Cairo University
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Risk assessment of some dairy products with special concern to safety issues

A Thesis Submitted by

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(B.V.Sc. Cairo University, 2016)

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

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Supervision sheet

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ABSTRACT

A total of 75 ice-cream samples and 75 white soft cheese samples (35 large scale and 40 small scale for each ice-cream and white soft cheese) were collected from street vendors, dairy shops and supermarkets in Cairo and Giza governorates. The collected samples were investigated for different types of hazards including, microbiological, chemical and physical. For microbiological examination; the mean count of total aerobic mesophilic bacteria, psychrotrophic bacteria, coliforms, fecal coliforms, yeast, mold and total staphylococci were 6.32 ± 5.8, 5.2 ± 4.5, 4.8 ± 4.4, 5.3 ± 4.7, 6.1 ± 5.8, 3.06 ±2.91, 5.13 ± 4.75, 2.55, 5.9 ± 5.6, 4.9 ± 4.5, 3.0 ± 2.6, 3.1 ± 2.9, 5.7 ± 5.2, 5.0 ± 4.5 for small and large scale ice-cream samples, respectively; and were 7.47 ± 6.98, 5.77 ±5.28, 6.70 ± 5.67, 5.33 ± 4.83, 7.71 ± 7.13, 3.43 ± 2.88, 6.30 ±5.99, 2.92 ± 2.53, 6.47 ± 5.71, 5.87 ± 5.39, 4.0 ± 3.89, 4.0 ± 3.93, 6.62 ± 5.08 ,5.5 ± 5.13 log cfu/g, for small and large scale white soft cheese samples, respectively. *S. aureus* was detected with incidence of 12.5%, 55.0%, 11.42% and 37.1% for small scale and large scale samples of ice-cream and cheese, respectively; while *E. coli*, salmonella, *L. monocytogenes* couldn't be detected in all examined samples. For chemical hazards; aflatoxin M1 was examined using a commercial enzyme-linked immunosorbent assay (ELISA) and the obtained results revealed that 80% of the examined ice-cream and white soft cheese samples were contaminated with a mean value of 24.31 ± 8.11 and 14.19 ± 2.48 ppt, respectively. Some of the organochlorine pesticides (OCPs) and Polychlorinated biphenyls pesticides (PCBs) were examined using Gas Chromatography (GC-MSMS); OCPs residues couldn't be detected in all ice-cream samples, while α-HCH, β-HCH, γ-HCH, P,P-DDT, o,p-DDT, p,p-DDE and Aldrin were detected in the examined white soft cheese samples with mean values of 0.02 ± 01, 0.03 ± 01, 0.03 ± 01, 0.03 ± 0.01, 0.05 ± 0.02, 0.01, 0.08 ppm., respectively. All of the the examined samples of ice-cream and white soft cheese were free from Polychlorinated biphenyl (PCBs) residues. The collected samples were exposed to physical inspection for the detection of physical hazards; hair, plastic and metal pieces were detected in some small scale samples of ice-cream and white soft cheese. The assessment of the existed hazards and their public health impact as well as the methods to to safe guard human health from the adverse effects of these hazards were fully mentioned and discussed.

Keywords, Risk, Biological Hazards, Chemical Hazards, Physical Hazards, Aflatoxin M1, Pesticide Residues, Ice-Cream, Soft Cheese, Salmonella, Listeria, *Staph. aureus.*

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Introduction

Milk and dairy products are vital sources of nutrition for many people. The demand for milk in developing countries is expected to increase by 25 percent by 2025. Consumption of dairy products has increased rapidly in recent decades in several parts of the developing world, driven by economic growth and rising income levels; this has been accompanied by major increases in production in several developing countries **(FAO, 2013).**

The microbiological hazards are most prevalent among the dairy supply chain. *Listeria monocytogenes, Staphylococcus aureus,* Salmonella, and pathogenic *Escherichia coli* were identified as the most important microbiological hazards in dairy products. Soft and semisoft cheeses are most frequently associated with *L. monocytogenes* and *S. aureus* enterotoxins, whereas raw milk is most frequently associated with pathogenic *E. coli* and Campylobacter spp. (**Van Asselt et al., 2017).**

The microbiological quality of white-brined cheeses can be influenced by numerous factors, including the quality of the milk, the use of pasteurization or thermization, various technological parameters, the level and type(s) of microbial contamination that occur throughout the manufacture and storage of the cheese and post-heat treatment contamination. Bacteriological quality of ice-cream reflects hygienic practice during production and is an indication of food safety **(Bintsis & Papademas, 2002 and Ambily & Beena, 2012).**

Primary sources of microbial contamination for ice-cream include water and raw milk, whereas secondary sources include flavoring agents, utensils and handling. Although pasteurization, freezing and hardening steps in production can estimate most of the microbial hazards, but still numerous health hazards are persistent due to various conditions **(Hossain et al., 2012)**.

Many outbreaks were associated with the consumption of cheese and ice-cream; the predominant involved microorganisms including Salmonella, *Listeria*

monocytogenes, Escherichia coli and *Staphylococcus aureus* **(EFSA, 2007; Cagri-Mehmetoglu et al., 2011** and **Domenech et al., 2013).**

Outbreaks of Salmonella have been reported in ice-cream and other frozen desserts in different parts of the world such as USA, UK and India**.** Nation-wide outbreak of Salmonellosis was more likely the result of contamination of pasteurized ice-cream premix during transport in tanker trailers that had previously carried non-pasteurized liquid eggs containing *Salmonella enteritidis*. In 2013, Raw Cashew cheese was linked to a multistate outbreak of *Salmonella Stanley* in California, this outbreak led to three hospitalizations, but no fatalities. Consumption of cheese in North Carolina caused an outbreak in 2016 with the strain of *Salmonella Typhimurium*, sixteen required hospitalization and eight out-of-state residents were also sickened **(Okojoh, 2006, CDC, 2014a** and **Edward et al., 2017)**.

Cheese-related *L. monocytogenes* outbreaks have had a relatively high fatality rate (15% to 30%), leading to increased public awareness of this infection. Clinically, *L. monocytogenes* causes sepsis and meningitis in immunocompromised individuals, and around 25% of the invasive listeriosis cases occur in pregnant women **(Wing & Gregory, 2002** and **FDA, 2012)**. Recently, the Centers for Disease Control and Prevention **(CDC, 2014b)** announced a multistate listeriosis outbreak due to cheese products in California and Maryland, of 58 listeriosis outbreaks reported during 1998–2014, a total of 17 (30%) were associated with soft cheese, and resulted in 180 illnesses, 14 fetal losses and 17 deaths. The proportion of listeriosis outbreaks linked to soft cheese made from pasteurized milk (12 outbreaks, 33%) was significantly higher during 2007–2014 than during 1998–2006 **(Jackson et al., 2018).**

Staphylococcus aureus is an important food-borne pathogen due to the ability of enterotoxigenic strains to produce staphylococcal enterotoxins (SEs) preformed in food **(Hennekinne et al., 2012)**. In 2013, a food poisoning outbreak caused by staphylococcal enterotoxins (SE) in ice-cream occurred in Germany, of

the 13 cases, seven cases were hospitalized; ice-cream was found to contain SE and high amounts of coagulase positive staphylococci **(Fetsch et al., 2014)**.

Yeasts and molds isolated from ice-cream have been mainly associated with the use of inadequately treated cane sugar, emulsifiers and flavourants and may also get entry in ice-cream from equipments, utensils, human hands and atmosphere **(Mathews et al., 2013** and **Edward et al., 2017).**

Based on literature and monitoring data, the most relevant chemical hazards in dairy products are aflatoxin M1, organochlorine pesticides (OCPs), organophosphorus pesticides (OPPs), dioxins, and dioxin-like compounds and residues of veterinary drugs. Chemical hazards primarily occur at the dairy farm and may accumulate during further processing **(Sajid et al., 2016** and **Van Asselt et al., 2017).**

The aflatoxins are groups of chemically similar toxic fungal metabolites produced by certain molds of the genus Aspergillus growing on a number of raw-food commodities. Aflatoxin M1 (AFM1) may be found in the milk of animals that are fed with aflatoxin B1 (AFB1) containing feed. Neither storage nor processing could destroy AFM1 and can be detected in dairy products submitted to pasteurization, sterilization process and also in fermented products **(Picinin et al., 2013** and **Ahmed et al., 2015).**

The presence of organochlorine pesticides (OCPs), neonicotinoid insecticides, and organophosphate pesticides (OPPs), in milk has been reported. OCPs, such as dichlorodiphenyltrichloroethane (DDT) compounds, hexachlorocyclohexane (HCH), and hexachlorobenzene (HCB), are the most common persistent in milk **(Tsakiris et al., 2013** and **Sajid et al., 2016)**. Because of their lipophilic characteristics, OCPs can accumulate in fat-rich tissues and be excreted in milk; thus, the presence of OCPs in dairy products is a potential exposure route for humans through the food chain **(Witczak & Abdel-Gawad, 2014; Witczak & Pohorylo, 2015** and **Yohannes et al., 2017).**

The cheese making process increased the residual concentration of OCPs in cheese compared to raw milk as the curding process concentrates the OCP residues resulted in a significant enrichment of the OCP residues in cheese **(Duan et al., 2018)**.

Organochlorine pesticides (OCPs) can alter the regulation of body weight and the hormone system, and disrupt the endocrine and reproductive systems, with potential toxic effects on humans and wildlife. More seriously, exposure to OCPs can result in birth defects, abortion, and damage to the immune and respiratory systems **(Mansour, 2009** and **Casals-Casas & Desvergne, 2011)**.

The occurrence of chemical residues in milk is a matter of public health concern, since dairy products are widely consumed by infants, children and many adults throughout the world. Therefore, many countries have enacted regulations that limit the level of chemical residues in milk and dairy products **(Jahed Khaniki, 2007).**

The most relevant physical hazards found in milk and dairy products are insects, metals, and plastics. Some of these components can cause serious health risks to the consumer, such as injury to the oral cavity, damage to the teeth, asphyxiation, internal bleeding, throat discomfort, dysphagia, regurgitation, and death **(Afoakwa et al., 2013** and **European Commission. 2015).**

Various trends are foreseen in the near future that may affect food safety, such as climate change, increased global trade, and changing consumer demands. The most important development is the further intensification of the dairy chain and higher milk production due to the growing demands; this may have positive effects for food safety as farmers can invest in improving their farms and will be more aware of possible food safety issues. However, an increased livestock population at the farm may also result in a raise in the occurrence of animal diseases and, consequently, more veterinary drug use. Dairy production follows several stages, along this dairy supply chain, food safety hazards may enter at various stages **(Noordhuizen & Metz 2005** and **Van Asselt et al., 2017)**.

Classical quality control methods only emphasized on hygienic quality of final products are inadequate to control hazards occurring at early stages of the process. Therefore, food safety must be ensured by a preventive approach, such as implementation of prerequisites programmes and application of procedures based on Hazard Analysis and Critical Control Point (HACCP) principles. The HACCP concept is a scientific approach to assess hazards associated with food production and establish control measures to ensure food safety. It is a preventive system that takes the whole chain of food production into consideration before biological, chemical and/or physical hazards affect the safety of food products **(Amagliani et al., 2012** and **Allata et al., 2017).**

Despite these quality control programs, food safety hazards may still be present; therefore, monitoring programs have been established to detect the possible presence of food safety hazards. These programs should be risk-based and focus on the most relevant food safety hazards, which will enhance the probability of detection**.** Risk assessment is the scientific process of determining the relationship between exposure to a given hazard under a defined set of conditions and the likelihood of an adverse health effect or disease. A microbial risk assessment is considered a scientific and methodical tool for preventing, regulating, and understanding the risk caused by hazardous microorganisms. Microbiological risk assessments have been subdivided into four steps, which comprise, hazard identification, hazard characterisation, exposure assessment and risk characterization **(McLauchlina et al., 2004** and **EPA, 2012)**.

Recent challenges to the food hygienists are finding the best ways to prevent the entry of microorganisms, destroy those that get in along with their enzymes, and prevent the growth and activities of those that escape processing treatments. The great recent challenge is the prevention of chemical hazards and the control of their sources **(Loralyn and Robert, 2009)**.

Therefore this study was planned out to throw light on the risk assessment of some dairy products in the Egyptian markets with special reference to some safety issues, through the following:

1- Investigation of the collected samples (75 small and large scale ice-cream as well as 75 small and large scale white soft cheese) for the presence of different types of food safety hazards.

2- Examination of some biological hazards.

- Enumeration of total aerobic mesophilic count (cfu/g.).
- Enumeration of total psychrophiles count (cfu/g.).
- Enumeration, isolation and identification of Coliformss.
- Isolation and identification of *E.coli.*
- Enumeration of total yeast and mold counts (cfu/g.).
- Enumeration of total Staphylococcal count (cfu/g).
- Enumeration, isolation and identification of *Stapylococcus aureus*.
- Isolation and identification of *listeria spp*.
- Isolation and identification of Salmonella organisms.

3- Detection of some chemical hazards:

- Determination of Aflatoxin M1 in the examined dairy products by using a commercial Enzyme-Linked Immunosorbent Assay (ELISA).
- Determination of Organochlorine pesticides by using GC-MSMS.

4- Physical examination of the collected samples for the presence of any physical hazards.

5- Determining the degree of acceptability of the examined dairy products Vs. the Egyptian standards.

6- Discussing the preventive and control measures which must be adopted to safeguard the consumer against the possible hazards, and safeguard the producer from economic losses.

Review Article

Risk assessment of some dairy products

Abstract

Risk assessment practice has steadily increased in prominence during the past several decades, as a risk manager in Governments and industry have thought to develop most effective ways to meet public demands for a safer and healthier environment. Several scientific disciplines have been mobilized to provide technical information about risk and Billions of Dollars have been expended to create this information and distill it in the context of Risk assessment. Research have begun to provide a new perspective on this problem by demonstrating the complexity of the concept (Risk) and the inadequacies of the traditional view of risk assessment as purely scientific enterprise.

Risk assessment is a term used to describe the overall process or method where; Identify Hazard and risk factors that have the potential to cause harm (Hazard Identification), Determine appropriate ways to eliminate the hazard or control the risk when the hazard cannot be eliminated (risk control) **(Rocourt et al., 2001)**.

Risk assessments are very important as they form an integral part of an occupational health and safety management plan. They help to create awareness of hazards and risk, identify who may be at risk (e.g., employees, cleaners, visitors, contractors, the public, etc.), determine whether a control program is required for a particular hazard, determine if existing control measures are adequate or if more should be done, prevent injuries or illnesses, especially when done at the design or planning stage, prioritize hazards and control measures and meet legal requirements where applicable.

Introduction

Milk and milk products are vital components of the food supply chain as milk is an excellent source of nutrients for humans; dairy products are considered as added-value products (e.g. lactose-free, Calcium-enriched), and dairy-based functional foods (e.g. with added probiotics, omega-3, phytosterols), **(O¨ zer and Kirmaci, 2010)**.

Among several milk products, cheese is widely popular in many countries because of the associated health benefits and flavor. The health benefits of cheese include natural probiotic and antitumor properties. Additionally, cheese is a rich source of dietary calcium, phosphorus, and proteins. Cheese making is a major industry worldwide, there are many varieties of cheese all over the world and much of it is still practiced on a relatively small scale **(Fox et al., 2004; Mozaffarian et al., 2010** and **USDA, 2011)**.

Frozen desserts like ice-cream received wide popularity and great acceptance from all age groups as it is a highly nutritious dairy product with delicious taste and flavor, high-energy foods due to its composition, which includes 10% fat, 20% total solids, vitamins A, B, C, D, E, K and the minerals calcium and phosphorus. It also contains 12-17% carbohydrates, 55-65% water and 0.2-0.5% emulsifiers and stabilizers. Fruits, nuts, candies and syrups are optionally added into ice-cream for flavor enrichment **(Damer et al., 2015** and **Edward et al., 2017)**.

The global demand for dairy products is rapidly increasing, as a result of the growing population and purchasing capacity. On the other hand**,** milk and milk products were exposed to different sources of contamination during the different stages of production, preparation, handling and distribution that possible contamination were existed either a microbiological, chemical or physical hazard which may affect a large population; consequently milk and dairy products must be assessed from time to another against the different types of hazards to ensure safety of consumers **(Papademas & Bintsis, 2010** and **Gerosa & Skoet, 2012)**.