TRACKING GROWTH OF LACTIC ACID BACTERIA IN ULTRA FILTERED MILK AND USING IT IN THE MANUFACTURE OF HARD CHEESE

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B.Sc. Agric. Sci. (Dairy Sci.), Fac. Agric., Fayoum Univ., (2021)

Thesis

Submitted in Partial Fulfillment of

the Requirements for the Degree of

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In

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Dairy Department

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SUPERVISION SHEET

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SUMMARY AND CONCLUSION

The pre-acidification of UF milk using food-grade organic acids is explored as a potential strategy to enhance the activity of LAB by lowering the initial pH and, consequently, reducing the UF milk's BC. **Therefore, this study aimed to:**

1- Tracking the growth and kinetics of selected LAB strains in UF cow's milk pre-acidified with edible organic acids (such as lactic, acetic or citric acid), to overcome the challenges posed by the high BC of UF milk when used in hard cheese manufacturing.

2- Investigate the effect of pre-acidifying UF cow milk to pH 6.00 using lactic, acetic, or citric acid on the physicochemical, microbiological, rheological, and sensory properties of Ras cheese throughout a 90-day ripening period. Additionally, this part of the study aimed to evaluate the feasibility of using pre-acidification with organic acids as a practical approach to improve ripening behavior and the overall quality of Ras cheese made from UF milk.

To achieve these objectives, the study was divided into two main **parts as follows**:

Part I: Tracking of some LAB strains in cow, UF, and pre-acidified UF milk throughout incubation time

Five milk treatments were prepared to investigate the impact of pre-acidification on UF milk's fermentation kinetics: cow's milk (C), UF milk concentrated three-fold without acidification (UF), and UF milk pre-acidified to pH 6.00 with lactic (T_1), acetic (T_2), or citric (T_3) acids. Each treatment was inoculated at 1% with one of five lactic acid bacteria, *Lb. acidophilus*, *Lb. bulgaricus*, *Lb. paracasei*, *Lb. casei* or *Str. thermophilus*, and incubated at each strain's optimal temperature. Samples were withdrawn at 0, 2, 4, and 8 h to measure titratable acidity TA%, pH, and strain viable counts (log cfu g^{-1}). From these data we calculated acid development rate and pH changing rate (ΔpH).

The statistically analyzed results revealed the following:

1. In the case of inoculation with *Lb .acidophilus*:

- At 0 h, the highest TA% was recorded in treatment T_1 (0.53%), while the lowest was in the control sample C (0.21%). After 8 h, TA% was highest in T_2 (0.67%) and lowest in UF (0.28%). The rate of acidity development %was highest in T_2 and lowest in UF.
- Initial pH was highest in the UF (6.86) and lowest in the pre-acidified treatments (6.00). After 8 h, UF still had the highest pH, while T_1 (lactic acid) showed the lowest value. The greatest ΔpH was observed in T_1, and the smallest in UF.
- At 0 h, The SVCs was highest in T_1 and lowest in the control sample C. After 8 h, the highest count was observed in T_2 and the lowest in C.

2. In the case of inoculation with *Lb .bulgaricus*:

- At 0 h, TA% was highest in T_1 (0.47%) and lowest in UF (0.25%). After 8 h, TA% in T_1 reached 0.77%, while the control sample C reached only 0.29%. The highest acid development rate % was observed in T_2 (acetic acid), and the lowest in C.
- The initial pH was highest in UF (6.83) and lowest in the acidified treatments (6.00). After 8 h, UF maintained the highest pH (6.79), and the lowest values were recorded in T_2 and T_3 (5.55). The largest ΔpH occurred in T_2 and T_3, while the smallest change was in UF.
- The highest initial SVCs was recorded in T_1 and the lowest in UF. After 8 h, the highest count was again in T_1, and the lowest in C.

3. In the case of inoculation with *Lb .paracasei*:

- At 0 h, TA% was highest in T_1 (0.54%) and lowest in the control C (0.23%). After 8 h, TA% in T_1 reached 0.72%, while C reached only 0.30%. The acid development rate %was highest in T_2 and lowest in UF.

- Initial pH was highest in UF (6.88) and lowest in the acidified treatments (T_1, T_2, T_3) (6.00). After 8 h, UF still had the highest pH (6.73), while the lowest value was in T_1 (5.35). The highest ΔpH (0.65) was observed in T_1 (lactic acid), while the smallest (0.15) was in UF.
- The highest SVCs at 0 h was recorded in T_3 (citric acid), and the lowest in C. After 8 h, T_2 had the highest count and C the lowest.

3.In the case of inoculation with *Lb .casei*:

- In T_3 (citric acid), TA% increased from 0.50% at 0 h to 0.75% after 8 h, while in C, it increased from 0.24% to only 0.30%. The highest rate of acid development %was observed in T_3 and the lowest in UF.
- The initial pH was highest in UF (6.94) and lowest in the acidified treatments (6.00). After 8 h, UF still showed the highest pH (6.84), and the lowest was in T_1 (5.45). The greatest ΔpH was recorded in T_1 (lactic acid), and the smallest in UF.
- The SVCs at the beginning and end of incubation was highest in T_3 and lowest in the control C.

5. In the case of inoculation with *Str .thermophilus*:

- At 0 h, TA% was highest in T_2 (acetic acid) (0.53%) and lowest in C (0.24%). After 8 h, TA% in T_2 reached 0.66%, while in C it only reached 0.28%. The highest acid development rate% was observed in T_1 and the lowest in C.
- The initial pH was highest in UF (6.91) and lowest in the acidified treatments (6.00). After 8 h, UF still had the highest pH (6.73), while the lowest was in T_2 (5.55). The greatest ΔpH (0.45) was observed in T_2, and the smallest (0.18) in UF.
- After 8 h, the highest SVCs was recorded in T_3, and the lowest in C.

In summary, pre-acidification of UF milk to pH 6 with lactic, acetic, or citric acids uniformly accelerated acidification rates, steepened pH declines, and elevated

LAB counts across all five strains. This simple adjustment effectively counteracts UF milk's high BC, enabling its routine use in hard and semi-hard cheese manufacturing without compromising fermentation kinetics or microbial viability.

Conclusion

Based out finding, we can conclude that, the pre-acidification process could a useful method for overcoming the BC of the high concentrated UF milk. And as consequent, the high concentrated UF milk could be used in the manufacture of the hard and semi-hard cheese. And we will show that in the second part of this work.

Part II: Characteristics of Ras cheese made from cow and ultrafiltrated milk with or without pre- acidification by some organic acids

The study investigated the manufacture of Ras cheese using UF cow's milk, focusing on the effects of pre-acidification with selected organic acids, lactic, acetic, and citric on cheese ripening. Five experimental treatments were designed: one with regular cow's milk (C_1), one with UF milk without acidification (C_2), and three with UF milk pre-acidified to pH 6.00 using the respective acids (T_1, T_2 and T_3). Comprehensive physicochemical, sensory, and microbiological analyses were conducted at intervals throughout the 90-day ripening period. In addition, rheological properties were assessed at the end of the ripening period. The statistically analyzed results revealed the following:

1. Gross chemical composition of experimental Ras cheese during ripening period

1-The pH values of all cheese samples declined progressively during ripening, indicating ongoing acidification. At the fresh stage, control samples (C_1 and C_2) had significantly higher pH values than the acid-treated ones, with T_2 (acetic acid) exhibiting the lowest initial pH. The high BC of UF milk was evident in C_2, which maintained higher pH values than other treatments throughout ripening.

2-Titratable acidity % followed an inverse trend to pH, increasing steadily across all samples during ripening. Acidified treatments, especially T_2 and T_3, demonstrated significantly higher acidity levels than the controls.

3-Moisture content decreased gradually in all treatments during ripening. Initially, no significant differences were observed; however, by day 90, acid-treated cheeses exhibited greater moisture loss than the control groups C_1 and C_2. C_2 consistently retained the highest moisture content.

4-Fat content increased in all samples throughout ripening. While acid-treated and control samples had comparable fat contents at the start, by the end of ripening, T_2 had the highest fat percentage.

-Fat-to-dry matter (F/DM) ratios also increased during ripening, again reflecting moisture loss. The UF cheese without acidification (C_2) recorded the highest F/DM by day 90, whereas acidified samples showed lower values.

5-Protein content exhibited a similar increasing trend, rising across all samples as ripening progressed due to moisture reduction. T_3 achieved the highest final protein concentration, followed by T_2 and T_1. The control (C_1) maintained the lowest protein levels.

-The water-soluble nitrogen (WSN) content of Ras cheese, expressed as a percentage of total nitrogen, showed notable variations across different treatments at the fresh stage of ripening. The control samples (C_1 and C_2) and acid-treated samples (T_1, T_2, and T_3) did not display significant differences in WSN%, but a general trend was observed. WSN% ranged from 0.31% to 0.38%, with the highest in T_1 (0.38%) and the lowest in the traditional control sample C_2 (0.31%). Throughout the ripening process, all samples exhibited an increase in WSN%. By the end of 90 days, T_3 showed the highest WSN% (0.57%), indicating more intense proteolysis, followed by T_1 and T_2. The C_2 sample exhibited a WSN% of 0.46%, similar to the traditional control.

-The percentage of water-soluble nitrogen to total nitrogen (WSN/TN%) varied significantly among different treatments at the fresh stage, with the highest value recorded

in T_3 (8.96%), followed by C_1, T_1 and T_2. The control sample C_2 had the lowest WSN/TN% (7.65%). all samples showed an increase in WSN/TN%, which is a key index of proteolysis and significantly affects cheese flavor, texture, and nutritional quality. By day 90, T_1 exhibited the highest WSN/TN% (11.35%), suggesting that the acid treatments accelerated proteolysis. These findings indicate that organic acids help release nitrogenous compounds and promote microbial activity, leading to better flavor development.

6-The ash content of Ras cheese showed no significant differences between some samples at the fresh stage. Ash content ranged from 4.55% to 5.42%, with the highest in T_2. Over the ripening period, ash content increased due to moisture loss, which concentrates minerals in the cheese. By day 90, T_3 had the highest ash content (7.63%), followed by T_2, while sample T_1 had a lower ash content.

7-Salt content varied significantly at the fresh stage, with T_3 having the highest salt concentration (2.87%) and C_1 the lowest (2.33%). As ripening progressed, salt content increased in all samples. At day 90, T_3 still had the highest salt content (5.79%), while T_1 had the lowest (3.56%).

8-The fatty acid profile of Ras cheese showed extensive lipolysis during ripening, with notable differences in fatty acid concentrations across treatments. Butyric acid, associated with cheese flavor, was highest in T_3. Other short- and medium-chain fatty acids followed a similar trend, with T_3 consistently showing the highest concentrations. The C_2 sample, produced from UF milk without pre-acidification, had the lowest concentrations of fatty acid.

2. Textural profile analysis (TPA) of experimental Ras cheese

-Textural profile analysis revealed significant differences in hardness, adhesiveness, and chewiness among the cheese samples. T_2, made with acetic acid pre-acidified UF milk, had the highest hardness, while T_1, made with lactic acid, had the lowest. These differences in texture were likely influenced by the type of acid used during pre-acidification .T_1 had

the lowest values for most textural parameters, suggesting a softer texture, while T_3 exhibited firmer and more resistant textures.

3. Microbiological examinations of experimental Ras cheese during ripening period

1- Microbiological examination showed that total viable counts (TVCs) were higher in the control and acid-treated samples compared to C_2 at the fresh stage. TVCs increased during the first 30 days of ripening, but a decline was observed thereafter, particularly in samples treated with organic acids. By day 90, the acid-treated samples had lower TVCs compared to the control samples, supporting the role of pre-acidification in enhancing microbial control and uniform ripening.

2- At the fresh stage of ripening, significant differences in LAB count were observed across all Ras cheese samples. The highest LAB count was found in the C_2 sample, followed by T_1, T_3, T_2, and the lowest in the control sample (C_1). By the 15th day, LAB counts increased in all samples. At the end of the 90-day ripening period, the highest LAB count was observed in T_2, followed by C_1 T_3, and C_2, while T_1 had the lowest LAB count.

3-Regarding spore-forming bacteria (SFB), significant differences were noted between the C_1 sample and other treatments at the fresh stage. The highest SFB count was found in C_2, followed by the control sample, with acid-treated samples exhibiting lower counts. Over the ripening period, a decline in SFB counts was observed in all samples, with more pronounced reductions in the acidified samples. By the end of the ripening period, the acidified samples exhibited significantly lower SFB counts, with T_1 showing the most substantial reduction.

4-All cheese samples were free from coliform bacteria, yeasts, and molds throughout the ripening period.

4. Organoleptic properties of experimental Ras cheese during ripening period

Sensory evaluations revealed differences in flavour intensity, body and texture, colour, and overall acceptability among the cheese samples. The T_1 sample, made with lactic acid, showed the highest flavour intensity score, followed by T_2 and T_3. The C_1 sample had the highest overall flavour intensity. In terms of body and texture, T_1 exhibited the highest score among the acid-treated samples, while the C_1 sample had the best overall texture. Colour and appearance were highest in the C_1 and C_2 samples. Overall, while there were no significant differences in sensory scores between the C_1 sample and the acid-treated samples, the influence of milk type and processing on acceptability was evident in the comparison between C_1 and C_2.

Conclusion

Based on the results of this study, it can be concluded that pre-acidification to a target pH of 6.00 significantly improved the physicochemical properties, including increased moisture retention, protein content, and titratable acidity, while reducing the buffering effect commonly observed in UF milk. Among the treatments, the lactic acid cheese (T_1) exhibited the most favorable outcomes, showing enhanced lipolysis as indicated by elevated free fatty acid levels, improved texture characteristics such as hardness and cohesiveness, and superior microbial safety with high LAB counts and reduced spoilage organisms. Sensory analysis confirmed that T_1 closely matched the traditional cheese control (C_1) in terms of flavor and overall acceptability, making it a suitable alternative for high-quality Ras cheese production. These findings suggest that lactic acid pre-acidification not only accelerates ripening kinetics but also contributes to better flavor development, texture, and microbial stability in UF Ras cheese. Therefore, this approach can be recommended as a practical and economical method for improving the functionality and consumer acceptance of UF-based Ras cheese in commercial dairy processing.

Recommendations:

1. Pre-acidification of UF milk using food-grade organic acids (lactic, acetic, or citric acid) is strongly recommended prior to inoculation with LAB cultures. This process significantly reduces the initial pH and overcomes the high BC of UF milk, facilitating improved acidification rates and enhancing microbial activity during fermentation.
2. Further studies are recommended to optimize critical pre-acidification factors such as acid concentration, target pH (below or above 6.00), and acid combination effects to maximize starter culture activity and improve cheese ripening kinetics while maintaining desirable sensory characteristics. Future studies are recommended to assess how pre-acidification affects sensory attributes (taste, aroma, mouthfeel) and functional properties (texture, meltability) of the final dairy products to ensure that technological improvements do not compromise consumer acceptance
3. The pre-acidification strategy should be applied in the production of hard and semi-hard cheeses (e.g., Ras cheese), where controlled acidification and enhanced LAB activity are essential for proper texture and flavor development. This can help reduce ripening time and improve yield consistency.
4. It is advised to conduct pilot and industrial-scale production trials using lactic acid pre-acidification to evaluate process scalability, cost-effectiveness, and impact on large-scale Ras cheese production and shelf life.
5. Broader consumer sensory evaluations are suggested to assess market acceptance of Ras cheese produced using pre-acidified UF milk, ensuring that technological improvements align with consumer preferences.
6. Future research should explore the impact of pre-acidification on the nutritional value and potential functional properties (e.g., bioactive peptides) of Ras cheese, contributing to the development of health-promoting dairy products