.06 ± 0.06^{a}	N.D
Threonine	0.09 ± 0.07^{a}	0.86 ± 0.09^{b}	0.06 ± 0.08^{c}
Glycine	0.04 ± 0.06^{a}	0.03 ± 0.05^{b}	2.48 ± 0.04^{c}
Hydroxyproline	N.D	0.04 ± 0.06^{a}	2.41 ± 0.08^{a}
Alanine	0.08 ± 0.04^{b}	0.03 ± 0.06^{a}	2.87 ± 0.09^{c}
Proline	0.06 ± 0.07^{a}	0.04 ± 0.09^{c}	2.44 ± 0.11^{b}
Tyrosine	0.03 ± 0.16^{c}	0.01 ± 0.14^{b}	3.31 ± 0.09^{c}
Valine	0.08 ± 0.06^{a}	0.03 ± 0.10^{c}	3.41 ± 0.12^{c}
Isoleucine	0.05 ± 0.11^{b}	0.01 ± 0.15^{a}	2.74 ± 0.13^{c}
Leucine	0.09 ± 0.01^{c}	0.07 ± 0.12^{a}	2.78 ± 0.10^{b}
Phenylalanine	0.07 ± 0.14^{a}	0.05 ± 0.16^{b}	3.95 ± 0.21^{c}
Tryptophan	0.01 ± 0.19^{b}	N.D	2.41 ± 0.05^{a}
Methionine	0.04 ± 0.02^{a}	0.03 ± 0.06^{c}	1.45 ± 0.21^{a}

Remarks: N.D: Not Detected,

*Mean ± standard deviation with different superscripts within the same column are significantly different at ($p<0.05$).

Lysine and threonine are classified as hydrophilic amino acids. The continuous phase experiences an accumulation of hydrophilic amino acid groups, which is then followed by the phases of creaming and sedimentation. The hydrophilic amino acids that exhibited the greatest values in the continuous phase group were glutamic acid (2.32 ± 0.21 g/100 g), aspartic acid (2.19 ± 0.03 g/100 g), and arginine (2.06 ± 0.06 g/100 g) g/100g. Glutamic acid and aspartic acid are classified as non-essential amino acids, whereas arginine is classified as an essential amino acid. The high capacity of hydrophilic amino acids in the continuous phase can be

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attributed to several factors. Firstly, this phase is distinct from the fat component. Additionally, the polar nature of most hydrophilic amino acids renders them attracted to the continuous phase, which is characterised by high moisture content, as noted by (Gaonkar & McPherson, 2006). Furthermore, the presence of robust hydrogen and van der Waals bonds has contributed to a strong bond between the hydrophilic amino acid component and the continuous phase, as observed by (Walnofer & Horax, 2005).

The amino acids that follow glycine and extend up to methionine are classified as hydrophobic amino acids. The present group exhibits restricted solubility in aqueous solutions and possesses a non-polar nature. According to the data presented in Table. 2, it can be observed that three amino acids with hydrophobic properties exhibited the most elevated values. These amino acids are phenylalanine (3.95 ± 0.21 g/100 g), tyrosine (3.31 ± 0.09 g/100 g), and alanine (2.87 ± 0.09 g/100 g). Phenylalanine and tyrosine are classified as essential amino acids, whereas alanine is classified as a non-essential amino acid. The sedimentation process is favoured by a high proportion of hydrophobic amino acids, as suggested by the findings of Damodaran & L. Parkin (2019) and Wu et al. (2020) . This observation aligns with the research conducted by Okorie SU et al. (2014) on the properties of tiger nut milk and the literature review compiled by Qamar et al. (2020) on the utilisation of nuts and oilseeds as plant-based milk alternatives.

The phenomenon of sedimentation is intricately linked to the concept of the excluded volume effect, which is commonly observed in protein unfolding, protein denaturation, and the development of quaternary structures (Gaonkar & McPherson, 2006; Mcclements, 2015). Upon exposure to a temperature of 72 ^{o}C for 15 s, the inherent protein present within KSM undergoes a process of denaturation. The progressive elevation of thermal conditions up to the point of pasteurisation has resulted in a decrease in the stability of the hydrogen bonds and electrostatic interactions inherent in KSM. The destabilisation of hydrogen bonds is attributed to exothermic factors. Consequently, hydrophobic interactions that are endothermic and non-polar factors between amino acids are more influential in promoting droplet aggregation and minimising direct interaction with water (Dickinson, 2010). The ongoing procedure has resulted in the unravelling of the protein from a homogeneous KSM to a complex folded quaternary configuration, as per the findings of (Jiang et al., 2018). The aforementioned circumstance is concomitant with a decrease in the interfacial area between the protein and water (Fitzsimons et al., 2007; Lakemond et al., 2000). Consequently, the agglomerated clusters featuring an intricately folded quaternary configuration exhibit intermolecular attraction and coalesce, leading to sedimentation.

Conclusion

The present analysis has revealed that the separation phase of pasteurised KSM can be attributed to two factors: an excessive crude fat value in relation to crude protein and an imbalance between hydrophobic and hydrophilic amino acid values. In light of this, it is pertinent for the present investigation to persist in discerning the optimal methodology for generating KSM that exhibits exceptional stability and has undergone the process of pasteurisation.

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