Yield and Processing Properties of Concentrated Yogurt Manufactured from Cow’s Milk: Effects of Enzyme and Thickening Agents

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Abstract. The objective of the experiment was to investigate the yield and processing properties of concentrated yogurt manufactured from local cow’s milk with the addition of microbial transglutaminase enzyme (mTGase) and several thickening agents. Concentrated yogurt was manufactured from local fresh milk, which were previously processed into plain yogurt by adding starter culture of lactic acid bacteria. The enzyme and four thickening agents (pectin, carrageenan, xanthan, and inulin) were added before the fermentation process. The amount of mTGase was 0.03% (w/w), while the amount of each thickening agent was 1.5g/100g of milk. Partial removal of whey was conducted by modified in-bag straining method using nylon bags. Data was analyzed by the procedure of generalized linear model. Overall, the present study showed that yield and the processing properties of concentrated yogurt can be improved by the addition of enzyme (mTGase) and thickening agents: inulin, carrageenan, xanthan, and pectin. The use of xanthan resulted in the highest yield, whereas the use of inulin and mTGase produce yogurt curd with low syneresis and high water holding capacity.

Keywords: concentrated yogurt, fermented milk, whey separation, local cow’s milk.

1. Introduction
Concentrated yogurt, or strained yogurt, is one of fermented milk products popular in many countries. The manufacture of concentrated yogurt from fresh yogurt requires partial whey separation in order to obtain soft, creamy-like, and nutrients-densed products [1]. Along with yogurt and other fermented milk products, concentrated yogurt can be considered as one of functional milk products [2], with longer shelf-life and broader application than fresh yogurt. Concentrated yogurt can be manufactured from whole milk of cows, goats or sheep, or recombined milk using different manufacturing methods [3].

In this study, the microbial transglutaminase enzyme (mTGase) and several carbohydrate-based thickening agents, including carrageenan, pectin, inulin and xanthan, were added to the raw material, which was local cow’s milk) in an effort to improve yield and processing characteristics of concentrated yogurt. mTGase is a transferase enzyme that able to accelerate the crosslinking of protein by forming intramolecular iso-peptide bonds [4]. The application of this enzyme in foods is wide,
including milk products. For example, incorporation of mTGase during the manufacture of concentrated yogurt from goat milk improve the consistency and firmness of the products [5]. Carragenan, xanthan, inulin and pectin are common food thickening agents, and also serve as stabilizers [6]. The addition of 0.01% xanthan and carrageenan improve the viscosity, with less syneresis, of yogurt [7]. Fat-free goat milk yogurt with pectin (0.5% w/v) has firmer curd but the presence of lumps was detrimental to the sensory properties, whereas the effects of pectin in combination with carrageenan (0.1% w/v) on the properties of yogurt were subtle [8].

Yield and processing properties are important variables in the manufacture of concentrated fermented milk products. Yield indicates the amount of product that can be obtained, which relates to the efficiency of the manufacturing processes. Processing properties, such as the relative proportion of free whey, pH of yogurt curd, syneresis and water holding capacity, are important to evaluate the manufacturing conditions which affects the quality properties of the final products. To date, limited information is available on the suitability of local fresh cow’s milk in Indonesia for manufacturing concentrated yogurt. Therefore, this study was aimed to determine the quality and processing characteristics of concentrated yogurt manufactured from local cow’s milk. Our general hypothesis was that the use of mTGase and different thickening agents would improve yield and have impact on processing properties during manufacture of concentrated yogurt. The final goal of this study is to establish a standardised procedure to manufacture concentrated yogurt from local cow’s milk with functional characteristics.

2. Methodology

Milk preparation
Unpasteurised fresh milk of morning milking was purchased directly from a local dairy farmer in Banyumas area, Central Java, Indonesia. The milk was taken to the laboratory in a milkcan, and pasteurised in a stainless steel container at 85°C for 15 minutes; then the milk was cooled down to 40°C.

Manufacture of yogurt and concentrated yogurt
Plain yogurt was manufactured from the milk by adding a previously activated lyophilised yogurt starter culture containing L. bulgaricus, S. thermophylus, and L. acidophylus. Fermentation of the milk was performed at 38°C for 4 hours. The resulting plain-fresh yogurt was used to manufacture concentrated yogurt using in-bag straining or Berge method [9] with some modifications. A special tool was created to accelerate partial whey separation from plain yogurt, which consists of a nylon bag and a PVC tube. The whey was allowed to separate for 18 hours under cold temperature. The resulting paste-like product (yogurt curd) was considered as plain concentrated yogurt.

In the production of yogurt as mentioned above, the addition of mTGase (0.03% w/w) and carbohydrate-based thickening agents: inulin, carrageenan, xanthan and pectin (1.5g/100g milk) to the milk was done before the fermentation stage. The milk was conditioned for 30 min before adding starter culture. All treatments were replicated four times.

Measurement of yield and processing properties
In order to evaluate the effects of mTGase and different thickening agents during manufacture of concentrated yogurt from cow’s milk, several measurements were made. These included yield, whey, degree of acidity (pH) of yogurt curd and whey, syneresis and water holding capacity (WHC), and titratable acidity) of yogurt curd.

Yield was calculated as the relative proportion of yogurt curd to the plain yogurt weight after the addition of mTGase or thickening agents. The weighing of yogurt curd was done after 18 hours in-bag straining for partial whey removal. After 18 hours straining, the free whey pooled at the container of the bottom of the strainer was collected and weighed. Whey percentage was calculated as the ratio of free whey (g) to the plain yogurt (g) multiply by 100. The pH values of whey and yogurt curd were determined by a pH meter that have been calibrated using standard buffer solution pH 4.0 and 7.0.

Syneresis of yogurt curd was determined using centrifugation procedures [10] with some modifications. Approximately 10 g of yogurt curd sample was weighted and placed in a centrifuge
tube, then centrifuged for 10 min at 2500 rpm under room temperature. The supernatant or expelled whey was poured into a beaker glass and weighed (g). Syneresis (%) was calculated as the proportion of supernatant to the weight of sample, multiplied by 100. Subsequently, the WHC of yogurt curd was calculated as the weight of yogurt curd sample minus weight of supernatant divided by weight of yogurt curd sample, then multiplied by 100. Titratable acidity was determined by titrating sample with 0.1M NaOH and phenolphthalein as indicator. Sample of yogurt curd, about 10 g, was previously diluted with 10 mL distilled water. The titratable acidity was expressed as g/100g lactic acid using formula = (volume of NaOH x 0.9)/weight of sample [11].

**Experimental design and data analysis**

All treatments were arranged in a completely randomized design with five treatments. Each treatment has four replications. Data obtained were analysis statistically using a generalized linear model (GLM) of a statistic software.

3. **Result and Discussion**

The main purpose of adding mTGase and thickening agents were to improve yield and textural properties of concentrated yogurt. Figure 1. shows that yield and the amount of free whey released were significantly (P<0.05) affected by the addition of mTGase and thickening agents. The yield ranged from 29.71±6.59% to 80.98±6.08%, with overall average 55.38±5.33%. Xanthan was able to retain whey more effectively than other stabilizers, hence it produced highest yield and lowest free whey. On the other hand, adding mTGase produced lowest yield, followed by pectin, inulin and carrageenan. The use of pectin produced yogurt curd with noticeable lumps.

Degree of acidity or pH, along with titratable acidity, is an important parameter of fermentation process during manufacture of concentrated yogurt. Yogurt and concentrated yogurt has lower pH than milk due to the accumulation lactic acid produced by lactic acid bacteria. Data shows that the use of mTGase and thickening agents resulted in slight changes of pH of curd and whey (Figure 2.), where the use of inulin produced yogurt curd and whey with noticeably lower pH (P<0.05) than other treatments. The pH of yogurt curd and whey range from 3.06±0.02 to 3.89±0.16, and 3.15±0.12 to 3.61±0.11 with overall average of 3.61±0.08 and 3.46±0.10, respectively.

Syneresis and WHC of yogurt curd indicates the ability of yogurt curd (gel) to hold or retain water in its gel. Results show that the syneresis of yogurt curd with carrageenan was highest (57.38±3.99%), and that with mTGase was lowest (19.94±5.00%) (Figure 3.). The overall average of syneresis for all treatments was 29.07±5.08%. Accordingly, the WHC of yogurt curd with mTGase was highest (82.1±5.00%) and that with carrageenan was lowest (42.6±3.99%). Overall, the average WHC of yogurt curd was 70.93±5.80%.

The titratable acidity of yogurt curd measures the amount acids produced during fermentation of milk. In this study, the titratable acidity of yogurt curd ranged from 0.96±0.06 to 1.51±0.33 g/100g lactic acid, with overall average of 1.30±0.16 g/100g lactic acid (Figure 4.). The use of mTGase and different thickening agents has significant (P<0.05) effects on the titratable acidity of yogurt curd. Addition of inulin, mTGase and pectin produced yogurt curd with higher titratable acidity than addition of carrageenan and xanthan.

The results presented in this study (Figure 1) suggest that addition of thickening agents, xanthan in particular, was able to improve yield during manufacture of concentrated yogurt. Previous report [7] also showed that the use of xanthan (0.01%) was more effective in improving viscosity and reducing syneresis of yogurt than the use of carrageenan. Both xanthan and carrageenan were recommended to improve rheological properties of yogurt. The application of xanthan as a thickening agents and stabilizer in yogurt is suggested because it is stable across a wide pH range, tolerance of high salinity, fast-hydrating, and easily dissolved at room temperature [12]. Other thickening agents that is carrageenan, has been known to have a wide application to thicken instant drinks and dairy desserts because it has a strong water-binding properties [13]. Overall, the addition of thickening agents prior to fermentation of the milk was able to retain some whey, and hence higher yield. The product posses desirable textural characteristics, that is soft and creamy with paste-like consistency.
Figure 1. Yield of yogurt curd and whey percentage during manufacture of concentrated yogurt from cow’s milk.

Figure 2. Degree of acidity (pH) of yogurt curd and whey during manufacture of concentrated yogurt from cow’s milk.

Figure 3. Syneresis and water holding capacity of yogurt curd during manufacture of concentrated yogurt from cow’s milk.
Figure 4. Titratable acidity of yogurt curd during manufacture of concentrated yogurt from cow’s milk

As presented in Figure 2, the effects of enzyme and thickening agents on the pH of yogurt curd and whey was minimal. The pH of yogurt curd in the present study (3.61) was slightly lower than that of goat milk yogurt treated with mTGase (3.8) [5]. Overall, the pH of both yogurt curd and whey was below the isoelectric point of casein (pH 4.6) [14]. At this point, the net negative charge on casein is reduced, hence the electrostatic repulsion between casein molecules is also decrease, which result in an increase of casein to casein attractions. The low pH of yogurt curd and whey can be attributed directly to the accumulation lactic acids produced by lactic acid bacteria from lactose during the course of fermentation. The accumulation of lactic acids is essential for the formation of yogurt texture, taste, also and contribute to the shelf life of the product. A previous study demonstrated that inulin improved yogurt gel textures without altering the pH, particularly in low fat or fat-free yogurt [15].

As shown in Figure 3, the addition of mTGase was able to produce yogurt curd with lowest syneresis and highest WHC. This evidence can be related to the the capability of mTGase in facilitating the formation of intramolecular cross-linking of milk protein, resulted in the formation of large molecular weight polymers. In turn, this contributed to the improvement of gel strength and less syneresis in yogurt as has been reported by previous study [16]. It is also apparent from this study that inulin is a potential thickening agent to improve processing properties during manufacture of concentrated yogurt. This can be related to the degree of chain polymerization of inulin, which able to form microcrystals. During processing, these microcrystals interacts with each other forming small aggregates, which encapsulate great amount of moisture [15]. In addition, the use of pectin and xanthan during manufacture of concentrated yogurt are also potential to maximize WHC, and at the same time minimize syneresis. In general, the WHC values of yogurt curd presented in this study was higher than WHC value of Greek-style yogurt added with pectin and whey protein concentrate (~56%) [17], which could be due to the amount of thickening agent added to the product.

Both syneresis and WHC are important parameters for yogurt and concentrated yogurt, because both are prone to structural arrangement. Yogurt curd or gels can be considered as a type of soft solid, which is relatively dynamic and susceptible to structural rearrangements[14]. Previous study showed that increasing the amount of total solids to the milk contributes to the increase of WHC and lower syneresis and porosity of yogurt, because it decreases the attractive forces between micelles of casein [8]. Furthermore, spontaneous syneresis (wheying-off) can be lowered to less than 5% by addition of albumin, whey protein concentrate, or sodium caseinate [18].
Based on the data presented in Figure 4, the use of mTGase, inulin and pectin produced yogurt curd with similar titratable acidity. The use of xanthan resulted in the lowest titratable acidity, followed by the use of carrageenan. The overall titratable acidity of yogurt curd (1.30 g /100 g lactic acid) is similar to the that of yogurt produced from sheep milk, 1.00 to 1.26% lactic acid[19] and goat milk yogurt with mTGase, 1.34 to 1.45% lactic acid [5].Titratable acidity of total acidity refers to the measurement of the total acid concentration contained within a food product, and more closely related to flavor than pH [20]. Based on national standard of Indonesia for yogurt (SNI 2981:2009), fresh yogurt should have titratable values between 0.5 to 2.0 [21].

4. Conclusion

Based on the evidence presented in this study, it can be concluded that concentrated yogurt with desirable quality characteristics can be manufactured from local fresh cow’s milk. The use of mTGase and thickening agents are recommended to improve yield and water holding capacity, and reduce syneresis of yogurt curd during manufacture of concentrated yogurt from cow’s milk. The use of xanthan resulted in the highest yield, whereas the use of inulin and mTGase produce yogurt curd with low syneresis and high water holding capacity. Further investigation is needed to study the effect of thickening agents on the rheological, microstructure and sensory properties of concentrated yogurt. As well, the nutritional composition and shelf life of the product still need further investigation.

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