بسم الله الرحمن الرحيم

شبكة المعلومات الجامعية

شبكة المعلومات الجامعية

التوثيق الالكتروني والميكروفيلم

شبكة المعلومات الجامعية

جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها
علي هذه الأقراص المدمجة قد أعدت دون أية تغيرات

يجب أن

تحفظ هذه الأقراص المدمجة بعيدا عن الغبار

Cairo University
Faculty of Veterinary Medicine
Department of Food Hygiene and Control

Cairo University

Microbial Profile and Aflatoxin M_1 in Some Dairy Products and Cheese Analogues

Thesis submitted by

Abdel-Kareem Hamed Adam Abdel-latif

(B.V.Sc. Cairo University, 2017)

For the Degree of M.V.Sc.
(Hygiene and Control of Milk and its Products)

Under supervision of

Prof. Dr. Salwa Ahmed Mohamed Aly

Professor of Milk Hygiene and control
Faculty of Veterinary Medicine
Cairo University

Dr. Mena Fouad Saad

Assistant Professor of Milk Hygiene and control
Faculty of Veterinary Medicine
Cairo University

(2021)

Cairo University
Faculty of Veterinary Medicine
Department of Food Hygiene and Control

Cairo University

Supervision sheet

Prof. Dr. Salwa Ahmed Mohamed Aly
Professor of Milk Hygiene and Control
Faculty of Veterinary Medicine
Cairo University

Dr. Mena Fouad Saad
Assistant Professor of Milk Hygiene and Control
Faculty of Veterinary Medicine
Cairo University

(2021)

Cairo University
Faculty of Veterinary Medicine
Department of Food Hygiene and Control

Cairo University

Name: Abdel-Kareem Hamed Adam Abdel-latif.
Date and place of birth: 25/4/1994, Fayoum.
Nationality: Egyptian
Degree: Master's degree in Veterinary Medical Sciences.
Specialization: Food Hygiene and Control (Milk and its Products)
Supervision:

Prof. Dr. Salwa Ahmed Aly	Professor of Milk Hygiene and Control, Faculty of Veterinary Medicine, Cairo University.
Dr. Mena Fouad Saad	Assistant Professor of Milk Hygiene and Control, Faculty of Veterinary Medicine, Cairo University.

Title of the thesis: Microbial Profile and Aflatoxin M_1 in Some Dairy Products and Cheese Analogues

ABSTRACT

Dairy products have a significant value in human nutrition but may harbor risk to public health, therefore monitoring of dairy products is highly essential to safeguard consumers. This thesis aimed to investigate the possible microbiological hazards as well as Aflatoxin M_1 in some dairy products. A total of 300 samples (50 each of cheese analogue; flavored yogurt; flavored drinking yogurt; kariesh cheese; white soft cheese, and Ras (Roumy) cheese) were collected from street vendors, dairy shops, and supermarkets at three various districts, El-Fayoum governorate, Egypt. The obtained results revealed that aerobic spore formers, *Bacillus cereus* (*B. cereus*), total staphylococci, total coagulase-positive staphylococci (TCPS), coliforms (MPN/g), and total yeast & mold were present in percentages of 36, 4, 30, 0, 0 and 24% in the examined cheese analogue samples ;66, 6, 58, 18, 64 & 30% in flavored yoghurt samples; 88, 8, 42, 0, 32 and 54% in flavored drinking yogurt samples; 76, 6, 100, 20, 88 and 78% in kareish cheese samples; 56, 8, 96, 20, 76 and 36% in white soft cheese samples, and 86, 6, 100, 22, 86 and 36% in the examined Ras cheese samples. *Bacillus cereus* isolates were confirmed and tested for enterotoxigenic genes via biochemical and molecular approaches using Polymerase Chain Reaction (PCR). The *nhe* gene is the most detected gene (100%) in all of the examined isolates, followed by *cytK* (52.6%), and *ces* (5.3%) genes. The *hbl* and *bceT* genes could not be detected in all of the examined isolates. *Escherichia coli* and *salmonella* failed to be detected in all of the examined samples (100.0% compliance with the Egyptian Standard Regulation). Also, *Pseudomonas aerogenosa* could not be detected in any of the examined samples. Aflatoxin M_1 (AFM_1) was evaluated in kariesh cheese, flavored drinking yogurt & cheese analogue using a commercial enzyme-linked immunosorbent assay (ELISA) method. The obtained data revealed that 80% of the analyzed kareish cheese samples were contaminated with aflatoxin M_1 in amount between 59.1 - 875.4 ng/kg which exceeds European Community (EC) regulation limits. In conclusion, the results of this investigation clarify the potential risk associated with the consumption of the examined products. As the consumers prefer safe and healthy products, several control measures in the food chain should be incorporated in the dairy industry to ensure the safety and quality of dairy products.

Keywords: Dairy products, cheese analogues, Microbiological hazards, *Bacillus cereus* enterotoxigenic genes, Spoilage microorganisms, Aflatoxin M_1, ELISA method.

Dedication

I dedicate this work to whom my heart feels thanks and love; to my great lovely father,

to my great lovely mother,

to my brother (Amr) and my sister (Rana),

to my kind uncle,

and to my grandmothers' souls;

for their unwavering support, encouragement, and unconditional love.

ACKNOWLEDGEMENT

First of all, I am very thankful to ***Allah,*** *our gracious and merciful GOD for helping me in accomplishing my thesis.*

No words can adequately express my heartfelt appreciation and great gratitude to my supervisor ***Prof. Dr. Salwa Ahmed Aly****, Prof. of Milk Hygiene, Faculty of Veterinary Medicine, Cairo University who offered me a lot of her time and who devoted her experience to provide me with the best possible pieces of advice and suggestion to this work.*

I am extremely thankful to my supervisor ***Dr. Mena Fouad Saad,*** *Assistant Professor of Milk Hygiene, Faculty of Veterinary Medicine, Cairo University for his encouragement, enthusiasm, dynamism, valuable help, and great patience during work.*

Deep gratitude to ***Dr. Rasha Sayed-ELAhl,*** *Chief researcher in Department of Mycology and Mycotoxins, Animal Health Research Institute (AHRI), Agriculture Research Center (ARC), Egypt for her valuable help, great patience during work, and active participation in AFM_1 analysis.*

Deep gratitude to my friend ***Mahmoud Gamal****, Lecturer Assistant of Biochemistry and Molecular Biology, Faculty of Veterinary Medicine, Cairo University for his active participation in the molecular identification work.*

Appreciation also goes to ***all members of Food Hygiene and Control Department, Faculty of Veterinary Medicine, Cairo University*** *for their encouragement and help.*

CONTENTS

List of tables

List of figures

CHAPTER (1)

INTRODUCTION

INTRODUCTION

Dairy products are common food consumed by people of all age groups, especially children. They are highly nutritious and provide micro and macronutrients essential for the growth and maintenance of human health. However, they are not only nutritious for humans but also, excellent growth substrates for microorganisms **(Fusco *et al*., 2020).**

Food safety is a pressing issue for governments, food processors, retailers, and consumers worldwide; this sense of urgency is in the dairy industry, particularly in developing countries. There are three categories of food safety concerns in the dairy industry. These include physical hazards, biological hazards; primarily food-borne pathogens, and chemical hazards, that cover a broad range of contaminants, including toxins produced by microorganisms found in the environment as mycotoxins **(Sandrou & Arvanitoyannis, 2000 and WHO, 2012)**.

Owing to insufficient animal health control, inadequate training of farmers & dairy processing workers about dairy hygiene, and weakness in the cold chain during production processes & storage; milk and dairy products are considered a high-risk category for potential microbial contamination **(Ruegg, 2003; Aaku *et al.*, 2004 and Chizari *et al*., 2008).** A significant economic loss at various stages of the milk products processing chain is driven by microbial contamination of milk. Official data from World Bank indicate that 20.0% of milk production in developing countries is lost due to early spoilage. The estimated average milk spoilage of one liter per farm per day is about 17% wastage of the milk produced. This is a significant loss with an economic opportunity of $12 per month **(Bonfoh *et al*., 2003 and Bayemi *et al*., 2007).**

Cheese is one of the most popular dairy products in Egypt. It supplies the body with protein, fat, vitamins, and minerals. Cheese intake is related to improving bone health and a lower risk of cardiovascular disease and

hypertension. There are wide varieties and kinds of cheese such as soft, Kariesh, Ras, and cheese analogue **(Al-Gamal, *et al.*, 2019).**

Currently, the shift in attention among people is towards plant-based substitutes to replace animal products that provide various health benefits when consumed rather than calorie-rich food, which causes diseases **(Erin *et al.*, 2021).**

The term, plant-based, is wider as it focuses on the consumption of foods primarily from plants (fruit, vegetables, nuts, and oil) but can include small quantities of food from animal origins such as milk, eggs, meat, and fish. Those who follow a plant-based diet might choose to substitute animal products for vegetable options, without the permanent restriction of animal foods. Plant-based diets have become popular as a means of reducing the environmental footprint of the diet and promoting human health and animal welfare. Although the percentages of vegetarians and vegans are low compared to omnivores, their numbers have increased significantly in the last years. The use of non-animal food products other than meat alternatives is also increasing and this tendency constitutes an opportunity for the food industry **(El-Badry & Raslan, 2016 and Alcorta *et al.*, 2021).**

Cheese analogues which are known as imitation cheese, cheese alternative, cheese substitutes, or cheese-like products are new products made by blending individual constituents, including non-dairy fats or proteins, together with a flavor system simulating as closely as possible natural product. They are widely chosen due to their cost-effectiveness, attributable to the simplicity of manufacture, their textural and functional properties (easily meltable, cuttable & stretching) as well as the replacement of selected milk ingredients by cheaper vegetable products. Moreover, there is an ever-increasing interest among consumers in food products, which contain less total fat, saturated fat, cholesterol, and calories. Cheese analogues may be regarded as engineered products. They made from plant origin was expected to fulfill the

people's need for cheese, and as alternative cheese made from cow's milk. There is a wide range of plant-based versions of soft cheese in the Egyptian markets. Some people are allergic to cow's milk and can have a potentially dangerous allergic reaction to it. For people, who are lactose intolerant or sensitive to dairy products, switching to alternatives can help to avoid symptoms and keep their digestive system healthy **(Bachmann, 2001 and Short *et al.*, 2021)**.

Contamination of cheese with microbial pathogens could initiate from several sources during cheese production, for example, starter culture, cheese vat, floor, brine, packaging material, curd cutting knife, cheesecloth, production room air, and refrigerating room as well as post-pasteurization contamination. This may be as a result of cross-contamination of finished product with the raw product, inadequate sanitation procedures in the plant environment, or inadequately sanitized equipment **(Aini *et al.*, 2019).** Because of their unique composition and properties, cheese represents an excellent growth media for many spoilage and pathogenic microorganisms including; spore-forming bacteria, *Staphylococcus aureus*, *Salmonella*, *Escherichia coli* O157:H7 during cheese processing, which cause economic consequences and many food-borne outbreaks to consumers **(Landgraf and Destro, 2013; Szabolcs, 2014; Kyoung-Hee *et al.*, 2016 and Cancino-Padilla *et al.*, 2017).**

Fermented milk products are of considerable economic and dietary importance to consumers. They are prepared by controlled fermentation of milk to produce acidity and flavor to the desired level. Fortunately, the dominant bacteria in fermented kinds of milk were progressive types such as **lactic acid** streptococci and lactobacilli, which generally reduce the spoilage and pathogenic organisms. Fermentation was used to control the growth of harmful bacteria and some pathogens Yoghurt is considered the most public and commercial