



Assiut University



Faculty of Veterinary Medicine
Department of Food Hygiene

Antimicrobial effect of Reuterin and Nisin and their improvement effect on locally manufactured soft cheese quality

Presented by

Rasha Fawzy Eid Ghetany

(B.V.Sc., 2008)

(M.V.Sc., 2019)

For

The degree of Ph. D.

(Milk Hygiene)

Under the Supervision of

Prof. Dr. Ahmed Abdel-Hameid Ahmed

Professor of Milk Hygiene
Faculty of Veterinary Medicine
Assiut University

Prof. Dr. Wallaa Farouk Amin

Professor of Milk Hygiene
Faculty of Veterinary Medicine
Assiut University

Dr. Manal Mohamed Amin

Senior Researcher of Milk Hygiene
Animal Health Research Institute
Assiut

2022 A.D. – 1444 A.H.

CONTENTS

Subject	Page
INTRODUCTION	1
REVIEW OF LITERATURE	5
MATERIALS AND METHODS	38
RESULTS	61
DISCUSSION	88
CONCLUSION	105
SUMMARY	107
REFERENCES	112
ARABIC SUMMARY.	

List of tables

Table 1. Statistical analytical results of total bacterial count in the examined soft cheese samples.....	61
Table 2. Frequency distribution of positive soft cheese samples based on their total bacterial count	61
Table 3. Statistical analytical results of coliforms count in the examined soft cheese samples	62
Table 4. Frequency distribution of positive soft cheese samples based on their coliforms count	62
Table 5. Statistical analytical results of fecal coliforms count in the examined soft cheese samples	63
Table 6. Frequency distribution of positive soft cheese samples based on their fecal coliforms count	63
Table 7. Statistical analytical results of <i>E. coli</i> count recovered from the examined soft cheese samples	64
Table 8. Frequency distribution of positive soft cheese samples based on their <i>E. coli</i> count	64
Table 9. Statistical analytical results of <i>Staph. aureus</i> count recovered from the examined soft cheese samples	65
Table 10. Frequency distribution of positive soft cheese samples based on their <i>Staph. aureus</i> count	65
Table 11. Enterotoxigenic <i>Staph.aureus</i> isolated from the examined Domiati cheese samples	66
Table 12. Enterotoxigenic <i>Staph.aureus</i> isolated from the examined Kareish cheese samples	67

Table 13. Enterotoxigenic <i>Staph.aureus</i> isolated from the examined Tallaga cheese samples	68
Table 14. Incidence of anaerobic bacteria in the examined soft cheese samples	69
Table 15. Statistical analytical results of yeasts count in the examined soft cheese samples	70
Table 16. Frequency distribution of positive soft cheese samples based on their yeasts count	70
Table 17. Statistical analytical results of molds count in the examined soft cheese samples	71
Table 18. Frequency distribution of positive soft cheese samples based on their molds count	71
Table 19. Effect of Reuterin and Nisin on total bacterial count of Domiati cheese during storage period	72
Table 20. Effect of Reuterin and Nisin on coliforms count of Domiati cheese during storage period	73
Table 21. Effect of Reuterin and Nisin on yeast count of Domiati cheese during storage period	74
Table 22. Effect of Reuterin and Nisin on mold count of Domiati cheese during storage period	74
Figure 1. Total bacterial count (log) of Domiati cheese (control one and those supplemented with Reuterin and Nisin) during storage period.....	75
Figure 2: Coliforms count (log) of Domiati cheese (control one and those supplemented with Reuterin and Nisin) during storage period.....	76
Figure 3: Yeast count (log) of Domiati cheese (control one and those supplemented with Reuterin and Nisin) during storage period.....	77
Figure 4: Mold count (log) of Domiati cheese (control one and those supplemented with Reuterin and Nisin) during storage period.....	78

Table 23. Total bacterial count of Domiati cheese stored in whey containing Reuterin and Nisin.....	79
Table 24. Coliforms count of Domiati cheese stored in whey containing Reuterin and Nisin	80
Table 25. Yeast count of Domiati cheese stored in whey containing Reuterin and Nisin	81
Table 26. Mold count of Domiati cheese stored in whey containing Reuterin and Nisin	81
Figure 5: Total bacterial count (log) of Domiati cheese (control one and those stored in supplemented whey with Reuterin and Nisin).....	82
Figure 6: Coliform count (log) of Domiati cheese (control one and those stored in supplemented whey with Reuterin and Nisin).....	83
Figure 7: Yeast count (log) of Domiati cheese (control one and those stored in supplemented whey with Reuterin and Nisin).....	84
Figure 8: Mold count (log) of Domiati cheese (control one and those stored in supplemented whey with Reuterin and Nisin).....	85
Table 27: Na Cl % and Titratable acidity % of manufactured cheese during storage period.....	86
Figure 9: Na Cl % and Titratable acidity % of manufactured cheese during storage period.....	87

Summary

One hundred and fifty random samples of soft cheeses represented by Domiati, Kareish and Tallaga (50 samples each) were collected from different local retails, dairy shops and street vendor in Assiut city, Egypt. A survey was conducted to determine the microbiological quality of the examined soft cheese samples.

Domiati cheese was prepared from pasteurized milk containing 10% sodium chloride. reuterin and nisin (1.4 g/l and 0.1 mg/l) were added. Cheese batches were stored in their whey at room temperature and examined periodically every week till 8 weeks.

The obtained results showed that:

1- Microbiological quality of soft cheese samples:

• Total aerobic count:

In soft cheeses aerobic bacteria were detected in 100% with average count values of 4.2×10^4 , 2.6×10^5 and 6.1×10^4 CFU/g in Domiati, Kareish and Tallaga cheese, respectively.

• Total coliforms and faecal coliforms count:

Coliforms were detected in 70, 86 and 76% of the examined soft cheese samples with average count values of 8.5×10^2 , 3.3×10^3 and 2.0×10^3 CFU/g in Domiati, Kareish and Tallaga cheese, respectively.

Fecal coliforms were present in 66, 74 and 70% of the examined soft cheese samples with average count values of 5.3×10^2 , 9.7×10^2 and 8.4×10^2 CFU/g in Domiati, Kareish and Tallaga cheese, respectively.

- ***E. coli* count:**

E. coli was isolated from 32, 50 and 42 % of the examined soft cheese samples with average count values of 3.0×10^2 , 4.0×10^2 and 4.4×10^2 CFU/g in Domiati, Kareish and Tallaga cheese, respectively.

- ***Staph. aureus* count:**

Staph. aureus was isolated from 74, 72 and 64% of the examined soft cheese samples with average count values of 1.8×10^3 , 1.7×10^3 and 8.7×10^2 CFU/g in Domiati, Kareish and Tallaga cheese, respectively. Enterotoxins (A, B, C and D) of *Staph. aureus* isolated from the examined Domiati, Kareish and Tallaga cheese samples, respectively, were found in 17.7, 25 and 16.6%, respectively.

- **Anaerobic bacteria:**

Anaerobic bacteria were found in 46, 66 and 54% in Domiati, Kareish and Tallaga cheese samples, respectively.

- **Yeast and mold count:**

Yeasts were present in 32, 60 and 56% of the examined soft cheese samples with average count values of 2.3×10^2 , 5.4×10^2 and 8.2×10^2 CFU/g in Domiati, Kareish and Tallaga cheese, respectively.

Molds were present in 46, 54 and 46% of the examined soft cheese samples with average count values of 3.8×10^2 , 4.1×10^2 and 3.9×10^2 CFU/g in Domiati, Kareish and Tallaga cheese, respectively.

2- Improve the microbial quality of cheese by reuterin and nisin:**• Total bacterial count:**

Total bacterial count reached a maximum of growth rate at 3rd week in control cheese batch while all enriched cheese showed gradual reduction in counts, till the reduction% reached 100% in the 5th week for reuterin and nisin enriched cheese batches, while the reduction was faster for the combination batch (100% reduction in the 4th week).

While in cheese samples stored in enriched whey, aerobic bacterial count reached a maximum of growth rate at 3rd week in control batch and 2nd week in all cheese of enriched whey batch, then reduction in growth rate was observed till reached 0 log CFU/g for all cheese of enriched whey batch.

• Total coliforms count:

Coliforms were not detectable in 3rd week of storage in Domiati cheese supplemented with reuterin and nisin, on the other hand coliforms were not detectable at the 2nd week of storage in Domiati cheese supplemented with combination of reuterin and Nisin, while were still present in control ones till 3rd week. Results showed that adding reuterin, nisin and combination of reuterin and nisin to cheese produced a reduction % of 7.27 and 18.18%, 7.27 and 17.63% and 9.09 and 100% for zero and 2nd week of storage, respectively.

Moreover, coliforms were not detectable in 4th week of storage in all cheese of enriched whey batch, while were still present in control ones till 4th week. Results showed that adding reuterin, nisin and combination of reuterin and nisin to whey produced a reduction % of 2.58 and 100%, 1.72 and 100% and 7.73 and 100% for zero and 4th week of storage, respectively.

- **Yeast and mold count:**

The reduction % of yeast count in reuterin enriched cheese batch were 19.35 and 31.37%, while that in nisin enriched cheese batch 7.26 and 25.98% and in combination of reuterin and nisin enriched cheese batch 19.35 and 36.76% for 1st and 8th week of storage, respectively.

The reduction % of mold count in reuterin enriched cheese batch were 7.26 and 23.73%, while that in nisin enriched cheese batch 7.26 and 15.25% and in combination of reuterin and nisin enriched cheese batch 7.26 and 26.55% for 1st and 8th week of storage, respectively.

Moreover, the reduction % of yeast count in Domiati cheese of reuterin enriched whey were 16.19 and 34.23%, while that in Domiati cheese of nisin enriched whey 15.47 and 32.80% and in Domiati cheese of the combination of reuterin and nisin enriched whey 24.10 and 39.96% for 1st and 8th week of storage, respectively.

While the reduction % of mold count in Domiati cheese of reuterin enriched whey were 13.43 and 21.13%, while that in Domiati cheese of nisin enriched whey 11.19 and 20.25% and in Domiati cheese of the combination of reuterin and nisin enriched whey 23.88 and 28.17% for 1st and 8th week of storage, respectively.

- **NaCl%:**

Salt content showed gradual increase in all cheese batches till reach 14.12, 14.35, 14.29 and 14.30% for control, reuterin, nisin and combination of reuterin and nisin enriched cheese, respectively by the end of the 8 weeks storage.

- **Titrateable acidity%:**

Titrateable acidity % of fresh cheese was increased by increasing the storage period to reach maximum values 1.62, 1.53, 1.56 and 1.61 % for control, reuterin, nisin and combination of reuterin and nisin enriched cheese, respectively by the end of the storage period (8 weeks).