Kafrelsheikh University
Faculty of Veterinary Medicine
Food Control Department

Using of natural antimicrobials for improving the hygienic status of fermented dairy products

Thesis presented by

Asmaa Mustafa Ismail Abou Zied

B. V. Sc., Fac. Vet. Med., Kafrelsheikh Univ, (2010)

M. V. Sc., Fac. Vet. Med., Kafrelsheikh Univ, (2015)

Under Supervision of

Dr. Ibrahim Ibrahim AL–Hawary

Dean of Faculty of Aquatic and Fisheries Sciences

Professor of Milk Hygiene

Kafrelsheikh University

Dr. Azza Marghani Mohamed Deeb

Professor of Milk Hygiene
Faculty of Veterinary Medicine
Kafrelsheikh University

Dr. Walaa Mohamed Elkassas

Senior Researcher of Food Hygiene, Animal Health

Research Institute (Kafr El-Sheikh branch)

Submitted to

Fac. Vet. Med., Kafrelsheikh Univ.

Food Control Department

(Milk Hygiene)

For obtaining Ph.D. degree in Veterinary Medical Sciences

(2022)

List of Contents

LIST OF TABLES

LIST OF FIGURES AND PHOTO

7-Summary

A total of 100 samples of kariesh cheese and balady yoghurt (50 samples each) were randomly collected from different localities in Kafr El-Sheikh city, Egypt. All samples were transferred to the laboratory in an insulated ice-box with minimum of delay.

1. Bacteriological evaluation:

1.1. Staphylococcal count:

Staphylococci were detected in 48 (96%) and 40 (80%) of examined kariesh cheese and yoghurt samples with mean values of $5.4\times 10^4 \pm 1.5\times 10^4$ CFU/g and $2.4\times10^3 \pm 0.13 \times 10^3$ CFU/g, respectively.

S. aureus was detected in 5 (10%) and 2 (4%) of the examined kariesh cheese and yoghurt samples, respectively**.** The virulence factor (*clf*A) of 638 pb was detected in one isolate of *S. aureus* which isolated from yoghurt samples.

1.2. Total coliforms count:

Coliforms were detected in all examined kariesh cheese samples, with mean value of $3.2\times 10^7 \pm 1.9\times10^7$ MPN/g. Whereas 45 (90%) of the examined yoghurt samples were positive for coliforms, with an average of $3.6\times10^5 \pm 2.4\times10^5$ MPN/g.

The highest frequency distribution of coliforms in the examined kariesh cheese samples was 44% which lied within the interval 10^3 -< 10^5MPN/g. While, the highest frequency distribution of the examined yoghurt samples (36%) lied within 3 -< 10^3MPN/g

E. coli was isolated from 10 (20%) and 7 (14%) of examined kariesh cheese and yoghurt samples, respectively**.** The isolated *E. coli*

strains were serotyped to O146:H5 (2,2), O111:H2 (2,2), O111:H5 (2 from kariesh cheese), O125:H11 (1 from yoghurt), O114:H2 (1 from kariesh cheese), O26:H10 (1 from yoghurt), O1:H2 (1 from kariesh cheese), O158:H7 (1 from yoghurt), O119:H7 (1 from kariesh cheese) and O166:H2 (1 from kariesh cheese).

2. Effect of natural antibacterial agents on survival of *S. aureus* and *E. coli* in manufactured yoghurt:

The capacity of antibacterial activities of natural preservatives like lysozyme (native and heated lysozyme), probiotics or their mixtures to inhibit the growth of both food-borne pathogens (*S. aureus* and *E. coli*) in manufactured yoghurt.

Yoghurt batches (1, 2, 3, 4, 5,& 6) which contain:

batch 1: LAB classic starter cultures. batch 2: LAB classic starter + LZ.

batch 3: LAB classic starter + HLZ. batch 4: ABT-5 culture.

batch 5: ABT-5 culture+ LZ. batch 6: ABT-5 culture + HLZ.

Every batch was doubled, one inoculated with *S. aureus* and the other inoculated with *E. coli* at the rate of 7.39 and 8.3 log 10 CFU/g, respectively.

2.1. pH value:

The pH values in yoghurt batches (1, 2, 3, 4, 5 & 6) inoculated by *S. aureus* and *E. coli* were gradually decreased as the storage period extend from an initial values at termination of fermentation (curd time) 5.61, 5.48, 5.03, 5.26, 5.38 and 5.43 to 4.00, 3.83, 3.30, 3.44, 3.65 and 3.70 at the day 14^{th} of storage period (4 °c), respectively, for yoghurt batches inoculated by *S. aureus*. While, yoghurt batches inoculated by *E. coli*, pH values were

5.34, 5.21, 5, 5.02, 5.12 and 5.2 initially and decreased to 3.92, 3.64, 3.40, 3.48, 3.61 and 3.63 at the end of storage period, respectively.

2.2. Antibacterial activity:

2.2.1. *S. aureus*:

The viable counts of *S. aureus* in all manufactured yoghurt batches decreased as the storage period extended. The counts decreased from the inoculation count (7.39 log 10 CFU/g) to 5.73, 4.76, 2.92 and 3.2 log 10 CFU/g in batches (1, 2, 5& 6). Whereas, *S. aureus* growth couldn't be detected in yoghurt batches (3 & 4) on the 14^{th} day of refrigerated storage.

2.3. E. coli:

In all manufactured yoghurt batches, the viable counts of *E. coli* decreased during the refrigerated storage period from the initial count (8.3 log 10 CFU/g) to 5, 4.49, 2.07, 2.77 and 3.41 log 10 CFU/g in batches (1, 2, 4, 5& 6). However, *E. coli* growth was not detected at day 14^{th} of refrigerated storage only in batch 3.