Quality attributes of probiotic-enriched chocolate: A preliminary study

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Abstract. Research on the development of foods containing probiotics is growing, such as research in the incorporation of probiotics in chocolate. Nevertheless, the quality attributes of the probiotic-enriched chocolate have not been fully explored. This present study is a preliminary study to the impact of ingredients proportion and processing method on the quality attributes of probiotic milk chocolate, more specifically on moisture content, appearance, hardness, rheological behavior, and microstructural characteristics. In this study, four different formulations and processing methods were involved. Skim milk powder was used as a freeze-dried probiotic replacer as it has high similarity in terms of physical characteristics. The result showed that chocolate containing freeze-dried probiotic powder replacer (Choc B) tended to have the comparable hardness and L*values to the chocolate reference (Choc A). However, hardness and L* values of milk chocolates enriched with freeze-dried probiotic powder replacer formulated with milk butter (Choc C and Choc D) were lower than that of chocolate reference (Choc A). Concerning the moisture level and flow parameters, Choc C exhibited higher moisture content and flow parameters values than other chocolates. Microstructural visualization showed that all chocolates exhibited a low level of agglomeration degree with particle sizes in the range of 19-32 µm.

1. Introduction
Chocolate is one of the most popular confectionery products in the world, and regardless of the level of chocolate consumption per capita chocolate is highly demanded by consumers at various ages both in developed and developing countries [1,2]. The popularity of chocolate is high due to its characteristics in terms of smoothness, melting properties, flow behavior, taste, and aroma. Apart from this, chocolate, especially dark chocolate, also contains many antioxidants and polyphenols beneficial for human health [3,4] and becomes one of the reasons people eating chocolates.

Nowadays, public awareness of healthy products is increasing. This condition encourages chocolate producers to continuously innovate and develop their products. Some research innovations have been performing, for example, making chocolate with reduced-fat/sugar [5], replacing sucrose with alternative ones considered as healthy sugar [6,7], and another attention-gaining way is adding probiotic
to chocolate [8-11]. The incorporation of probiotics to chocolate is vastly challenging. This is because probiotics have to survive during food processing [12]. Besides, adding probiotics might change the characteristics of chocolate. This does not only influence consumer preference but also the processing method and handling properties applied [13,14]. Therefore, investigating the impact of probiotics addition on the chocolate characteristics is the main aim of this study.

This study is a preliminary study of the addition of freeze-dried probiotics into chocolate. Thus, in this study, instead of using freeze-dried probiotics, a material replacer with high similarity is used. In this preliminary study, skim milk powder is added as a freeze-dried probiotic replacer considering its similarity in terms of physical characteristics. The formulation and processing method are the two main factors used as variables in this study.

2. Materials and Methods

The ingredients used were cacao mass made of beans obtained from Gunung Kidul, Yogyakarta, while sucrose, cacao butter, full cream milk powder, skim milk powder, and milk butter were purchased in local Supermarkets in Yogyakarta.

2.1 Chocolate processing

Chocolates were produced following this formulation (table 1). Chocolates were processed using a stone melange suitable for the small scale chocolate producer [15]. The method used can be seen in table 2.

| Table 1. Chocolate formulation. |
|---------------------------------|
| Ingredient                      | Proportion (gr) |
|                                 | Choc A | Choc B | Choc C | Choc D |
| Cacao mass                      | 250    | 250    | 250    | 250    |
| Sucrose                         | 250    | 250    | 250    | 250    |
| Cocoa butter                    | 250    | 250    | 250    | 250    |
| Full cream milk powder          | 250    | 200    | 150    | 150    |
| Skim milk powder                | -      | 50     | 50     | 50     |
| Milk butter                     | -      | -      | 50     | 50     |

| Table 2. Chocolate processing method. |
|---------------------------------------|
| Processing stages                     | Choc A | Choc B | Choc C | Choc D |
| Mixing process                        | All ingredients | All ingredients, except skim milk powder, were mixed | All ingredients, except skim milk powder and milk butter, were mixed | All ingredients, except skim milk powder and milk butter, were mixed |
| Grinding process Sequence of tempering temperatures 2nd mixing process | 17 hours | 17 hours | 17 hours | 17 hours |
|                                    | 50°C - 27°C - 29°C - | 50°C - 27°C - 29°C - | 50°C - 27°C - 29°C - | 50°C - 27°C - 29°C - |
|                                    | Skim milk was added (before the end of the tempering process). | Milk butter and skim milk were added (before the end of the tempering process). | Milk butter and skim milk were added (before the end of the tempering process). | Milk butter and skim milk were added. The mixture was ground for 1 hour and added before the end of the tempering process. |
| Molding process                     | 5-gr mould | 5-gr mould | 5-gr mould | 5-gr mould |
2.2 Analytical methods

2.2.1 Moisture content. Moisture content was determined by the thermogravimetric method. Approximately 3 grams of chocolate were weighed. Afterward, the sample was heated at 105°C for 24 hours. Subsequently, it was cooled in the desiccator for 1 hour and weighed again as the final mass of the sample. Water evaporated in this process was stated as moisture content (%). Measurements were done in triplicate.

2.2.2 Particle size. Particle size observation was carried out using an OptiLab Microscope. About 0.5 grams of molten chocolate was diluted in 10 ml of cooking oil and heated in the oven at 55°C for 1 hour. A drop of diluted chocolate was then put on the glass slide. Subsequently, a coverslip was placed on the sample before the observation. Measurements were done in triplicate.

2.2.3 Color. The color of the chocolates was measured using a colorimeter (Minolta CR-400) which was calibrated using a white reference standard. The color parameters were expressed in the L*a*b* color space system. L* represents lightness (luminance ranging from 0 (black) to 100 (white)), a* indicates the level of green to red, and b* represents blue to yellow. Measurements were done in triplicate.

2.2.4 Hardness. The hardness of chocolates was determined at room temperature of 20°C. Brookfield Texture Analyser with probe TA39 with a diameter of 2 mm was used. The probe speed was 0.5 mm/second with a pressing target of 3 mm. Measurements were done in triplicate.

2.2.5 Rheological behavior. Casson yield value, Casson viscosity, and thixotropy were determined using Viscometer (Brook Field type of RV 9). The obtained data were plotted to the Casson model. Casson yield value and Casson plastic viscosity were determined using equation 1, while thixotropy was determined by calculating the difference between ramp up and ramp down shear stress at 5 s⁻¹.

\[ \sqrt{\tau} = \sqrt{\tau_{CA}} + \mu_{CA} \cdot \sqrt{\gamma} \]  

Equation 1

\( \tau \) is shear stress, \( \tau_{CA} \) is Casson yield value (Pa), \( \mu_{CA} \) is Casson viscosity (Pa.s) and \( \gamma \) is the shear rate.

2.3 Data analysis

The data of this study were analyzed using SPSS software version 22.0. The researchers used one-way analysis of variance (ANOVA) with a significance level of 5% to test differences in moisture content, color, and hardness. For testing the homogeneity of variances, the Levene Test was performed. Meanwhile, Tukey test was used to determine the differences among samples and Principal Component Analysis (PCA) was used to visualize relationships among the samples.

3. Results and discussion

3.1 The relationship between chocolate characteristics

Principal Component Analysis (PCA) plots in figure 1 (a) and (b) show a general overview of the relationship among chocolates. PCA explained 88% of the variance in the first to factors, with PC 1: 67% and PC 2: 21%. The chocolates were clustered into three groups. In the same group, Choc A and Choc B had a propensity to have similar characteristics, characterized by their relatively high values of hardness and lightness (L*) exhibiting relatively high positive values in PC 2. In the second cluster, Choc C was characterized by its high value of Casson Yield Value, Casson Viscosity, Thixotropy, and moisture content exhibiting high values in PC 2. The third cluster (Choc D) was characterized by its low value of hardness exhibiting high negative values in PC 1 and PC 2.
3.2. Physicochemical characteristics

3.2.1 Moisture content. The moisture content of chocolates affects the level of chocolate grittiness, hardness, flow properties, and influences the color of chocolates, to some extent [1,13,16,17]. Chocolate containing high moisture content tends to have higher hardness and viscosity value. Due to agglomerates formed, high moisture content chocolate also exhibits a higher level of grittiness [2,18]. Besides, due to the occurrence of sugar bloom which is commonly observed in high-moisture chocolate, moisture content also determines the appearance of chocolate [1].

The results in table 3 shows that the chocolate samples tended to have a relatively higher moisture content (3.06%-3.88%) than dark chocolates sweetened with sucrose (0.7%) [18] and dark chocolate sweetened with palm sap sugar (1%-1.5%) [16]. The relatively high level of moisture observed in the chocolate could be due to the high moisture content of the ingredients used and the processing method applied. The latter determines the amount of moisture that can be evaporated during chocolate processing [19]. Aside from this, the method used for moisture determination might also affect the values.

| Chocolate Samples | Moisture Content (%) | Color L* | Color a | Color b | Hardness (N/mm²) |
|-------------------|----------------------|----------|---------|---------|-----------------|
| Choc A            | 3.12 ± 0.10a         | 38.65 ± 0.56a | 18.06 ± 0.53a | 18.70 ± 0.94a | 5.93 ± 0.37b    |
| Choc B            | 3.12 ± 0.26a         | 39.53 ±1.21a  | 18.08 ± 0.64a | 19.17 ± 0.86a | 4.94 ± 1.89ab   |
| Choc C            | 3.88 ± 0.08b         | 38.66 ± 0.76a  | 17.57 ± 0.13a | 18.33 ± 0.46a | 2.86 ± 1.23a    |
| Choc D            | 3.06 ± 0.08b         | 37.75 ±1.07b  | 18.41 ± 0.52a | 19.31 ± 0.81a | 3.06 ± 0.31ab   |

Different superscripts in the same column indicate significant differences (p < 0.05) among samples.

3.2.2 Particle size. The particle size of chocolate affects the grittiness of the chocolate. Besides, as discussed in several works, particle size influences the color, hardness, and viscosity of chocolate [1,16]. In this study, the particle size of chocolate was measured and visualized using a normal light microscope.
In general, it could be concluded that the range of chocolate particle sizes among samples was comparable (figure 2). It can be seen in figure 2 that Choc A, Choc B, Choc C, and Choc D had a particle size range of 25-27 µm, 20-27 µm, 19-29 µm, and 22-32 µm, respectively.

Furthermore, it can be seen in figure 2 that all chocolates exhibited agglomeration. This phenomenon occurred due to the presence of relatively high moisture which sticks the particles to each other. In the previous study by Saputro [18] reported that due to high moisture content, palm sap sugar-sweetened chocolate exhibited particle agglomeration.

Figure 2. The particle size of probiotic-enriched chocolates processed using alternative processing

3.2.3 Color. Color, in combination with the glossiness, determines the appearance of chocolate that influences consumer preferences. Color is not only influenced by the particle size of the chocolate [20], but also by the surface roughness of the chocolate [21]. Afoakwa [20] reported that chocolate with fine particles has more particle interactions than that with coarser particles, resulting in a lighter color. It can be seen in table 3 that the color of the chocolates was not significantly different (p<0.05). Since the particle size of the chocolates were in a similar range figure 2, slight difference values in the color parameters could be associated with the surface roughness of the chocolates. In his study, Briones [21] found that chocolate with a smooth surface tended to have a brighter color.

3.2.4 Hardness. Similar to the color of chocolate, with the same level and type of fat, the hardness of chocolate is determined by particle size. It was reported by Afoakwa [20] that smaller particle size resulted in higher particle-particle interactions, creating harder chocolate. Besides, hardness, to some extent, is also influenced by moisture content. The higher the moisture content, the higher the hardness was observed in palm sap sugar-sweetened chocolate [18]. It can be seen in table 3 that the hardness of Choc A, Choc B, and Choc D was not significantly different (p<0.05). On the other hand, Choc C, even though the value was quite low (2.86 ± 1.23 N/mm²), was comparable to Choc B and Choc C (p<0.05).
This phenomenon showed that in this study, moisture content only had a little influence on the hardness of chocolate. With regard to the influence of particle size, since all the chocolates exhibited a comparable range of particle sizes (figure 2), it seemed that particle size did not have a significant effect.

3.2.5 Rheological behavior. The rheological behaviour of chocolate determines the mouthfeel of chocolate during consumption [5]. To easily quantify the value of rheological behavior parameters, the Casson model is commonly used. It can be seen in figure 3 that all chocolates, except Choc C, exhibited a comparable Casson yield value and Casson viscosity, while the thixotropy value of the chocolates varied among samples. Moreover, it can be seen in figure 3 that the Casson yield value and Casson viscosity of Choc C were higher than those of other samples. This phenomenon could be due to the presence of higher moisture content in Choc C (table 3). Beckett [2] stated that for every 0.3% extra moisture left in chocolate, an extra of 1% fat should be added to reach similar flow properties to the one without 0.3% extra moisture. Due to the presence of relatively high moisture in all chocolates, homogeneous chocolates could not be created, resulting in chocolates with high thixotropy values.

![Figure 3. Comparison of flow properties of probiotic-enriched milk chocolates produced using alternative processing.](image)

Different superscripts per parameter indicate significant differences (p < 0.05) among samples

4. Conclusion

The incorporation of freeze-dried probiotic powder replacer and the applied processing method influenced the characteristics of milk chocolate to some extent. From the study, it was found that milk chocolate made without milk butter exhibited the closest characteristics to the chocolate reference. Moreover, the addition of a freeze-dried probiotic powder replacer at the end of the tempering process was the best option. To have a better understanding of the impact of the processing method, further study related to viability assessment of probiotics is required.

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