Acidity dynamic in low-fat yogurt with the addition of potato starch modified by bacterial amylases

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Abstract. Potato starch was modified by two multienzyme bacterial preparation of Amylosubtilin® and α-amylase of Bacillus licheniformis at various enzyme concentrations. Eight types of modified starch were obtained and further applied to the mixture of low-fat yoghurt. In this research, we examined the acidity dynamic throughout the fermentation process of low-fat yogurt. It was found that the addition of modified potato starches with the concentration of 2% (w/w) did not affect the acidity dynamic compared to the native starch.

1. Introduction
Yogurt is well known for its various health benefits for human. Commonly the fat content of yogurt somewhere around 3.25% while another types of yogurt are low fat (0.5% to 2% milk fat) and nonfat (less than 0.5% milk fat). Lesser fat content is more preferred due to health concerns [1]. The loss of fat content in yogurt resulted in undesirable characteristics that might be affecting the acceptance of yogurt to the consumer. Those characteristics for instance are lack of firmness, difference in taste, flavor and aroma [2]. One of the solution to overcome the undesirable characteristics of low fat yogurt is by adding the fat replacer. While decreasing the calorific value of food, fat replacer can be used to solve some problems of physical and organoleptic originating from low fat final products [3]. Fat replacers consist of mixtures of lipid-originated fat substitutes, protein- or carbohydrate-originated fat mimetics, or their combinations [4]. In this research we used potato starch modified by bacterial amylases as fat replacer in low fat yogurt. Previous study [5] had revealed the effect of these modified starches in low fat yogurt on textural and sensory characteristic while in this paper we focused on the modified starches addition effect on the acidiy dynamic during fermentation process.

2. Materials and Methods
2.1. Enzyme preparation
Amylosubtilin and α-amylase of B. licheniformis preparation and amylase activity detection are described in the earlier studies [6].

2.2. Starch modification
Native potato starch (according to GOST P52791-2007 standard) was used for the research. Modification of these starches were based on the application of amylase of B. licheniformis (BI) and Amylosubtilin® (AM) of different concentrations (Table 1). Modification was carried out in water (30
g/100 ml), pH = 7, 40°C for 1 hour. The hydrolysis reaction was stopped with the addition of concentrated sulfuric acid (pH = 2). The starch was afterwards separated from the liquid by filtration and dried at 40°C.

**Table 1.** Code of modified starches

| Starches | Amylase activity (U/g starch) | Amylosubtilin (g/100 ml reaction mixture) | Amylase B. licheniformis (ml/100ml reaction mixture) |
|----------|-------------------------------|-----------------------------------------|--------------------------------------------------|
| AM-0.05  | 0.415                         | 0.01                                    | -                                                |
| AM-0.1   | 0.83                          | 0.0201                                  | -                                                |
| AM-0.05  | 4.15                          | 0.1005                                  | -                                                |
| AM-1     | 8.3                           | 0.201                                   | -                                                |
| BI-0.05  | 0.415                         | -                                       | 0.05                                             |
| BI-0.1   | 0.83                          | -                                       | 0.1                                              |
| BI-0.5   | 4.15                          | -                                       | 0.5                                              |
| BI-1     | 8.3                           | -                                       | 1                                                |

2.3. **Yogurt preparation**
Pasteurized cow’s milk (according to GOST 31450-2013 standard) subjected to centrifugation at 3000 g. Analysis of the physicochemical properties of milk was tested on a Klever-2M quality analyzer, according to the method recommended by the manufacturer. *Lactobacillus delbrueckii subsp. bulgaricus* strain 51 (isolated from Gastrofarm®), *Bifidobacterium BB-12 ™*, and *Streptococcus thermophilus* TH-4 (isolated from Bifiform Baby®) were used as the yogurt starter. Overnight yoghurt culture was grown on low-fat milk at 37°C for 16 hours. Milk without starch was used as a control, starch was added to the test samples at a concentration of 2% (w/w), the milk was heated to gelatinize the starch with constant stirring. After cooling the milky-starch mixture, the starting culture of yogurt was added to it in the amount of 5% (v/v). Fermented milk was incubated at 37 °C.

2.4. **Acidity analysis**
Measurements of pH were carried out by using a digital pH meter (HI 2216, Hanna Instruments, Germany).

3. **Results and Discussion**

**Table 2.** Initial chemical composition of the milk

| Chemical composition | Concentration (%) |
|----------------------|-------------------|
| Fat                  | 1.71              |
| Protein              | 3.31              |
| Lactose              | 4.6               |
| Salt                 | 0.74              |

Chemical composition of the milk used for the fermentation was shown in Table 2. The fat content was 1.71% thus the milk was considered low fat. Also the milk had sufficient amount of protein and lactose.
as the starting capital for the bacteria to ferment. It is already well known that lactose degradation of LAB resulted in lactic acid primarily and other acids [7].

![Figure 1. Acidity dynamic of low-fat yogurt with addition of amylosubtilin-modified starches.](image)

All of the treatments’ pH were around 6.6 as shown in Figure 1. As the fermentation progressed, the pH decreased for all treatments. In the fifth hour of fermentation there are slight differences in the pH of several treatments, the lowest pH was native starch (5.19) and the highest pH was A-0,1 (5.59). This could be caused by the difference of amylose and amylopectin ratio on the starches based on previous study [6]. Since some LAB produce amylase enzyme [8], the difference of amylose content in the starches might differentiate the time and concentration of acid production. Eventually all the treatments of amylosubtilin-modified starches resulted in pH of 4.5 at the 8th hour of fermentation.

![Figure 2. Acidity dynamic of low-fat yogurt with addition of amylase-modified starches.](image)

Compared to previous treatments of amylosubtilin, the treatments of amylase-modified starches shown in figure 2 did not present any significant differences. All of the treatments’ final pH are around 4.5 at the 8th hour.
4. Conclusion
The addition of modified potato starches with the concentration of 2% (w/w) did not affect the acidity dynamics during fermentation process of low-fat yogurt compared to the addition of native starch. The fermentation time for the low fat yogurt with the addition of bacteria modified starches is eight hours.

5. References

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