HARMONIZATION OF MICROBIOLOGICAL METHODS TO MONITOR QUALITY AND SAFETY IN THE DAIRY PRODUCTION CHAIN

By

AMAL ABD ALLAH ABD EL-MONEM HEGAZY
B.Sc. Agric. Sc. (Dairy Science and Technology), Ain Shams University, 2007

A thesis submitted in partial fulfillment
of
the requirements for the degree of

MASTER OF SCIENCE

in

Agricultural Science
(Dairy Science and Technology)

Department of Food Science
Faculty of Agriculture
Ain Shams University

2013

Approval Sheet

HARMONIZATION OF MICROBIOLOGICAL METHODS TO MONITOR QUALITY AND SAFETY IN THE DAIRY PRODUCTION CHAIN

By

AMAL ABD ALLAH ABD EL-MONEM HEGAZY
B.Sc. Agric. Sc. (Dairy Science and Technology), Ain Shams University, 2007

This thesis for M.Sc. degree has been approved by:

Dr. Taha Abd El-Halim Nassib ....
Prof. Emeritus of Dairy Microbiology, Faculty of Agriculture, Mansoura University.

Dr. Youssef Morsy El-Kenany ....
Prof. of Dairy Microbiology and Technology, Faculty of Agriculture, Ain Shams University.

Dr. Mohamed Abd El-Razek El-Nawawy ....
Prof. Emeritus of Dairy Microbiology, Faculty of Agriculture, Ain Shams University.

Dr. Yehia Abd El-Azim El-Samragy ....
Prof. Emeritus of Dairy Microbiology and Technology, Faculty of Agriculture, Ain Shams University.

Date of Examination: 11 / 6 / 2013

HARMONIZATION OF MICROBIOLOGICAL METHODS TO MONITOR QUALITY AND SAFETY IN THE DAIRY PRODUCTION CHAIN

By

AMAL ABD ALLAH ABD EL-MONEM HEGAZY

B.Sc. Agric. Sc. (Dairy Science and Technology), Ain Shams University, 2007

Under the supervision of:

Dr. Yehia Abd El-Azim El-Samragy

Prof. Emeritus of Dairy Microbiology and Technology, Food Science Department, Faculty of Agriculture, Ain Shams University (Principal Supervisor).

Dr. Mohamed Abd El-Razek El-Nawawy

Prof. Emeritus of Dairy Microbiology, Food Science Department, Faculty of Agriculture, Ain Shams University.

Dr. Ali Abd El-Aziz Ali

Prof. of Dairy Microbiology and Technology, Food Science Department, Faculty of Agriculture, Ain Shams University.

.

ABSTRACT

Amal Abd Allah Abd El-Monem Hegazy: Harmonization of Microbiological Methods to Monitor Quality and Safety in the Dairy Production Chain. Unpublished M.Sc. Dissertation, Department of Food Science, Faculty of Agriculture, Ain Shams University, 2013.

The current study was designed to study the effect of some parameters on the uncertainty of the results of the **ISO 7932:2004** and **10272-1:2005** methods used for *Bacillus cereus* and *Campylobacter Spp.* as compared with PCR protocol that can be used for the rapid detection of *B. cereus* and *Campylobacter jejuni* in milk and milk products. So we studied the effect of some parameters of the **ISO 7932:2004** and **10272-1:2005** for *B. cereus* enumeration and *C. jejuni detection* compared with PCR direct detection method for non-emetic *B. cereus* and hipO gene for *C. jejuni.*

The parameters such as type of medium, dilution solution type, incubation time, milk fat, type of coagulation, heat treatment, of milk and sodium chloride concentration in rennet coagulated milk were tested on the enumeration of *B. cereus*.

The results show that Mannitol Egg Yolk Polymyxin agar (MYP) appears to be a reliable medium for the isolation of *B. cereus* group, use buffered sodium chloride peptone solution as dilution solution in this method to give better recovery, the best incubation time is from 16 to 24 h, there was negative effect of fat but the medium had higher effect.

Assess the level of *B. cereus* contamination in some Egyptian dairy products using **ISO 7932:2004** method and compared with the PCR detection method. To assess the level of *B. cereus* contamination in some Egyptian dairy products in great Cairo governorate a total of

50 raw milk and 211 different cheese types (Low salt (Tallagah, Feta and Kariesh cheese), high salt (Istanboly) samples were randomly collected and analyzed using **ISO 7933:2004** method and compared with the PCR detection method. The *B. cereus* counts in 55% of the milk samples were lower than 10^5 cfu/ml and 23% of the samples contained 10^7 cfu/ml or higher. In this study Nhe-B gen was found in 45% of the 50 samples. Moreover 71% Istanbuly and Bramily cheese and 64% of feta and Talaga cheese contained lower than 10^5 cfu /g. *C. jejuni* contamination in the same Egyptian dairy products collected from great Cairo governorate showed that 6% of raw milk samples, 9% of low salt cheese samples and 2% of karish cheese samples. In conclusion, the methods described in this study can help to optimize the detection of the 2 organisms in dairy production chain. The next logical step is the application of the current methods to the analysis of contamination in a factory setting during processing runs.

Key words:

B. cereus, *C. jejuni*, **ISO 7932:2004**, **ISO 10272-1:2005**, PCR, Dairy products.

ACKNOWLEDGEMENT

All praises are due to God, who blessed me with kind professors and colleagues, and gave me the support to produce this thesis.

I would like to express my profound gratitude and sincere appreciation to **Prof. Dr. Yehia A. El-Samragy** Prof. of Dairy Microbiology and Technology, Faculty of Agriculture, Ain Shams University, for his kind supervision, valuable guidance and his kind encouragements for me during the present investigation.

I wish also to express my deepest thanks for **Prof. Dr. Mohamed A. El-Nawawy**, Prof. of. Dairy Microbiology, Faculty of Agriculture, Ain Shams University, for his kind attention and efforts made through the course of the experiments, useful comments and editing this thesis.

Sincere thanks and deepest gratitude to **Prof. Dr. Ali A. Ali**, Prof. of Dairy Microbiology and Technology, Faculty of Agriculture, Ain Shams University, for his supervision, guidance, continuous support in the course of this investigation and help during the preparation and writing this manuscript.

Thanks to **Dr. Howida El-Shazly** Assistant Prof. of dairy technology, Food Technology Inst., Agric. Res. Center for her support during the PCR analysis.

I would also like to express my gratitude for all staff members of Microbiological Resources Center **(Cairo-MIRCEN)**, and of Department of Food Science, Faculty of Agriculture, Ain shams University, Cairo, Egypt for their fruitful cooperation and friendships.

I would also like to thanks the Monitoring and Quality Assurance in the Food Supply Chain Network of Excellent **(MoniQA)** (contract no. FOOD-CT-2006-36337) for the financial support in this work.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. INTRODUCTION

Milk can harbor a variety of microorganisms and can be an important source of foodborne pathogens. The presence of foodborne pathogens in milk can be due to direct contact with contaminated sources in the dairy farm environment and to excretion from the udder of an infected animal. The dairy industry should be concerned about dairy food safety because outbreaks of disease in humans have been traced to the consumption of unpasteurized milk and have also been traced back to pasteurized milk. Unpasteurized milk is consumed directly by dairy producers and their families, farm employees and their families, neighbors, and raw milk advocates. In Egypt unpasteurized milk is consumed directly by a large segment of the population via consumption of several types of cheeses manufactured from unpasteurized milk. Entry of foodborne pathogens via contaminated raw milk into dairy food processing plants can lead to persistence of these pathogens in biofilms, and subsequent contamination of processed milk products and exposure of consumers to pathogenic bacteria. The final outcome of this cycle is a constantly maintained reservoir of foodborne pathogens that can reach the human population by direct contact, ingestion of raw contaminated food (raw milk or cheese made with raw milk), or contamination during the processing of milk.

The challenges to providing a safe and nutritious food supply are complex because all aspects of food production—from farm to fork— need to be considered. Given the considerable national/international demand and expectations for food safety and the formidable challenges of producing and maintaining a safe food supply, food safety research and educational programs have been taken on a new urgency.

As the system of food production and distribution changes, the food safety system needs to change with it. A strong science-based approach that addresses all the complex issues involved in continuing to improve food safety and public health is necessary to prevent foodborne illnesses. Not only must research be conducted to solve complex food safety issues, but also results of that research must be communicated effectively to producers and consumers. Research and educational efforts identifying potential on-farm risk factors will better enable dairy producers to reduce/prevent foodborne pathogen contamination of dairy products leaving the farm. Identification of on-farm reservoirs could aid with implementation of farm-specific pathogen reduction programs.

The rapid detection of pathogens, and other microbial contaminants, in food is crucial for ensuring the safety of consumers and for many countries, preserving the export market. Traditional methods for detecting foodborne pathogens often rely on time-consuming growth in culture medium followed by isolation, biochemical identification and in some cases, serology. Recent advances in technology have led to the development of many quantitative, semi-quantitative and qualitative rapid methods that make detection and identification faster, more convenient, more sensitive and more specific than many conventional assays. These “rapid methods” cover a huge array of tests including miniaturised biochemical kits, antibody and DNA-based tests and assays that are modifications of conventional tests aimed at speeding up analysis. Some of these assays have also been automated to reduce operator or handling errors. Most of the rapid assays to detect specific pathogens in foods do however require growth in an enrichment medium before analysis.

Almost all rapid methods are designed to detect a single target, making them ideal for the quick screening of large numbers of food samples. A positive result following the use of a rapid method is only regarded as presumptive and must be confirmed by standard methods.