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Characterization of Liquid Skim Milk Fortified with Whey-Mangosteen Pericarp (*Garcinia mangostana*) Extract Solution

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Abstract. Mangosteen pericarp has many health benefits. The types of mangosteen-based functional foods and drinks are still very limited, because the phenolic components of the mangosteen pericarp extract are easily damaged and cannot be fully absorbed by the body. Whey protein in milk is believed to function as a carrier for food. This whey protein can form complexes with phenolic components and can improve its physical and functional properties. In producing functional foods that are more stable, whey complexes - extracts of mangosteen pericarps that have been formed are fortified into skim milk. Addition of whey-mangosteen pericarp extract solution not significantly affected (\(\alpha = 0.05\)) to creaming, sedimentation, and solubility of skim milk. However, there was enlargement in particle size of each concentration. The best treatment in this study was the concentration of adding 5% whey-extract of mangosteen pericarp extract with a phase separation value of 7.33\% ± 0.70, the value of solubility reached 92.90 ± 4.86, the sedimentation value reached 0.0211 ± 0.007, and the average particle diameter was 3.9 \(\mu\)m

Keywords: Stability, Whey, Mangosteen pericarp, extract
1. Introduction

Mangosteen pericarp contains several phenolic components, including: xanthons and their derivatives, anthocyanins, and several types of flavonoids [1]. Xanthon antioxidants are believed to be beneficial as anti-inflammatory, anti-bacterial and anti-allergic agent to fight the development of cancer cells [2]. In addition, flavonoids acts as antibacterial, antifungal, anticancer and antiviral agent [3,4].

Fortification process with the addition of mangosteen skin extract presents a challenge, as the components of mangosteen pericarp extract have not been fully absorbed by the body due to conditions of gastric pH in digestive system. Therefore, it is necessary to protect these bioactive compounds to increase their stability, solubility and bioavailability in the body [5]. One way to protect these bioactive compounds is conducted by the formation of complexes with whey protein.

Whey protein is one component of protein that can bind and form complexes with phenolic components, and is believed to be a delivery agent or carrier in the food system as it is more consumable in nanocapsule form [6]. The largest content of whey protein includes β-lactoglobulin and α-lactalbumin [7]. Based on molecular docking tests, whey protein can interact with xanthone components through hydrogen bonds, van der waals, and hydrophobic interactions [8].

Fortification of whey solution and mangosteen pericarp extract is performed into liquid skim milk to diversify dairy products and alternative products containing antioxidants (from mangosteen pericarp extract). In this study, skim milk was administered due to its lower fat content compared to pure milk as milk fat can potentially increase blood sugar in consumers [9].

Mixing whey with casein protein will add stability to the whey complex with phenolic components [10]. However, the addition of plant phenolic components to skim milk will affect the colloidal bond and system found in milk, due to changes in protein content to complex bonds that occur between protein molecules and polyphenol components from mangosteen pericarp extract [11]. Therefore, it is necessary to understand the proper concentration in the addition of mangosteen pericarp extract, to produce a more stable fortified milk. Furthermore, in the next research the stable fortified milk could increase the bioavailability of mangosteen pericarp extract and potential as anti-hyperglycemia agent.

2. Material and Method

2.1. An Ingredients

The utilized ingredients were: mangosteen pericarp powder obtained from UPT Materia Medica, Batu City, East Java; pasteurized skim milk (Diamond), distilled water, 96% ethanol, and whey protein isolate powder (Chemistry and Biochemistry Laboratory, Agricultural Technology Faculty, Brawijaya University).

2.2. A Tool

The tools utilized in this study were: heat mantles (Clarkson), soxhlet (Gerhardt), filter paper (Wattman), cotton, electric stoves (Maspion, Indonesia), magnetic stirrers (Scilogex), spoons, analytical scales (Denver instrument M-310, USA), beaker glass, spectrophotometer (Lan Optic), thermometer, rotary evaporator (color palmer), test tube (Iwaki, Indonesia), erlenmeyer (Iwaki, Indonesia), centrifuge, desiccator (Nalgene), vortex (LW scientific) , electric oven 220V (Memmert), plastic bottle, centrifuge tube, and ultra turrax (IKA)

2.3. Research Design

The research method in this final project applies the Complete Random Design with one factor such as: the concentration of adding the solution of whey-mangosteen pericarp extract into skim milk with five variations (0, 5, 10, 15, 20 and 25%) with four replications.

2.4. Research Stage

The first stage of this study was a preliminary study to determine the best concentration of the addition of mangosteen pericarp extract into the whey solution. The addition of 3 concentrations of mangosteen pericarp extract at 0.025%, 0.05% and 0.1% is administered into whey solution. Whey solution is obtained by mixing whey powder into hot water at 55°C, with a ratio of 1:20. Heating is performed to open the structure of whey protein to easily bind the phenolic components to the extract of mangosteen pericarp. Based on the parameters, the emulsion stability index and antioxidants value were obtained as the best treatment, indicating that whey solution was mixed with mangosteen pericarp extract in as much as 0.05% (v/v)

The extraction of mangosteen pericarp is conducted by weighing 30 grams of mangosteen pericarp powder. Mangosteen pericarp powder is wrapped with cotton and filter paper, and placed into soxhlet flask. The socletation process was carried out at 70°C for 5 hours by adding the ethanol solvent (Andayani, 2015). The extract solution was then
evaporated by setting a rotary evaporator at 45°C and at a speed of 40 rpm.

Mangosteen pericarp extract solution was obtained with a modification of the Thongkaew method [12]. Aquades was heated to 55°C, and whey powder was included with a ratio of 1:20. Then a 0.05% mangosteen pericarp extract was added. The mixture of whey and mangosteen pericarp extract was stirred by setting Ultra Turrax at 7,600 rpm for 15 minutes, with a temperature of 50-55°C. The solution is then filtered by using wattman filter paper no. 40. Whey-mangosteen pericarp extract was obtained and mixed into skim milk with a concentration of 0%; 5%; 10%; 15%; 20%; 25% (v / v). Homogenization of milk was performed by setting Ultra turrax at a speed of 7,300 rpm for 15 minutes at room temperature. The solution was placed into erlenmeyer and stored at low temperature using modified Leong method [13].

2.5. Parameter Analysis

Liquid skim milk which fortified with whey-mangosteen pericarp extract was analyze with several tests including: sedimentation [14], solubility [15] and creaming [16], material identification using FTIR [17], particle size [18] and microstructural test [19].

2.6. Data Analysis

Sedimentation, solubility and creaming data were analyzed by ANOVA, if the obtained data was significantly different, the LSD test is performed (Smallest Significant Difference) using a confidence interval of 5%. Whereas, FT-IR, particle size and microstructure data was describe in descriptive analysis.

3. Results and Discussion

3.1. Characteristics of Whey-Mangosteen Extract using Fourier Transform-Infra Red (FTIR) Method

The utilization of FTIR in the characterization of the whey complex and extract of mangosteen pericarp will qualitatively determine the characteristics of the functional groups (Van der Ven, 2002). Comparison of spectra produced by FTIR analysis on pure isolate whey protein with spectral results in the mangosteen pericarp extract whey solution and comparison of the spectrum of mangosteen pericarp extract with whey and mangosteen pericarp extract are depicted in Figure 1.

In the spectrum of whey protein pure isolate, a peak at wavelength of 1638.0 cm⁻¹ appears in the form of amide I. In contrast, the spectrum of mangosteen pericarp extract and whey-solution indicates a peak at a wavelength of 1658.78 cm⁻¹. The results of this FTIR spectrum are in accordance with the statement of Mehana et al [20], which states that there is an increase or a significant shift in peak position due to the hydrogen bond between all phenolic components and whey protein. This peak shift refers to the existence of a carboxyl bond between the phenolic component and the amide group on protein. This finding is also supported by Wu [21], stating that changes in the position of amide band I are due to differences in β-sheet confrontation in protein molecules.

Based on molecular docking analysis, the bonds that occur between whey proteins and phenolic components in mangosteen pericarp extract tend to be weak. Estimation of binding affinity between xanthone components found in mangosteen pericarp extract and whey protein, tends to be low, demonstrating result of: -5.7 Kcal / mol on α-Laktalbumin, and -6.6 Kcal/mol on xanthone molecules with β-Lactoglobulin [8]. This weak bond is marked by the absence of peaks in the wavelength range of 1750 cm⁻¹. [20] stated that a strong interaction in the complex consisting of phenolic components with protein components, will tend to decrease the number of NH2 groups with a peak at a wavelength of 1741 cm⁻¹.
In Figure 1 (b), there are several bands that describe the structure of the polyphenols in the solution of the whey-extract of mangosteen pericarp. Ribbons with wavelengths of 2962.66 cm⁻¹ and 2875.86 cm⁻¹ indicated CH strain by alkanes, in which the spectrum of pure mangosteen pericarp extract contained peak formation at wavelengths of 2963.70 cm⁻¹ and 2855.07 cm⁻¹ [22]. The existence of the polyphenol structure is also indicated by the presence of C = C bonds. This C = C strain occurs in the wavelength range of 1661-1626 cm⁻¹. This C = C strain is seen in the band of mangosteen pericarp extract solution in the form of a peak at wavelength of 1658 cm⁻¹ and in the band of mangosteen pericarp extract, at a wavelength of 1644.53 cm⁻¹. However, the band formed by aromatic components at this wavelength is not too strong; thus, if there are other components such as proteins, the picture of the protein component will be more visible [23].

The interaction of polyphenols with globular proteins can affect their affinity depending on the molecular size of polyphenols. The larger size of the polyphenol molecule, will cause greater tendency in the formation of complexes with proteins [24]. The interaction between proteins and polyphenols is found on the surface of the protein molecule, which occurs due to adsorption at the interface of the protein with polyphenols. Previous research demonstrated a significant change in the distribution of shape and intensity of the amide I band after the interaction between molecules [26, 27]. Result of sedimentation test are presented at Figure 2.

The results of analysis of variance (ANOVA) indicated that the addition of a whey-extract of mangosteen pericarp extract to a concentration of 25% did not present a significant effect (P≤0.05) on sedimentation of skim milk. However, the phenomenon causes the increasing value of sedimentation with the addition of whey-extract solution of mangosteen pericarp. The decreasing levels of polyphenols forms the low sediment. According to previous research, the addition of a polyphenol component with 0.1% content in skim milk will cause a decrease in solubility of the components of milk protein, affecting the formation of sediments [12]. In addition, the concentration of the polyphenol component added to this study ranged from 0.0025 to 0.0125%.

There is a tendency for an increase in sedimentation value in skim fortified milk proportional to the increase in the concentration of whey-extract solution of mangosteen pericarp to skim milk. This is in accordance with the previous research, stated that the tannin component can increase leading to conformational changes in both protein and tannin molecules. This finding has an impact on reducing the solubility of the two components [28]. Meanwhile, the decreasing value of sedimentation at a concentration of 20% and 25% is due to differences in high level of whey protein and casein protein.

3.3. Solubility
Solubility of protein is tested by a thermodynamic parameter demonstrating the concentration of protein in saturated solution which is in balance with the solid phase [29]. If viewed from a thermodynamic, solubility is the concentration of required solute to achieve equilibrium between the phase of aggregation of a solute and its liquid phase [30]. The results of the analysis of variance (ANOVA) demonstrated that the addition of a solution of whey-mangosteen pericarp extract had no significant effect (P≤0.05) on the solubility of fortified skim milk. The absence of significant differences in this study was due to the polyphenol component added to skimmed fortified milk at the tolerable limit of milk protein. The research conducted by Thonkaew [12] states that the polyphenol component is advisable added at least 0.1%, up to 0.3% to significantly produce different changes in protein solubility.

Figure 3 demonstrates an increasing trend in the value of soluble skim fortified milk with a solution of mangosteen pericarp and whey extract. The increasing level in solubility value is thought to be due to the reduced concentration of solute components contained in fortified skim milk. The whey solution

Fig. 2. Sedimentation of Liquid Skim Milk which Fortified with Whey-mangosteen Pericarp Extract
and mangosteen pericarp extract contains less dissolved components compared to skim milk. The whey and mangosteen pericarp extract contains of 0.05% mangosteen pericarp concentrate and 5% whey protein powder (w/v), while skim milk contains about 20% dissolved solids (w/v). This result causes greater concentration by adding whey and mangosteen pericarp extract, dissolving fewer components in skim milk changing the size of the component in skim milk to be in the form of solvent (water). According to Lu and Bhimji [31], this ratio of dissolved components to solvents plays an important role in determining the solubility of a mixture, where a less soluble condition increasing the capacity of water to interact with the dissolved component.

The creaming value in this study represents the number of serum layers formed. Greater serum formed will decrease the colloidal stability. According to SPX [32], the value of the creaming index of a stable system is less than 10%, indicating that skim milk is fortified with a solution of mangosteen pericarp extract to a concentration of 25% in stable condition during separation phase. The amount of added polyphenol components, and the presence of complexes between polyphenol components and whey protein are considered to play a role in maintaining colloidal stability from skim milk. These two factors play a role in separation phase because the polyphenol component can cause bridges between casein micelles and other protein molecules. The bridge between these molecules will initially produce haze [33]. These binding molecules connected to each other, form larger particles, generating protein precipitation. According to Tadros [27], a molecular system with nano and micro size will show little to no phenomena of separation phase.

3.5. Particle Size Distribution

Measurements of the particle size of fortified skim milk with a solution of whey and mangosteen pericarp extract were conducted by utilizing laser technology with the Cilas 1090 tool. The average size of distribution in milk protein molecules was highly dependent on season and lactation trends, and there were differences between each individual cow. In addition, the measurement results will be greatly influenced by measurement techniques.

The particle size distribution of skim fortified milk is indicated by the values of D10, D50, and D90, indicating that 10% of the microparticles in the emulsion will have a smaller size than the value of D10. The D50 value indicates that 50% of the microparticles in the emulsion have a smaller size than the D50 value. The D90 value indicates that 90% of the microparticles in the mixture have a size below the D90 value. Thus, only 10% of the microparticles in the mixture will have a size above the D90 value [34].

The mean diameter of skim milk added to whey and mangosteen pericarp extract has increased significantly when compared to controlled skim milk. This finding is due to polyphenol component mixed into skim milk causing aggregation affecting the size of the particles. In accordance with the statement of Pascal [35], in a colloidal system with a high protein concentration, the polyphenol component will form a bridge between proteins before reaching its saturation point. In addition, the heat treatment and formation of casein complexes with whey protein on casein micelles affects the increase in diameter and particle
size range. This complex between whey proteins and casein in can provide estimates of other complex size ranges bound by casein micelles [36].

Table 1. Particle Size Distribution in Skim Milk Differentiated with Whey and Mangosteen Pericarp Extract

| Whey-mangosteen | D10 (µm) | D50 (µm) | D90 (µm) | Particle size distribution | Diameter average (µm) |
|------------------|---------|---------|---------|--------------------------|---------------------|
| extract concentration (%) |         |         |         |                          |                     |
| 0% (control)     | 0.06    | 0.08    | 0.11    | 0.59                     | 0.08                |
| 5%               | 3.90    | 4.49    | 6.83    | 0.64                     | 3.20                |
| 10%              | 8.05    | 10.44   | 11.97   | 0.38                     | 11.17               |
| 15%              | 21.67   | 26.19   | 42.10   | 0.78                     | 22.78               |
| 20%              | 18.07   | 23.68   | 33.70   | 0.66                     | 20.78               |
| 25%              | 6.03    | 7.47    | 10.44   | 0.59                     | 7.21                |

The reduction in the mean particle size of skim milk fortified with a solution of whey-extract of mangosteen pericarp concentrations of 25% and 20% is due to the increase in the whey protein component, accompanied by a reduced component of casein in milk. Casein has a much larger size than whey protein, ranging from 50 to 500 nm, with an average particle size of 120 nm. Whereas, whey protein particle size ranges from 100 to 160 nm [37, 38].

The results obtained from the particle analysis test indicated that there was no large particle size distribution in each treatment. According to McClements [39], there are two things that can determine the particle size distribution in a mixture, such as the volume fraction of the dispersed phase and the interaction of colloids that can naturally occur. In a mixture with relatively high fat concentrations (Ø> 0.45), the size of these fat molecules will have a large impact on the overall particle size.

3.6. Microstructural Observation

Observation of this microscopic structure is required to determine the impact of the interaction of protein complexes with polyphenols [12]. On observation by applying a light microscope, fortified skim milk was directly placed on the preparation and observed by using 1000x magnification. At this magnification, casein micelles will separate from the solvent component, generally ranging from 50 to 500 nm.

In control skim milk (0%), the aggregate formed is relatively small and spread evenly. The aggregate size is relatively uniform. When compared with the control group, adding whey and mangosteen pericarp extract can affect the size of the skim milk protein. The size of the aggregate from fortified skim milk with a solution of whey and mangosteen pericarp extract relatively increases the concentration of solution of whey and mangosteen pericarp extract. There is a form of large globule that is not uniform which indicates low homogeneity in the sample.

The size of the aggregate size in skim milk is very closely related to the type and composition of proteins from the added polyphenol component. Larger form of micelles are formed due to a reduced component of casein which cannot cover all phenolic components in milk. This is in accordance with the statement of Jobsti, et al [40] which states that the comparison of the number of caseins with polyphenol components directly impacts the size of the casein radius. More casein in the complex will reduce the size of the casein radius due to low polyphenol concentrations. Casein components can form a more compact complex, to minimize interactions between components. A more compact complex between casein and phenolic components in mangosteen pericarp extract will produce a smaller molecular picture.

Fig. 5. (A) control skim milk (0%); fortified skim milk with mangosteen pericarp extract (B) 5%; (C) 10%; (D) 15%; (E) 20%; (F) 25%, 1000x magnification with a light microscope

During the homogenization process, several different complexes between milk protein and polyphenol components from mangosteen pericarp extract will be formed forming insoluble aggregates. The phenolic component contains several rings that can act as ligands with several binding sites. The interaction with globular protein can lead to a variety of diverse aggregate properties, depending on the molar ratio and the concentration of protein used [41].

At the addition concentration of mangosteen pericarp extract (20% and 25%), a chain-like shape was
formed, with a very clear shape in microstructure from the treatment (with 25% addition). The chain formed consists of protein aggregates that stick together, caused by interactions between protein aggregates due to the addition of polyphenols. Polyphenols that have several binding sites, will be able to bind the two adjacent protein molecules, leading to the compact structure of milk protein. Higher concentration of phenolic components will saturate the binding site of the polyphenols forming a bridge between proteins. These protein aggregates will then be bound and become visible by naked eye [35].

4. Conclusions

There was different characteristic between skim milk and skim milk fortified with a solution of whey-mangosteen pericarp extract. Addition of mangosteen pericarp extract to whey solution did not have a significant effect (α = 0.05) during the separation phase (creaming), sedimentation, and solubility, due to complex polyphenol component and formation between whey protein and mangosteen pericarp extract. However, there is an increase in particle size in skim milk with the addition of a whey solution to mangosteen pericarp extract and an insignificant microstructure change. The polyphenol component can bind to several different protein molecules, causing crosslinking and bridging, generating a greater size of the protein molecule. The best treatment obtained from this study is skim milk with 5% fortification of mangosteen pericarp extract.

Acknowledgement

The authors wish to thank the Brawijaya University Grant “Hibah Peneliti Pemula 2017” for support this work.

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