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Technological Feasibility and Sensory Evaluation of Lactose-Free Fresh Cheese in Vietnam

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Abstract

Lactose intolerance is prevalent in Asia, affects approximately two-thirds of the Asian population. Therefore, the market for lactose-free dairy products, which can provide essential macro and micro-nutrients to lactose-intolerant people especially in Vietnam, holds significant promised. In this study, the lactose-free cheese production from Vietnamese raw milk was developed. The conditions of commercial lactase enzyme for lactose hydrolysis were investigated. Lactic acid bacteria were added to the lactose-free milk to reduce the pH and increase the texture and aroma of the product. As results, lactase enzyme should be used at the concentration of 1 mL/L of raw milk in 90–120 min at 40–42oC to totally hydrolyse lactose. The detailed fatty acid profile of lactose-free cheese was identified, where the saturated fatty acids are dominant (palmitic acid: 59,245 µg/g cheese, myristic acid: 21,408 µg/g cheese, and stearic acid: 16,828 µg/g cheese). The sensory analysis results showed that the sweetness between the lactose-free and normal fresh cheese is significantly different, and the sensorial committee preferred lactose-free cheese to normal fresh cheese. Nevertheless, new cheese product does not contain lactose is completely feasible in terms of technology and the established process could be reliably scaled up to pilot scale prior to commercialization.

Keywords: Lactose-free cheese, lactic acid bacteria, lactose-intolerance, sensory analysis.

1. Introduction

Lactose is the main sugar in milk and in dairy products. However, this sugar could not be directly absorbed by the human body, that could lead to the clinical condition named lactose intolerance. This is a prevalent worldwide symptom, that occurs from 57 – 65% of the world population, and up to 70% in Asia [1]. Common signs of lactose intolerance include abdominal pain, bloating, flatulence, diarrhea, borborygmi, nausea, and vomiting [2]. Regarding the prevalence of this symptom, many strategies have been developed to eliminate the presence of lactose in milk. Currently, three primary methods are widely employed: enzymatic hydrolysis, membrane filtration, and fermentation [3]. Most studies focused on using β-galactosidase enzyme (commonly known as lactase) to hydrolyse lactose to glucose and galactose. These products are normally sweeter than non-treated dairy products [4]. In the production of lactose-free milk by lactose hydrolysis, manufacturers typically rely on two primary techniques: batch and aseptic processing [3].

In a study on the Spanish market, Rodríguez *et al.* [5] analysed the nutrition of 327 lactose-free labelling products. No significant differences were found between the nutritional composition of lactose-free and traditional products, suggested that the array of lactose-free products was enough, both in quantity and quality, to satisfy the dairy needs of lactose intolerants in Spain [5]. Furthermore, sensory analysis is frequently applied to evaluate the consumer acceptance for lactose-free products [3, 6, 7]. Previously, the volatile compound profile and the corresponding sensory attributes of lactose-free kefir were compared with the traditional one. The consumer test of 256 Polish consumers (from 65–76 years old) showed that the right balance between sweet and sour taste influenced hedonic acceptability of lactose-free kefir by the elderly, making these taste attributes key factors in their overall liking of the product [6]. Cincotta *et al.* [7] compared the lactose-free and the traditional mozzarella cheese by their aroma compounds and sensory features. It is reported that after 13 days of storage, bitter and acid taste and yoghurt odours were described in lactose-free mozzarella cheese. The differences between the sensory features of two cheese types could be due to the proteolytic activity of the lactase enzyme [7].

In Vietnam, with the increase of the cheese demand, local dairy manufacturers take attention on the production of cheese products, that are currently mainly imported. Some studies have been carried out to investigate the ability to use Vietnamese raw milk for fresh cheese production [8-10]. Nguyen *et al.* [10] reported the milk coagulation properties for quark

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cheese processing and identified the appropriate conditions: heat treatment at 60 °C for 15 minutes, coagulation temperature at 40 °C, and pH 5.5, $CaCl_2$ concentration at 0.04 g/L [10]. In another study, Hoang *et al.* [8] showed that the pre-fermentation using *Streptococcus thermophilus* at 10^6 CFU/mL at 40 °C for 115 minutes could improve the curd yield and the flavour of fresh cheese [8]. The hydrolysis of lactose using Lactozyme enzyme was reported by Ngo *et al.* [11] The hydrolysis was carried out at 30–35 °C for 3h, pH 7, and the degree of hydrolysis was obtained over 80% [11]. However, the number of research on lactose-free cheese in Vietnam is limited.

The objectives of this study are: i) to investigate the appropriate conditions for lactose hydrolysis; ii) to apply those conditions to the production of lactose-free cheese at lab scale; iii) to evaluate the sensory of this product.

2. Materials and Methods

2.1. Materials

Raw milk used in this study was collected in Phu Dong village, Hanoi from February to September 2025. Milk was stored at 4–6 °C after collection, and then transported to Laboratory of Food Technology, Hanoi University of Science and Technology. Milk's quality was then analysed in accordance with TCVN 7405:2018.

NOLA™Fit5500 (β-galactosidase enzyme), provided by CHR Hansen, Denmark, was used to hydrolyse lactose in milk. STI-12 (*Streptococcus thermophilus*), provided by CHR Hansen, Denmark, was used with recommended concentration of 100U/1000 L milk. MARZYME® SUPREME DS 5 GAL (protease enzyme origin *Rhizomucor miehei*), provided by Dupont Danisco, Denmark, was used for milk coagulation.

$CaCl_2$ (80% purity) was obtained from Solvay, Italy. Lactic acid solution (88% purity) was obtained from PURAC® FCC, Thailand.

2.2. Methods

2.2.1. Investigation of lactose hydrolysis conditions

500 ml of raw milk was prepared in a 1 L container. The raw milk was then pasteurised at 65 °C for 15 minutes before cooling to 40 °C as described in previous study [10]. NOLA™Fit5500 was added at different concentrations (0.25, 0.5, 0.75, and 1 mL/L milk). The hydrolysis was carried out at 40 °C and different durations (30, 45, 60, 75, 90, and 120 minutes). After lactose hydrolysis, NaOH 1N was added until pH equal 11 to stop the reaction. Lactose hydrolysis efficiency was then calculated based on the following formula:

$$H\% = \frac{M2}{M1}.100\%$$

where:

M_1: initial lactose mass

M_2: hydrolysed lactose mass

2.2.2. Lactose-free and reference cheese production

500 ml of raw milk was prepared in a 1 L container. The raw milk was then pasteurised at 65 °C for 15 minutes before cooling to 40 °C.

- For lactose-free cheese: The hydrolysis of lactose was carried out with the presence of NOLA™Fit5500 (1 mL/L milk) at 40 °C for 90 minutes. STI-12 (0.1 U/L milk) was then added to the lactose-free milk. When pH reached 5.5, $CaCl_2$ (0.3 g/L milk) and MARZYME® SUPREME DS 5 GAL (48 IMCU/L milk) were used for coagulation at 38–40 °C.

- For reference cheese: Lactic acid was added to the raw milk until pH reached 5.5. The coagulation was carried out at 38–40 °C with the presence of $CaCl_2$ (0.3 g/L milk) and MARZYME® SUPREME DS 5 GAL (48 IMCU/L milk).

After coagulation, the curd was cut into 1 cm^3 cubes and gently stirred at 38 °C before draining. The curd was transferred to molds with a pressure of 265 kg/m^2 for 1h. Lactose-free cheese was then withdrawn from molds, wrapped in plastics bags, and stored at 4 °C.

2.2.3. Sensory analysis

Sixty participants from 18–25 years old were asked to evaluate both regular and lactose-free cheese samples using sensory scoring method. The evaluation criteria included: colour attributes, surface characteristics, smoothness by looking, fresh milk flavour, buttery flavour, creamy flavour, yogurt flavour, freshness, stickiness, solubility, smoothness, flexibility, saltiness, sourness, sweetness, vanilla flavour, richness, salty aftertaste, sour aftertaste, sweet aftertaste, fatty aftertaste, and overall liking. For overall liking, the score was from 1 to 9 while for other criteria, the score was from 0 to 5.

2.2.4. Analysis methods

Density, pH, and total dry matter of raw milk were analysed according to the TCVN 7405:2018 (ISO 6731:2010). Titratable acidity was measured according to AOAC 947.05. Protein and fat content were determined using Kjeldahl method (ISO 8968-1:2014) and Gerber method (ISO 19662:2018), respectively.

Textural properties were analysed by texture analyser TA.XT plus (Stable Micro System, Godalming, UK) according to Nguyen *et al.* [10]. Texture profile analysis (TPA) included the assessment of the following characteristics: hardness, springiness, and cohesiveness.

The fatty acids profile of the lactose-free cheese was analysed using gas chromatography coupled with mass spectrometry (GC-MS) in accordance with Kourimska *et al.* [12]. The milk fat is separated by using the acid-butyrometric method. The clear fat is removed from the butyrometer with a syringe. 50 µl was taken for the re-esterification. The fat is diluted in 0.5 ml of methanol and then methylated with 0.5 ml of methanolic base. The mixture is heated for 5 minutes in water bath with temperature 65 °C. Then the samples are cooled, and the methyl esters of fatty acids are extracted in 1.5 ml of hexane. A saturated salt solution is also added for better separation of the hexane phase during the centrifugation. The samples were centrifuged at 5000 RPM for 10 minutes, then 1 ml of the hexane layer is transferred into the vial for GS/MS. The analysis was performed using an Agilent DB-5MS column (30m x 250µm x 0.25µm). The starting temperature of the column is at 70 °C, and the injection temperature is at 230 °C. Injected volume is at 1 µl. Helium was used as the carrier gas at a flow rate of 1 ml/min.

Lactose concentration was determined through the amount of glucose obtained after lactose hydrolysis using the D-Glucose assay kit (GOPOD) Format (Megazyme, Ireland). The limit of detection (LOD) of this kit is 40 mg/L. First, milk protein was precipitated according to Vu *et al.* [13]. The glucose concentration was then determined following the instructions of the manufacturer. Lactose concentration was then calculated by the formula:

$$\text{Lactose}\left(\frac{\text{g}}{1000\ \text{g}}\right) = \text{glucose}\left(\frac{\text{g}}{\text{L}}\right) \times \frac{342}{180} \times \frac{1}{1{,}032}$$

where:

180: Molecular weight of glucose

342: Molecular weight of lactose

1,032: Density of milk

Cheese recovery yield is calculated by the ratio of the mass of cheese obtained to the initial mass of milk.

2.2.5. Statistical analysis

All experiments were performed in triplicate. The obtained results were statistically analysed at the significance level p equal 0.05 using Microsoft Excel software. Mean values and standard deviations were calculated, the values were compared by ANOVA method.

3. Results and Discussion

3.1. Quality of the Raw Milk

After collection, raw milk properties were analysed and presented in Table 1. These results demonstrated that the raw milk collected from Phu Dong village met the demand of milk used for cheese production in Vietnam.

Table 1. Physicochemical parameters of raw milk

Parameter	Raw milk	Vietnamese standard TCVN 7045:2018
Dry matter (%)	12.77 ± 0.46	11.5
Fat (%)	3.94 ± 0.39	3.2
Protein (%)	3.14 ± 0.16	2.8
Acidity (°T)	17.8 ± 0.7	16 - 21
Sensory	White colour, characteristic light aroma, homogeneous solution and no impurities	From white to light cream colour, characteristic odour, homogeneous solution, no impurities visible to the naked eye

3.2. Enzymatic Hydrolysis of Lactose in Vietnamese Bovine Milk

Fig. 1 demonstrates a decrease in lactose content across all four concentrations of lactase over time. The higher the lactase concentration, the higher amount of lactose was hydrolysed. After 90-minute reaction, all lactose was hydrolysed with lactase concentration of 1 mL/L, while the concentration of lactose reduced by 88%, 72%, and 58% with lactase concentration of 0.75, 0.5, and 0.25 mL/L, respectively. This result is similar to previous studies. Czyzewska *et al.* [14] reported the encapsulation of NOLA™Fit5500 to obtain lactose-free milk at low temperature. The complete lactose decomposition was observed after 30 minutes with enzyme concentration of 1.5 g/L [14]. Moreover, application of NOLA™Fit5500 at high enzyme to substrate ratio of 80 lactase units/g lactose resulted in hydrolysis degree of 96% after 7h at 45–50 °C [15]. In a study on lactose-free cheese production from sheep's milk, Pulinas *et al.* [16] investigated the use of lactase enzyme at different concentrations of 0.7, 0.9, and 1.1 g/L. Results showed that at concentration of 1.1 g/L, lactose concentration in milk reduced to below the limit of detection of 0.04 g/L [16].

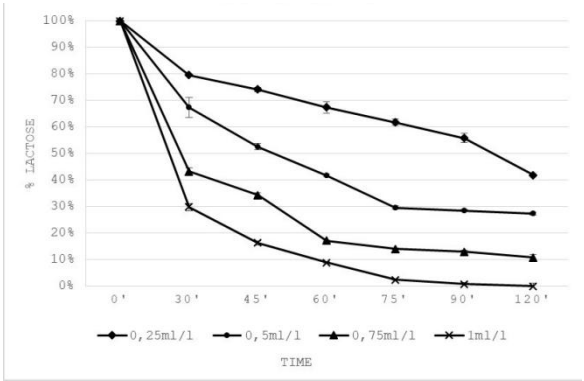


Fig. 1. Lactose content in samples treated with lactase enzyme at various concentrations and times

3.3. Characteristics of Lactose-Free Cheese

3.3.1. Physicochemical characteristics of lactose-free cheese

After identifying the using conditions of lactase enzyme, the manufacture of lactose-free cheese was carried out using either lactic acid bacteria or lactic acid to reduce the pH of the milk.

Table 2. Physicochemical parameters of reference and lactose-free cheese

	Reference cheese	***Lactose-free cheese***
Cheese recovery yield (%)	15.15 ± 0.14	17.18 ± 0.27
Moisture content (%)	47.82 ± 0.23	49.34 ± 0.36
Fat content (%)	20.90 ± 0.12	20.35 ± 0.15
Soluble protein content (%)	20.40 ± 0.21	20.46 ± 0.18
Lactose content (%)	3.64 ± 0.1	-

Overall, there were no significant differences in fat content and soluble protein content between the reference cheese and the lactose-free cheese. The lactose-free cheese met the requirement of no detectable lactose, while the reference cheese had a lactose content of 3.5–3.8%. In fact, the preheating process during lactose hydrolysis pretreatment in the lactose-hydrolysed cheese caused increased interactions between casein and whey proteins, enhancing the whey-holding capacity of the cheese [17], thus the cheese recovery yield of lactose-free cheese was higher than that of reference cheese.

Table 3 shows the fatty acid profile of lactose-free cheese. Saturated fatty acids (SFA) are the main fatty acids, in which palmitic, myristic, and stearic are the most abundant (59,245 µg/g cheese, 21,408 µg/g cheese, and 16,828 µg/g cheese, respectively). Among unsaturated fatty acid, oleic, and linoleic are the predominant (16,471 µg/g cheese, and 13,460 µg/g cheese, respectively). Similar content of saturated and unsaturated fatty acids was reported in normal cheese from different milk sources [18-20]. Regarding the nutritional value, a lactose-free product should not only be free of lactose but also match the nutritional profile of products containing lactose. Our results suggested that this lactose-free cheese could meet the nutritional requirements for commercial un-ripened cheese in Vietnam as described in national regulation QCVN 5-3:2010/BYT.

Table 3. Fatty acid profile of lactose-free cheese

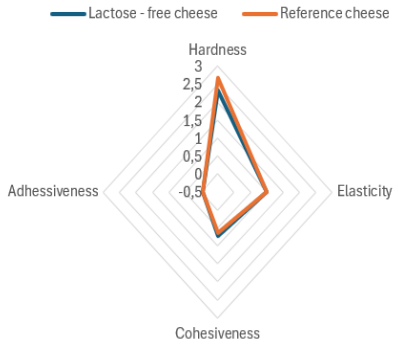
Fatty acid	**Content (µg/g)**	**Fatty acid**	**Content (µg/g)**
Butyric acid	875	cis-9_oleic acid	16,471
Decanoic acid	2,421	Erucic acid	62
Undecanoic acid	61	Linoleic acid	13,460
Lauric acid	5,371	Arachidonic acid	204
Tridecanoic acid	52	cis11,14-eicosadienoic acid	118
Myristic acid	21,408	cis-11-eicosanoic acid	245
Myristoleic acid	2,184	Linolenic acid	2,381
Pentadecanoic acid	1,544	Behenic acid	7
cis-10_pentadecenoic acid	1,216	Tricosanoic acid	2
Palmitic acid	59,245	cis-5,8,11,14-eicosictraenoic acid	159
Palmitoleic acid	3,576	cis-5,8,11,14,17-eicosapentaenoic acid	61
Heptadecanoic acid	635	Stearic acid	16,828
cis-10_heptadecenoic acid	668	trans-9_eladiic acid	5,946

3.3.2. Structural characteristics of lactose-free cheese

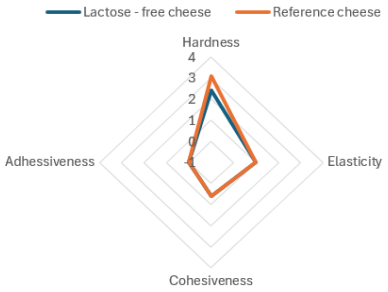
The lactose-free cheese was significantly softer than the reference cheese after 24h and 72h of storage (Fig. 2). This softness can be explained by the higher moisture content in the lactose-free cheese. It is well-known that increasing moisture content in cheese leads to a decrease in hardness. McMahon *et al.* [21] reported that increasing moisture content through whey separation and pressing resulted in both softer and stickier cheese [21].

The reference cheese showed a heterogeneous and fragmented structure (Fig. 3). However, the lactose-free cheese exhibited a homogeneous structure (Fig. 4) due to the starter culture's ability to ferment milk uniformly, resulting in a consistent composition. The starter culture also contributed to a firmer cheese structure by producing substances that bind the components together. This finding is supported by Kongo *et al.* [22] who reported that exopolysaccharides produced by starter

cultures increased viscosity and firmness, improving the product's structure [22].



a. Structure of the lactose-free cheese after 24 hours



b. Structure of the lactose-free cheese after 72 hours

Fig. 2. Structural properties of the lactose-free cheese after 24 and 72 hours



Fig 3. The structure of cheese reference cheese before and after being subjected to the force of the texture probe



Fig 4. The structure of lactose-free cheese before and after being subjected to the force of the texture probe

Texture profile analysis revealed that the lactose-free cheese exhibited significantly improved structural properties, as evidenced by increased hardness and cohesiveness, compared to the reference cheese (Table 4). Thus, the simultaneous addition of NOLA™Fit5500 and STI-12 during milk pretreatment can enhance the structure of lactose-hydrolysed cheese.

Table 4. Hardness, elasticity, and cohesiveness of cheese

	Reference cheese	**Lactose-free cheese**
Hardness (g/cm^2)	2.663 ± 0.076	2.835± 0.084
Elasticity (cm)	0.999 ± 0.001	0.999 ± 0.001
Cohesiveness	0.626 ± 0.064	0.813 ± 0.037

3.3.2. Sensory analysis of lactose-free cheese

Sixty participants from 18–25 years old participated in the evaluation of lactose-free and reference cheese. It is shown that the lactose hydrolysis process did not significantly alter the overall sensory profile of the cheese, except for sweet aftertaste (0.89 and 0.53 in lactose-free cheese and reference cheese, respectively) (df = 1, F-value = 3.868, p–value = 0.018) (Fig. 5), and a notable increase in sweetness, which was twice as high in the lactose-free cheese (1.32) compared to the reference cheese (0.68) (df = 1, F-value = 12.406, p–value = 0.001) (Fig. 6). The similar influence of sweetness could be observed in previous studies [4, 6, 7].

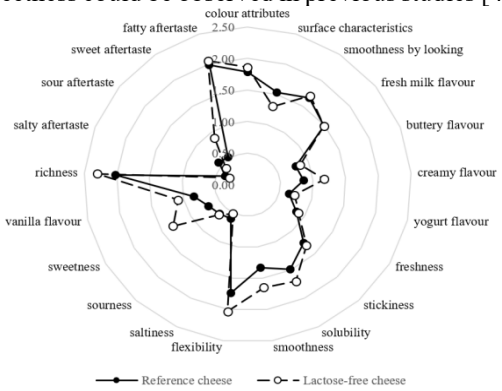
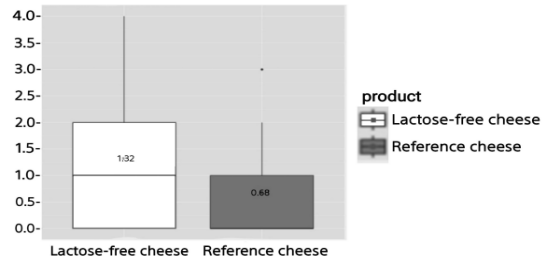
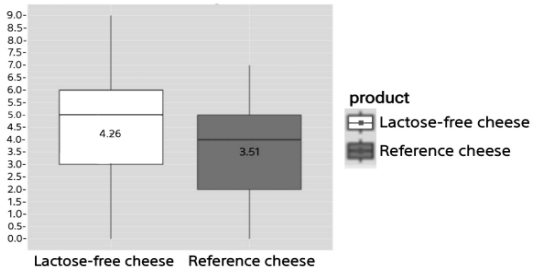


Fig 5. Sensory profile of lactose-free and reference cheese

Lactose-free cheese was significantly preferred by consumers, achieving an overall liking score of 4.26 compared to 3.51 for reference cheese (df = 1, F-value = 4.527, p–value = 0.036). This finding aligns with previous studies, indicating increased snacking frequency and a preference for sweeter, processed foods [23]. The lactose-free products are normally sweeter than non-treated dairy products [4]. In Vietnam, sweetness was reported to be one of the key sensory attributes driving consumer preference [24]. Thus, the higher sweetness level of lactose-free cheese likely contributed to its greater consumer acceptance, especially for young Vietnamese people.



a) Sweetness taste mark of lactose-free and reference cheese



b) Liking mark of lactose-free and reference cheese

Fig. 6. Sweetness and liking mark of lactose-free and reference cheese

4. Conclusion

The optimal duration of lactase treatment and concentration for complete hydrolysis of lactose in raw milk were identified. Besides, the fatty acid profile of lactose-free cheese was defined, that is similar to other cheese products. Regarding the simultaneous addition of lactase and lactic acid bacteria *S. thermophilus*, the structure of lactose-free cheese exposed a homogeneous structure rather than reference cheese. In addition, sensory test revealed the preference of consumer to lactose-free cheese, characterized mostly by the increase of sweetness, that is appropriate to Vietnamese young consumers.

In conclusion, this study is a preliminary step to carry out the lactose-free cheese production process at pilot scale before being developed into a product for the Vietnamese market.

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