Use of Some Lactobacillus Strains to Improve Soft Cheese Quality

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Abstract: UF-white soft cheese was made from concentrated buffalo’s milk using some lactobacilli strains as adjunct culture to enhance its flavour. Five treatments were made. The first was a control one with normal cheese starter (Lactococcus lactis spp. lactis and Lactococcus lactis spp. cremoris, 1:1). The other four treatments T1, T2, T3 and T4 were made by using the selected four lactobacilli strains Lactobacillus plantarum, Lactobacillus delbrueckii ssp. bulgaricus, Lactobacillus rhamnosus and Lactobacillus helveticus. All cheeses were stored at 15°C for 28 days for pickling and were periodically examined chemically, microbiologically organoleptically and for pickling indices. Results indicated that UF-white soft cheese (control) contained slightly lower total nitrogen, soluble nitrogen, non protein nitrogen, soluble tyrosine and soluble tryptophan ratio than in the other cheese treatments. The rate of accumulation of total volatile fatty acids increased with the increase of the storage period. The highest number of lactic acid bacterial count was presented in cheese made with Lactobacillus delbrueckii ssp. bulgaricus (T2). Lactobacillus helveticus gained the highest score level at the end of pickling period. It could be concluded that for the fast UF-white soft cheeses consumption, addition of Lactobacillus helveticus as starter culture is recommended. On the other hand, for better quality UF-white soft cheeses of heat treated milk according to the latest legislation, adjunct culture of Lb. helveticus, Lactobacillus plantarum and Lactobacillus delbrueckii ssp. bulgaricus can be recommended either for fresh or pickling cheese consumption.

Keywords: Lactobacillus plantarum, Lactobacillus delbrueckii ssp. bulgaricus, Lactobacillus rhamnosus, Lactobacillus helveticus, Protease activity, UF-white soft cheese, Cheese pickling.

INTRODUCTION

In cheese manufacture, the proteolysis of casein is thought to play a pivotal role because amino acids resulting from proteolysis are the major precursors of specific flavour compounds, such as various alcohols, aldehydes, acids, esters and sulfur compounds [1]. The specificities of cell envelop protease play an essential role in the production of bitter peptides [2]. The use of Lactic acid bacteria (LAB) strains deficient in peptidase activity have also indicated that peptidases, are involved in the degradation of bitter peptides; these peptidases therefore impact the development of the organoleptic quality of the milk product [3,4]. For economic reasons, several approaches were exploited to accelerate the cheese pickling process. These have included methods such as elevation of storage temperature, addition of proteinases, the use of bacteriophage-encoded lysis or lytic bacteriophages and the addition of selected nonstarter LAB or lactobacilli adjuncts to cheese [5-7]. Enriching the proteolytic of LAB potential by constructing a recombinant starter strain expressing peptidases derived from L. helveticus or L. delbrueckii subsp. lactis under a constitutive or inducible promoter can also be used to accelerate cheese proteolysis and, hence, the pickling process [4, 8-13]. However, while it can be concluded that balanced proteolysis is important for flavour formation and especially in prevention of bitterness in cheese, it is the conversion of the free amino acids, rather than proteolysis / peptidolysis that controls the rate of flavour formation from proteins [1].

In addition to the good viability in the intestine, technological properties are a prerequisite for potential use of the strains as probiotic culture in cheese.
The addition of probiotic cultures was tested in several cheeses. These included Cheddar [14, 15], Gouda [16] and soft cheeses [17-19]. However, several studies in which commercial or noncommercial Lactobacillus adjuncts were used have been published Fox and Mesweeney [20] and Fox et al. [21] in which, low numbers of selected mesophilic lactobacilli were added to the cheese milk. There is general agreement that the lactobacilli modify proteolysis, in particular, they result in a higher concentration of free amino acids and improve the cheese sensoric quality.

In view of the foregoing, the present study was carried out to investigate the use of some selected lactobacilli strains as adjunct culture for improving soft cheese pickling made from buffaloes milk concentrated by ultrafiltration (UF).

MATERIALS AND METHODS

Materials: Four lactobacilli strains were obtained from the Food Sci. Dept., Faculty of Agriculture, Ain Shams University. These strains were: Lactobacillus rhamnosus NRRL B-445, Lactobacillus plantarum NRRL B-4004, Lactobacillus delbrueckii ssp. bulgaricus and Lactobacillus helveticus CNRZ32. Microbial rennet powder obtained from (Valley Research Inc., USA), was used for pre cheese rennetate coagulation at the rate of 1g / 100 liter. The pre cheese buffalo’s milk rennetate was obtained from the Dairy Products Unit at the Animals Production Research Institute, Ministry of Agriculture, Giza, Egypt. The Ultrafiltration was carried out to concentration factor of ~ 4.5, using tubular concentration module DC2, supplied by amicon Corporation, USA, at 4 bar pressure and 50°C. The average composition of the used rennetate was: 35.85% total solids, 15.44 total protein, 14.58% fat, 4.63% lactose and 1.20% ash and pH value 6.56.

Cheese Manufacture: UF soft cheese manufacture was carried out as given by El-Shibiny et al. [22] with some modifications; the rennetate was salted at a level of 3 % sodium chloride, heat treated 72°C for ~15 seconds then cooled to 37°C. Five UF-soft cheese treatments were conducted; the first was inoculated with 1% active cheese culture (Lactococcus lactis ssp lactis and Lactococcus lactis ssp cremoris, 1:1) and served as a control. The other four portions were inoculated with 2% of the LAB strains (which were previous by selected from the first part). Namely Lactobacillus plantarum NRRL B-4004, Lactobacillus delbrueckii ssp. bulgaricus, Lactobacillus rhamnosus NRRL B-445 and Lactobacillus helveticus CNRZ32. Representing UF-soft cheese treatments: control, T1, T2, T3 and T4, respectively. To all treatments 0.02% of calcium chloride was added, left at 37°C for one hr, rennet was then added (with the level which completes coagulation within 40 minute). The UF soft cheese was immediately distributed into a number of plastic containers (each for chemical, microbiological and sensory examinations). The containers were incubated at the same temperature (37°C) to complete the suitable curd formation. The top of the curd in each container was covered with a volume of pasteurized salted permeate (3%) height ~1 cm then tightly closed with its lids and stored at 15 ±2°C for four weeks. Three replicates were carried out for each treatment. The resultant cheese was examined when fresh and after 1, 2, 3 and 4 weeks.

Physico Chemical Properties: Moisture content was determined in both milk and cheese samples and total nitrogen (TN) of milk and cheese samples was determined by semi-micro kjeldhal distillation as methods described in Association of Official Analysis Chemists [23], lactose content was determined according to Barnett and Abd El-Tawab method [24], titratable acidity (TA) for milk and cheese samples. The results were expressed as lact acid percentages and the fat content using Gerber tube for milk or cheese and soluble nitrogen (SN) for cheese samples were determined by semi-micro kjeldhal distillation method by Ling [25], the soluble tyrosine and tryptophan contents were measured according to Vakaleris and Price [26] and total volatile fatty acid (TVFA) it was determined in cheese sample according to the method described by kosikowski [27], the value was expressed as ml of 0.1N NaOH/100g cheese.

Microbiological Analysis: Lactic acid bacterial count (LABC) was enumerated according to Elliker et al. [28], the plates were incubated at 32°C for 48 hrs. Coliforms counts were enumerated using violet red bile agar medium as reported by American public health association [29], the plates were incubated at 37°C for 48 hours. Moulds and yeasts were determined on potato dextrose agar medium as suggested by Beaver and Bollard [30], the plates were incubated at 25-27°C for 4 days. Aerobic sporeforming counts were determined according to Murrell et al. [31].

Sensory Evaluation: Cheese samples were organoleptically scored for flavour (50 points), body and texture (40 points) and appearance (10 points), according
to the score card suggested by Davis, [32]. Samples were judged by the staff members of the Dairy Science Department, National Research Centre.

**Statistical Analysis:** Statistical analysis was performed according to SAS Institute [33], using General Linear Model (GLM) with main effect of treatments. Duncan’s multiple range was used to separate among of three replicates at p=0.05.

**RESULTS AND DISCUSSION**

**Physico Chemical Properties**

**Moisture Content:** Moisture content of white soft cheese made from buffalo’s milk retentate by the using of some lactobacilli strains is shown in fig. 1. The Moisture content of fresh cheeses were 64.54, 63.88, 63.88, 64.00 and 63.81% for control, T1, T2, T3 and T4 cheeses, respectively. The results indicated that control treatment had the highest moisture content. While, white soft cheese treatments with lactobacilli strains had lower moisture content being the lowest in T4 made with *Lactobacillus helveticus*. Moisture data revealed a significant (p ≤ 0.05), difference among control and treatments with lactobacilli strains as well as among the examined treatments with starter. The lower moisture content of treatments with starters is due to the higher acidity development attained due to starter growth. The higher acidity in the milk reduces the coagulation time and decreases the moisture content [18, 19].

**Lactose Content (%)**: It could be seen from the data given in fig. 2. That lactose content in the different UF-white soft cheeses gradually decreased throughout the pickling period. The lactose content in different cheese treatments ranged from 3.97 to 4.34 % in fresh cheese and from 1.05 to 1.67 % after 28 days of storage.

Results also indicated that the rate of decrease in lactose content in the control was lower as compared with other cheese treatments, which might be due to the addition of lactobacilli adjunct Cultures (*Lactobacillus rhamnosus, Lactobacillus plantarum, Lactobacillus delbrueckii ssp. bulgaricus and Lactobacillus helveticus*), which were different in their ability to hydrolyze lactose.

**Titratble Acidity (TA %):** Changes in titratable acidity (TA) of white soft cheese made from buffalo’s milk retentate as treated with selected lactobacilli strains are presented in fig. 3. The results showed that titratable acidity of fresh UF-white soft cheese samples were 0.17, 0.22, 0.20, 0.23 and 0.20 % for control, T1, T2, T3 and T4 cheeses, in order. The corresponding values of acidity after 4 weeks of pickling were 1.91, 2.49, 2.15, 2.21 and 2.91 % for the same treatments, in the same order. All white soft cheese with selected adjunct cultures exhibited obviously higher acidity values than that in control cheese.

The obtained data also indicate that white soft cheese made with added *Lactobacillus rhamnosus* (T3) had the highest TA while, control cheese (without adjunct starter) had the lowest TA, when fresh and along pickling period. There were slight differences in TA between T2 and T3 cheese treatments, since it increased from 0.20 to 2.15% and from 0.23 to 2.21 %, respectively, throughout the pickling period (28days).

These results are in harmony with those obtained by EL-Abd *et al.* [18], El-Zayat and Osman [19] and Melanna *et al.* [34], who found that the use of different starter cultures in Domiatu cheese manufacture increased the acidity development in the product.

**Total Nitrogen (TN %):** Total nitrogen in UF-white soft cheeses with and without (control) selected bacteria are recorded in fig. 4. These results indicated that TN content in all the experimented UF-soft cheeses gradually increased with the advancement of the pickling period due to the decrease in the moisture contents in cheese. Total nitrogen content ranged from 2.52 to 2.65 % after 28 days of pickling period in all cheeses being made with selected bacteria.

**Ripening Indices of Cheese**

**Soluble Nitrogen (SN %):** Fig. 5. Includes the soluble nitrogen in the experimental UF soft cheese during the pickling period at 15°C for 28 days. It is clear from these results in all cheese treatments, that the rate of accumulation of SN increased with the prolongation of the pickling period. This was attributed to the rate of proteolysis throughout the storage period.

In fresh cheeses samples SN contents were 0.174, 0.194, 0.184, 0.206 and 0.195 % for control, T1, T2, T3 and T4 cheese, respectively. These results indicated that cheeses of T3 had the highest SN content compared to other treatments when fresh, while T3, T4 were highest after 28 days.

From the aforementioned results it could be seen that addition of adjunct strains in soft cheeses resulted in an increase in SN content. This increase could be due to the activity of proteases and peptidases released from the experimental strains, which resulted in higher proteolysis in cheese.
Fig. 1: Moisture content during pickling of white soft cheese made from buffalo's milk retentate with adding adjunct cultures. (for abbreviation see materials and methods)

Fig. 2: Lactose content during pickling of white soft cheese made from buffalo's milk retentate with adding adjunct cultures

Fig. 3: Titratable acidity during pickling of white soft cheese made from buffalo’s milk retentate with adding adjunct cultures
Soluble Tyrosine and Tryptophan: It could also be noticed from figs. 6, 7, that, soluble tyrosine and tryptophan (mg/100g) gradually increased as the time of pickling passed.

The obtained results indicated that UF-white soft cheese (control) contained lower soluble tyrosine and tryptophan ratio than in the other cheese treatments made with the experimental adjunct strains.

Total Volatile Fatty Acids Content (TVFA): Results in fig. 8. Included the TVFA in both of UF-white soft cheese (control) and cheese with experimental adjunct strains during the pickling period at 15°C for 28 days. Obtained results indicated that, the rate of accumulation of TVFA increased with the increase of the pickling period in all cheese treatments. Values of TVFA in fresh samples were 17.66, 18.46, 18.16, 18.03 and 18.63 ml of 0.1 N Na OH/100g cheese for control, T1, T2, T3 and T4 cheeses, in order. The corresponding values for pickling cheese after 4 weeks were 113.76, 121.63, 127.25, 116.68 and 156.27 ml of 0.1 N Na OH/100g cheese for same treatments in the same order. From these data, it could be noticed that among treatments, T4 possessed the highest value of TVFA, while control had the lowest. Furthermore, T1, T2, T4 cheeses showed highly significant (p ≤ 0.05), difference in TVFA than the control and T3 cheese.
Fig. 6: Soluble tyrosine content during pickling of white soft cheese made from buffalo's milk retentate with adding adjunct cultures.

Fig. 7: Soluble tryptophan content during pickling of white soft cheese made from buffalo's milk retentate with adding adjunct cultures.

Fig. 8: Total volatile fatty acids (TVFA) content during pickling of white soft cheese made from buffalo's milk retentate with adding adjunct cultures.
Fig. 9: Lactic acid bacterial count (LAB) (log cfu/g) during pickling of white soft cheese made from buffalo's milk retentate using adjunct cultures

Although, T1 cheese was manufactured with added traditional starter culture but still has lower TVFA value. These observations could be due to the different lipolytic activity of starter adjunct cultures.

**Microbiological Examination**

**Lactic Acid Bacterial Count (LABC):** Data presented in fig. 9, illustrated the changes in lactic acid bacterial counts (log cfu/g) of UF-white soft cheese during the pickling period at 15°C for 28 days. The log of lactic acid bacterial counts of UF-white soft cheese were 6.93, 7.49, 7.91, 7.44 and 7.12 log cfu/g for control, T1, T2, T3 and T4 treatments, respectively when fresh. However, the lactic acid bacterial counts gradually increased till the first 14 days of pickling period, then slight decreased at the end of this period (28days), results indicated that the log of lactic acid bacterial counts in control was less than that in all of the other cheese treatments. This might be attributed to the addition of experimental adjunct strains to all treatments (except control one) markedly increase in the viable lactic acid bacterial count in fresh and along the storage of cheese, as compared with control cheese. Moreover, the highest number of LAB was detected in cheese made with *Lactobacillus delbrueckii ssp. bulgaricus* (T2). This could be due to the higher rate of strains growth and higher ability to resist cheese conditions compared to other treatments.

During the storage, LAB counts of all cheese samples gradually increased with the prolonging of pickling period till the end of first 14 days of pickling. After that, LAB counts were gradually decreased till the end of pickling period. At the end of storage period (28 days), the populations of LAB were amounted to be 4.41, 6.50, 5.67, 5.47 and 4.72 (log cfu/g) in control, T1, T2, T3 and T4 cheese, respectively. Treatment (T2) with *Lactobacillus delbrueckii ssp. bulgaricus* of UF-white soft cheese still had the highest LAB counts compared to other all cheese treatments. Similar results were recorded by Abdeen [35], who reported that during the storage of cheese, the total bacterial counts slightly increased during the first period of storage, then gradually decreased till the end of the storage period at 15°C this could be attributed to the development of the acidity in cheese.

**Coliforms, Aerobic Sporeforming Bacterial Counts, Yeasts and Molds:** Effect of some lactobacillus cultures on coliforms, aerobic sporeforming bacterial and yeasts and molds count in UF-white soft cheese during pickling period was examined. They not detected in all cheese treatments either when fresh or during the pickling period. This might be due to results of high hygienic condition during making steps and pickling period and the development in the acidity in cheeses when fresh and during the pickling period.

**Sensory Evaluation:** Results in fig. 10, show that general acceptability of cheeses as total scores were 86.5, 89.5, 92.3, 92.4 and 94.5 points, respectively, for fresh cheese samples of control, T1, T2, T3 and T4, cheeses these data indicated that T2 gained the highest acceptability among all fresh cheese samples. On the other hand, the control cheese sample gained the lowest score, being significantly (p < 0.05), different than those cheese treatments with experimental adjunct strains. Storage has affected the total acceptability of cheese properties.
In all cases the acceptability increased during the early stage of the storage and by extending the pickling period. The improvements were slow in control cheese treatment while, it was faster in the treatments with the experimental strains being faster in T4 with Lactobacillus helveticus. Stored cheese samples of T4 gained the highest score at the end pickling period. It could be concluded that for the fast UF-white soft cheeses consumption, addition of Lactobacillus helveticus as starter culture can be recommended. On the other hand, for better quality UF-white soft cheeses of heat treated milk according to the latest legislation, adjunct cultures of (Lactobacillus rhamnosus, Lactobacillus plantarum and Lactobacillus delbrueckii ssp. bulgaricus) can be recommended either for fresh or stored cheese consumption.

REFERENCES


