The quality of skim milk curd produced using biduri (Calotropis gigantea) latex as rennet replacement

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Abstract. Physical and chemical quality of milk curd produced using various biduri latex as rennet placement were investigated in skim milk-clotting. The materials used in this study were rennet, commercial skim milk and biduri latex powder. Four types of formula namely P0: rennet 0.02% + CaCO₃ 0.01%; P1: biduri latex powder 0.015% + CaCO₃ 0.01%, P2: biduri latex powder 0.020% + CaCO₃ 0.01% and P3: biduri latex powder 0.025% + CaCO₃ 0.01% were applied to produce milk curd. As quality parameter, weight and yield (based on dry matter content in %) curd, dry matter (DM), water, protein (Kjeldahl method), and fat content were evaluated. Result showed that fat content, weight and yield of curd were higher in milk curd produced with biduri latex compared to control, however protein content was lower. As the conclusion, the use of biduri latex up to 0.025% able to replace rennet for milk curd production which shows better in physical quality.

1. Introduction
Rennet has long been used as enzyme to agglomerate milk. Others than rennet, milk clot agent could be obtained from microbes and plants source [1]. Biduri (Calotropis gigantea) is a plant in Indonesia which quite large in population but still optimally use [2]. This plant, Biduri, produce latex that contain protease [3] and exopeptidase [4]. The proteinase of Biduri was reported to be able to hydrolyze casein, fibrinogen and fibrin [5].

The crude extract of Biduri plant latex has been characterized. The value of its protease activity was known and applied to agglomerate milk [6]. The crude enzyme of Biduri also able to coagulate casein and has same activity as renin [7]. Moreover, the addition of Biduri latex to produce milk curd shows higher activity compared to rennet, therefore it can be replacing the function of rennet [8].

According to the mentioned facts above, this study aimed to evaluate chemical and physical quality of curd skim milk which produced by various concentration of Biduri latex.

2. Materials and Methods

2.1. Materials
Commercial skim milk, rennet, Biduri latex powder and CaCO₃ were materials used in this study. The rennet was taken from abomasum according to Hertanto et al. [9] with modification. The modification
was filtration step by using filter paper (Whatman 41) in 1 ml microtube followed with stored at -21°C. The crude protease source of biduri latex was tapped from the young tissue of the stem then dried using oven at 40°C for 24 hours.

In this study, milk curds were produced using four formulas namely P0: Rennet 0.02% + CaCO$_3$ 0.01%; P1: Biduri latex powder 0.015% + CaCO$_3$ 0.01%, P2: Biduri latex powder 0.020% + CaCO$_3$ 0.01% and P3: Biduri latex powder 0.025% + CaCO$_3$ 0.01%.

2.2. Cheese making and physicochemical analysis
The cheese making in this study, was done according to Hutagalung method [10] with modification, by adding CaCO$_3$ in purpose to increase the curd. Following curd production, its moisture content was evaluated using gravimetric method. The DM was evaluated by sum of cheese weight subtracted with water content, in term of protein content it determined using Kjeldahl method [11] and the fat was evaluated with Soxhlet method [12]. Lastly, curd weight and yield were determined according to the Afiati method [13].

2.3. Data analysis
The experiment in this study was designed as complete random design. Data were analysed using variance analysis followed with Dunnet test [14].

3. Results and Discussion
The chemical and physical content of skim milk curd produced in this study was shown in Table 1. The results showed that biduri latex in P1, P2 and P3 had no significant effect (P>0.05) on moisture content and dry matter, however significant effects (P<0.01) were found on fat, protein, weight and yield of curd when compared to control (P0; rennet).

Protease enzyme in biduri latex has properties to cut peptide bonds of milk protein. This could occur due to protease cut the hydrophobic side of carboxyl and amino group, therefore water remains bounded to the hydrophilic side [10, 15]. Moreover, it is explained that N-terminal and C-terminal are part of casein which is hydrophobic [16]. The moisture content of milk curds produced in this study was ranged from 69.827 - 70.725%, and it is categorized as soft cheese due to its water content more than 61% [16]. Meanwhile, the application of biduri latex was not able to increase the dry matters of curd. This was in agreement with the previous studies [17, 18] which found that dry matter in curd was negatively correlated with moisture content. High level of water content would decrease the curd dry matter.

| Quality                     | P0          | P1          | P2          | P3          |
|-----------------------------|-------------|-------------|-------------|-------------|
| Moisture content (%)        | 70.456±0.540| 70.725±0.491| 70.326±0.359| 69.827±0.355|
| Fat content (%)             | 0.570±0.039$^a$ | 0.699±0.048$^b$ | 0.572±0.020$^b$ | 0.564±0.045$^b$ |
| Protein content (%)         | 23.82±0.432$^a$ | 21.66±0.627$^b$ | 22.66±0.462$^b$ | 22.68±0.442$^b$ |
| Drymatter content (%)       | 29.543±0.540 | 29.275±0.491 | 29.674±0.359 | 30.173±0.355 |
| Curd weight (g)             | 106.73±7.708$^a$ | 137.305±5.992$^b$ | 142.359±5.499$^c$ | 158.799±7.41$^d$ |
| Curd rendement (%)          | 31.515±1.974$^a$ | 40.191±1.719$^b$ | 42.233±1.319$^c$ | 47.910±2.193$^d$ |

$^a$Significant (P<0.01)
$^d$Different superscripts on the same line show significant differences (P<0.01)

The fat content of curd in this study was significantly higher (P <0.01) in P1 and P2 compared to P0. This was in accordance with Witono [2] which reporting that use of coarse latex extract increase fat content of goat milk. The fat content of cheese produced in this study was ranged from 0.564 to 0.699% and categorized as skim milk cheese because fat content is less than 10% [16]. We also found that the protein content of skim milk curd produced with biduri latex was significantly lower (P<0.01) compared to P0 in this study.
It could be explained due to the biduri latex used in this study was in form of extract, then the concentration amount was low. As stated by Geantaresa and Supriyanti [19] tenzyme activity depends on enzyme concentration and its substrate. If the substrate concentration is low, then the enzyme activity became low as well. The protein content of cheese produced in this study was ranged from 21.66 - 23.82%. This value is lower than protein content of cheese in the previous study, i.e. 23.50% [20]. Based on the protein content determined by the Indonesian National Standard (SNI), the protein content found in this study is above the minimum threshold that is 19.5% [20].

The further analysis found that weight and yield of curd in P1, P2 and P3 were significantly higher (P<0.01) compared to P0. Curd obtained by clotting casein from skim milk occur in the presence of rennet and protease enzymes [21], due to the the coagulation of milk casein [22]. The amount of protease will increase the amount of curd [23]. In this study we found the weight of curd produced is in line with the increasing amount of biduri latex used. Moreover, it is also in accordance with the results of protease activity test of biduri which showed that the proteolytic activity was better than rennet [6]. Thus, all treatments showed an increase in the yield of curd obtained when compared to P0. The yield was affected by curd weight which is in agreement with the previous result [24], that there is a relationship between weight and yield of curd of 0.1% papain and 5% lemon juice. Curd yield produced in this study was ranged from 31.515 to 47.910 g, it is indicated that curd yield produced by biduri latex had a high quality of cheese [25].

4. Conclusion
Based on the current study, it is concluded that fat content, weight and yield of curd were higher with the addition of biduri latex than controls, but protein content was lower. The addition of biduri latex extract to 0.025% can replace rennet with better physical quality.

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