

Acceleration of White Brine Cheese by Microbial Lipase Enzyme

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Abstract

The aim of this work was to determine some physicochemical (Titratable acidity%, Lactose%, Dry matter%, Salt%, Fat %) and to measure proteolysis by Water soluble Nitrogen (WSN %) and Tri-choloro acetic acid- Soluble Nitrogen (TCA-SN %) of white brine cheese accelerated by microbial lipase enzyme at two levels (1% and 2%) during 90 days of ripening period (5, 45 and 90 days). Titratable acidity%, fat%, dry matter% and WSN% changed significantly ($P<0.05$) during ripening but lactose%, salt% and NPN% did not differ significantly ($P<0.05$) during ripening. Dry matter%, fat% and lactose% decreased during ripening but titratable acidity%, WSN% and NPN% increased throughout the ripening period. A longer ripening period could produce a better quality of final product.

Keywords: Acceleration, White brine cheese, Microbial lipase enzyme, proteolysis, physicochemical

Introduction

White brine cheese is traditionally produced in almost all parts of Iran, especially in north-west of Iran. It mainly produced from sheep and goat's milk or mixture of two. This type of cheese is very popular and familiar in many Middle Eastern countries. World cheese production is almost 14 million metric tons per year (Aydemir et al., 2001), approximately 75% of which is ripened for periods ranging from 3 weeks to more than 2 years. White brine cheese is semi-hard, salty, slightly piquant in flavor and cubic or rectangular in shape. Much of this cheese is ripened for 3 months or more in order to develop a desired flavor and texture. Cheese storage represents a significant proportion of the total cost which is 0.25 to 1.0 US \$/mo/kg. The major cost incurred involves the cost of providing refrigerated storage as well as the cost of the investment covering the value of material held in the inventory. Accelerated ripening of cheese has the potential for saving the industry hundreds of millions of dollars annually, and has therefore been of great interest. Methods which are currently being evaluated include starter culture modification, elevated or programmed ripening temperatures, addition of exogenous enzymes, and combination thereof. None of these methods have been totally successful in duplicating the changes that occur in cheeses during natural ripening.

For example, the use of elevated temperatures often results in microbial spoilage and development of flavor defects not characteristic of white brine cheese. Modification of lactic acid cultures by physical, chemical or genetic means has not yet produced a system

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capable of controllability and reproducibility giving the required balanced cheese flavor and texture. Similarly, exogenous enzyme addition has been generally unsuccessful because enzymes with the correct specificities of action have not been available. Thus, proteolysis and lipolysis are major sources of cheese flavor and odour compounds. The enzymatic processes must occur in a coordinated way to give each cheese type its unique and appreciated sensory characteristics.

In this study, we focus to monitor some considerable physico- chemical and proteolysis specifics of white brine cheeses containing different proportion of fungal lipase enzyme during 90 days of ripening period.

Material and methods

Cheese making

Ewe's milk from the zandy breed was supplied from a farm in Varamin. Experimental cheese samples were made in three replication at the Tehran Pegah dairy plant (Tehran, Iran). White brine cheese was produced using raw milk. The raw milk was warmed to 36°C, and then added microbial lipase (2% and 1%) (Fluka, Swiss) and coagulated with microbial rennet (Hansen, Denmark) for 45 minutes. After curdling, the curd was cut into cubes of approximately 1 cm³ and left to rest for 15 minutes. The slab curd was placed on a mesh table and weighted for draining. After whey separation was completed, the curd was cut into large cubes (approximately 10×10×7 cm³) and immersed in brine with 22% concentration for about seven hours at room temperature. The cheese blocks were placed into a tin plate container with brine salted to about a 12% concentration. The container was sealed and stored for 90 days (Shahab lavasani et al., 2012).

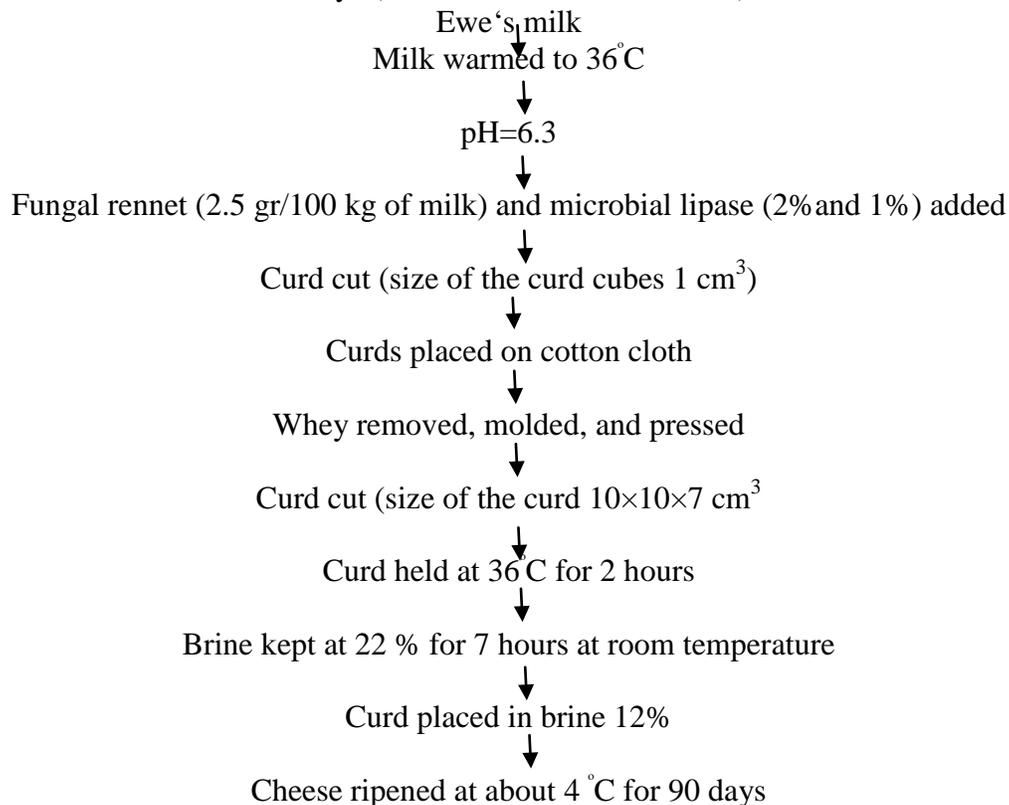


Figure 1. Protocol for the production of white brine cheese using microbial lipase

Chemical analysis

Samples of cheese were analyzed for pH (Metrohm Model 632 pH-Meter; Switzerland) and percentages of Titratable acidity, Lactose, Dry matter, Salt and Fat after 5, 45 and 90 days of ripening (AOAC, 2000)

Nitrogen Fractionation

Water – Soluble N (WSN) and N Soluble in 12% tri-chloroacetic acid (TCA-SN) were determined in aliquots of Water-Soluble Extract (WSE) prepared as described by authors, except that the cheese: water ratio was 1:5, a Sorvall Omni-mixer (Dupont company, Newton, CT, USA). Was used for homogenization and the supernatant obtained was filtered through whatman No. 42 filter paper.

The TCA-SN fraction was obtained by mixing 10 ml of WSE with 10 ml of 24% (W/V) aqueous solution of TCA, holding the mixture at room temperature for 1 hour and then filtering it through whatman No. 42 filter paper.

Statistical analysis

The data were statistically analyzed using a completely randomized design (CRD) with three replications. Data were subjected to analysis of variance using the SAS statistical software package (SAS, 1988). Mean comparison was performed with LSD's test at the $P < 0.05$ level of significance.

Results

Table 1. Presents the chemical composition of traditional white brine cheese with containing different concentrations of microbial lipase enzyme. According to statistical analysis, the percentage of titratable acidity of all treatments showed significance differences ($P < 0.05$) during 90 days of ripening period while there were no significance differences ($P > 0.05$) among all treatments. The percentage of titratable acidity of all samples increased until the 90th day of ripening, while pH values decreased. The highest content of titratable acidity was attributed to treatment B and the lowest content of acidity was associated to treatment C. The percentage of titratable acidity of treatment B was slightly greater than treatment A and titratable acidity of treatment A was greater than treatment C.

Lactose content of all treatments did not showed significant differences ($P > 0.05$). Lactose content of all samples decreased until 90th day of ripening.

According to statistical analysis, dry matter (%) of all samples showed significance differences ($P < 0.05$) during 90 days of ripening period while all treatments did not showed any significance differences ($P > 0.05$). dry matter (%) of all samples decreased during 90 days of ripening period.

According to statistical analysis, there were no significant differences ($P > 0.05$) with due attention to salt content. Total fat (%) of all samples showed significance difference during 90 days of ripening period and decreased gradually during ripening.

Table 1. physicochemical properties of white brine cheese[†] with and without microbial lipase enzyme addition[‡]

| Days of ripening | Treatments | Physicochemical properties | | | | | |
|------------------|------------|----------------------------|-------------------------|------------|--------------------------|-----------|--------------------------|
| | | pH | Titratable acidity% | Lactose % | Dry matter% | Salt% | Fat% |
| 5 | C | 6.7±0.025 ^a | 0.04±0.03 ^a | 0.23±0.00 | 41.38±0.085 ^a | 9.83±0.07 | 14.28±0.065 ^a |
| | A | 6.79±0.00 ^a | 0.04±0.005 ^a | 0.21±0.015 | 41.31±0.08 ^a | 9.76±0.06 | 14.19±0.07 ^a |
| | B | 6.85±0.015 ^a | 0.036±0.00 ^a | 0.23±0.01 | 41.39±0.11 ^a | 9.76±0.02 | 14.35±0.055 ^a |
| 45 | C | 6.68±0.025 ^b | 0.19±0.015 ^b | 0.21±0.005 | 39.97±0.19 ^b | 9.81±0.05 | 13.42±0.17 ^b |
| | A | 6.75±0.09 ^{bc} | 0.19±0.005 ^b | 0.21±0.02 | 39.87±0.03 ^b | 9.85±0.07 | 13.44±0.29 ^b |
| | B | 6.75±0.025 ^{bc} | 0.2±0.0012 ^b | 0.21±0.00 | 39.88±0.22 ^b | 9.89±0.01 | 13.47±0.17 ^b |
| 90 | C | 6.67±0.05 ^d | 0.2±0.01 ^c | 0.22±0.01 | 37.30±0.15 ^c | 9.91±0.03 | 13.78±0.03 ^c |
| | A | 6.79±0.07 ^d | 0.22±0.01 ^c | 0.2±0.00 | 37.2±0.42 ^c | 9.83±0.11 | 13.27±0.62 ^c |
| | B | 6.51±0.07 ^d | 0.25±0.02 ^c | 0.22±0.005 | 37.17±0.025 ^c | 9.88±0.01 | 12.79±0.045 ^c |

[†]Means in each column without a superscript did not differ significantly (P>0.05)

[‡]Cheese: C, without microbial lipase enzyme addition as a control sample; A, containing 0.1% microbial lipase enzyme addition ; B, containing 0.2% microbial lipase enzyme addition

Table 2. gives the mean percentages for water-soluble nitrogen (WSN) and TCA-SN throughout the ripening of white brine cheese. WSN (%) of all samples showed significance (P<0.05) differences during 90 days of ripening period. WSN (%) of all treatments increased until the end of ripening period. TCA-SN of all samples did not show any significance differences during 90 days of ripening period.

Table 2. proteolysis indices of white brine cheese[†] with and without microbial lipase enzyme addition[‡]

| Days of ripening | Treatments | Proteolysis indices | |
|------------------|------------|-------------------------|--------------|
| | | WSN% | NPN% |
| 5 | C | 0.33±0.005 ^a | 0.006±0.0000 |
| | A | 0.32±0.003 ^a | 0.004±0.0005 |
| | B | 0.33±0.01 ^a | 0.005±0.0000 |
| 45 | C | 0.42±0.009 ^b | 0.004±0.0005 |
| | A | 0.41±0.01 ^b | 0.005±0.001 |
| | B | 0.4±0.01 ^b | 0.004±0.0005 |
| 90 | C | 0.51±0.01 ^c | 0.004±0.0005 |
| | A | 0.55±0.05 ^c | 0.004±0.0005 |
| | B | 0.63±0.03 ^c | 0.004±0.0005 |

[†]Means in each column without a superscript did not differ significantly (P>0.05)

[‡]Cheese: C, without microbial lipase enzyme addition as a control sample; A, containing 0.1% microbial lipase enzyme addition ; B, containing 0.2% microbial lipase enzyme addition

Discussion

According to physicochemical characteristics, the increase in titratable acidity during 60 days of ripening in brine was due mainly to the near completion of lactose fermentation and the liberation of amino and free fatty acids following proteolysis and lipolysis. Similar to our result Shahab Lavasani et al., (2011) reported that lactose is converted into lactic acid during cheese-making by the starter culture. The loss of lactose content was attributed to lactose fermentation because lactose fermentation was occurred during ripening period hence, lactose is converted into lactic acid during cheese-making by the starter culture. Decrease in dry matter content of white brine cheese generally was attributed to water-soluble proteins and peptides passing from the cheese matrix; this decrease may be due to proteolysis phenomena and the release of new ionic groups. These results agree with those reported by Shahab Lavasani et al., (2011). Salt is driven into cheese by the concentration gradient between the cheese blocks and brine. Increase in salt content during ripening could be attributed to higher water content, as salt penetrates the cheese matrix in water (Shahab Lavasani et al., 2011). Changes in fat

content during storage could be due to a decrease in total solids and lipolysis. The breakdown of the protein network plays an essential role in the development of textural properties and in the release of free amino acids. Those amino acids are then available for secondary catabolic reactions, which are of great importance in the production of sapid compounds. Some peptides may impart a bitter flavor to cheese, if proteolysis is not well balanced, leading to the accumulation of an excess of hydrophobic peptides of intermediate size (Saldo et al., 2002).

According to proteolysis indices, the changes of WSN were attributed to hydrolysis of proteins to WSN compounds and to the diffusion of these products into brine (Shahab Lavasani et al., 2012). The depth of proteolysis was measured by NPN. Extending the ripening time leads to an increase in protein degradation in cheeses (Shahab Lavasani et al., 2012).

Conclusion

This study has explained that white brine cheese containing microbial lipase enzyme like other types of ripened cheese, requires maturation to develop the required properties. Proteolysis occurred slowly and a longer ripening period could produce a better quality of final product and treatment B was the best among all treatments.

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