RESEARCH ON THE INFLUENCE OF TECHNOLOGICAL FACTORS ON THE QUALITY OF KEFIR FROM MILLET *(PANICUM MILIACEUM)*

Tran Thi Thanh Thao
Master of chemical technology
Faculty of Chemical Engineering
Viet Tri University of Industry,
Lam Thao, Phu Tho, Vietnam
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Abstract

In this study, the effects of technological factors on the quality of kefir yogurt from millet (*Panicum Miliaceum*) were investigated. The nutritional composition of millet was analyzed, the factors influencing the fermentation process of millet yogurt were identified. These influencing factors were determined, including a raw material-to-water ratio of 1/1 (g/ml), 4% added sugar, 6% added starter culture, and the fermentation time of 28 hours (based on the volume of the milk mixture).

Keywords: Yogurt, kefir grains, millet, fermentation, cereal, starter yeast.

Introduction

Malnutrition remains a major public health concern in many developing countries, particularly among children [1]. Low birth weight or malnourished children often suffer from compromised immune systems, making them more susceptible to infectious diseases and increasing the risk of mortality [2]. According to the United Nations Standing Committee on Nutrition, one of the key factors influencing the nutritional status of young children is the quality of complementary foods and functional foods [3].

In recent years, the global consumption of fermented products has been increasing due to their health-promoting effects and associated functional properties [4–8]. Fermented products contain probiotics that confer various health benefits to consumers [9,10]. These microorganisms may be naturally present in food or may be introduced as starter cultures after pre-treatment or cooking of the product [11]. Fermentation is also regarded as a traditional, long-standing, and cost-effective method of food preservation [12].

Kefir is a traditional fermented dairy beverage originating from the Caucasus Mountains. Today, kefir products are among the most widely consumed probiotic foods globally [5,13,14]. Kefir grains are composed of symbiotic lactose-fermenting and non-lactose-fermenting yeasts, as well as lactic acid bacteria, all embedded in a matrix of polysaccharides and proteins known as kefiran, which is believed to exhibit anticancer properties [4,13,14]. Thus, kefir is a nutrient-rich functional food that benefits the immune and anti-inflammatory systems, and it has been used as a dietary supplement for individuals with immunodeficiency syndromes, chronic fatigue syndrome, herpes, and cancer [4,5]. Moreover, the consumption of kefir delivers beneficial bacteria, yeasts, vitamins, minerals, fatty acids, and complete proteins, all of which are associated with a variety of health-promoting effects, including anti-inflammatory, anticancer, antimicrobial, and antidiabetic properties; reduction of serum cholesterol levels; improved digestion and gut health; reduced blood pressure; and regulation of reactive oxygen species [4,9,13].

The increasing prevalence of lactose intolerance, dairy allergies, and cholesterol-related health concerns has driven a growing consumer trend toward non-dairy alternatives to cow's milk. Furthermore, the rising popularity of plant-based diets, with increasing numbers of vegan and vegetarian consumers, underscores the global significance of plant-based probiotic products [15]. In response, various kefir-like formulations have been proposed, utilizing plant-based milk and alternative carbohydrates to produce diverse non-dairy probiotic beverages with high added value, which may be classified as functional foods [14,16].

Proso millet *(Panicum miliaceum)* is the sixth most important cereal crop globally and belongs to the Poaceae family. Nutritionally, millet is comparable to other staple cereals such as wheat and rice [17]. It is a gluten-free, nutrient-dense grain that may help prevent and manage various health conditions, including hypertension, cardiovascular diseases, cancer, and tumors. Millet also supports digestion and serves as a source of dietary fiber for gut health [16]. In addition, millet provides antioxidant compounds that help protect human health from serious diseases and play critical roles in the immune system [18]. However, millet is currently used mainly in traditional grain-based forms, and its potential for broader applications remains underexplored. Furthermore, domestic research on this nutritious cereal is still limited.

Therefore, the objective of this study is to investigate the effects of technological parameters on the quality of yogurt produced from millet.

Materials and Methods

Materials and Chemicals

The millet used in this study was pre-dehulled glutinous yellow millet, sourced from Hạt Xanh Organic Store, originating from Nghệ An province, Vietnam. The kefir starter culture was supplied by Nihon Kefia Co., Ltd. (Japan). The sugar used in the formulation was refined white crystalline sugar, with a saccharose content ≥ 99.5%, moisture content ≤ 0.2%, and ash content ≤ 0.03%.

Physicochemical Analysis of Yogurt Samples pH Measurement: The pH values of the samples were determined using a handheld Aqualytic pH meter (Taiwan).

Soluble Solid Content (°Brix): Soluble solids were measured using a handheld refractometer (Agator, Japan) [19–21].

Total Solid Content: Determined by oven-drying the samples to constant weight at 102 ± 2°C [22].

Preparation of Yogurt

Starter Culture Activation: All glassware used in the cultivation of kefir starter was sterilized at 121°C for 15 minutes. A total of 20 mL of boiled and cooled water (to 40°C) was used to dissolve the powdered kefir starter, and the solution was left to rest for 1 hour. Subsequently, 500 mL of unsweetened pasteurized milk was heated to 40°C. The activated kefir culture was then added to the milk at the same temperature and mixed thoroughly using a plastic spoon for homogenization. The mouth of the glass container was covered with cheesecloth to allow air circulation. The mixture was fermented at ambient temperature for 24 hours. After fermentation, the container was sealed and stored in a refrigerator. As the kefir grain population increased with repeated culturing, the volume of fresh milk was adjusted proportionally.

Yogurt Production from Millet: Millet grains were soaked in water, drained, and blended with water at an appropriate grain-to-water ratio using a household blender. The resulting mixture was filtered to obtain millet milk. Sugar was added to the millet milk, which was then boiled and cooled to 40°C. The activated starter culture was added at an appropriate ratio, and the mixture was incubated for fermentation. After fermentation, the yogurt samples were evaluated for pH, soluble solids, and sensory characteristics.

Homogeneity Assessment

Sample homogeneity was assessed following a modified version of the method by Hinds et al. [23]. Suspension stability was evaluated based on variations in total solid content at different sampling locations within the product. The relative standard deviation (RSD) was calculated to assess the distribution of solids using the following formula:

$$RSD = \frac{SD}{X}$$

Where: SD = standard deviation; X = average solid content measured at three different sampling positions

Sensory Evaluation: Ranking Preference Test

Samples were coded with three-digit random numbers. A panel of five assessors participated in the sensory evaluation. A four-point ranking scale was used, with rank 1 representing the most preferred sample and rank 4 the least preferred. Sensory attributes assessed included color, aroma, taste, texture, and overall acceptability..

Results and discussions

Effect of the Ingredient-to-Water Ratio

An appropriate millet-to-water ratio is essential to obtain milk extract with desirable quality for yogurt production. Experimental observations indicated that a ratio of 1:1 (g/mL) provided optimal results, producing a yogurt with suitable consistency and acidity, without phase separation. In contrast, increasing the water proportion led to excessive whey separation, paler color, and increased sourness in the final product.

As shown in Table 1, increasing the water content significantly reduced the total soluble solids in both pre-fermentation and post-fermentation samples. The pre-fermentation milk exhibited mildly acidic pH values, ranging from 5.57 to 5.99, across all ingredient-to-water ratios. All samples demonstrated satisfactory fermentation capability, as evidenced by a marked decrease in pH values after fermentation. Notably, the ingredient-to-water ratio did not significantly affect the final pH of the fermented samples, with post-fermentation pH values ranging from 3.27 to 3.37.

Furthermore, the sensory evaluation in Table 1 revealed that the 1:1 (g/mL) ratio was the most preferred across all criteria, including color, aroma, taste, and texture. Based on these findings, the 1:1 millet-to-water ratio was deemed optimal and subsequently selected for further experiments in the study.

***Table 1.* Brix Value, pH, and Sensory Evaluation of Yogurt Samples**

Ingredient-to-Water Ratio		1/1	1/1.5	1/2	1/2.5	1/3	1/4	1/5
Bx	**Before**	23,60	21,25	18,83	17,33	15,83	13,83	6,93
	After	18,10	14,16	7,50	6,83	4,33	6,16	5,91
pH	**Before**	5,99	5,57	5,60	5,57	5,56	5,60	5,64
	After	3,28	3,27	3,27	3,37	3,29	3,24	3,37
Color		4,00	1,75	2,50	3,00	2,75	1,75	2,25
Aroma		3,25	1,50	2,00	2,25	2,75	2,25	1,50
Flavor		3,25	2,50	2,00	1,75	2,75	1,75	1,25
Consistency		3,25	2,00	2,25	2,75	3,00	1,50	2,00
Mean Value		**3,44**	**1,94**	**2,19**	**2,44**	**2,81**	**1,81**	**1,75**

Investigation of the Effect of Inoculum Ratio

The proportion of added starter culture has a significant influence on the final product. If the inoculum ratio is too low, the fermentation time becomes excessively long, increasing the risk of contamination by undesirable microorganisms, which can spoil the product. Conversely, a high inoculum ratio can shorten the fermentation time but may increase production costs due to higher input of starter culture. Observations of the samples indicate that an inoculum ratio of 6% yields yogurt with a moderate consistency, no phase separation, and a pleasantly mild sour aroma post-fermentation. Lower ratios (2–4%) result in foaming and whey separation, reducing the uniformity and overall quality of the yogurt. When the inoculum ratio increases to 7%

and 8%, phase separation and gas bubble formation become more severe, causing the product to lose its homogeneity.

Table 2 shows a clear variation in total soluble solids content before and after fermentation. Most yogurt samples after fermentation exhibited soluble solids content ranging from 13.33 to 15.66 °Brix. The highest difference in soluble solids content was observed in the sample supplemented with 6% starter culture. Furthermore, as the inoculum ratio increased, the pH of the final yogurt samples decreased. The results indicate that the addition of 2% to 5% starter culture was insufficient for complete fermentation, as evidenced by relatively higher pH values. In contrast, the pH decreased significantly in samples supplemented with 6% inoculum. However, further increasing the inoculum ratio beyond 6% did not significantly improve fermentation efficiency. Based on these results, the yogurt sample with 6% starter culture was rated the most favorable across all sensory attributes, including color, aroma, flavor, and texture (Table 2).

***Table 2.* Brix Value, pH, and Sensory Evaluation of Yogurt Samples**

Inoculum ratio, %		**2**	**3**	**4**	**5**	**6**	**7**	**8**
Bx	**Before**	17,00	15,66	15,33	16,66	17,00	16,00	16,00
	After	15,66	13,66	13,33	14,66	14,83	14,66	13,33
pH	**Before**	5,96	5,88	5,87	5,82	5,99	5,84	5,79
	After	4,95	4,79	4,70	4,55	4,37	4,42	4,40
Color		2,25	3,25	2,00	3,25	3,50	2,00	1,00
Aroma		1,50	2,25	2,75	3,00	3,75	1,50	1,50
Flavor		1,00	2,00	2,25	2,75	3,00	1,25	1,50
Consistency		1,00	2,25	1,25	2,25	3,25	1,00	1,00
Mean Value		**1,44**	**2,44**	**2,06**	**2,81**	**3,38**	**1,44**	**1,25**

Thus, the yogurt sample fermented with 6% inoculum exhibited superior quality across all evaluated parameters. Therefore, the 6% inoculum ratio was selected for subsequent experiments.

Investigation of the Effect of Fermentation Time

Fermentation time has a direct impact on both the efficiency of the fermentation process and the quality of the final yogurt product. Preliminary evaluations of the product state revealed that insufficient fermentation time resulted in phase separation, a sour odor, and in some cases, the formation of surface films. Fermenting for 28 hours yielded a yogurt with desirable homogeneity, moderate sourness, and no phase separation. Conversely, prolonged fermentation led to syneresis (whey separation), excessive foaming, and an overly sour odor, all of which negatively affected product quality.

Most pre-fermentation milk extracts had similar pH values, ranging from 3.63 to 4.14. As shown in Table 3, fermentation times between 16 and 24 hours resulted in only slight decreases in pH, indicating that the fermentation process had not yet fully progressed.

***Table 3.* Brix Value, pH, and Sensory Evaluation of Yogurt Samples**

Fermentation time (hours)		**16**	**20**	**24**	**28**	**32**	**36**	**40**
Bx	**Before**	14,67	17,67	17,33	14,67	15,67	17,33	17,67
	After	12,67	14,33	15,60	11,67	11,67	15,67	15,67
pH	**Before**	4,94	4,69	4,63	4,91	4,90	4,54	4,85
	After	4,04	4,11	4,00	3,63	3,70	3,97	4,14
Color		2,00	1,75	3,00	3,75	3,00	2,75	2,25
Aroma		1,25	1,25	1,75	3,25	2,75	2,00	2,00
Flavor		1,25	1,25	1,25	3,00	2,25	1,25	1,25
Consistency		1,00	1,75	2,50	3,25	2,00	2,25	2,00
Mean Value		**1,38**	**1,50**	**2,13**	**3,31**	**2,50**	**2,06**	**1,88**

When the fermentation time was increased to 28 hours, the greatest difference in pH was observed, indicating the highest fermentation efficiency. Additionally, the total soluble solids (TSS) content in fermented yogurt samples over the fermentation period of 16 to 40 hours ranged from 11.67 to 15.67 °Bx. The most significant difference in °Bx values before and after fermentation occurred between 28 and 32 hours (Table 3). Sensory evaluation results also demonstrated that the yogurt fermented for 28 hours achieved the highest scores in terms of color, aroma, taste, and overall acceptability (Table 3). Therefore, the optimal fermentation time was determined to be 28 hours.

Investigation of Saccharose Content Added to Fermented Milk

Experimental observations indicated that at 2–4% added sugar, the yogurt exhibited a high viscosity and mild sourness. At the 4% level, the yogurt achieved a balanced structure and flavor. However, at 6% and above, phase separation began to appear, and the sweetness became more pronounced. When the saccharose concentration reached 8% or higher, water separation increased significantly, compromising product homogeneity, and the sweetness became excessively strong compared to the typical taste profile of yogurt.

The pH values of the milk prior to fermentation ranged from 5.81 to 6.02, indicating a relatively stable and near-neutral environment. After fermentation, as the saccharose content increased, the pH ranged from 4.36 to 4.91, showing a trend toward higher pH values, meaning the resulting yogurt was less acidic. Lower sugar concentrations (2–6%) led to more acidic products, resulting in a distinct sour taste, while higher sugar levels (8–12%) produced less acidic and sweeter yogurts, which might lack the traditional sour profile characteristic of yogurt.

Table 4 shows a decreasing trend in °Bx values as the saccharose content increased. This could suggest that higher sugar concentrations may hinder fermentation efficiency or result in incomplete sugar consumption. Sensory evaluation results revealed that the sample containing 4% added sugar received the highest scores in all criteria, including color, aroma, taste, texture, and overall preference.

Thus, based on the combined evaluation of physical and sensory parameters, the yogurt sample formulated with 4% saccharose was considered the most suitable.

***Table 4*. Brix Value, pH, and Sensory Evaluation of Yogurt Samples**

Sugar Content, %		**2**	**4**	**6**	**8**	**10**	**12**
Bx	**Before**	20,3	20,67	20,33	23,33	23,67	26,67
	After	15,67	16,33	17,33	20,67	21,83	24,50
pH	**Before**	5,82	6,02	5,86	5,85	5,84	5,81
	After	4,36	4,38	4,41	4,71	4,76	4,91
Color		2,60	3,60	2,40	3,20	3,40	3,40
Aroma		2,20	3,60	3,40	2,80	2,80	3,40
Flavor		1,40	3,00	1,60	2,20	1,80	1,60
Consistency		3,20	3,60	2,00	3,20	3,20	3,40
Mean Value		**2,35**	**3,45**	**2,35**	**2,85**	**2,80**	**2,95**

Conclusions

The study has investigated and analyzed the factors affecting the fermentation process of yogurt made from millet seeds. These influencing factors were identified as follows: a raw material-to-water ratio of 1/1 (g/ml), a sugar supplementation ratio of 4%, a yeast inoculum ratio of 6%, and a fermentation time of 28 hours (based on the volume of milk mixture).

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