Assessment of the microbiological quality of spices and herbs commercialized in Lebanon

A Thesis presented to

The Faculty of Nursing and Health Sciences

At Notre Dame University-Louaize

In partial fulfillment

Of the Requirements for the Degree

Master of Sciences

In Food Safety and Quality Management

By

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June 2020

Notre Dame University-Louaize

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Acknowledgments

I would express my sincere gratitude to my thesis advisor Dr. Layal Karam, Assistant Professor of Food Safety and Quality Management at the faculty of Nursing and Health Sciences, for her continuous guidance for me through this present work with pragmatism and passion.

I would also like to thank my committee members, Dr. Hussein Hassan, Associate professor of Food Science and Technology, associate chairperson of the Department of Natural Sciences at the Lebanese American University, and Dr. Rima El Hage, Head of Microbiological Food department at Lebanese Agricultural Research Institute, who contributed to its multidisciplinary aspect and I am gratefully indebted for their valuable comments on this thesis.

I would like to thank Notre Dame University (NDU) and Lebanese American University (LAU) for allowing me to undertake this work.

I am also grateful to Dr. Michel Afram and the Lebanese Agricultural Research Institute-Fanar (LARI) for supporting the laboratory work.

Special thanks go to Mrs. Samar Merhi, for guiding me through the statistical analysis part.

Finally, my gratitude goes to those who are most proud of this accomplishment. My family, thank you for being so proud of me.

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List of abbreviations

EU: European Union

U.S.: United States

U.S.A.: United States of America

ESA: European Spices Association

ICMSF: International Commission on Microbiological Specifications for Food

LIBNOR: Lebanese Standards Institution

ISO: International Organization for Standardization

WHO: World Health Organization

FAO: Food and Agriculture Organization

LARI: Lebanese Agricultural Research Institute

FSMS: Food Safety Management System

HACCP: Hazard Analysis and Critical Control Points

GHPs: Good Hygienic Practices

GMPs: Good Manufacturing Practices

RTE: Ready to Eat

HDL: High Density Lipoprotein cholesterol

LDL: Low Density Lipoprotein cholesterol

kGy: KiloGray

TAMB: Total Aerobic Mesophilic Bacteria

BPW: Buffered Peptone Water

PCA: Plate Count Agar

TBX: Tryptone Bile Glucuronic agar

VRBL: Violet neutral Red Bile Lactose agar

IS: Iron Sulfite agar

SC: Sulfite Cycloserine agar

DG 18: Dichloran-18% Glycerol agar

XLD: Xylose Lysine Deoxycholate modified agar

SS: Salmonella Shigella agar

TSA: Tryptone Soy Agar

ANOVA: One Way Analysis of Variance

AOAC: Association of Official Agricultural Chemists

Abstract

Spices and herbs are widely used in almost all types of food preparation and their microbial contamination may pose spoilage and public health risk. Thus, the aim of this study was to assess the microbiological quality of spices and herbs commercialized in Lebanon. A total of 480 samples of thirteen most commonly consumed types of spices and herbs were collected at two complete sets at three months interval. Each type was purchased in 5 common brands from 4 categories: packaged in companies with Food Safety Management System (FSMS), packaged in companies without FSMS, packaged imported, and unpackaged. A composite sampling approach was applied and 96 composite samples were tested for moisture content, total aerobic mesophilic bacteria TAMB, coliforms, *Escherichia coli, Salmonella,* sulfite-reducing anaerobic bacteria, *Clostridium perfringens,* yeasts and molds. The moisture profile of all samples was adequate except for sumac. All samples were negative for *Salmonella*. However, *E. coli* was found in one paprika sample at a count of 2.2 log CFU/g. Total aerobic mesophilic bacteria, sulfite reducing anaerobic bacteria, coliforms, *C. perfringens*, yeasts and molds were found in 88.5%, 42.7%, 14.6%, 17.7% and 54.2% of the samples, respectively. According to the International Commission on Microbiological Specifications for Foods (ICMSF), 1.0%, 2.1%, 7.3% of the samples were unacceptable for TAMB, coliforms, yeasts and molds, respectively. According to the Lebanese Standards, 1.0%, 4.2%, 6.3%, 1.0% and 7.3% of the samples had unacceptable counts of TAMB, coliforms, sulfite reducing anaerobic bacteria, *E. coli*, yeasts and molds, respectively. Among the four categories, imported samples had the best microbiological quality, followed by locally packaged in companies with FSMS, then locally packaged in companies without FSMS and the poorest microbiological quality was for the unpackaged spices and herbs. This study highlighted the importance of storage conditions, good hygienic practices, process controls and FSMSs in the spices and herbs sector.

Keywords: Food safety; food spoilage; spices; herbs; Food Safety Management System

Chapter 1

1. Health benefits of spices and herbs

Spices and herbs are used as ingredients in food, alcoholic beverages, cosmetics and medicine. The trend towards the use of spices and herbs is to produce more appealing food with reduced salt or sugar content (Kurian, 2012). Moreover, many of them (ginger, coriander, turmeric and chili) increase pancreatic secretions, amylase activity, bile secretion and improve the digestion of dietary fats and carbohydrates (Dog et al., 2006). In addition, spices are protective against cardiovascular diseases. Allicin is an active compound found in garlic powder that reduce the total cholesterol, low density lipoprotein (LDL) cholesterol and triglycerides levels (Tapsell et al., 2006). Moreover, the consumption of ginger is associated with the decrease of LDL cholesterol and increase in high density lipoprotein (HDL) cholesterol (Gujral et al., 1978). Spices and herbs such as turmeric, ginger, chili, cumin, rosemary and thyme provide phytochemical substances that protect against skin, prostate and gastric cancers (O'Mahony et al., 2005; Tapsell et al., 2006). Additionally, phenolic compounds contained in spices and herbs, such as phenols, quinones, flavones, tannins, terpenoids and alkaloids possess antiadhesive properties. They prevent the adhesion of microbes to the host tissue and prevent infection. Moreover, several studies demonstrated the antimicrobial activity of garlic, basil, thyme and clove (Adler & Beuchat, 2002; Ahmad et al., 2005; Giordani et al., 2004; Harris et al., 2000; Hersch-Martínez et al., 2005; Opalchenova & Obreshkova, 2003; Udupa et al., 2006). On the other hand, aromatic plants have been used for treatment of acute and chronic respiratory disorders. Previous reports demonstrated the beneficial effects of thyme and basil against bronchitis and asthma (Gruenwald et al., 2005). Furthermore, the consumption of herbs reduces the risk of neurological diseases, clears the mind and improves the memory. Epidemiological studies reported a correlation between the consumption of turmeric and sage, and low risk of neurological diseases such as Alzheimer's dementia among elder populations (Lim et al., 2001; Perry et al., 2003).

2. Imports and Exports of spices and herbs

Globally, the herbal market is estimated around $60 billion (Sharma, 2006). Moreover, the market of spices and herbs is growing significantly at a rate of 6% per annum between 2013 and 2017. With more than 500 million consumers, the EU is the most important market for spices and herbs, followed by North America and East Asia. Between 2009 and 2013, the volume of imports in Europe increased 4.1% per year (Figure 1). The largest supplier of spices and herbs to the European market is China (20%) followed by India (8.7%), Vietnam (7.7%), Indonesia (4.0%), Brazil (3.1%) and Peru (2.4%) (Figure 2). However, Europe produce chili and peppers, anise, fennel, badian and coriander, and other spices. The increase of production of spices and herbs in Europe was 5.2% per year between 2009 and 2012 (Eurostat, 2014).

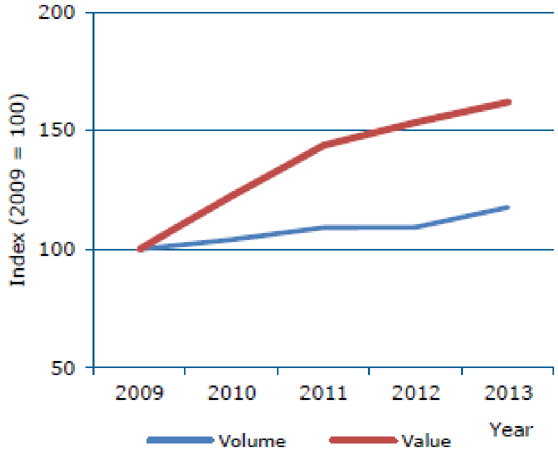

Figure 1: Increase in value and volume of EU imports of spices and herbs between 2009 and 2013.
Source: Eurostat, 2014

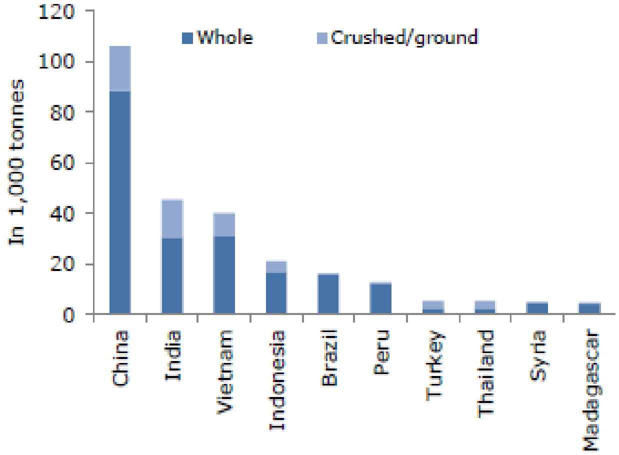

Figure 2: Developing countries suppliers of spices and herbs to the EU in 2013.
Source: Eurostat, 2014

In United States, the consumption of spices and herbs had increased in the last decades. Between 2013 and 2017, U.S. imported 47,226 metric tons of herbs valued at U.S. $ 294 million per year. The major supplier of herbs for U.S. was India (27%). During 2017, U.S. imported 1.8 million metric tons of spices estimated at U.S. $ 7.6 billion (Nguyen et al., 2019). Currently, the major suppliers of spices for the U.S. market are China, India, Turkey, Spain and Peru (Figure 3).

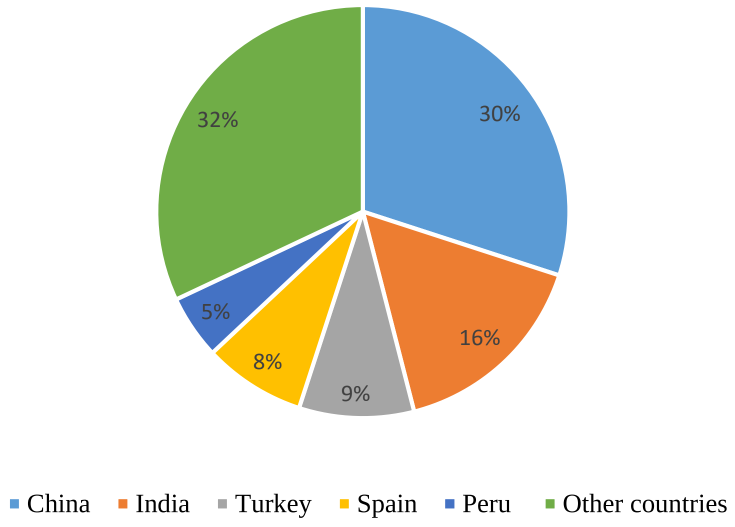

Figure 3: U.S. major imports of spices from suppliers.
Source: The United State Department of Agriculture, Foreign Agricultural Service (USDA FAS), 2018

Spices and herbs market in Asia is expected to grow of 6.1% from 2018 to 2023 due to the consumer focus towards natural products and increase of demand for convenience foods. Asian countries, mainly India and China, export celery, cumin, chili, coriander, cardamon, oregano and pepper to Europe and North America regions (Nguyen et al., 2019).

On the other hand, Lebanon imports thyme from Syria (more than 70%) and Jordan (20%). In 2008, the exports of thyme and oregano was valued at U.S. $ 1.9 million. Moreover, in 2011, 561 tons of thyme were exported from Lebanon to U.S.A., EU, Canada, Australia and Saudi Arabia. Furthermore, Lebanese production and exportation of wild sage varied annually between 600 and 800 tons (Hamade, 2016).

3. Contaminants in spices and herbs

Spices and herbs are classified as low water activity food. Moreover, they are mainly cultivated in poor hygienic conditions, warm and humid climate which may facilitate the growth of pathogenic microorganisms (Moreira et al., 2009). Table 1 summarizes the characteristics of microorganisms that may frequently contaminate spices and herbs (Mckee, 1995).

Table 1: Microorganisms that frequently contaminate spices and herbs.

Microorganism	Characteristics
Salmonella	Persists in low water activity food for a long period of time. Consumption of spices and herbs contaminated with *Salmonella* have been implicated in foodborne Salmonellosis outbreaks.
Bacillus cereus	Spores can survive for months and years in low water activity food. Toxin producing human pathogen that may cause diarrheal and emetic forms of food poisoning.
Clostridium perfringens	Spore forming bacterium that can survive normal heating and multiply in large numbers of viable bacterial cells due to inappropriate cooking or storage. Large numbers of spores cause diarrhea and necrotic enteritis foodborne diseases.
Coliforms	Indicators of unhygienic conditions.
Escherichia coli	Indicators of fecal contamination.
Yeasts and molds	Survive in inadequate storage conditions: Humidity and high temperature. Fungal contamination cause food spoilage and may pose a public health risk due to the ability of some species to produce toxicogenic substances such as aflatoxins.
Aerobic mesophilic bacteria	Indicators of poor quality of spices and herbs.

4. Regulations and standards related to spices and herbs

In order to ensure the microbial safety of spices and herbs, microbiological criteria have been set. The table below represents the maximum acceptable limits for the most commonly tested microorganisms set by the Lebanese Standard Institution (LIBNOR), the International Commission on Microbiological Specifications for Foods (ICMSF) and the European Spices Association (ESA) (Table 2).

Table 2: Spices and herbs maximum acceptable limits set by ICMSF, LIBNOR and ESA for the most commonly tested microorganisms.

Parameters / Maximum acceptable Limits set by (log CFU/g)	*Salmonella*	Sulfite reducing anaerobic bacteria	Total Aerobic Mesophilic bacteria (TAMB)	*Escherichia coli*	Coliforms	*Clostridium perfringens*	Yeasts and molds
ICMSF (International Commission on Microbiological specifications for foods)	should be absent in 25g	-*	6	3	4	3	4
LIBNOR (Lebanese Standards Institution)	Should be absent in 25g	2	5.7	1	3	-*	4
ESA (European Spices Association)	Should be absent in 25g	-*	9	2	-*	3	6

*No limit found.

5. Assessment of the microbiological quality of spices and herbs in different countries

Spices and herbs are defined as whole, broken or ground natural substances added to RTE with no further need for cooking. The consumption of contaminated spices and herbs may contribute to severe human illnesses. Therefore, several studies conducted in Europe, Asia, United States and Middle East region reported the poor microbiological quality of spices and herbs due to significant microbial contaminations with pathogenic microorganisms.

In Spain, the microbial quality of 53 samples of spices, herbs, herbs mixture and spices blends was assessed. All samples were tested for aerobic mesophilic bacteria, Enterobacteriaceae, *Escherichia coli, Salmonella, Listeria monocytogens, Staphylococcus aureus, Shigella spp., Yersinia intermedia* according to ISO methods. It was found that all samples were negative for *Salmonella, E. coli* and *Listeria monocytogens*. Thyme, Basil, cinnamon, clove, ginger, turmeric, aniseed and dry parsley were free of contamination. However, spices and herbs were contaminated with the following pathogenic bacteria: *S. aureus, Yersinia intermedia, Shigella spp., Enterobacter spp.* The authors suggested the need to provide a control system and improve the sanitary conditions while production to ensure the safety of spices and herbs (Sospedra et al., 2010). On the same scope, Sagoo et al., (2009) collected spices and herbs from retail (132 samples) and production premises (2833 samples) in United Kingdom. *Salmonella, E. coli, Bacillus cereus* and *Clostridium perfringens* were detected and enumerated according to Health Protection Agency (HPA) Standard Microbiological Methods. *Salmonella* was detected in 1.5% and 1.1% of samples at production and retail, respectively. It was found that 96.0% of retails samples and 92.0% production batches were of acceptable quality. According to EC Recommendation 2004/24 and European Spices Association, unacceptable counts of *E. coli (*≥2 log CFU/g), *B. cereus* (≥ 5 log CFU/g) and *C. perfringens* (≥ 3 log CFU/g) were found in 3.0% of spices and herbs. The results reported in this study emphasized the importance of the application of good hygienic practices and correct food handling to reduce the risk of contamination (Sagoo et al., 2009). A study performed by Witkowska et al., (2011) investigated the quality of commercial spices and herbs, evaluated the effect of simulated industrial chilled temperature and storage process on survival of microflora. One hundred eighty samples of thirty types of spices and herbs collected from the Irish market were tested for TAMB, *B. cereus, S. aureus, Salmonella, Pseudomonas spp., Listeria monocytogens*, Enterobacteriaceae, yeasts and molds according to AOAC and the ICMSF methods. Spices and herbs having the highest microbial loads were subjected to simulated typical heating process and two different media were used (sterile water and commercial chilled ready meal) to evaluate the influence of food matrix on survival of microflora. The results showed that 20.0% of the samples had counts of TAMB above the maximum limit set by ICMSF (>6 log CFU/g). Moreover, Spore forming bacteria, *Pseudomonas spp.*, Enterobacteriaceae and molds were detected in 80.0%, 33.0%, 23.0%, and 50.0% of spices respectively. However, the heat processing steps during production and chilled temperature storage were not sufficient for the elimination of microorganisms in highly contaminated spices. This study suggested the application of good hygienic practices, storage at appropriate temperature of RTE meals containing spices, implementation of hazard analysis and critical control points (HACCP) at all stages of production (Witkowska et al., 2011). A recent study in Germany was done to investigate the survival of *B. cereus, B. thuringiensis* spores and *S. aureus* cells in the following dry condiments: allspices, cinnamon, nutmeg, oregano, paprika, parsley and pepper. Samples were artificially contaminated by a dry spiking method using sand as carrier matrix and as control. The results showed that the storage at room temperature of low moisture condiments do not affect the survival of spores, but significantly affect the survival of non-spore forming bacteria. Therefore, steam treatments applied may inactivate *S. aureus* cells, but do not significantly affect spore forming bacteria (Dinh Thanh et al., 2018).

In Thailand, the prevalence of *C. perfringens* in 100 dried pepper samples was investigated using multiplex PCR method. In this study, *C. perfringens* was detected in 86.0% of the samples analyzed. These findings showed that the application of good hygienic practices and appropriate method reduce the risk of contamination (Tassanaudom et al., 2017). In India, 154 random samples of packaged and non-packaged spices and herbs were tested for aerobic mesophilic bacteria, *S. aureus*, *B. cereus*, *C. perfringens*, coliforms, Enterobacteriaceae, *Salmonella*, *Shigella spp.*, yeasts and molds. The non-packaged samples showed higher levels of contamination with *B. cereus*, Enterobacteriaceae and molds than those packaged. According to ICMSF specifications, TAMB counts exceeded 6 log CFU/g in 51.0% of the samples. Molds were found in 97.0% of spices and 2.6% were contaminated with *Salmonella* and *Shigella.* Spices and herbs consumed in India were of poor microbiological quality and may be a major source of contamination of ready to eat food (Banerjee & Sarkar, 2003). A similar study in Iran assessed the microbial counts of aerobic mesophilic bacteria, coliforms, *Escherichia coli* and molds in packaged and non-packaged black pepper, caraway, cow parsnip, curry, garlic powder, red pepper, sumac and turmeric. It was shown that 63.2%, 23.4%, 24.8% and 21.9% of the samples had unacceptable counts of aerobic mesophilic bacteria, *E. coli*, coliforms and molds. On the other hand, non-packaged spices showed higher counts of coliforms and *E. coli* than those packaged. (Koohy-Kamaly-Dehkordy et al., 2013).

In addition to that, a study made in Brazil analyzed 233 samples of spices and herbs for aerobic mesophilic bacteria, coliforms, *B. cereus, S. aureus* and *Salmonella* according to the American Public Health Association guideline. Data showed that 5.6% of the samples were contaminated with *Salmonella* and had unacceptable counts of coliforms. Black pepper and cumin had the poorest microbiological quality, and bay leaves were the least contaminated. These findings proved that the application of irradiation treatments ensure the safety and improve the quality of spices and herbs (Moreira et al., 2009). According to these results, good hygienic practices and food safety measures should be applied to reduce the risk of contamination and improve the microbiological quality of spices and herbs.

In a study conducted by Hashem & Alamri in Saudi Arabia, the total fungal load was determined in 138 samples of 15 types of spices and herbs using standard dilution plate method. The results showed that ginger, fenugreek, fennel, thyme, red pepper, sweet cumin and aniseed were highly contaminated with fungi. In contrast, cinnamon, green cumin, pepper, green cardamom, caraway and cloves were moderately contaminated. However, the lowest contamination levels were found in sumac. These findings proved the strong antifungal activity of sumac. This study recommended the use of sumac in RTE food (Hashem & Alamri, 2010). A similar study was conducted in West Africa on 66 samples of spices and herbs. A significant occurrence of molds was reported. This study showed that water activity in spices, processing steps and storage temperature must be controlled to reduce the growth of molds (Akpo-Djènontin et al., 2018).

6. Objectives, research question and hypothesis of the study

Our knowledge of the microbiological quality of spices and herbs in Lebanon is based on very limited data. Thus, the aims of this study were to assess the microbiological quality of dried herbs and spices commercially available in the Lebanese market and compare the microbiological quality of spices and herbs locally packaged in companies with Food Safety Management System (FSMS), locally packaged in companies without FSMS, packaged imported, and unpackaged.

Our research question: “Are the levels of microbial contaminants in spices and herbs commercialized in Lebanon in compliance with the limits set by the Lebanese and international standards?

Our hypothesis is: The microbiological quality of spices and herbs packaged imported and locally packaged in companies with FSMS is better than those packaged in companies without FSMS or unpackaged.

Chapter 2

1. Introduction

Spices and herbs are natural products valued for their distinctive flavor, color, and aroma, and are among the widely used ingredients in food preparation. Spices and herbs may provide protection against cancer, cardiovascular diseases, inflammation, diabetes and hypertension (Tapsell et al., 2006). They are natural flavor enhancers and good alternatives for reduced-salt ready meals (Mitchell et al., 2013). In the recent years, the global exports and imports of these commodities had grown and their usage as main ingredients in food had increased because of their preservative effect and health benefits. In 2013, EU imported 520,000 tons of spices and herbs valued at over € 1.8 billion and the European market is expected to grow by 7% in the next five years (Eurostat, 2014). In 2014, Lebanon exported 548 tons of thyme mix (known as Zaatar mix) and oregano for a value of U.S. $ 1.9 million (Hamade, 2016).

Generally, spices and herbs grow in temperate and tropical regions. They are mainly produced in China, India, Indonesia, Brazil, Mexico and Egypt (FAO, 2000). Moreover, the production of these commodities in poor hygienic conditions, warm and humid climate may increase the risk of contamination with aerobic mesophilic bacteria, *Salmonella*, *Escherichia coli*, coliforms, *Clostridium perfringens*, yeasts and molds (Banerjee & Sarkar, 2003; Gambacorta et al., 2019). Therefore, the use of contaminated spices and herbs in ready to eat food contribute to food spoilage and cause foodborne outbreaks (Mckee, 1995; Sagoo et al., 2009). Between 2007 and 2010, 475 laboratory cases of Salmonellosis outbreaks were confirmed in the United States due to the consumption of contaminated paprika, black pepper, white pepper and red pepper (FDA, 2013; Zhang et al. 2017). Between 1969 and 2003, 95% of food containing contaminated spices with *Salmonella* were recalled in United States (Vij et al., 2006). *Salmonella* may contaminate spices and herbs due to its ability to survive in low water activity food for a long period of time and requires low doses to cause acute human illness (Van Doren et al., 2013). In France in 2007, 19 foodborne outbreaks were reported due to the consumption of spices and herbs contaminated with *C. perfringens* (EFSA, 2013). Several studies conducted in India (Banerjee & Sarkar, 2003), United Kingdom (Sagoo et al., 2009), Thailand (Tassanaudom et al., 2017), and Greece (Cosano et al., 2009) reported the presence of *C. perfringens* in cinnamon, black pepper, red pepper, cumin and saffron. *C. perfringens* can survive normal heating process and multiply in large numbers of viable bacterial cells due to inappropriate heating, cooling and storage conditions causing food poisoning (Tassanaudom et al., 2017). The contamination of spices and herbs with coliforms and *Escherichia coli* indicates the presence of fecal contamination and unhygienic practices while cultivation, harvesting, drying, grinding, transportation and handling (Vitullo et al., 2011). After harvesting, the traditional way of drying spices and herbs under sunlight may expose them to high risk of contamination with aerobic mesophilic bacteria. Previous studies reported high counts of TAMB in black pepper (Banerjee & Sarkar, 2003), paprika (Gonzalez et al., 2017), cinnamon (Koohy-Kamaly-Dehkordy et al., 2013) and cumin (Witkowska et al., 2011), as indicator of poor quality of these spices. Additionally, high counts of yeasts and molds were found in spices and herbs due to the storage temperature and long preservation time in humid conditions (Akpo-Djènontin et al., 2018; Banerjee & Sarkar, 2003; Cosano et al., 2009; Dghaim et al., 2017; Koohy-Kamaly-Dehkordy et al., 2013). These high levels of contamination cause food deterioration and pose a serious problem due to the ability of some species to produce toxicogenic substances such as aflatoxins (Hashem & Alamri, 2010; Witkowska et al., 2011). To control microbial contamination, microbiological criteria have been set by the European Spices Association (ESA), European Union (EU), International Commission on Microbiological Specifications for Foods (ICMSF), World Health Organization (WHO), Food and Agriculture Organization (FAO) and Lebanese Standards Institution (LIBNOR).

Previous studies conducted in Brazil (Moreira et al., 2009), United Kingdom (Sagoo et al., 2009), Germany (Dinh Thanh et al., 2018), Spain (Sospedra et al., 2010), Dublin (Witkowska et al., 2011), Italy (Vitullo et al., 2011), Argentina (Aguilera et al., 2005), West Africa (Akpo-Djènontin et al., 2018), India (Banerjee & Sarkar, 2003), Iran (Koohy-Kamaly-Dehkordy et al., 2013), Dubai (Dghaim et al., 2017) and Saudi Arabia (Hashem & Alamri, 2010) assessed the microbiological quality of retail spices and herbs and investigated the counts or the presence of *Salmonella*, aerobic mesophilic bacteria, *Escherichia coli*, *Bacillus cereus*, Enterobacteriaceae, coliforms, Sulfite reducing anaerobic bacteria, *Clostridium perfringens*, *Cronobacter spp.*, *Shigella spp.*, *Pseudomonas spp.*, *Listeria monocytogens*, *Staphylococcus aureus*, yeasts and molds. In Lebanon, published studies investigated the incidences of mycotoxins in spices and herbs (El Darra et al., 2019; Gambacorta et al., 2019). However, no studies have been done to assess the microbiological quality of spices and herbs consumed in Lebanon. Furthermore, spices and herbs are highly consumed in Mediterranean diet and added to several dishes such as salads, sauces, chicken, and meat with no further treatment /processing kill step. Therefore the key objectives of this study were to (i) to evaluate the microbiological quality of the most consumed spices and herbs that can be added to RTE food; (ii) assess the compliance of spices and herbs commercialized in Lebanon to international and local standards; (iii) compare the microbiological quality of spices and herbs locally packaged in companies with FSMS, locally packaged in companies without FSMS, packaged imported and sold unpackaged in bulk.

2. Materials and methods

2.1. Sampling and sample preparation

A total of 480 samples of spices and herbs were collected at two complete sets at three months interval (different production dates). The first collection was performed between May and June 2019 and the second collection was between September and October 2019. Thirteen most commonly consumed types that can be added to RTE food were collected including: Cinnamon, black pepper, sumac, cumin, sesame (hulled non-roasted), dried mint, paprika, white pepper, thyme mix (known as Zaatar mix), red chili, oregano, thyme and garlic powder. Those types were provided by an online survey (n=355 participants) (SurveyMonkey Inc., San Mateo, California, USA). Each type was purchased from 4 categories: Packaged in companies with FSMS, packaged in companies without FSMS, packaged imported brands, and in bulk. Sumac, sesame, dried mint and thyme mix were not available from imported brands. All samples were collected in their original retail package to simulate consumer normal purchasing procedure. Packaged samples from each category were collected in 5 common brands from supermarkets, and unpackaged retail spices were purchased from local markets covering different areas in Lebanon. All samples were transported to the Lebanese Agricultural Research Institute (LARI) accredited under the ISO/IEC 17025 standard (ISO/IEC 17025:2005) and analyzed within 24 hours. A composite sampling approach was applied. The five individual samples of each brand (20g each) were mixed in one sample (100g) before analysis. A total of 96 composite samples were analyzed (13 types x 4 categories x 2 collections and 4 types were not available in imported brands). This approach was used to screen a higher number and assess the overall status of spices and herbs samples available in the Lebanese market even though it may dilute the contamination levels that may be found in each individual sample (Raad et al., 2014).

2.2. Microbiological analysis

Initial suspension (1:10 dilution) was prepared by adding 10g of each composite sample to 90 ml Buffered Peptone water BPW (0.1% w/v) (Scharlau, Barcelona, Spain) in sterile stomacher bag and homogenized using a stomacher lab blender (Interscience, Saint Nom , France) for 30s. Serial dilutions were prepared and enumerated by spread plating (0.1ml) or pour-plating (1ml) methods.

Pour-plating was used for the enumeration of : i) Total aerobic mesophilic bacteria on Plate Count Agar PCA (Scharlau, Barcelona, Spain) followed by incubation at 30°C for 72h according to ISO 4833-1:2013; ii) *Escherichia coli* on Tryptone Bile Glucuronic Agar TBX (Scharlau, Barcelona, Spain) and the plates were incubated at 44°C for 24h (ISO 16649-2:2001); iii) Coliforms on crystal violet neutral red bile lactose agar VRBL (Scharlau, Barcelona, Spain) followed by incubation at 37°C for 24h. Red colonies surrounded by reddish zones of precipitated bile were considered typical colonies. Atypical colonies were inoculated into tubes of brilliant green lactose bile broth (Scharlau, Barcelona, Spain) and incubated for 24h at 37°C. Tubes with gas formation were considered positive according to ISO 4832:2006; v) Sulfite reducing anaerobic bacteria on Iron Sulfite agar IS (Scharlau, Barcelona, Spain). An overlay was added followed by incubation at 37°C for 24h in anaerobic jar (Mitsubishi gas, Tokyo, Japan) according to ISO 15213:2003; vi) *Clostridium perfringens* on sulfite-cycloserine agar (SC) (Scharlau, Barcelona, Spain) according to ISO 7937:2004. The plates were overlaid with the same agar and incubated at 37°C for 24h in anaerobic conditions. Black colonies were selected and inoculated into Thioglycollate medium (Scharlau, Barcelona, Spain) for 24h at 37°C in anaerobic conditions.5 drops of the Thioglycollate culture were transferred to Lactose Sulfite medium (Scharlau, Barcelona, Spain) and incubated for 24h at 46°C. Tubes with gas production were considered positive.

Yeasts and molds were enumerated by spread-plating method on Dichloran-18% glycerol agar (DG 18) (Deben Diagnostics Ltd, Suffolk, UK). The plates were incubated at 25°C for 2 to 5 days in accordance with ISO 21527-2:2008.

Salmonella was isolated and identified according to ISO 6579-1:2017. A pre-enrichment culture was prepared by adding 25g of sample in 225 ml BPW (0.1% w/v) (Scharlau, Barcelona, Spain) followed by incubation at 37°C for 24h. The primary enrichment culture was inoculated in Muller Kauffman Tetrathionate Novobiocin broth (Scharlau, Barcelona, Spain) and Rappaport Vassiliadis Soy (Scharlau, Barcelona, Spain) enrichment broth and incubated for 24h at 37°C and 41°C, respectively. The enrichment cultures were streaked onto two plates of Xylose Lysine Deoxycholate Modified Agar (XLD) (Scharlau, Barcelona, Spain) and Salmonella Shigella Agar (SS Agar) (Scharlau, Barcelona, Spain) and the plates were incubated for 24h at 37°C. Black colonies with transparent hallow suspected to be *Salmonella* were inoculated on nutrient agar (TSA) (Scharlau, Barcelona, Spain) for 24h at 37°C. Presumptive *Salmonella* colonies were identified using serological test consisting of poly H antigen agglutination with Poly O (Remel, Thermo Fisher, Lenexa, KS) and biochemical test consisting of API 20E test kit (BioMerieux, Marcy l'Etoile, France).

2.3. Moisture content

The moisture content was determined by drying approximately 1g of each individual sample at 105°C for 24h (Sospedra et al., 2010).

2.4. Statistical analysis

Each composite sample was tested in duplicate at each collection point. The microbial count colony-forming unit (CFU per g) was converted to logarithmic scale. Means and standard deviations were calculated. Statistical analysis was performed with IBM SPSS version 22.0 (IBM SPSS Statistics for Windows, version 22.0, Armonk, NY). Kruskal-Wallis test was carried out to assess the relationship between moisture content and microbial growth. ANOVA was used to assess the significance of difference in moisture content among different collections, categories and types of spices and herbs. Chi- square test (χ^2) was performed to assess the significance of difference in microbial contamination among different categories and types of spices and herbs. Mann-Whitney U test was used to assess the significance of difference between first and second collection for microbial growth. A P-value <0.05 was considered significant.

3. Results and discussion

3.1. Microbiological quality of spices and herbs per type of microorganism

The microbiological quality of spices and herbs was assessed according to the limits set by the International Commission on Microbiological Specifications for foods (ICMSF, 2005) and the Lebanese Standards (NL 615:2002; NL 546:2004; Ministry of Agriculture, 1207.40, 322/323:2015) (Table 3).

Table 3: Microbiological criteria of spices and herbs set by ICMSF and Lebanese standards.

Microorganisms	Microbiological quality (log CFU/g) of spices and herbs					
	ICMSF			Lebanese standards		
	Acceptable (m[a])	Marginally acceptable (m-M [b])	Unacceptable (M [c])	Acceptable (m[a])	Marginally acceptable (m-M [b])	Unacceptable (M [c])
Total aerobic mesophilic bacteria	4	4-6	6	4.7 [d]	4.7-5.7 [d]	5.7 [d]
Sulfite reducing anaerobic bacteria	- [e]	- [e]	- [e]	1	1-2	2
Clostridium perfringens	1	1-3	3	- [f]	-[f]	-[f]
Escherichia coli	1	1-3	3	0 [g]	0-1 [g]	1 [g]
Coliforms	1	1-4	4	2 [h]	2-3 [h]	3 [h]
Salmonella	Absent in 25 g	N/A [i]	Detected in 25g	Absent in 25g	N/A [i]	Detected in 25g
Yeasts and molds	2	2-4	4	3 [j]	3-4 [j]	4 [j]

[a] Limit below which samples were considered acceptable.

[b] Counts between m and M were considered marginally acceptable.

[c] Limit above which samples were considered unacceptable.

[d] Limits for all samples except sesame: m=3 and M=4 log CFU/g (Ministry of Agriculture, 1207.40, 322/323:2015) and garlic powder: m= 5 and M= 6 log CFU/g (NL 615:2002).

[e] No recommended limit according to ICMSF (ICMSF, 2005).

[f] No recommended limit according to Lebanese standards (NL 615:2002; NL 546:2004; Ministry of Agriculture, 1207.40, 322/323:2015).

[g] Limits for all samples except garlic powder: m=1 and M=2 log CFU/g (NL 615:2002).

[h] Limits for all samples except sesame: m=1 and M=2 log CFU/g (Ministry of Agriculture, 1207.40, 322/323:2015).

[i] N/A: Not Applicable

[j] Limits for all samples except sesame: m=2 and M= 3 log CFU/g (Ministry of Agriculture, 1207.40, 322/323:2015).

TAMB results showed that, 51.0% (49/96) and 70.8% (68/96) of the samples were acceptable, 47.9% (46/96) and 28.1% (27/96) were marginally acceptable, according to ICMSF and Lebanese standards, respectively. 1.0% (1/96) of the samples were rejected according to both standards. Among the four categories, the highest percentage of acceptable counts was found in imported samples (100%) (18/18), followed by locally packaged in companies with FSMS (50.0%) (13/26), then locally packaged in companies without FSMS (38.5%) (10/26) and the lowest percentage was for unpackaged spices and herbs (30.8%) (8/26). According to ICMSF and Lebanese standards, variations in aerobic mesophilic bacteria count from acceptable to marginally acceptable were found between the two collections in spices and herbs locally packaged in companies with FSMS (black pepper, cumin, dried mint, thyme mix and thyme), locally packaged in companies without FSMS (black pepper, dried mint, paprika and thyme) and unpackaged (cinnamon, dried mint, sesame, white pepper and red chili).The highest count of aerobic mesophilic bacteria was found in garlic powder locally packaged in companies with FSMS at the second collection (6.1 log CFU/g) and it was unacceptable for both standards. However, the lowest mean count of TAMB (1.5 log CFU/g) was observed in sumac sample (Figure 4). Similar results were reported in Iran, where garlic powder (6.15 log CFU/g) and sumac (4.71 log CFU/g) showed the highest and lowest mean count of aerobic mesophilic bacteria, respectively (Koohy-Kamaly-Dehkordy et al., 2013). The high counts in garlic powder may be attributed to the traditional method of grinding and air-drying garlic slices in large bulk bins under uncontrolled temperature conditions, which increase the growth of aerobic mesophilic bacteria and affect the quality (Park et al., 2019). On the other hand, the presence of phenolic compounds in sumac may be associated with the low detected counts in those samples. It was found that hydrosable tannins compose the highest percentage of sumac and may be the main antimicrobial agent (Sakhr & El Khatib, 2020).

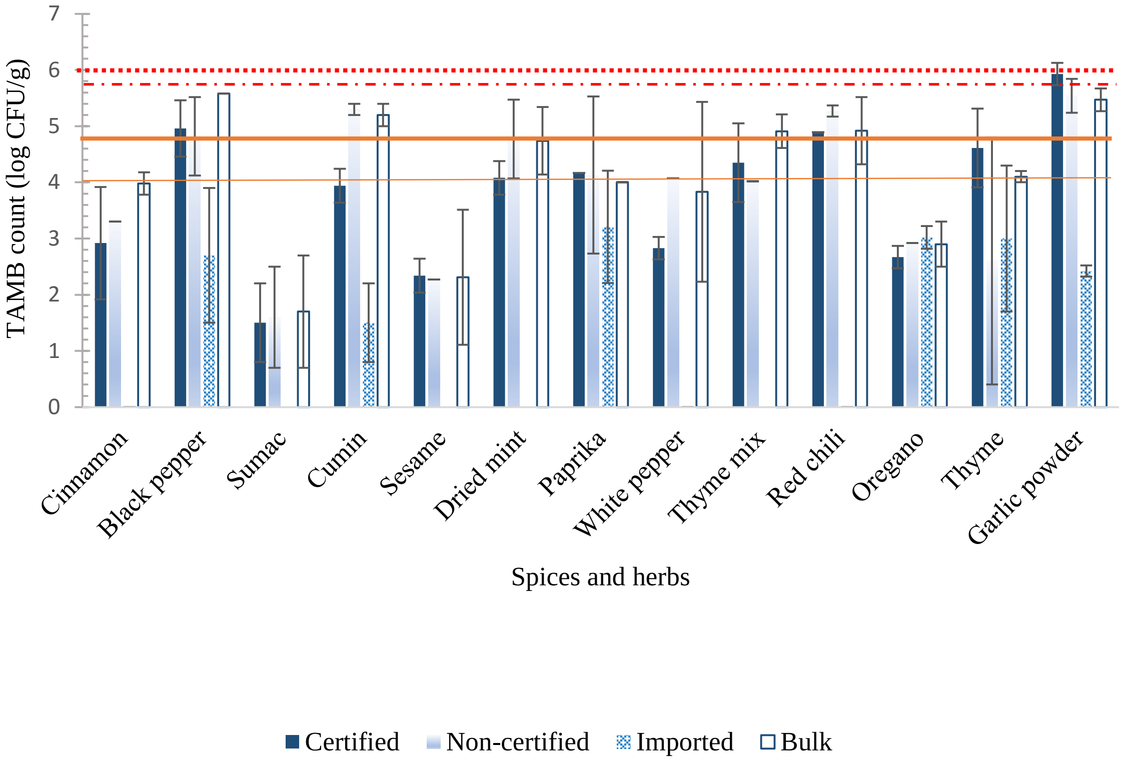

Figure 4: Microbiological quality (log CFU/g) of spices and herbs for total aerobic mesophilic bacteria. Data shown represent the mean ± standard deviation of data of duplicate sample taken from two collection points. The lines: (——) represents the limit (m= 4 log CFU/g) below which values were considered acceptable according to ICMSF, (·······) represents the limit (M= 6 log CFU/g) above which values were considered unacceptable according to ICMSF, (▬▬) represents the limit (m= 4.7 log CFU/g) below which values were considered acceptable according to Lebanese standards for all spices except sesame and garlic powder (m= 3 and 5 log CFU/g, respectively), (— · —) represents the limit (M= 5.7 log CFU/g) above which values were considered unacceptable according to Lebanese standards for all spices except sesame and garlic powder (M= 4 and 6 log CFU/g, respectively). According to each standard, the values between the solid and dashed lines were considered marginally acceptable.

For coliforms, according to ICMSF and Lebanese standards, 85.4% (82/96) and 86.5% (83/96) were acceptable, 12.5% (12/96) and 9.4% (9/96) were marginally acceptable, 2.1% (2/96) and 4.2% (4/96) of the samples were rejected, respectively. No coliforms were detected in imported spices and herbs. Eighty-eight point five % (23/26) of samples locally packaged in companies with FSMS, 80.8% (21/26) of samples locally packaged in companies without FSMS and 76.9% (20/26) of unpackaged samples had acceptable counts. According to ICMSF and Lebanese standards, variations in coliforms count were observed in the same type of spices and herbs ranging from acceptable to marginally acceptable between two collections for cumin locally packaged in companies with FSMS, paprika locally packaged in companies without FSMS and unpackaged paprika and thyme mix. Coliforms were not detected in any cinnamon, black pepper, sumac, sesame, white pepper, red chili, oregano, thyme and garlic powder samples from the 4 categories. According to ICMSF and Lebanese standards, unacceptable counts were found at the first collection in dried mint locally packaged in companies without FSMS (4.1 log CFU/g), unpackaged dried mint (3.5 log CFU/g) and at both collections in unpackaged cumin (4.3 log CFU/g and 3.4 log CFU/g) (Figure 5). Similar findings were reported in India (Banerjee & Sarkar, 2003) and Brazil (Moreira et al., 2009), where coliforms counts were unacceptable in 50.0% and 20.0% of cumin samples, respectively. The unhygienic conditions while grinding cumin seeds may contribute to high contamination counts with pathogenic bacteria (Sharma et al., 2016). Furthermore, the high contamination of packaged and unpackaged dried mint available only in the local market, may be due to the use of untreated sewage from Litany River for irrigation and poor hygienic practices while harvesting, grinding and handling of herbs cultivated in Lebanon (Halablab et al., 2011; Tortora et al., 2011).

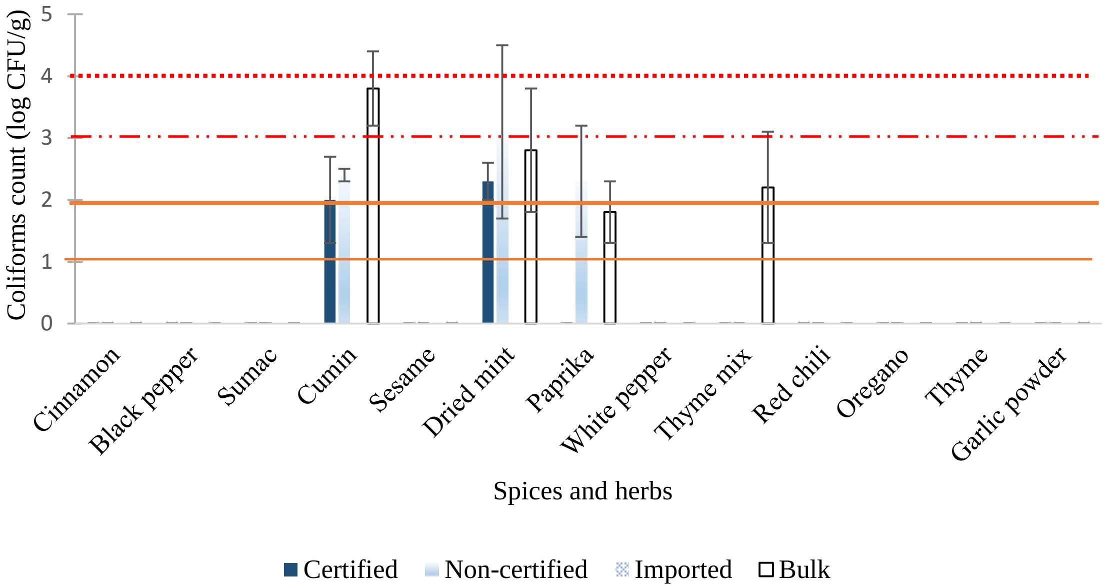

Figure 5: Microbiological quality of spices and herbs for coliforms counts (log CFU/g). Data shown represent the mean ± standard deviation of data of duplicate sample taken from two collection points. The lines: (——) represents the limit (m=1 log CFU/g) below which values were considered acceptable according to ICMSF, (······) represents the limit (M= 4 log CFU/g) above which values were considered unacceptable according to ICMSF, (——) represents the limit (m= 2 log CFU/g) below which values were considered acceptable according to Lebanese standards for all spices except for sesame (m= 1 log CFU/g), (—··—) represents the limit (M= 3 log CFU/g) above which values were considered unacceptable according to Lebanese standards for all spices except for sesame (M= 2 log CFU/g). According to each standard, the values between the solid and dashed line were considered marginally acceptable.

Our results showed that sulfite reducing anaerobic bacteria were detected in 42.7% (41/96) of the analyzed samples. According to Lebanese standards, 57.3% (55/96), 36.5% (35/96) and 6.3% (6/96) were acceptable, marginally acceptable and unacceptable, respectively. The percentage of acceptable counts were higher in imported samples (88.9%) (16/18) compared to locally packaged and unpackaged spices and herbs (50.0%) (39/78). All categories showed variations in counts between first and second collection. For locally packaged in companies with FSMS (cinnamon, black pepper), locally packaged in companies without FSMS (sesame, white pepper, thyme), imported (paprika, garlic powder) and unpackaged (dried mint, paprika, oregano) the sulfite reducing anaerobic bacteria count varied from acceptable to marginally acceptable. The samples found to be unacceptable were locally packaged in companies without FSMS (cinnamon at first collection, cumin at both collections) and unpackaged (black pepper at first collection, cinnamon at both collections). Among all tested samples, no counts were detected in any sumac and red chili samples in all categories (Figure 6). Sulfite reducing bacteria are indicators of *Clostridia* contamination, spread in the environment and may contaminate spices and herbs during poor handling, uncontrolled drying process, grinding or storage conditions which may explain the high counts in locally packaged in companies without FSMS and unpackaged samples. Moreover, this group of bacteria may cause food spoilage and contribute to foodborne outbreaks (Cetinkaya et al., 2012).

In this study, *C. perfringens* were detected in 17.7% (17/96) of the samples at counts ranging between 1 and 2.6 log CFU/g (<3 log CFU/g). The percentage of acceptable counts was higher in imported spices and herbs (94.4%) (17/18) than those locally packaged and unpackaged (79.5 %) (62/78). The results showed that the levels of contamination varied from acceptable to marginally acceptable in the locally packaged in companies with FSMS (cinnamon, thyme mix, thyme), locally packaged in companies without FSMS (cinnamon, thyme mix), imported (paprika) and unpackaged samples (cinnamon, cumin, paprika, thyme and garlic powder). No sample was in the unacceptable range. The highest count of *C. perfringens* was found in unpackaged cinnamon (2.6 log CFU/g) at the second collection, and no counts were detected in any black pepper, sumac, sesame, dried mint, white pepper, red chili and oregano samples in all categories (Figure 7). In the present study, the incidence of *C. perfringens* was similar to those reported in India (Banerjee & Sarkar, 2003) and Argentina (Aguilera et al., 2005) where 17.0% (26/154) of the samples including black pepper, cinnamon, cumin, garlic powder, red chili and 12.2% (14/115) of red chili samples were contaminated, respectively. The presence of *C. perfringens* in spices and herbs may pose a serious public health risk. A previous study demonstrated the ability of this spore former bacteria to survive when added to RTE food and germinate during inappropriate storage at room temperature or inappropriate cooling process which may increase the growth of vegetative bacterial colonies and cause foodborne outbreaks (Luo et al., 2017).

Among all tested microorganisms, unpackaged samples showed lower percentages of acceptability as compared to locally packaged ones except for sulfite reducing anaerobic bacteria and *C. perfringens*, where the 3 local categories showed similar acceptability. This result may be related to the aerobic conditions of storage in open containers that provide unfavorable growth conditions for the anaerobic Sulfite reducing bacteria and *C. perfringens* (Juneja et al., 1994).

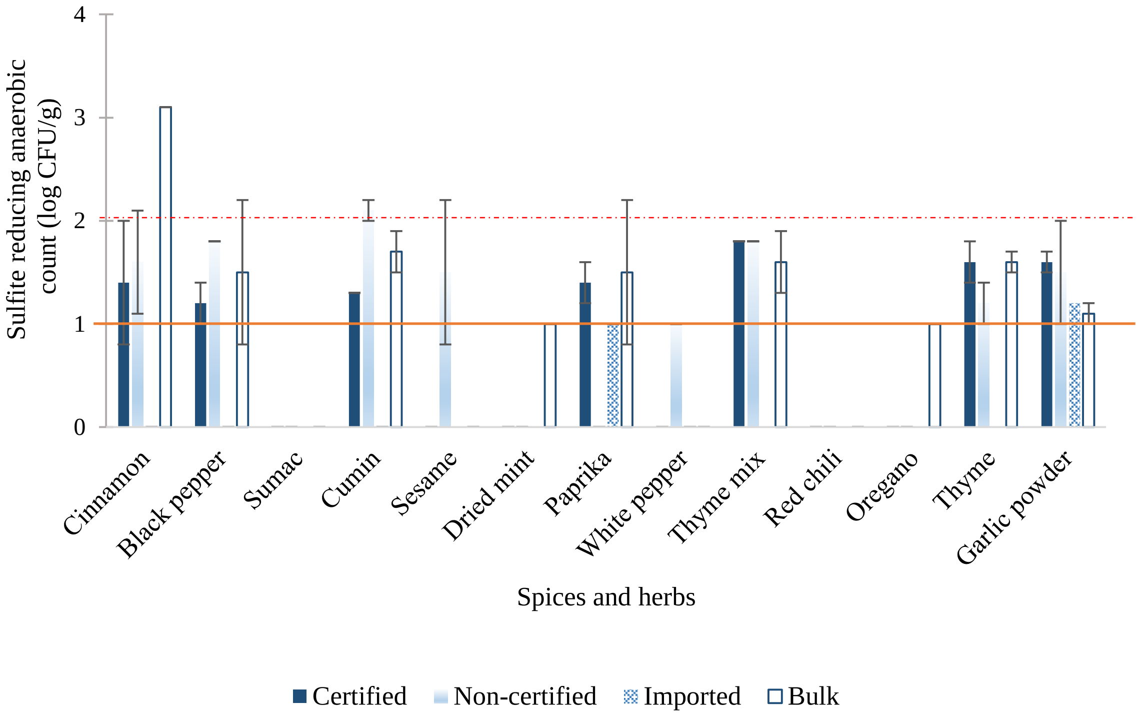

Figure 6: Microbiological quality of spices and herbs for sulfite reducing anaerobic bacteria counts (log CFU/g); Data shown represent the mean ± standard deviation of data of duplicate sample taken from two collection points. The lines: (——) represents the limit (m= 1 log CFU/g) below which values were considered acceptable according to Lebanese standards, (– · –) represents the limit (M= 2 log CFU/g) above which values were considered unacceptable according to Lebanese standards. The values between the solid and dashed line were considered marginally acceptable.

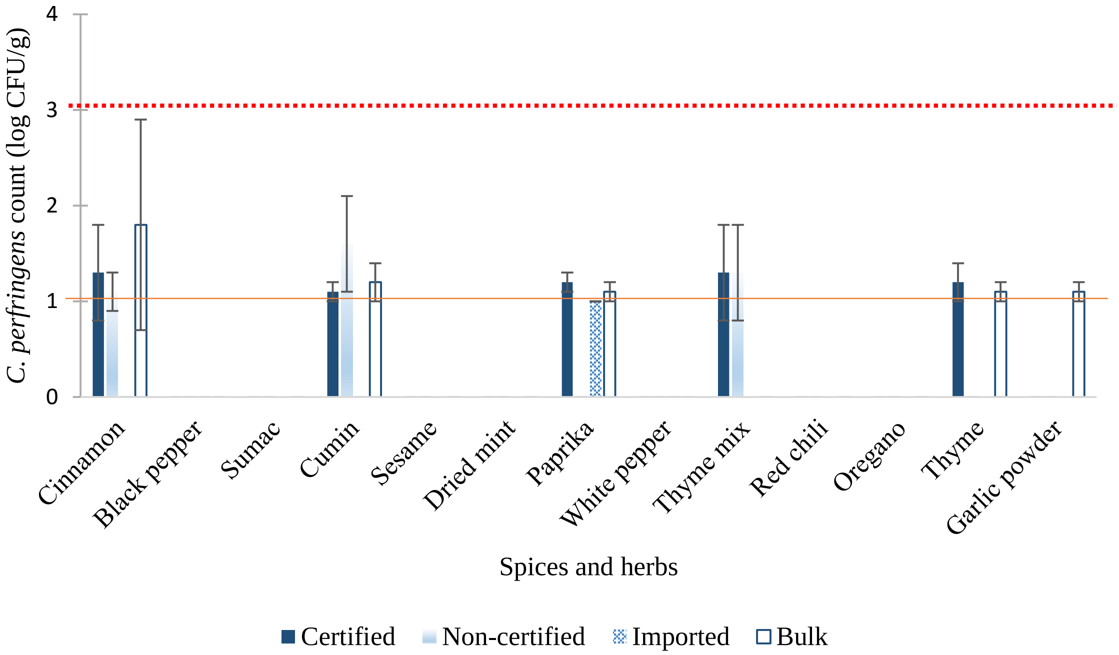

Figure 7: Microbiological quality of spices and herbs for *C. perfringens* counts (log CFU/g); Data shown represent the mean ± standard deviation of data of duplicate sample taken from two collection points. The lines: (———) represents the limit (m= 1 log CFU/g) below which values were considered acceptable according to ICMSF, (········) represents the limit (M= 3 log CFU/g) above which values were considered unacceptable according to ICMSF. The values between the solid and dashed line were considered marginally acceptable.

In the current research, yeasts and molds were found in 54.2% (52/96) of the analyzed samples. According to ICMSF and Lebanese standards, 45.8% (44/96) and 66.7% (64/96) of the samples were acceptable, while 46.9% (45/96) and 26.0% (25/96) of the samples were marginally acceptable, respectively. However, 7.3% (7/96) of the samples exceeded the maximum limit set by both standards. Fungal growth was absent in all imported samples. This finding can be related to the application of treatments such as irradiation, steam or heat which may inhibit the growth of yeasts and molds in imported spices and herbs during storage period and thus improve their quality (Nathawat et al., 2013). Furthermore, 42.3% (11/26), 34.6% (9/26), 23.1% (6/ 26) of samples were of acceptable quality from companies with FSMS, without FSMS, and when sold unpackaged, respectively. Remarkably, the counts of yeasts and molds varied from acceptable to marginally acceptable according to ICMSF and Lebanese standards between the two collections in samples locally packaged in companies with FSMS (black pepper, sesame, paprika, white pepper, thyme mix, red chili, oregano, thyme), locally packaged in companies without FSMS (black pepper, cumin, paprika, white pepper, thyme mix, oregano, garlic powder) and unpackaged (paprika, red chili, oregano, garlic powder). Moreover, no yeasts and molds counts were detected in any sumac samples in all categories. However, unacceptable counts were found in dried mint (both collections) and red chili (second collection) locally packaged in companies without FSMS and in unpackaged dried mint (first collection), white pepper (second collection), thyme mix (first collection) and thyme (second collection). The highest count was detected in unpackaged thyme at the second collection (5.0 log CFU/g) (Figure 8). The low counts of yeasts and molds in sumac were reported in several studies. In Iran (Koohy-Kamaly-Dehkordy et al.,

2013) and Saudi Arabia (Hashem et al., 2010), sumac was the lowest contaminated spice with counts of 1.36 log CFU/g and 1.7 log CFU/g, respectively. Data from the literature reported the strong antifungal effect of phenolic compounds such as xanthones contained in sumac which reduce its affinity to be contaminated (Nasar-Abbas & Halkman, 2004; Sakhr& El Khatib,2020).The high fungal loads in samples packaged in companies without FSMS and unpackaged may be due to the uncontrolled processing, inappropriate storage conditions in high environmental temperature and humidity (Gambacorta et al., 2019). Our findings were similar to that reported in Saudi Arabia, where thyme showed counts of yeasts and molds higher than 3 log CFU/g and was classified among the most contaminated herb (Hashem & Alamri, 2010). However, in Dubai, the lowest counts were found in thyme and were associated to the strong antimicrobial activity of thymol, terpens, eugenol and flavones against fungi (Dghaim et al., 2017). A study conducted in Croatia investigated the factors that may affect the effectiveness of antimicrobial agents in herbs (Jerković et al., 2001). It was reported that season's fluctuations, geographical areas of cultivation, time of harvesting, environmental conditions, drying conditions and age of the plant affect the composition and concentration of essential oils. These findings may explain the variations in yeasts and molds counts in thyme reported in different countries. Therefore, the presence of high counts of fungi in spices and herbs would be of particular importance due to the ability of some species such as *Aspergillus, Fusarium* and *Penicillium* to produce secondary metabolites knows as mycotoxins. Data from the literature showed that mycotoxins are potentially harmful due to their ability to persist during processing and cannot be eliminated while cooking or heating of the food (Romagnoli et al., 2007; Raad et al., 2014). A previous study showed the multi-mycotoxins occurrence in spices and herbs consumed in Lebanon (El Darra et al., 2019). The results showed that 80.0% of spices and herbs analyzed were contaminated with at least one type of mycotoxins in particularly those stored in uncontrolled conditions. Other researchers reported that black pepper and paprika consumed in Lebanon were highly contaminated with *Alterania* mycotoxins (Gambacorta et al., 2019). These findings emphasize the importance of the application of good manufacturing practices and controlled storage conditions of spices and herbs to reduce the risk of contamination with fungi and exposure to mycotoxins.

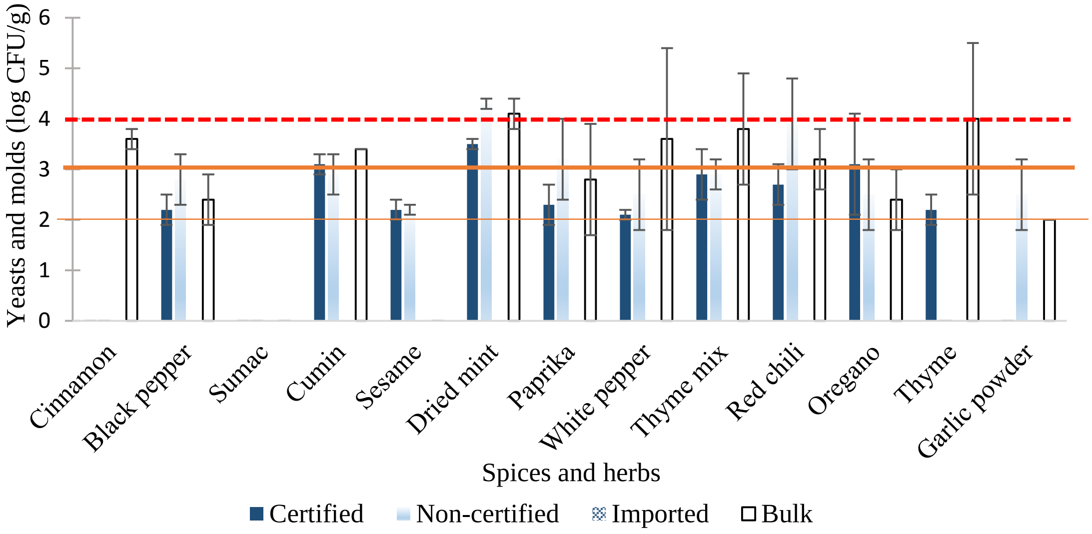

Figure 6: Microbiological quality of spices and herbs for yeasts and molds counts (log CFU/g); Data shown represent the mean ± standard deviation of data of duplicate sample taken from two collection points. The lines: (——) represents the limit (m= 2 log CFU/g) below which values were considered acceptable according to ICMSF, (——) represents the limit (m= 3 log CFU/g) below which values were considered acceptable according to Lebanese standards for all spices except sesame (m= 2 log CFU/g), (- - - -) represents the limit (M= 4 log CFU/g) above which values were considered unacceptable according to both ICMSF and Lebanese standards for all spices except for sesame (M=3 log CFU/g according to Lebanese standards).According to each standard, the values between the solid and dashed line were considered marginally acceptable.

In the current study, *E. coli* was found in paprika samples locally packaged in companies without FSMS with a count of 2.2 log CFU/g at the second collection. This level was marginally acceptable according to ICMSF but exceeded the maximum limit set by Lebanese standards. Similar results were reported in India where *E. coli* was found in one garlic sample among 154 analyzed samples of 27 types of spices and herbs with a count of 2.36 log CFU/g (Banerjee & Sarkar, 2003). However, in Iran (Koohy-Kamaly-Dehkordy et al., 2013) and Turkey (Kahraman & Ozmen, 2009), *E. coli* was found in 23.4% (black pepper, garlic powder, red chili) and 34.7% of the samples (black pepper, red pepper), respectively. These high incidences may be an indicator of fecal contamination of water used during cultivation and irrigation. The presence of antimicrobial phenolic compounds in spices and herbs may reduce *E. coli* counts, as investigated by Cetin-Karaca and Newman (2015). They reported that all strains of *E. coli* had a consistent antimicrobial sensitivity towards eugenol, quercetin, thymol and thymoquinone available in spices and herbs (Cetin-Karaca & Newman, 2015).

All analyzed samples fulfilled the ICMSF and Lebanese standards for the absence of *Salmonella*. Our findings were in agreement with others who reported the absence of this pathogen in saffron, paprika, black pepper, white pepper, red pepper, thyme, cumin, dried mint, cinnamon and oregano (Cosano et al., 2009; Moreira et al., 2009; Sospedra et al., 2010; Vitullo et al., 2011; Witkowska et al., 2011). However, in Brazil (Moreira et al., 2009) and United Kingdom (Sagoo et al., 2009), *Salmonella* was reported in black pepper, cumin, and dried mint . Due to high tolerance to desiccation stress, *Salmonella* may survive for a long period of time in low water activity food such as spices and herbs (Hara-Kudo et al., 2006). The presence or absence of *Salmonella* may be related to processing steps, storage temperature, its survival in dry products and concentration of antimicrobial compounds in spices and herbs. Previous studies demonstrated the antimicrobial effect of the phenolic compounds extracted from spices and herbs; eugenol, linalool, terpens, thymol and cinnamic aldehyde against *Salmonella* species (Cetin-Karaca & Newman, 2015; Tajkarimi et al., 2010).

Among all tested microorganisms, the highest percentage of unacceptability was reported in yeasts and molds (7.3%), followed by sulfite reducing anaerobic bacteria (6.3%), then coliforms (4.2 %), total aerobic mesophilic bacteria (1.0 %) and *E. coli* (1.0%) according to Lebanese standards. The variations in microbial counts between first and second collection may be explained by temperature fluctuation, humidity and different environmental conditions between seasons.

In the present study, the difference in counts of TAMB, coliforms, sulfite reducing anaerobic bacteria, *C. perfringens*, yeasts and molds between different types of spices and herbs was statistically significant ($P<0.05$) ($P=0.000$, $P=0.000$, $P=0.000$, $P=0.001$ and $P=0.005$, respectively). Moreover, there was a statistically significant difference ($P<0.05$) in counts of aerobic mesophilic bacteria, sulfite reducing anaerobic bacteria, yeasts and molds among the four categories ($P=0.000$, $P=0.027$, $P=0.000$, respectively). However, this difference was not statistically significant ($P>0.05$) for *C. perfringens* and coliforms ($P=0.359$ and $P=0.457$, respectively). For all microorganisms, the variation in microbial counts between first and second collection was not statistically significant ($P>0.05$).

3.2. Microbiological quality per categories and types of spices and herbs

Overall, data showed that spices and herbs imported from France showed lower counts of aerobic mesophilic bacteria, sulfite reducing anaerobic bacteria, *C. perfringens* and no contamination with coliforms, *E. coli, Salmonella*, yeasts and molds. One of the reasons for such result may be the application of good hygienic practices (GHPs), good manufacturing practices (GMPs) during production, harvest, post-harvest including curing, bleaching, drying, grading, packing, transportation and storage. Moreover, imported spices and herbs may be subjected to sterilization treatments by heat, steam, dehydration, desiccation or irradiation such as gamma rays, X-rays, accelerated electrons for microbial decontamination, disinfestation and shelf life extension (Bourgeois et al., 1995; Kirkin et al., 2014; Koohy-Kamaly-Dehkordy et al., 2013). Sharma (2006) reported that irradiation doses of 5 KiloGray (kGy) and 10 KiloGray (kGy) eliminated fungal contamination and total bacteria count, respectively (Sharma, 2006). A previous study compared the effectiveness of irradiation and fumigant ethylene oxide on pepper, paprika, onion powder and garlic powder (Farkas, 1998). The irradiation at a dose of 10 kGy was more effective than fumigant (800g/6h) and eliminated the counts of aerobic mesophilic bacteria, spore forming bacteria, fungi, coliforms, *E. coli* and *Salmonella* in all samples, without significantly affecting the flavor, color and aroma of spices. For the other categories, the acceptable counts in samples locally packaged in companies with FSMS were higher than those locally packaged in companies without FSMS followed by unpackaged ones for all microorganisms except for sulfite reducing anaerobic bacteria and *C. perfringens*. The poor microbiological quality of unpackaged spices and herbs may be due to the uncontrolled handling/storage in open bags. Our findings were in agreement with others who highlighted the importance of the implementation of food safety management systems which include the principles of hazard analysis and critical control points (HACCP), ensure the application of good hygienic practices (GHPs), good manufacturing practices (GMPs), maintain a sustainable food safety performance, reduce food safety incidences, and control all stages of production to improve the safety and quality of the end product (El Darra et al., 2019; Sagoo et al., 2009).

Among all spices and herbs, paprika (*Capsicum annuum*) showed multiple contamination with all microorganisms except *Salmonella* (Table 4). The counts of TAMB, coliforms, sulfite reducing anaerobic bacteria, *C. perfringens*, yeasts and molds were ranging between acceptable and marginally acceptable. However, 12.5% (1/8) of paprika samples showed unacceptable count of *E. coli*. Previous reports showed that manual handling of paprika exposes this spice to a high risk of contamination due to unhygienic practices (Moreira et al., 2009; Velázquez et al., 2019; Witkowska et al., 2011). Cumin (*Cuminum cyminum*) was contaminated with all microorganisms except *E. coli* and *Salmonella*. The counts of coliforms and sulfite reducing anaerobic bacteria were unacceptable in 25.0% (2/8) of cumin samples. The normal grinding technique of cumin may increase the temperature of the seeds, alter the composition of essential oils (cuminaldehyde and monoterpene) and decrease their effectiveness against microorganisms (Sharma et al., 2016). Thyme mix was contaminated with all microorganisms except *E. coli* and *Salmonella*. Unacceptable counts of yeasts and molds were found in 16.7% (1/6) of thyme mix samples and no counts were within the acceptable range for sulfite reducing anaerobic bacteria. The contamination levels in thyme mix may be due to mishandling of thyme while mixing its ingredients such as sumac, sesame and salt. Dried mint (*Mentha sativa*) samples were contaminated with all microorganisms except *E. coli, Salmonella* and *C. perfringens*. Furthermore, 33.3 % (2/6) and 50.0% (3/6) of dried mint samples were unacceptable for coliforms and yeasts and molds, respectively. It was remarkable that all dried mint samples had no counts within the acceptable range for those 2 microorganisms. Dried mint and thyme mix are added to salads, sauces, chicken, meat, fish, sandwiches, pies and several Lebanese dishes. Therefore, the high contamination levels in dried mint and thyme mix with coliforms, spore-forming sulfite reducing anaerobic bacteria or *C. perfringens,* yeasts and molds may pose a public health risk in both RTE and non RTE products. While coliforms can be controlled in non RTE through proper heating, the spore formers and mycotoxins produced by yeasts and molds can survive normal cooking process (Hashem & Alamri, 2010; Tassanaudom et al., 2017; Witkowska et al., 2011). Previous studies in Lebanon reported the contamination of thyme mix samples with tentoxin (67.0 µg/kg) (Gambacorta et al., 2019) and Zearalenone (2.8µg/kg) (El Darra et al., 2019) produced by *Alterania* and *Fusarium* species, respectively. Moreover, high mean level of contamination (4286.9 µg/kg) with Fumonisin (FB_1 and FB_2), Deoxynivalenol, T-2, H-T2 toxins produced by *Fusarium* species and Zearalenone produced by *Alterania* species was found in dried mint samples (El Darra et al., 2019). On the other hand, thyme (*Thymus vulgaris*) was less contaminated than thyme mix in TAMB, sulfite reducing anaerobic bacteria, *C. perfringens,* yeast and molds and was not contaminated with *E. coli*, *Salmonella* and coliforms. 12.5% (1/8) of thyme samples were unacceptable for yeasts and molds. Cinnamon (*Cinnamomum zeylanicum*) and garlic powder (*Allium sativum*) were contaminated with all microorganisms except coliforms, *E. coli* and *Salmonella*. In addition, all cinnamon and garlic powder samples were acceptable for TAMB, yeasts and molds, respectively. However, 37.5% (3/8), 12.5% (1/8) of cinnamon and garlic powder samples showed unacceptable counts of sulfite reducing anaerobic bacteria and TAMB, respectively. Black pepper (*Piper nigrum*) and white pepper (*Piper nigrum*) were not contaminated with coliforms, *E. coli, C. perfringens* and *Salmonella*. In contrast, 12.5% (1/8) of black and white pepper samples showed unacceptable counts of sulfite reducing anaerobic bacteria and yeasts and molds, respectively. White pepper had higher acceptability for TAMB and sulfite reducing anaerobic bacteria, as compared to black pepper and similar acceptability was found for the others microorganisms. Similar results were reported by Witkowska et al., (2011) where the TAMB count in black pepper was 2.44 log CFU/g and undetectable in white pepper. This difference may be due to the harvesting methods; Black pepper are made from green berries dried using the natural sun heat which expose them to high risk of contamination. However, white pepper are produced from fully ripe pepper berries soaked in hot water and the skin is removed after drying which reduce the microbial contamination (Witkowska et al., 2011). Red chili (*Capsicum annum*) showed acceptable counts for all microorganisms except TAMB, yeasts and molds. The counts of TAMB ranged between acceptable and marginally acceptable. However, 12.5% (1/8) of red chili samples had unacceptable counts of yeasts and molds. Sesame (*Sesamum indicum*) samples were acceptable for all microorganisms except TAMB, sulfite reducing anaerobic bacteria, yeasts and molds where the counts varied between acceptable and marginally acceptable. No counts were found within the unacceptable range for all sesame samples. Other researchers demonstrated the strong antibacterial effect of sesamol, sesamin and sesamolin contained in sesame seeds (Mahendra Kumar & Singh, 2015).

Moreover, all oregano (*Origanum vulgare*) samples were acceptable for TAMB, coliforms, *E. coli, Salmonella* and *C. perfringens*. The counts of sulfite reducing anaerobic bacteria, yeasts and molds ranged between acceptable and marginally acceptable but no sample was unacceptable. Compared to thyme mix and thyme, oregano was the least contaminated herb with aerobic mesophilic bacteria, sulfite reducing anaerobic bacteria and *C. perfringens*. Oregano and thyme are known to contain antimicrobial agents such as thymol and carvacrol (Dghaim et al., 2017; Tajkarimi et al., 2010; Witkowska et al., 2011). Gutierrez et al., (2011) evaluated the inhibitory effect of the essential oils (EOs) of oregano and thyme against microbial growth when added to food (Gutierrez et al., 2011) and found that oregano EOs have the highest antimicrobial activity as compared to thyme. However, sumac (*Rhus coriaria*) was the only spice that showed acceptable counts for all microorganisms in all categories. The lowest levels of contamination in sumac samples may be due to their antimicrobial properties (Koohy-Kamaly-Dehkordy et al., 2013; Nasar-Abbas & Halkman, 2004; Sakhr & El Khatib, 2020).

It should be highlighted that all samples in this study were not subjected to any washing or treatment before analysis to minimize the natural residual antimicrobial activity of spices and herbs. An alternative method consisting of washing the samples was developed to reduce the inhibitory effect of naturally occurring substances. Vitullo et al., (2011) assessed the microbiological quality of 51 samples of herbs collected from Italy by using conventional testing method and “Washing and Shaking” protocol (WaS). They found that microbial counts, particularly *E. coli* and *B. cereus*, were higher when the WaS method was applied (Vitullo et al., 2011). Therefore, the microbiological counts observed in our study are expected to be higher if the same protocol was used to take into account the effect of naturally occurring substances in spices and herbs, but the conventional method assessed the microbiological quality of spices and herbs as consumed in our food.

Table 4: Percentage of acceptable and unacceptable counts for each microorganisms per type of spices and herbs according to Lebanese standards.

Spices/Herbs	Microorganisms	TAMB	Coliforms	*E. coli*	Sulfite reducing anaerobic bacteria	*C. perfringens* [c]	Yeasts and molds	*Salmonella*
Cinnamon	% of acceptable counts (n/N [a])	100(8/8)	100(8/8)	100(8/8)	37.5(3/8)	62.5(5/8)	75.0(6/8)	100(8/8)
	% of unacceptable counts (n/N [b])	0.0(0/8)	0.0(0/8)	0.0(0/8)	37.5(3/8)	0.0(0/8)	0.0(0/8)	0.0(0/8)
Black pepper	% of acceptable counts (n/N [a])	50.0(4/8)	100 (8/8)	100(8/8)	50.0(4/8)	100(8/8)	87.5(7/8)	100(8/8)
	% of unacceptable counts (n/N [b])	0.0 (0/8)	0.0(0/8)	0/0(0/8)	12.5(1/8)	0.0(0/8)	0.0(0/8)	0.0(0/8)
Sumac	% of acceptable counts (n/N [a])	100 (6/6)	100(6/6)	100(6/6)	100(6/6)	100(6/6)	100(6/6)	100(6/6)
	% of unacceptable counts (n/N [b])	0.0(6/6)	0.0(0/6)	0.0(0/6)	0.0(0/6)	0.0(0/6)	0.0(0/6)	0.0(0/6)
Cumin	% of acceptable counts (n/N [a])	50.0(4/8)	37.5(3/8)	100(8/8)	25.0(2/8)	37.5(3/8)	37.5(3/8)	100(8/8)
	% of unacceptable counts (n/N [b])	0.0(0/8)	25.0(2/8)	0.0(0/8)	25.0(2/8)	0.0(0/8)	0.0(0/8)	0.0(0/8)
Sesame	% of acceptable counts (n/N [a])	83.3(5/6)	100(6/6)	100(6/6)	83.3(5/6)	100(6/6)	50.0(3/6)	100(6/6)
	% of unacceptable counts (n/N [b])	0.0(0/6)	0.0(0/6)	0.0(0/6)	0.0(0/6)	0.0(0/6)	0.0(0/6)	0.0(0/6)
Dried mint	% of acceptable counts (n/N [a])	66.7(4/6)	0.0(0/6)	100(6/6)	83.3(5/6)	100(6/6)	0.0(0/6)	100(6/6)
	% of unacceptable counts (n/N [b])	0.0(0/6)	33.3(2/6)	0.0(0/6)	0.0(0/6)	0.0(0/6)	50.0(3/6)	0.0(0/6)
Paprika	% of acceptable counts (n/N [a])	87.5(7/8)	87.5(7/8)	87.5(7/8)	50.0(4/8)	50.0(4/8)	75.0(6/8)	100(8/8)
	% of unacceptable counts (n/N [b])	0.0(0/8)	0.0(0/8)	12.5(1/8)	0.0(0/8)	0.0(0/8)	0.0(0/8)	0.0(0/8)
White pepper	% of acceptable counts (n/N [a])	87.5(7/8)	100 (8/8)	100(8/8)	87.5(7/8)	100(8/8)	87.5(7/8)	100(8/8)
	% of unacceptable counts (n/N [b])	0.0(0/8)	0.0(0/8)	0.0(0/8)	0.0(0/8)	0.0(0/8)	12.5(1/8)	0.0(0/8)
Thyme mix	% of acceptable counts (n/N [a])	50.0(3/6)	83.3(5/6)	100(6/6)	0.0(0/6)	66.7(4/6)	33.3(2/6)	100(6/6)
	% of unacceptable counts (n/N [b])	0.0(0/6)	0.0(0/6)	0.0(0/6)	0.0(0/6)	0.0(0/6)	16.7(1/6)	0.0(0/6)
Red chili	% of acceptable counts (n/N [a])	37.5(3/8)	100 (8/8)	100(8/8)	100(8/8)	100(8/8)	50.0(4/8)	100(8/8)
	% of unacceptable counts (n/N [b])	0.0(0/8)	0.0(0/8)	0.0(0/8)	0.0(0/8)	0.0(0/8)	12.5(1/8)	0.0(0/8)
Oregano	% of acceptable counts (n/N [a])	100 (8/8)	100(8/8)	100(8/8)	87.5(7/8)	100(8/8)	75.0(6/8)	100(8/8)
	% of unacceptable counts (n/N [b])	0.0(0/8)	0.0(0/8)	0.0(0/8)	0.0(0/8)	0.0(0/8)	0.0(0/8)	0.0(0/8)
Thyme	% of acceptable counts (n/N [a])	87.5(7/8)	100(8/8)	100(8/8)	37.5(3/8)	75.0(6/8)	75.0(6/8)	100(8/8)
	% of unacceptable counts (n/N [b])	0.0 (0/8)	0.0(0/8)	0.0(0/8)	0.0(0/8)	0.0(0/8)	12.5(1/8)	0.0(0/8)
Garlic powder	% of acceptable counts (n/N [a])	25.0(2/8)	100(8/8)	100(8/8)	12.5(1/8)	87.5(7/8)	100(8/8)	100(8/8)
	% of unacceptable counts (n/N [b])	12.5(1/8)	0.0(0/8)	0.0(0/8)	0.0(0/8)	0.0(0/8)	0.0(0/8)	0.0(0/8)

[a] n/N: Number of acceptable samples / total number of samples in the four categories at each collection

[b] n/N: Number of unacceptable samples/ total number of samples in the four categories at each collection.

[c] Limit was not available in Lebanese standards and was used according to ICMSF (ICMSF,2005)

3.3. Moisture content of spices and herbs

The moisture profile was adequate with the specifications set by the European Spices Association (ESA, Quality minima document, 2018) and Lebanese standards (NL 9:1998, NL 10:1998, NL 70:1999, NL 194:2000, NL 615:2002, NL 678:2003, NL 544:2004, NL 546:2004, NL 534:2004, NL 331:2009) for all analyzed samples except sumac which had the highest mean of moisture (11.3%) exceeding the limits set by Lebanese standards (Table 5). Sesame had the lowest moisture content (2.4%). This difference in moisture between types of spices and herbs was statistically significant (P=0.000<0.05).

It was found that the moisture content of thyme locally packaged in companies without FSMS, unpackaged paprika and unpackaged red chili exceeded the legislation levels at one collection point (10.3%, 11.8% and 11.0% respectively). These variations in moisture between first and second collection may be due to climatic conditions, re-absorption of moisture, inappropriate storage of unpackaged samples in open containers, uncontrolled processing measures in companies without FSMS and long storage period of packaged spices and herbs. The difference in moisture content between first and second collection was not statistically significant (P=0.932). There was no statistically significant relationship between moisture and categories of spices and herbs (P=0.847). This relationship was not statistically significant for the growth of all tested microorganisms too (P> 0.05). While the moisture content is an important quality characteristic of spices and herbs, water activity is a better indicator that can be correlated to microbial growth (FDA,2018; Syamaladevi et al., 2016).

Table 5: Moisture content of spices and herbs (Mean values ± SD).

Spices/herbs	Maximum acceptable limit set by Lebanese standards [a] (%)	Maximum acceptable limit set by ESA [b] (%)	Moisture content (%)				
			Locally packaged in companies with FSMS	Locally packaged in companies without FSMS	Packaged imported	Bulk	Mean±SD
Cinnamon	14	14	8.0±0.2	8.8±1.7	7.7±1.3	7.2±1.7	7.9±1.2
Black pepper	13	12	10.0±0.8	9.4±0.9	10.7±0.4	8.9±0.8	9.7±0.9
Sumac	8	-	8.7±0.1 [c]	15.2±1.8 [c]	N/A [d]	9.9±0.9 [c]	11.3±3.2 [c]
Cumin	13	13	6.8±0.1	8.3±0.6	7.3±0.4	7.4±0.4	7.5±0.7
Sesame	8	-	2.1±0.4	2.6±1.1	N/A [d]	2.6±0.6	2.4±0.6
Dried mint	13	13	7.3±0.4	7.4±0.8	N/A [d]	8.0±1.7	7.6±0.9
Paprika	11	11	6.2±0.5	4.7±0.6	4.7±1.5	8.4±4.8	6.0±2.5
White pepper	14	12	9.7±0.4	9.5±0.3	10.0±0.0	9.0±1.4	9.6±0.7
Thyme mix	10	12	5.5±0.3	5.5±0.6	N/A [d]	5.4±1.2	5.5±0.6
Red chili	11	11	6.9±0.8	6.7±1.5	8.5±0.7	8.1±4.2	7.5±1.9
Oregano	10	12	7.3±0.3	7.2±1.5	6.6±0.7	8.6±0.7	7.4±1.1
Thyme	10	12	9.6±0.0	9.4±1.3	8.3±0.1	7.8±2.6	8.8±1.4
Garlic powder	8	6.5	3.8±0.5	4.1±0.6	4.9±0.6	2.6±1.2	3.9±1.1

[a] Cinnamon (NL 534:2004), black pepper (NL 70:1999),sumac (NL 678:2003), sesame (NL 331:2009), cumin (NL 9:1998), paprika and red chili (NL 544:2004), white pepper (NL 194:2000), garlic powder (NL 615:2002), thyme, thyme mix, oregano (NL 546:2004) and dried mint (NL 10:1998)
[b] European Spices Association, Quality minima document, 2018
[c] Exceeded the maximum limit set by Lebanese standards
[d] N/A: Not Applicable

3.4. Limitations of the study

Due to the availability of equipment and limited resources, moisture content was measured instead of water activity and thirteen most commonly consumed types were analyzed. A composite sampling approach was applied to screen a higher number and assess the overall status of spices and herbs samples available in the Lebanese market even though it may dilute the contamination levels that may be found in each individual sample.

4. Conclusion

In this research, the microbiological quality of thirteen most commonly consumed types of spices and herbs commercialized in Lebanon was assessed. The microbiological quality was in the following decreasing order (from highest to lowest): imported brands, locally packaged in companies with FSMS, locally packaged in companies without FSMS, and unpackaged samples. In the local market, spices and herbs sold in bulk (open containers) are highly susceptible to microbial contamination. The hygienic, environmental and sanitation conditions during processing, distribution and storage of unpackaged spices and herbs must be controlled to prevent high microbial counts. In this regard, food handlers, suppliers and consumers should be aware of the appropriate handling and storage practices to reduce the risk of contamination. Our findings also suggest the need of the implementation of a food safety management system, application of good hygienic practices, good manufacturing practices, performance of routine inspection, testing of the end product ensure the microbial safety at all stages of production to improve the quality of ready to eat spices and herbs. In addition, the application of irradiation, heat or steam treatments may help to inhibit the growth of pathogenic and spoilage microorganisms.

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