Yogurt-like Product from Fermented Maize
Part I: Selection of Starter and Fermentation Conditions

Sản xuất đồ uống lên men lactic từ ngô: Phần I: Lựa chọn chủng khởi động và điều kiện lên men

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Abstract

Fermented starchy foods and beverages play an important role in the diet of many countries. Maize is used as the main raw material and considered as non-allergenic food. This study focussed on the selection of lactic starter and fermentation conditions in order to control product quality. Six single lactic acid bacteria were inoculated in maize suspension (20% dry matter content). The cell density varied between 10^5 and 10^7CFU.mL^{-1}, the fermentation temperature ranged from 26 to 42°C during 18h. Different biochemical and sensorial characteristics were evaluated. The results showed that Lactobacillus plantarum NCDN4 *is the most suitable strain for production of yogurt-like beverage. The used cell density was 10^6CFU.mL^{-1} and fermentation occurred at 32°C for 12h. Final obtained product was biochemically comparable to popular drinking yogurt with pH 4.23±0.03, titratable acidity 2.83 ±0.03 (g lactic acid.L^{-1}) with desirable taste, texture and aroma.*

Keywords: Non-dairy yogurt, Fermented maize, Lactic acid bacteria

Tóm tắt

Sản phẩm lên men từ tinh bột đóng vai trò quan trọng trong dinh dưỡng hàng ngày của nhiều quốc gia. Ngô được coi là nguồn nguyên liệu thực phẩm an toàn không gây dị ứng. Nghiên cứu này nhằm lựa chọn chủng khởi động và điều kiện lên men phù hợp để sản xuất đồ uống thanh trùng từ dịch ngô nếp. Sáu chủng vi khuẩn lactic được cấy vào dịch ngô (20% chất khô) với: mật độ cấp giống 10^5-10^7CFU.mL^{-1}, nhiệt độ lên men 26-42°C trong thời gian tối đa 18h. Các mẫu được phân tích các tính chất hóa lý và cảm quan. Kết quả cho thấy chủng Lactobacillus plantarum NCDN4 *có khả năng lên men tốt nhất trong điều kiện 32°C trong 12h ở mật độ 10^6CFU.mL^{-1}. Sản phẩm thu được có tính chất cảm quan tốt, pH 4.23±0.03, axit tổng 2.83 ±0.03 (g axit lactic.L^{-1}).*

Từ khóa: sữa chua không sữa, ngô lên men, vi khuẩn lactic

1. Introduction

Fermented cereal foods are widely consumed worldwide, especially in South America, Central Africa and West Africa countries [1]. The main used grains as raw materials are maize, sorghum and millet. These products are consumed in the form of cooked porridge, beverage (diluted with ice and/or sugar addition) and often in the form of yogurt [2]. Ogi and Mawe are the most popular starchy products in Africa are obtained by fermentation of a suspension of wet-milled cereal. Traditional production process includes: washing and soaking cereal kernels (24 hours), wet-milling, water mixing and fermentation (48-72h) [3].

Lactic fermentation plays an important role in the production of the above products. The action of lactic acid bacteria during fermentation is associated with the development of the aroma, taste and texture of the final product, but also with the maintenance of good food safety due to produced antibiotics, and organic acids [4]. Fermentation also reduces the toxin in the product such as cyanide in cassava. It also boosts the nutritional value of the product by reducing antinutritional compounds such as phytate, increasing the solubility of certain minerals such as iron. The latest research by Gullon et al. (2015) [1] on the effectiveness of using Akpan (the drink product from Ogi) to human intestinal microflora demonstrated that Akpan was metabolized after 44 h of fermentation, exerting a prebiotic effect, similar with the observation for fructooligosaccharides[1]. The lactic bacteria often in these traditional products belong to the genus *Lactobacillus*, *Streptococcus* and *Leuconostoc* [5]. Despite the fact that starch is the major component of cereal substrate, a few amylolytic strain was reported in Mawe and Ogi products [5].

Research studies on lactic fermented cereal foods are so limited and focused towards its basic

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description, as well as to its sensory quality and nutritional attributes [3].

In Vietnam, study on cereal products such as maize has been developed by many research groups, but the segment of beverage products, especially lactic fermented beverages from starchy sources, has not yet been focussed. According to modern rhythms, consumers require more convenient, natural and safe products. Instead of using chemical-based additives, the market demand of organic/bio-based food products increases rapidly. Maize is considered like a non-allergenic food. It is suitable for lactose maldigestion on diet or protein allergenic people. These fermented cereal beverages contain less sugar low fat, as well as free of cholesterol and saturated fats. The fermentation and cooking process increase the flavour and the ability of carbohydrate metabolism.

As presented in the overview, there was a limited quantity of publications reported systematically the production of maize yoghurt-like product using starter and fermentation control. Therefore, this study focussed on the selection of lactic starter and on fermentation process control to create a desired flavour and taste for the product. In addition, research on lactic fermented beverages from maize also provides new directions for the cereal processing, especially maize, which helps diversify the application of this familiar crop.

2. Materials and Methods

2.1. Maize and lactic bacteria

Waxy maize (*Zea mays* var. *ceratina*) was directly purchased from farmer in Son Duong district, Tuyen Quang province, Vietnam. Dried maize (water content 13.92±0.12%, 100kg totally) was stocked in dry place for all the experiments. The biochemical compositions of waxy maize (in % dry basis) were as follow: carbohydrate (71.06±0.05), total protein (9.62±0.03), lipid (5.38±0.09), fibre (3.09±0.04) and ash (1.92±0.01).

The used microorganisms include six single probiotics strains supplied by different institutes: *Lactobacillus rhamnosus* GG, *L. casei* 334, *L. casei* BL23, *L. brevis* 105135, (University of Burgundy, Dijon – France); *L. plantarum* NCDN4 (Institute of Food Industry - Vietnam); *L. acidophilus* VAST (Vietnam Academy of Science and Technology).

2.2. Preparation of maize suspension for fermentation

The maize kernels were soaked in boiling water and kept at 30°C during 18h. The ratio of maize/water was 1/2. And then the drained grains were wet-milled with ratio 1/3 of grains/water. The obtained suspension was filtered by N°120 sieve before liquefied by enzyme (Spezyme Xtra - Genencor USA – 0.02%w/w) during 1h at 70°C then 5 minutes at 95°C for enzyme inactivation and sterilization. The maize suspension was added by sucrose to reach 20% dry matter content and its compositions (in % dry basis) were as follow: carbohydrate (77.70±0.05), total protein (8.70±0.02), lipid (1.20±0.03), fibre (3.10±0.02) and ash (1.70±0.01).

2.3. Preparation of inoculum and fermentation

Lactic acid bacteria were grown on MRS broth at 37°C for overnight and then centrifuged at 10,000g for 5 min. The pellet was washed two times with NaCl 9‰ then resuspended in NaCl 9‰ at appropriate concentration before inoculating. In order to select the starter the most adapted for fermentation of starchy substrate, six lactic acid bacterial strains were inoculated at 10^6CFU.mL^{-1}. The fermentation was conducted during 18h at 37°C. The best strain was selected based on following: (i) acceleration of metabolic activities (acidification and pH decrease) and (ii) formation of the desired sensory characteristics.

Once the starter was selected, the different fermentation conditions were examined. Cell density of starter varied between 10^5 and 10^7CFU.mL^{-1}; fermentation temperature and time were changed from 26 to 42°C and from 6h to 18h, respectively. After fermentation, the suspension was sterilised at 95°C during 15 minutes then cooled down to 20°C for the final product and assessment.

2.4. Physical, biochemical, microbiological and sensory analysis

Dry matter content was determined using the AACC Method 44-15A [6]. Titratable acidity was determined by neutralization with 0.1MNaOH until a pH of 8.5 was obtained; results were expressed as lactic acid equivalent (1mL NaOH 0.1M=0.009g lactic acid) [7]. Starch and reducing sugar (as glucose equivalent) were quantified by Graxianop method [8]. Total protein, lipid were determined by Kjeldahl and Soxhlet methods, respectively [8]. The cell concentration was assessed by absorbance at 600 nm [7]. After fermentation, cooking and cooling to 20°C, the products (identified by unique three-digit codes each) were evaluated for maize odour, sour and sweet tastes, texture (viscosity) and overall liking by 30 panellists using nine-point hedonic scales, where 9=extremely like and 1=extremely dislike [9].

2.5 Statistical analysis

The means and standard deviations were determined for all the measurements from at least

three replicates. The significant difference of mean values was assessed with one-way analysis of variance (ANOVA) followed by Duncan's test using SPSS software at a significance level of $p < 0.05$.

3. Results and discussion

3.1. Starter selection

Starters were added in maize suspension at 10^6CFU.mL^{-1} and 37°C. pH, acidity (in lactic acid equivalent) then were determined after 6, 12 and 18 h of fermentation and sensorial characteristics to describe for final product.

Fig. 1 presented the evolution of suspension pH as a function of fermentation time and bacteria strains. As expected, the pH decreased during fermentation. In the first 6h, the pH decreased slowly, however, the chute was clearly observed between 6h and 12h before reached steady phase. These first corresponded to adaptation phase of lactic bacteria, after that due to the increase of biomass, the pH downed and organic acids were produced massively before appearance of cell inhibition by accumulation of produced acids. Considering the velocity of pH decrease, six starters can be divided into two distinct groups, one cause the rapid chute of pH and another less fast. Four strains can be added into the first group: *L. casei* 334, *L. casei* BL23, *L. acidophillus* VAST and *L. plantarum* NCDN4 which made pH fall from 6.60 to 4.25-4.33 during 12h. For the same period, two strains: *L. brevis* 105135 and *L. rhamnosus* GG reduced pH only to 4.93-5.15. After 18h fermentation, the difference of pH of maize suspension between six strains became negligible; the final pH reached the same range about 4.00. The results of titratable acidity (lactic acid equivalent) strengthened these observations for pH evolution. Parallel to pH decrease, during the fermentation, the acid content in maize suspension raised and reached finally at 18h about 3.32-3.41g.L^{-1}. There was only one strain, *L. brevis*105135 which presented less effective for acid production (<3g.L^{-1} at 18h). After 12h of fermentation, considering the produced acid content, the lactic bacteria would be divided into two groups like those presented above. The first group (*L. casei* 334, *L. casei* BL23, *L. acidophillus* VAST and *L. plantarum* NCDN4) had capacity to produce nearly 3g.L^{-1} (2.77-2.97) acid while the second group produced only 2 g.L^{-1} (2.00-2.06).

The use of starter cultures of lactic acid bacteria during fermentation must take into account the criteria for improvement of process and product quality through firstly an acceleration of the metabolic activities (here is acidification). Based on the Fig. 1, after 12 hours of fermentation, there were four strains (*L. casei* 334, *L. casei* BL23, *L. acidophillus* VAST and *L. plantarum* NCDN4) that met this criterion (rapid decrease of pH and high acid production). However, the sensorial characteristics of product were very important factors that determined qualities of products, so the five sensorial characteristics: sweetness, sourness, maize odour, fermented odour (mainly undesirable odour) and viscosity (texture) of product were examined and the results were presented in Fig. 2. Regarding this Fig., the texture of six products had no significant difference. This observation was confirmed by viscosity measurement. The product viscosities had the same value of about 20mPa.s although the *L. brevis* strain has capacity to produce extracellular polysaccharides. This number corresponds to most of quotidian drinking yogurts. The sweetness characteristic presented with no significant difference between six products. In this case, it was necessary to focus on two main characteristics which are maize odour and fermented odour.

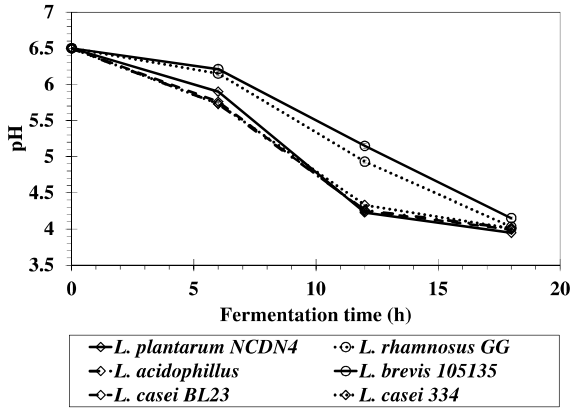

Fig. 1. pH of maize suspension as a function of fermentation time

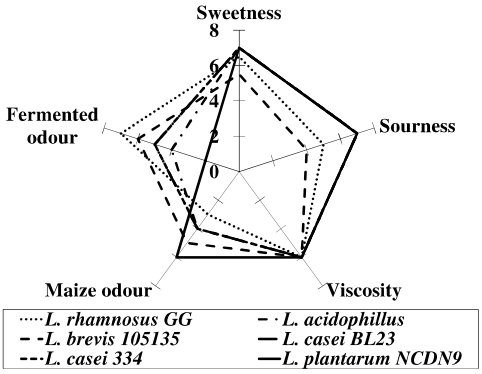

Fig. 2. Sensorial analysis of yogurt-like product fermented by different lactic strains

The final product, as expected, must retain the natural odour of raw material (maize) and exhibit at minimal undesirable odours due to fermentation. The *L. plantarum* NCDN4 perfectly answered these two

criteria (the solid line in Fig. 2). It possessed the high level of maize odour (6 points comparing to 4 for the others) and very low intensity of undesirable fermented odours (2 points comparing to 6 for the others). The fermented beverage product with *L. plantarum* NCDN4 had the harmony of sweetness and sourness, moderate viscosity and especially retained the natural aroma of waxy maize. In some previous publications, some others lactic bacteria were isolated from traditional products with spontaneous fermentation such as *L. brevis, L. acidophilus* or used for starter culture *L. casei* [1]. However *L. plantarum* is found and used the most for cereal fermented products [4,7].

Considering the criteria for starter selection presented above (i) acceleration of metabolic activities (acidification and pH decrease) and (ii) formation of the desired organoleptic characteristics, the *L.plantarum* NCDN4 strain presented as the most suitable for production of non-dairy yogurt-like beverage from maize and therefore was selected for next experiments.

3.2. Determination of suitable fermentation conditions

3.2.1. Cell density of starter

The starter *L. plantarum* NCDN4 was inoculated at 10^5, 10^6 and 10^7CFU.ml^{-1}. The fermentation was conducted in 18h at 32°C. The evolution of pH and titratable acidity were shown in Fig. 3. The pH of suspension decreased over time but can be divided into three phase explained in section 3.1. The final pH reached around 4 and the acid content (lactic acid equivalent) was 3.2g.L^{-1} in average. At the lowest cell density of inoculum, the fermentation started slower than those of higher cell densities. At 12 h of fermentation, pH was 4.4±0.06 and lactic acid content was 2.66±0.04 g.L^{-1}. At 10^6CFU.ml^{-1}, after 12h, the pH decreased faster, from 6.65 to 4.20, the acid content was 2.81g.L^{-1}. At the highest cell density, the pH of fermented suspension fall under 4.00 after only 12h of fermentation.

In addition, the sensorial analysis was carried out; it was found that at 10^5CFU.ml^{-1} the product had slightly sourness but high intensity of sweetness, lack of taste harmony due to low microbial density and slow fermentation. In contrast, the 10^7CFU.ml^{-1} density provoked the fermentation too fast; therefore the obtained product was assessed with great sourness and less sweetness. The fermentation rapidly converted sugar into organic acids and degraded the sweetness-sourness harmony of product and caused a small extend of spicy taste. With 10^6CFU.ml^{-1} of lactic bacteria, the final product had a harmony of sweetness and sourness, slightly maize flavour and fermented aroma.

The biochemical analysis of several popular products (drinking yogurts) in Vietnamese market demonstrated that the pH and titratable acidity varied from 4.14 to 4.43 and from 2.17 to 2.93g.L^{-1} respectively (unpublished data). In the literature, Akissoé et al. (2015) [3] studied the acceptance of European and African consumer on Akpan product (made from fermented white maize and sorghum) showed that the most accepted one had pH 4.0-4.2 and total acidity (lactic acid equivalent) of 0.23-0.26%. According to these observations, the cell density of starter inoculated in maize suspension was chosen at 10^6CFU.ml^{-1} for the best quality of cereal fermented beverage.

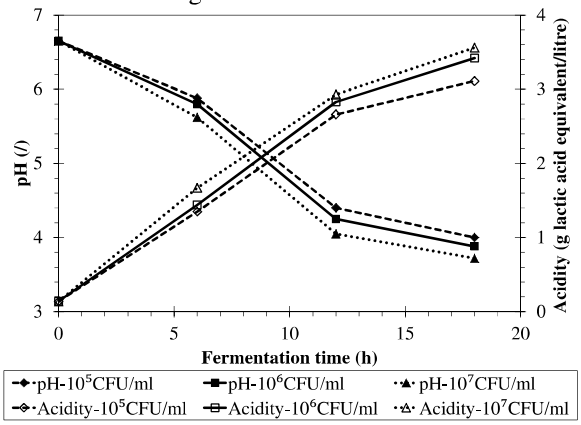

Fig. 3. Impact of cell density of starter on pH and acidity of fermented suspensions

3.2.2. Fermentation time and temperature

In order to determine the shortest fermentation time for suitable quality of product, various biochemical and sensorial characteristics of fermented product were evaluated at 6h, 12h and 18h of fermentation at 32°C with 10^6CFU.ml^{-1} of inoculum. After 6h of fermentation, the pH decreased from 6.65 to 5.79±0.03, the acid content increased by 1.16g.L^{-1} to 1.40±0.03. These values are not enough for starchy fermented products which need more acid and lower pH to inhibit pathogenic microorganism. Considering the criteria in the previous chapter, the duration of fermentation can be fixed at 12h whereas the pH and acidity were respectively 4.23±0.03 and 2.83±0.03g.L^{-1} (lactic acid equivalent). This condition provided the product a good taste and odour, a suitable pH and acidity for beverage conservation and consumption. Beyond this time of fermentation, pH continued to decrease (to 3.88±0.05, acid content 3.42±0.04g.L^{-1} at 18h). The prolongation of fermentation time caused product taste too acidic. The beverage lost sweetness, natural maize aroma and had important undesirable odours. Thus, 12 hours

of fermentation was chosen as duration of fermentation of maize suspension. Comparing the African traditional process to produce starchy fermented beverages, it shortened from 2 to 6 folds of fermentation time [2].

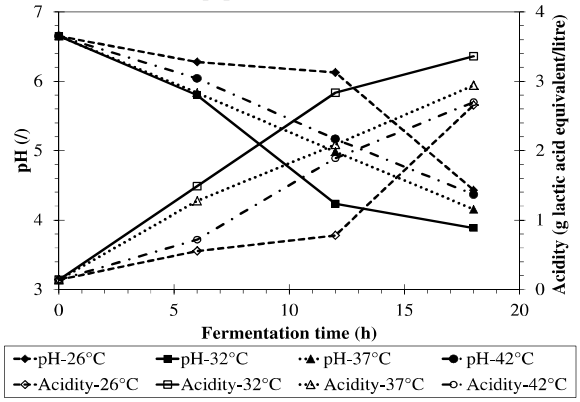

Fig. 4. Impact of fermentation temperature on pH and acidity of fermented suspensions

After selection of cell density of starter and fermentation time, it is necessary to determine the fermentation temperature. Results for different fermentation temperature at 26, 32, 37 and 42°C were presented in Fig. 4. Although the *L. plantarum* NCDN4 strain is mesophilic, the experience was still carried out at 26°C in order to avoid the undesirable odours due to a fast fermentation. However, the obtained results showed that the fermentation occurred very slowly. After 12h of fermentation the pH was only 6.13±0.16 and acidity was 0.78±0.05g.L^{-1}. These results were far from expected criteria. In addition, the sensorial characteristics were not satisfactory. For 32°C which is in the optimum range of temperature for *L. plantarum* NCDN4. The pH decreased faster those at 26°C. After 12h pH reached 4.2 and acid content was 2.88g.L^{-1}. The obtained product had a good sensory evaluation for taste, texture and aroma. When the fermentation temperature raised to 37 and to 42°C, the bacteria activities decelerated as results of obtained pH was higher and acidity was lower than those at 32°C. This observation can be explained by the out of optimum temperature of *L. plantarum* NCDN4 so the pH and acidity at 37 and 42°C were respectively 4.83±0.15 and 5.16±0.20; 2.10±0.11 and 1.89±0.09. That is the reason why these temperatures were not suitable for fermentation of maize suspension by *L. plantarum* NCDN4. These results were strengthened by different studies in literature. Gullon et al. 2015 [1] used *L. casei* CNCM-I 4592 for Akpan production, the fermentation was realised at 35°C. Agati et al. 1998 [10] produced Ogi (mainly fermented maize) by *L. fermentum* through the fermentation at 30°C. For conclusion, the most reasonable fermentation conditions were 32°C during 12h.

4. Conclusion

This study has determined *L. plantarum* NCDN4 as the lactic bacteria in order to produce the yogurt-like beverage from waxy maize. The maize suspension was fermented with cell density of 10^6CFU.mL^{-1} during 12h at 32°C. Final product was biochemically comparable to popular drinking yogurts with pH 4.23±0.03, titratable acidity 2.83 ±0.03 (g lactic acid.L^{-1}), desirable taste and texture.

Acknowledgments

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