



# Microbiological Evaluation Of Raw Milk And Soft Cheese In Sohag Governorate.

PhD. V. Sc. Thesis Presented by

### Fadel Abdel-fattah Mohammed

B.V.Sc. Assiut University (2008) M.V.Sc. Assiut University (2016)

## Under the supervision of

## Prof. Dr. Wallaa Farouk Amin

Professor of Milk Hygiene Faculty of Veterinary Medicine Assiut University

## Dr. Eman Mokhtar Shaker

Assistant Professor of Milk Hygiene Faculty of Veterinary Medicine Sohag University.

## Prof. Dr. Hassan Mohammed Gad Al-Rab

Emeritus Prof. of Milk Hygiene Animal Health Research Institute Sohag

1442 H – 2020 A.D.

# CONTENTS

Introduction 1
Review Of Literature 5
Materials and Methods 61
Results 78
Discussion
Conclusion and Suggestions 119
Summary 121
References 126
Arabic Summary1

# List of Tables

No.	Table	Page
(1)	Statistical analytical results of total aerobic count in the examined samples.	78
(2)	Frequency distribution of the positive samples based on their total aerobic count.	79
(3)	Statistical analytical results of total coliforms count in the examined samples.	80
(4)	Frequency distribution of the positive samples based on their total coliforms count.	81
(5)	Statistical analytical results of faecal coliforms count in the examined samples.	82
(6)	Frequency distribution of the positive samples based on their faecal coliforms count.	82
(7)	Statistical analytical results of <i>E. coli</i> count in the examined samples.	83
(8)	Frequency distribution of the positive samples based on their <i>E. coli</i> count.	83
(9)	Incidence of anaerobic bacteria in the examined samples.	84
(10)	Statistical analytical results of psychrotrophic count in the examined samples.	85
(11)	Frequency distribution of the positive samples based on their psychrotrophic count.	86
(12)	Statistical analytical results of <i>Staph. aureus</i> count in the examined samples.	87
(13)	Frequency distribution of the positive samples based on their <i>Staph. aureus</i> count.	87
(14)	Statistical analytical results of total yeasts count in the examined samples.	88
(15)	Frequency distribution of the positive samples based on their total yeasts count.	89

(16)	Statistical analytical results of total molds count in the examined samples.	90
(17)	Frequency distribution of the positive samples based on their total molds count.	91
(18)	Statistical analytical results of total yeast and mold count in the examined samples.	92
(19)	Frequency distribution of the positive samples based on their total yeast and mold count.	93
(20)	Effect of Nigella sativa seed oil (NSSO) on total bacterial count in Domiati cheese.	94
(21)	Effect of Nigella sativa seed oil (NSSO) on total coliforms count in Domiati cheese.	94
(22)	Effect of Nigella sativa seed oil (NSSO) on <i>E. coli</i> count in Domiati cheese.	95
(23)	Effect of Nigella sativa seed oil (NSSO) on psychrotrophic count in Domiati cheese.	95
(24)	Effect of Nigella sativa seed oil (NSSO) on <i>Staph. aureus</i> count in Domiati cheese.	96
(25)	Effect of Nigella sativa seed oil (NSSO) on total yeast and mold count in Domiati cheese.	96
(26)	Na Cl % and Titratable acidity % of manufactured cheese.	98

# List of Figures

No.	Figure	Page
(1)	Microbial counts (log) in Domiati cheese (control and supplemented with 0.1% and 0.2% NSSO).	97

## **Summary**

A total of 250 samples; 150 samples of raw milk (50 samples each from dairy farms, dairy shops and street venders), 50 samples each of Domiati and Kareish cheese were randomly collected from dairy farmers, farmer's houses, dairy shops, street venders and groceries in Sohag, Egypt. A survey was conducted to determine the microbiological quality of the examined milk and soft cheese samples.

Domiati cheese was prepared from pasteurized milk containing 10% sodium chloride. Nigella sativa seed oil (0.1 and 0.2%) was added. Cheese batches were stored in their whey at room temperature and examined periodically every week at day (0, 7, 14, 21, 28, 35 and 42).

#### The results obtained showed that:

#### 1-Microbiological quality of raw milk and soft cheese samples:

#### • Total aerobic count:

All the examined samples of raw milk were contaminated with aerobic bacteria with average count values of  $3.8 \times 10^8$ ,  $1.05 \times 10^{10}$  and  $1.17 \times 10^{10}$  CFU/ml for dairy farms, street venders and dairy shops, respectively. In soft cheeses aerobic bacteria were detected in 100% with average count values of  $6 \times 10^7$  and  $3.1 \times 10^9$  CFU/g in Domiati and Kareish cheese, respectively.

#### • Total coliforms and faecal coliforms count:

Coliforms were detected in 96, 84 and 96% of the examined raw milk samples with average count values of  $2.2 \times 10^5$ ,  $1.6 \times 10^6$  and  $1.5 \times 10^6$  CFU/ml for dairy farms, street venders and dairy shops, respectively. In soft cheese coliforms were detected in 26 and 94% with average count values of  $1.7 \times 10^3$ 

and 4.1 x10<sup>4</sup> CFU/g in Domiati and Kareish cheese, respectively.

Fecal coliforms were present in 50, 44 and 54% of the examined raw milk with average count values of  $1.6 \times 10^3$ ,  $2.1 \times 10^3$  and  $1.8 \times 10^3$  CFU/ml for dairy farms, street venders and dairy shops, respectively. In soft cheese fecal coliforms were detected in 12 and 86% with average count values of  $2.3 \times 10^2$  and  $1.3 \times 10^3$  CFU/g in Domiati and Kareish cheese, respectively.

#### • *E. coli* count:

*E. coli* was isolated from 12, 20 and 24% of raw milk samples with average count values of  $1.4 \times 10^2$ ,  $2.6 \times 10^2$  and  $2.2 \times 10^2$  CFU/ml for dairy farms, street venders and dairy shops, respectively. In soft cheese *E. coli* was detected in 4 and 34% with average count values of  $1.3 \times 10^2$  and  $4.9 \times 10^2$  CFU/g in Domiati and Kareish cheese, respectively.

#### • Anaerobic bacteria:

Anaerobic bacteria in raw milk samples were present in 34, 42 and 48% in dairy farms, street venders and dairy shops, respectively. While in soft cheese were found in 32 and 38% in Domiati and Kareish cheese samples, respectively

#### • Psychrotrophic count:

All the examined samples of raw milk were contaminated with psychrotrophic bacteria with average count values of  $1.6 \times 10^6$ ,  $6.1 \times 10^6$  and  $4.2 \times 10^6$  CFU/ml for dairy farms, street venders and dairy shops, respectively. In soft cheese psychrotrophic bacteria were detected in 50 and 88% with average count values of  $1.4 \times 10^4$  and  $1.14 \times 10^5$  CFU/g in Domiati and Kareish cheese, respectively.

#### • Staph. aureus count:

*Staph. aureus* was isolated from 34, 44 and 48% of raw milk samples with average count values of  $8.1 \times 10^3$ ,  $9 \times 10^3$  and  $2.2 \times 10^4$  CFU/ml for dairy farms, street venders and dairy shops, respectively. In soft cheese *Staph. aureus* was detected in 40 and 42% with average count values of  $1.9 \times 10^5$  and  $1.1 \times 10^5$  CFU/g in Domiati and Kareish cheese, respectively.

#### • Yeast and mold count:

Yeasts in raw milk samples were present in 40, 42 and 46% with average count values of  $1.2 \times 10^3$ ,  $2.5 \times 10^3$  and  $2.9 \times 10^3$  CFU/ml for dairy farms, street venders and dairy shops, respectively. In soft cheese yeasts were detected in 66 and 70% with average count values of  $2.7 \times 10^4$  and  $6.6 \times 10^5$  CFU/g in Domiati and Kareish cheese, respectively.

Molds were present in 38, 32 and 28% of raw milk samples with average count values of  $4.1 \times 10^2$ ,  $6 \times 10^2$  and  $1.09 \times 10^3$  CFU/ml for dairy farms, street venders and dairy shops, respectively. In soft cheese molds were detected in 32 and 22% with average count values of  $1 \times 10^4$  and  $4.3 \times 10^5$  CFU/g in Domiati and Kareish cheese, respectively.

Yeasts and molds were present in 40, 42 and 46% of raw milk samples with average count values of  $1.6 \times 10^3$ ,  $2.9 \times 10^3$  and  $3.4 \times 10^3$  CFU/ml for dairy farms, street venders and dairy shops, respectively. In soft cheese yeasts and molds were detected in 66 and 70% with average count values of  $3.16 \times 10^4$  and  $8.01 \times 10^6$  CFU/g in Domiati and Kareish cheese, respectively.

# 2-Evaluation of antimicrobial potential of *Nigella Sativa Seed Oil* (NSSO) in Domiati cheese:

#### • Total bacterial count:

Total bacterial count reached a maximum of growth rate after 21 days in all samples, then reduction in growth rate was observed till reached 8.74 log CFU/g for 0.2% NSSO, 9.76 log for 0.1% NSSO and10.81 log for control by the end of day 42 of storage at room temperature. Results showed that adding NSSO (0.1 or 0.2%) to cheese produced a reduction % of 5.29, 9.71% and 8.82, 19.15% for zero and  $42^{nd}$  day of storage respectively.

#### • Total coliforms count:

Coliforms were not detectable in 21<sup>st</sup> day of storage at room temperature in soft cheese supplemented with 0.2% NSSO, while were still present in 0.1% and control ones. Results showed that adding NSSO (0.1 or 0.2%) to cheese produced a reduction % of 14.9, 7.5% and 22.65, 100% for zero and 21<sup>st</sup> day of storage respectively.

#### • *E. coli* count:

*E. coli* rapidly lost its viability in cheese made from milk containing 0.2% NSSO to be undetectable by  $14^{\text{th}}$  day, while it could be detected in that made by 0.1% NSSO and control ones till  $21^{\text{st}}$  day. Results showed that adding NSSO (0.1 or 0.2%) to cheese produced a reduction % of 23.38, 15.25% and 35.06, 100% for zero and  $21^{\text{st}}$  day of storage respectively.

#### • Psychrotrophic count:

Psychrotrophic bacteria grew in enriched and control cheese till reached 4.45, 5.49 and 6.80 log CFU/g in 0.2%, 0.1% enriched cheese and control cheese, respectively. Results showed that adding NSSO (0.1 or 0.2%) to cheese produced a reduction % of 14.12, 19.26% and 19.21, 34.56% for zero and 42<sup>nd</sup> day of storage respectively.

#### • Staph. aureus count:

*Staph. aureus* counts reached the maximum at  $21^{st}$  day, then there was a dropping in all cheese batches till reached 4.00, 4.95 and 6.65 log CFU/g in 0.2%, 0.1% enriched cheese and control cheese, respectively by the end of day 42. Results showed that adding NSSO (0.1 or 0.2%) to cheese produced a reduction % of 4.61, 25.56% and 11.54, 39.85% for zero and  $42^{nd}$  day of storage respectively.

#### • Total yeast and mold count:

Total yeast and mold count showed growth from 7<sup>th</sup> day till reached 6.41 log CFU/g for 0.2% NSSO enriched cheese, 7.71 log for 0.1% NSSO enriched cheese and 9.61 log for control one by the end of day 42 of storage at room temperature. Results showed that adding NSSO (0.1 or 0.2%) to cheese produced a reduction % of 0, 19.77% and 4.49, 33.3% for 7<sup>th</sup> and 42<sup>nd</sup> day of storage respectively.

#### • Na Cl%:

Salt content showed gradual increase in all cheese batches till reached 7.4, 7.6 and 7.36% in control cheese, 0.1% NSSO enriched cheese and 0.2% NSSO enriched cheese, respectively by the end of day 42 of storage.

#### • Titratable acidity %:

Titratable acidity % was increased by increasing the storage period to reach maximum values 0.62, 0.60 and 0.48 % for control cheese, 0.1% NSSO enriched cheese and 0.2% NSSO enriched cheese, respectively by the end of day 42 of storage.