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Title:

The role of NARO lactic acid bacteria in improving smoothness of plant-based yogurt imitation

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Abstract: (Your abstract must use **Normal style** and must fit in this box. Your abstract should be no longer than 300 words. The box will 'expand' over 2 pages as you add text into it.)

Preparation of Your Abstract

1. The title should be as brief as possible but long enough to indicate clearly the nature of the study. Capitalise the first letter of the first word ONLY (place names excluded). No full stop at the end.
2. Abstracts should state briefly and clearly the purpose, methods, results and conclusions of the work.

Introduction: Clearly state the purpose of the abstract
Methods: Describe your selection of observations or experimental subjects clearly
Results: Present your results in a logical sequence
Discussion: Emphasize new and important aspects of the study and conclusions that are drawn from them

Abstract: This study aims to contribute an approach of improving the smoothness of fermented soymilk yoghurt imitation by effective use of applicable lactic acid bacteria (LAB) strain and proper fermentation condition. This study investigated the smoothness and quality of soymilk yoghurt imitations fermented by four LAB strains through image processing, physicochemical properties and taste analysis. By comparing the primary fermentation products of the four strains and the secondary fermentation products produced using the primary fermentation products with different refrigeration time as passage cultures, control strain could not produce fermented soymilk yoghurt imitation with good smoothness via technical improvement, while AL3G1 strain, AL21D1 strain, and AL28A1 strain, which were used to produce the secondary fermentation products fermented using their primary fermentation products refrigerated for four days, exhibited relatively good smoothness, as well as superior rheological properties and flavour quality. It will be beneficial to meet the strong demand for fermented soymilk product commercialization.

Introduction: This study aims to provide a solution to improve simply the smoothness of fermented soymilk yoghurt imitation without the use of any additives or EPS, which will be achieved by effectively utilizing applicable LAB strains and optimizing their fermentation conditions. Specifically, four LAB strains of *Lacticaseibacillus paracasei*, *Loigolac-tobacillus coryniformic*, *Weissella cibaria*, *Latilactobacillus sakei* will be selected for primary fermentation of soymilk. In addition, the primary fermented products will be stored under refrigeration at different times. Moreover, the primary fermented products with different refrigeration storage times will be used as passage cultures for secondary fermentation of soymilk. By analysing the physicochemical properties including pH, titratable acidity, and rheological properties and flavour quality of the primary and secondary fermentation products, the contributions of the four LAB strains to the fermented soymilk yoghurt imitation will be compared and evaluated, leading to the identification of applicable strains and their fermentation conditions that contribute to improving the smoothness of fermented soymilk yoghurt imitation. It is hoped this study will be beneficial to meet the strong demand for the commercialization of fermented soymilk products.

Methods: The LAB strains of Control (*Lacticaseibacillus paracasei*), AL3G1 (*Loigolactobacillus coryniformis*), AL21D1 (*Weissella cibaria*), AL28A1 (*Latilactobacillus sakei*) were selected from the "NARO Lactic Acid Bacteria Collection," which is independently integrated and centrally managed by the National Agriculture and Food Research Organization (NARO). Approximately 3,000 strains in the "NARO *Lactobacillus* Collection" are derived from food sources such as crops and fermented foods. The LAB culture of each strain was added into commercial plain soymilk at a concentration of 0.1% v/v, fermenting at 30 ℃ for 24 hours to produce the original yoghurt (Y0-1), which was considered as primary fermentation. Additionally, Y0-1 was refrigerated at 4 ℃ for 1 day (Y1-1), 4 days (Y4-1), and 7 days (Y7-1), which were analyzed to investigate the effect of refrigeration on the primary fermentation

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products. Furthermore, the primary fermentation products were respectively added at a concentration of 10% w/w into commercial plain soymilk as passage cultures, fermenting at 30 ℃ for 24 hours, to produce the secondary fermentation products, which were then com-pared with the primary fermentation products for investigating the effect of the primary fermentation product as passage culture on the yoghurt-mimic. As the samples, the primary fermentation products of Y0-1, Y1-1, Y4-1, Y7-1 as well as the secondary fermentation products of Y0-2, Y1-2, Y4-2, Y7-2, which were fermented by Y0-1, Y1-1, Y4-1, Y7-1 respectively, were analyzed by smoothness observation, physicochemical analysis and taste sensor analysis.

Results: The smoothness of yogurt imitations were assessed based on the status of unevenness and granularity of the surface after stirring. To objectively reflect the differences, ImageJ was used for image processing and numerical calculation of roughness area. As the results, it was shown that all primary fermentation products following different refrigeration time, which were fermented by the four LAB strains, exhibited uneven and irregular granular appearance. On the other hand, it was shown that the secondary fermentation products of Control strain's Y4-2 and Y7-2 still exhibited unevenness and granularity, while that of the other three strains in Y4-2 were obviously improved. Referring to physicochemical properties, in the primary fermentation products, the pH values of primary fermentation products of all types of strains decreased significantly after refrigeration, compared to the primary fermentation products that were not refrigerated. Compared to Control strain, the pH decreases in the other three strains were relatively smaller. Although the pH of the primary fermentation products of each strain showed significant changes after refrigeration, when using the primary fermentation products with different refrigeration times as passage cultures for secondary fermentation, the pH did not undergo significant changes in the secondary fermentation products, which meant that the improvement of smoothness of AL3G1, AL21D1, and AL28A1 in Y4-2 comparing to their Y0-2 and Y1-2 was not caused by pH changes. In terms of rheological properties, in the primary fermentation products, the G' of the fermentation products from Control and AL21D1 did not show any regular changes along with the refrigeration time increased, while the G' in AL3G1 and AL28A1 exhibited an increasing trend with prolonged refrigeration time. In the secondary fermentation products, it was shown that the longer the refrigeration time of the first fermentation product, the lower the G' of the second fermentation product in AL3G1, while the G' of the second fermentation products in the other three strains showed no regular changes. The same trend could also be found in G", but it is worth noting that the changes in G" tend toward stability in all strains, while the G's of AL3G1, AL21D1, Al28A1 exhibited an acute decline in storage elasticity as the shear stress increased, indicating that the fermentation products of these three strains tend toward a more fluid status under shear stress. When G" kept stable, the acute decrease in G' caused the increased tan value, indicating a tendency toward viscosity and fluidity. Regardless of the primary fermentation products used for secondary fermentation, the tan values of AL3G1, AL21D1, and AL28A1 were higher than that of control, which was considered being associated with the improved smoothness of the secondary fermentation products in these three strains. In terms of taste analysis, compared to the product fermented by Control, the sourness of AL3G1, AL21D1, AL28A1 showed reduced values. Furthermore, the bitterness and astringency of AL3G1, AL21D1, AL28A1 were improved. Surprisingly, the umami flavour of these three strains was enhanced, both in terms of their inherent taste and aftertaste.

Conclusions: This study evaluated the smoothness of the primary and secondary fermentation products fermented by four LAB strains and analysed the physicochemical properties and flavour quality in conjunction with smoothness. It was found that AL3G1, AL21D1, AL28A1 can improve the smoothness of fermented soymilk yoghurt imitation without the use of any additives through reasonable refrigeration methods, enhancing umami quality and reducing sourness of yogurt imitation. Compared to improving the smoothness of yoghurt by use of additives or high-cost exopolysaccharides, the approach of improving the smoothness of LAB fermented yoghurt imitation through processing techniques in this study is expected to be applied and popularized in future actual production.