PHYSICOCHEMICAL CHARACTERISTICS OF SOY-CHEESE WHEY USING *AVERRHOA BILIMBI* JUICE AS COAGULANT

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Abstract
The type and concentration of coagulant has been studied well as important factor that affected to the physicochemical characteristic of soy-cheese whey. This research used juice from Averrhoa bilimbi as coagulant to obtain whey from soy since commonly found in tropical area, low in price and no health risks for the use. This research aimed at analyze physicochemical characteristics of whey from soy-cheese that were coagulated with Averrhoa bilimbi juice using dilution and no dilution process. As much as 7% (v/v) Averrhoa bilimbi juice with dilution (1:1) and without dilution, and 5 ml of rennet (0.01% w/v) were used to obtain the whey. After incubation at 42^{o}C for 3 hours, the whey were separated by curd-hanging for 4-5 hours. The results showed that dilution treatment of Averrhoa bilimbi juice increased total solids, pH value and absorbance spectrum. The dilution also decreased whey volume and protein content of the soy-cheese whey. As conclusion, the concentration of Averrhoa bilimbi juice had remarkable effect to the physicochemical characteristics of the soy-cheese whey. Since physicochemical characteristics of whey gives important information to whey-based food industry, the utilization of natural coagulant such as Averrhoa bilimbi juice may open the knowledge for application to produce healthy food.

Keywords : *Averrhoa bilimbi*, soy-cheese whey, volume, protein, pH, total solid, spectral analysis

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1. INTRODUCTION

Soy whey are generated as a by-product from the coagulation process of protein soya in liquid. The phenomenon of coagulation is an important part in the formation of curd matrix which has implications on the quality of the final product such as soy-cheese curd. Beside curd's characteristic, information about the characteristics of whey formed can be used as a basis to determine the quality of curd, one of which is influenced by the type of coagulant.
The used of rennet in making soy-cheese can be combined with direct acidification (Rakhmah and Suryani, 2016) using local fruit *Averrhoa bilimbi* juice. *Averrhoa bilimbi* could potentially use as a natural coagulant because it contains high citric acid up to 92-133 meq acid/100 g of total solids resulting very sour taste (Prahadi et al., 2015), in addition, it also contains 25 mg/100 g vitamin C of fresh fruit (Agustin and Putri, 2014).
Moreover, the fruit is easy to obtain and have a low price. The utilization of *Averrhoa bilimbi* is still underutilized for food industry since the application of this fruit only to fulfill the local dishes' requirement (Windyastari et al., 2012).
Whey from of cow has been well studied (Widarta et al., 2016; Sumarmono and Suhartati, 2012) however, whey from soymilk were found to be rare (Aryanti et al., 2016). The optimal concentrations may accelerate the process for coagulation and the provide benefit to the quality of whey (Chipkah *et al.*, 2015; Fasoyiro, 2014; Purwadi 2008), therefore, the purpose of this study is to analyze the characteristics of physicochemical of soy-cheese whey using *Averrhoa bilimbi* juice as coagulant, with or without dilution treatment. Since the consumer's need toward healty food is now being increase, the natural coagulant such as *Averrhoa bilimbi* juice plays key role to whey-based food industry for producing healthy food.

2. MATERIAL AND METHODS

This research was conducted at the Laboratory of Food Chemistry and Nutrition, Faculty of

Animal and Agriculture Sciences, Diponegoro University and UPT Integrated Laboratory of Diponegoro University during November 2016 – Februari 2017.

Materials

Soymilk (USA Soybean No. 1) obtained from "Serasi" Tofu Factory Bandungan, Semarang, vegetable rennet "Davisco" obtained from Bintang Makmur Co. Ltd., Surabaya, *Averrhoa bilimbi* juice from local farm, Coomassie Brilliant Blue G 250 (C.I. 42655, Merck), ethanol (Emsure, Merck), acid phosphats and comersial ovalbumin were obtain from Laboratory of Food Chemistry and Nutrition, Faculty of Animal and Agriculture Sciences, Diponegoro University Semarang. pH meter (Hanna HI 8424, USA), electric oven (Memmert, Germany), and spectrophotometer (UV Mini Shimadzu 1280, Japan).

Methods

Preparation of *Averrhoa bilimbi* Juice as Coagulant

Averrhoa bilimbi juice methods was conducted using the method of Widarta et al. (2016). *Averrhoa bilimbi* freshly cut into a small pieces (5 mm for each pieces) and subsequently washed, drained, and blended with juicer using lowest speed and filtered using a paper cloth. The resulted liquid of *Averrhoa bilimbi* juice was used as solution of *Averrhoa bilimbi* juice.

Determining The Volume Concentration of *Averrhoa bilimbi* juice.

One to ten percent *Averrhoa bilimbi* juice were mixed with 0,01% rennet (w/v) tube added to 10 ml of soymilk and incubated at 42^{o}C for 3 hours. The whey was separated using paper cloth filter.

Based on this preliminary research, 7% *Averrhoa bilimbi* juice showed highest weight curd (17.97±0.84%), therefore 7% of *Averrhoa bilimbi* juice were used for entire research.

The dilution treatment was conducted by diluting *Averrhoa bilimbi* juice with water at ratio 1:1.

Separation of Soy-cheese Whey

Direct acidification methods based on Widarta et al. (2016) with minor modifications were used to separate whey from curd. Soymilk was made using dry soybeans and water's ratio at 1:10 (Suhaedi, 2003). Seven percent of *Averrhoa bilimbi* juice was added to 500 ml of pasteurized soymilk. Treatment for T1 used *Averrhoa bilimbi* juice without dilution and treatment for T2 used *Averrhoa bilimbi* juice with the ratio of juice and water at 1:1 (v/v). After 15 minutes, 5 ml of 0.01% (w/v) rennet was added in each samples followed by gentle stirring. The final mixture was incubated at 42^{o}C for 3 hours. The whey was separated by hanging for 4-5 hours using filter cloth. The obtained whey was measured for volume, pH, total solids, protein content and spectral performance.

Whey volume was determined using AOAC methods (1995). Total solids was analyzed using drying methods (Nawangsari et al., 2012) with minor modifications. pH value measurement was done using methods of Rukmi et al. (2015). Protein content was analyzed using Bradford method that has been done by Owusu-Apenten (2002). Spectral analysis test was conducted using method of Copriady et al. (2011) with minor modifications on the value of wavelength 190 nm to 750 nm.

Data analysis

Whey volume, total solids, pH and protein content were analyzed by t-test using α=5% using SPSS 20.0 for Windows and spectral analysis was analyzed descriptively.

3. RESULTS AND DISCUSSION

The results of the analyze physicochemical characteristics of soy-cheese whey after treatment are presented in Table 1. The dilution process of *Averrhoa bilimbi* juice as coagulant remarkably reduced whey volume from 71.50±1.35% to 57.30±2.64% (Table 1). This may be explained due to weak of bond among protein molecules (Fahmi et al., 2010). Furthermore, the lower pH value of coagulant

may increase the number of hydrogen bonds resulting in the rapid coagulation (Hsia et al, 2016; Anggraini et al., 2013). This study showed that 7% of coagulant's addition was optimally separate whey from the curd. It was understood that the optimal concentration of coagulant was important factor to achieve the optimum process of separation (Sulejmani and Hayaloglu, 2016; Arifiansyah et al., 2015; Anggraini et al., 2013). The obtained volume in this research was close to the result of previous researcher. Rekha and Vijayalakshmi (2010) was able to produced 81% of whey volumes using lemon, tamarind, garcinia, gooseberry and passion fruit as coagulant. In addition, the result was also comparable to the research of Chipkah et al. (2015) which produced 72% of whey volumes using coagulant from apple of Sodom (*C. procera*). This indicates that different types of fruit affected the volume of whey.

The dilution process of *Averrhoa bilimbi* as coagulant remarkably increased total solids of soy-cheese whey from 1.74±0.17% to 1.91±0.16%, or almost 20% (Table 1). This may be explained due to weak protein bond produced a clumps with loose matrix structure (Sumarmono and Suhartati, 2012; Fahmi et al., 2010; Obatolu, 2008). This is showed that the protein bond affected by pH of coagulant. Furthermore, the protein bond was influenced by the ratio of soy protein 11S/7S. Low ability of protein 11S made the low Water Holding Capacity (WHC) resulting in the decreased of gel strength (Zhao et al., 2016; Guo et al., 2012), thus providing dispersion of solid content in whey. The obtained total solids in this research can be compared to previous researcher. Tripathi and Chandra (2013) was able to produced 3% of total solids in the soy whey using $MgCl_2$ and $CaSO_4$ as coagulant. The total solids of whey using *Averrhoa bilimbi* juice as coagulant is much less than that of salt as coagulant. The high acidity of coagulant without dilution in this research was found (pH 1.60) that might generating high rate of proteolysis, resulting in the curd coagulation (Anggraini et al., 2013), thus the high acidity in coagulant retarded the process of generating total solid in whey.

The dilution process of *Averrhoa bilimbi* juice as coagulant remarkably increased pH of soy-cheese whey from 5.07±0.02 to 5.61±0.04 (Table 1). This may be explained due to the concentration of water (Aryanti et al., 2016), resulting in the breaking of peptide bond (Malaka, 2010). The obtained pH of whey in this research can be compared with previous researcher by Tamimi et al. (2015) stated that pH of soy whey had around 5.0. The pH changes during coagulation using *Averrhoa bilimbi* juice as coagulant are presented in Figure 1. The addition of coagulant, both dilution and without dilution decreased the pH of soymilk. The dilution of *Averrhoa bilimbi* juice increased pH of coagulant with a range of 0.44. Sulejmani and Hayaloglu (2016) stated that difference pH of coagulant with a range of 0.30 affected to an enormous impact on the amount of curd and whey. Furthermore, the higher pH of medium provided the more difficulty of separation process between curd and whey (Rohmatussolihat et al., 2015; Triyono, 2010).

In this research, coagulation was occurred before reaching of the isoelectric point 4.5-4.8 (Nishinari et al., 2014). This may be explained by the influence of heating process. This condition was appropriate with the opinion of Fox et al. (2017) stated that the coagulation might be occurred at pH 5.2 if the heating temperature was also applied.

Table 1. The results of the analyze of whey volume, total solids, pH and protein content of soy-cheese whey

Parameter	Types of Dilution Coagulant *Averrhoa bilimbi* Juice	
	Without Dilution	Dilution (1:1)
Whey volume (%)	$71,50 \pm 1,35^a$	$57,30 \pm 2,64^b$
Total solids (%)	$1,74 \pm 0,17^a$	$1,91 \pm 0,16^b$
pH	$5,07 \pm 0,02^a$	$5,61 \pm 0,04^b$
Protein contains (%)	$0,42 \pm 0,47^a$	$0,33 \pm 0,12^b$

Note: The data showed an average of ten repetitions. The column followed by different letters for each treatment, different significantly by T-test (P<0,05).

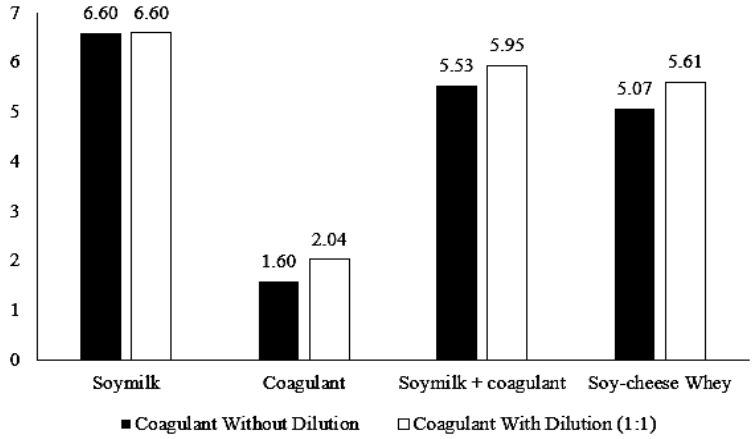

Figure 1. The pH changes during coagulation using *Averrhoa bilimbi* juice as coagulant

The heating process at the minimum of 65°C will formed aggregates, thus unfolded subunit oligomers rapidly (Peng et al., 2016; Guo et al., 2012). Furthermore, the H+ ions from the acid induced the disconnection of most peptide bond and formed the clump (Malaka, 2010; Triyono, 2010). This research showed that pH of the process to produced soy-cheese whey was classified as the minimum requirement to produced soy-cheese (Pearson, 1983).

The dilution of *Averrhoa bilimbi* juice as coagulant remarkably reduced of soy-cheese whey protein content from 0.42±0.47% to 0.33±0.12% (Table 1). It was stated by Aryanti et al. (2016) that the more percentage of proteins coagulated into curd, the less protein remaining in the whey. This may due to the acidity of *Averrhoa bilimbi* juice without dilution affected to the rapid coagulation of curd resulting in the less protein content in whey. However, the acidity may reduce the binding of protein in curd that might potentially increased the soluble protein in soy-cheese whey (Widarta et al., 2016). Furthermore, the curd matrix formed from *Averrhoa bilimbi* juice with dilution has a tenuous bond, so contain lots of water and have a high yield of curd (Sumarmono and Suhartati, 2012; Fahmi et al., 2010), thus the more protein trapped in curd (Karsono et al., 2010) resulting in the elss amount protein in soy-cheese whey. The protein content of whey produced from this research was lower than the results from previous researcher. Fahmi et al. (2010) stated that the contents of soy whey protein using $CaSO_4.2H_2O$ and CH_3COOH as coagulant was able to produced 0.85% and 0.73%, respectively. This may be explained by the influence of hydrogen ions from chemical coagulant.

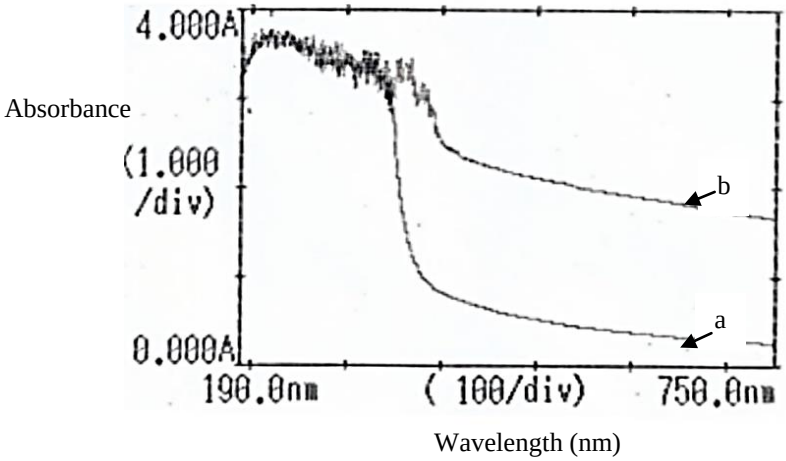

Figure 2. The graph of the spectral analysis on soy-cheese whey from addition of *Averrhoa bilimbi* juice as coagulant (a) without dilution and (b) with dilution

It has been understood that hydrogen ion might neutralized protein into isoelectric point (Aryanti et al., 2016; Prabhakaran et al., 2006). The different slope of the spectral analysis values could be detected on samples of the whey with and without dilution of coagulant (Fig. 2). The dilution of the coagulant remarkably delayed slope of the spectrum. This may be explained due to weak of bond protein matrix in curd remaining high content of unbound-protein in soy-cheese whey (Sumarmono and Suhartati, 2012; Fahmi et al., 2010), thus postponed the slope. Hardoko et al. (2003) stated that total soluble solid affected to the turbidity that might increase the absorbance. It has been understood that turbidity may indicated the effectiveness of the process of coagulation (Ashraf et al., 2016), resulting in the change of whey quality (Larasati et al., 2016; Effendi, 2003). Since unbound protein is unnecessary component in whey, the dilution of coagulant is not required.

4. CONCLUSIONS

The dilution of *Averrhoa bilimbi* juice increased total solids, pH and delayed the slope of spectrum, however decreased whey volume and protein content, thus the concentration of *Averrhoa bilimbi* juice affected to overal physicochemical characteristics of soy-cheese whey.

5. ACKNOWLEDGMENTS

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