

*Full Length Research Paper*

# An innovative microwave process for microbial decontamination of spices and herbs

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Accepted 20 November, 2012

The microbiological status of some dried spices and herbs from local markets in Jordan and the effect of home microwave on reducing their microbial load were evaluated. The results indicated that the sixteen tested samples were contaminated with aerobic mesophilic bacteria ranging from  $1.18 \times 10^3$  to  $5.1 \times 10^5$  CFU/g, mesophilic spore former bacteria  $1.2 \times 10^2$  to  $1.2 \times 10^4$  CFU/g, thermophilic spore former bacteria  $4.8 \times 10^2$  to  $1.2 \times 10^3$  CFU/g, fungi  $1.04 \times 10^3$  to  $2.2 \times 10^4$  CFU/g and coliforms  $< 10^2$  to  $3.44 \times 10^2$  CFU/g. *Salmonella* and *Staphylococcus aureus* were not detected. According to the international standards, 87.5% of the samples were unsatisfactory because of the large population of fungi (87.5%) and coliforms (37.5 %). Exposure of spices and herbs (dry and wet treatments) to microwave resulted in a reduction of the microbial population. In both treatments and at 30 s exposure, the total counts of fungi were decreased by 1-3 log cycle (D values = 0.188 to 0.450 min). Thermophilic spore former bacteria were reduced by 1-2 log cycle (D values = 0.165 to 0.357 min). This novel short period decontamination process could be suitable for use at home and at industrial levels. In addition, implementation of an authorized food control program could prevent the harmful contamination effect on human.

**Key words:** Spices and herbs, microwave, microbiological quality, D value, microbial decontamination.

## INTRODUCTION

Early cultures recognized the importance of using spices and herbs in preserving foods and for their medicinal value. Worldwide trade/commerce of spices has continuously increased from 209,293 tons in 2000 to 226,910 tons in 2004, which corresponds to a trade value of 625 Mio US\$ (FAO-STAT, 2005). The use of spice mixtures, aroma components and functional ingredients have increased in the food industry, especially in functional foods, ready-to-eat meals and highly spiced cuisine (Srinivasan, 2005; Subbulakshmi and Naik, 2002). Despite their contribution to taste, color and aroma in foods, these powders are known to be a significant source of micro-organisms, due to high microbial contamination caused by poor sanitary conditions during growing, harvest, processing and storage, which lead to an increased number of food-borne infections and intoxications (Buckenhueskes and Rendlen, 2004). Owing to their low moisture contents, spices are non-perishable commodities, but once they get in contact with water-rich food products, microbial

populations may develop quickly. Particular attention needs to be paid to the application of spices to ready-to-eat foods which are not subjected to further heat treatments (Little et al., 2003). The production of different types of spices in various areas of the world and aspects of microbiological contamination as well as sanitation technologies have already been reviewed in a number of previous papers (McKee, 1995; Subbulakshmi and Naik, 2002). The microbial populations are mainly composed of mesophilic and spore-forming bacteria, moulds and yeasts, among them are food spoiling and pathogenic genera like *Salmonella*, *Clostridium*, *Bacillus*, *Listeria* and *Staphylococcus* (ASTA, 2008; Banerjee and Sarkar, 2004; Candlish et al., 2001; McKee, 1995). During the last decade of the 20th century, food-borne infections and intoxications due to spices have increased in several European countries (Buckenhueskes and Rendlen, 2004). In particular, salmonellosis constitutes an important food-borne disease (Sagoo et al., 2009; Vij et al., 2006). Bet-

ween April and September 1993, a nationwide outbreak of salmonellosis with estimated 1000 cases of disease occurred in Germany which was traced back to contaminated paprika and paprika-powdered potato chips (Lehmacher et al., 1995). In addition, the growth of moulds creates serious problems in spices and herbs (Hashem and Alamri, 2010; Jorgensen, 2005). Mycotoxins have been classified as teratogenic, mutagenic and cancerogenic (Stark, 2005). Recently, the maximum tolerated mycotoxin contents in food and feed are regulated worldwide (FAO, 2004).

Several sanitation methods have been developed to reduce the microbial loads of spices. Fumigation with ethylene oxide has been proven to significantly reduce microbial population (Toofanian and Stegeman, 1988). However, it is generally considered a carcinogen and mutagen, particularly, when taken up via inhalation (Steenland et al., 2004). Therefore, the use of ethylene oxide for fumigation is banned in the European Union. Irradiation, including the application of gamma rays, electron beam and X-rays, has been shown to be an effective method for spices decontamination (Farkas, 2006). Although, ionizing radiation has proven to be an efficient, environmentally clean, energy effective and even officially approved method, it is scarcely used because of its poor consumer acceptance. Additionally, slight modifications in sensorial and anti oxidative properties have been observed (Suhaj et al., 2006). Steam treatments technique involving high temperature steam usually is an effective decontamination method, but is associated with discoloration and reduction of volatile oil contents (Almela et al., 2002). High hydrostatic pressures ranging from 100 to 1000 MPa provide a valuable tool for microbiologically safe and shelf stable fruit and vegetable products (Guerrero-Beltran et al., 2005). However, the inactivation of microorganisms is strongly dependent on water activity. Spice samples with water activities below 0.66 showed no reduction in the microbial count (Butz et al., 1994). Therefore, high pressure treatment is an unsuitable sanitation method in spice production.

Commercial and legal requirements regarding the safety, quality and storage of food products have still focused attention on the development and improvement of decontamination methods. Microwave processing and cooking of foods is a recent development, which is gaining momentum in household as well as large scale food applications (Akgul et al., 2008; Basaran and Akhan, 2010). Microwaves (MW) are defined as electromagnetic waves in the range of infrared and radio waves, with a wavelength ranging from 1 mm to 1 m and operating at a frequency of 300 MHz to 300 GHz (Thostenson and Chou, 1999). In the United States, two frequencies (915 and 2450 MHz) are designated by Federal Regulations for industrial application (Lau and Tang, 2002). Microwave energy has been used in food applications mainly for its heating properties. It seems natural, therefore, to adapt microwave radiation to pasteurize or even sterilize foods

at low temperatures in shorter times than required by conventional methods (Akgul et al., 2008). Despite some disadvantages, such as non-heterogeneous heating treatment, edge overheating, soggy texture and browning, MW processing can be regarded as an alternative approach for food stabilization, due to the possibility of treating the product inside the packaging and prepare convenience food (Giuliani et al., 2010). This technology allows a reduction in the warm up time and preserves the natural organoleptic characteristics of the juice. Due to the absorption of electromagnetic energy, temperature of material of high dielectric capacity increases after microwave pasteurization. Therefore, microwave pasteurization offers similar benefits to conventional methods, but with an improved product quality and reduced time of exposure to energy (Canımır et al., 2002). Processing of spices using microwaves is a newer dimension. This alternative methodology is preferred, due to the convenience and ease of handling (Behera et al., 2004). Microwave heating has been successfully used in food industry, including tempering, thawing, blanching, drying, roasting, inactivating of enzyme, pasteurization and sterilization of food products (Venkatesh and Raghavan, 2004; Yarmand and Homayouni, 2009). However, it is well known that it is effective against a wide range of microorganisms in various food systems, including *Escherichia coli* (Canımır et al., 2002), *Staphylococcus aureus* (Yeo et al., 1999), *Bacillus* spp. (Celandroni et al., 2004), *Campylobacter jejuni* (Uradzinski et al., 1997), *Pediococcus* spp. (Kozempel et al., 1997), *Saccharomyces cerevisiae* and *Lactobacillus plantarum* (Tajchakavit et al., 1998). In the literature, only one work has been reported by Aydin and Bostan (2006) on microbial decontamination of powdered black pepper (*Piper nigrum* L.) by using microwave. Finding a method to reduce, inhibit or kill the microorganism of spices and herbs before consumption is mostly required and needed, particularly, if they are added directly to prepared food without further cooking. On the other hand and at the time of this study, there is a lack of uniformed microbiological standards for dried spices and herbs at international (EC, 2004) and national levels. Thus, the aims of this study were to evaluate the microbiological status of some spices and herbs and to examine the suitability of microwave as a short time microbial decontamination method, for fungi and thermophilic spore former microorganisms that contaminate spices and herbs.

## MATERIALS AND METHODS

### Source of spices and herbs

A total of sixteen commercially dried spices and herbs, representing different types of ready to eat food, were collected from random local markets at Amman Governorate, Jordan during 2011.

### Microbiological methods

Microbiological evaluation of spices and herbs were conducted or enumerated according to Food and Drug Administration-FDA (1998) Bacteriological Analytical Manual and American Public Health Association APH - (CMMEF), Compendium of Methods for the Microbiological Examination of Foods (Downs and Ito, 2001). The initial microflora in the spices and herbs was determined by homogenizing 25 g of each sample with 225 ml of buffered peptone water to give 0.1 dilution ( $1:10^{-1}$ ). Serial dilutions up to  $1:10^{-6}$  were prepared and 1 ml aliquots were pour plated in duplicates for the isolation and enumeration of total aerobic plate count (ABC), coliforms (TC), fungi, yeast and mould (TF). In order to isolate and count the mesophilic spore former bacteria (MSFB) and thermophilic spore former bacteria (TSFB), a heat treatment was applied for the different dilutions ( $1:10^{-1}$  to  $1:10^{-5}$ ) at 95°C for 15 min in water bath and immediately cooled at 45°C (Speck et al., 1992). Standard plate counts agar for MSFB were incubated at 37°C and for TSFB were incubated at 55°C for 72 h. Colonies of the appeared bacteria were counted and calculated as an average of microbial count expressed as CFU/g sample. Concerning the identification of *S. aureus*, typical and atypical presumptive *Staphylococci* spp. Colonies were examined by Gram stain, coagulase, catalase and latex agglutination (Oxoid, FT0203) tests. For *Salmonella*, typical *Salmonella* spp. colonies were subjected to the further biochemical and serological characterization tests using Triple Sugar Iron agar (TSIA) (Merck 103915), Lysine Iron Agar (LIA) (Merck 111640) and agglutination (Oxoid FT 203) tests.

### Microwave irradiation

A home microwave oven 2450 MHz - 900 W, Kelvinator (model 1.5CU,FT) was used to study the effect of microwave radiation on reducing the microbial load of spices and herbs as dried (dry treatment) and in liquid solution (wet treatment). For dry treatment, 11 g of dried powder samples were arranged in a single layer Pyrex Petri dishes (9-cm diameter); for wet treatment, 11 g of dried samples were transferred into 250 ml Erlenmeyer flasks containing 99 ml of 0.1% phosphate peptone water. Samples for both treatment were placed on the center of the turntable plate and exposed to microwave for various duration (0, 15, 30, 45 and 60 s). Duplicate sample preparations were made for each time of exposure. After microwave processing time, the samples were left in the oven for 3 min to permit the internal heat equilibration, and then the samples were refrigerated immediately prior to further microbiological analysis. At each time intervals for tested sample, the average of duplicate total count of fungi and thermophilic spore former bacteria were determined and compared with control sample with-out microwave radiation. Survivor's curves were drawn by plotting recovered CFU/g of sample vs. microwave exposure times.

### Statistical analysis

The destruction effect of microwave radiation on the reduction of microorganisms count was calculated based on a first-order kinetic (Koutchma et al., 2001):

$$D = 2.303 / K_D \dots\dots\dots \text{Equation 1}$$

where decimal reduction time (D) is the time necessary for the disappearance of 90% of the initial bacterial population and  $K_D$  is the slope of a linear regression line of the plot relating microorganisms count to exposure time; the lower the D value, the higher the destruction efficiency. Portion of inactivation curves from 0 to 30 s were used for regression analysis (Excell, Microsoft Office 2000). Linear regression analysis was applied for inactivation curves of fungi, while exponential was chosen for TSFB because of the lower

total count.

Matched-pair t-test was computed for the statistical significance of D values at  $\alpha = 0.05$ : destruction effect of microwave on microorganisms in dry and wet treatments.

## RESULTS

### Microbiological quality of spices and herbs

The assessment of microbial quality of spices and herbs was carried out for the presence of *S. aureus*, *Salmonella*, coliforms, total aerobic count, total count of fungi, total count of mesophilic spore former bacteria and total count of thermophilic spore former bacteria (Table 1). The observed counts reflected the original microorganisms bioload and growth. The total aerobic viable counts was noticed in different spices and herbs at different levels, ranging from  $1.18 \times 10^3$  to  $5.1 \times 10^5$ . The highest mean count level was detected in mustard followed by black pepper, fenugreek, ginger, stevia, chilli pepper and great galangal. The lowest count was obtained in tea. Concerning the contamination of tested spices and herbs by mesophilic spore former bacteria, all tested samples were contaminated with a range from  $1.2 \times 10^2$  to  $1.2 \times 10^4$  CFU/g; mustard was the most contaminated spice. Regarding thermophilic spore former bacteria counts, 100% of the samples were contaminated ranging between  $4.8 \times 10^2$  and  $1.2 \times 10^3$  CFU/g and the highest counts were in oregano, mustard and oat. Contamination with fungi is common in herbs and spices of all types ranging between  $1.04 \times 10^3$  and  $2.2 \times 10^4$  CFU/g. The highest counts were determined in fenugreek and stevia. Coliforms were detected in the range of  $<10^2$  and  $3.44 \times 10^2$  CFU/g, the highest count was in white pepper and pimento. Pathogens such as *S. aureus* and *Salmonella* were not found.

### The effect of microwave on microbial load

To evaluate the effect of microwave on fungi and thermophilic spore former bacteria in spices and herbs, survivor's curves were drawn by plotting recovered CFU/g of sample against microwave exposure times. For dry and wet treatment of spices and herbs, average inactivation profiles for fungi (Figure 1) and for TSFB (Figure 2) were compared in all tested samples at different exposure time. In general, a decrease in the microbial load was noted as exposure time increased from initial time to 60 s.

A considerable linear reduction slope was obtained at 15 s exposure followed by a lower and semi constant rate from 30 to 60 s in both dry and wet treatments. For D-value calculation, a linear relationship between log survivors and treatment time is assumed, and is typically determined from the portion of an inactivation curve from 0 to 30 s (Table 2). A logarithmic increase response was observed with time, the total counts of fungi were mainly decreased by 1-3 log (Figure 1). Similarly, the TSFB was reduced by 1-2 log cycle (Figure 2). The distraction time

**Table 1.** Total count (CFU/g) of microorganisms detected in spices and herbs

Spice/herb common and scientific name	ABC	MSFB	TSFB	Fungi	Coliforms	<i>S. aureus</i>	<i>Salmonella</i>
Black pepper <i>Piper nigrum</i>	3.04x10 <sup>5</sup>	1.32x10 <sup>3</sup>	9.10x10 <sup>2</sup>	1.64x10 <sup>4</sup>	3.44x10 <sup>2</sup>	N.D	N.D
Chilli pepper <i>Capsicum annuum</i>	1.58x10 <sup>5</sup>	1.88x10 <sup>3</sup>	8.60x10 <sup>2</sup>	1.18x10 <sup>4</sup>	< 10 <sup>2</sup>	N.D	N.D
Cinnamon <i>Cinnamomum zeylanicum</i>	1.12x10 <sup>4</sup>	9.50x10 <sup>2</sup>	6.30x10 <sup>2</sup>	9.70x10 <sup>3</sup>	< 10 <sup>2</sup>	N.D	N.D
Clove <i>Eugenia caryophyllis</i>	1.30x10 <sup>4</sup>	1.12x10 <sup>3</sup>	5.30x10 <sup>2</sup>	1.40x10 <sup>4</sup>	< 10 <sup>2</sup>	N.D	N.D
Fenugreek <i>Trigonella foenumgraecum</i>	2.32x10 <sup>5</sup>	1.24x10 <sup>3</sup>	8.70x10 <sup>2</sup>	2.20x10 <sup>4</sup>	< 10 <sup>2</sup>	N.D	N.D
Ginger <i>Zingiber officinale</i>	2.12x10 <sup>5</sup>	1.40x10 <sup>3</sup>	6.80x10 <sup>2</sup>	1.82x10 <sup>4</sup>	1.20x10 <sup>2</sup>	N.D	N.D
Great galangal <i>Languas galangal</i>	1.16x10 <sup>5</sup>	9.20x10 <sup>2</sup>	4.10x10 <sup>2</sup>	1.04x10 <sup>3</sup>	< 10 <sup>2</sup>	N.D	N.D
Licorice <i>Glycyrrhiza glabra</i>	1.90x10 <sup>4</sup>	1.32x10 <sup>3</sup>	8.70x10 <sup>2</sup>	1.04x10 <sup>4</sup>	1.22x10 <sup>2</sup>	N.D	N.D
Mustard <i>Brassica alba</i>	5.10x10 <sup>5</sup>	1.24x10 <sup>4</sup>	1.20x10 <sup>3</sup>	1.10x10 <sup>4</sup>	< 10 <sup>2</sup>	N.D	N.D
Oat <i>Avena sativa</i>	1.64x10 <sup>3</sup>	8.70x10 <sup>2</sup>	1.10x10 <sup>3</sup>	1.04x10 <sup>4</sup>	< 10 <sup>2</sup>	N.D	N.D
Oregano <i>Origanum vulgare</i>	6.40x10 <sup>3</sup>	3.10x10 <sup>2</sup>	1.20x10 <sup>3</sup>	1.34x10 <sup>4</sup>	1.40x10 <sup>2</sup>	N.D	N.D
Pimento <i>Pimenta dioica</i>	4.10x10 <sup>3</sup>	1.20x10 <sup>2</sup>	9.10x10 <sup>2</sup>	1.30x10 <sup>4</sup>	3.18x10 <sup>2</sup>	N.D	N.D
Stevia <i>Stevia rebaudiana</i>	1.88x10 <sup>5</sup>	5.40x10 <sup>2</sup>	6.70x10 <sup>2</sup>	2.20x10 <sup>4</sup>	< 10 <sup>2</sup>	N.D	N.D
Tea <i>Camellia sinensis</i>	1.18x10 <sup>3</sup>	7.10x10 <sup>2</sup>	4.80x10 <sup>2</sup>	1.30x10 <sup>4</sup>	< 10 <sup>2</sup>	N.D	N.D
Turmeric <i>Curcuma longa</i>	1.22x10 <sup>4</sup>	8.50x10 <sup>2</sup>	5.10x10 <sup>2</sup>	1.52x10 <sup>4</sup>	< 10 <sup>2</sup>	N.D	N.D
White pepper <i>Piper nigrum</i>	5.10x10 <sup>4</sup>	9.70x10 <sup>3</sup>	8.10x10 <sup>2</sup>	1.22x10 <sup>4</sup>	3.24x10 <sup>2</sup>	N.D	N.D

ABC: Aerobic mesophilic bacteria; MSFB: mesophilic spore former bacteria; TSFB: thermophilic spore former bacteria; N.D: not detected

for fungi of dry spices and herbs samples range from 0.118 to 0.443 min and in the wet treatment 0.118 to 0.437 min. Most destruction of fungi was observed in stevia. D values for TSFB contaminated spices and herbs with dry treatment were between 0.165 and 0.357 min and for the wet treatment was 0.159 to 0.357 min. The highest destruction was obtained in chilli pepper. In this study, t-test analysis of the results at  $\alpha = 0.05$  indicated that there is no significant differences of the distraction effect of microwave on microorganism decontaminated spices and herbs for dry and wet treatments.

## DISCUSSION

### Microbiological quality of spices and herbs

Spices and herbs powder are known to be significant

source of microorganisms, due to high microbial contamination caused by poor sanitary conditions along the production chain. In this study, the microbial populations of all studied spices and herbs were mainly composed of mesophilic and thermophilic spore-forming bacteria, moulds and yeasts, among them are coliforms.

The two pathogens *S. aureus* and *Salmonella* spp. were not detected. The obtained results were similar to other microbiological assessment of spices and herbs by other researches in different countries. In 2004, Buckenhu"skes and Rendlen mentioned that herbs and spices were contaminated with total bacterial counts between 4 and 7 log CFU/g while moulds and coliforms were >2-5 log CFU/g. The microbial quality of 53 samples of spices and dry herbs collected from Spanish markets showed contamination of samples of spices with mesophilic aerobic counts (10%) and *Enterobacteriaceae* (20%).

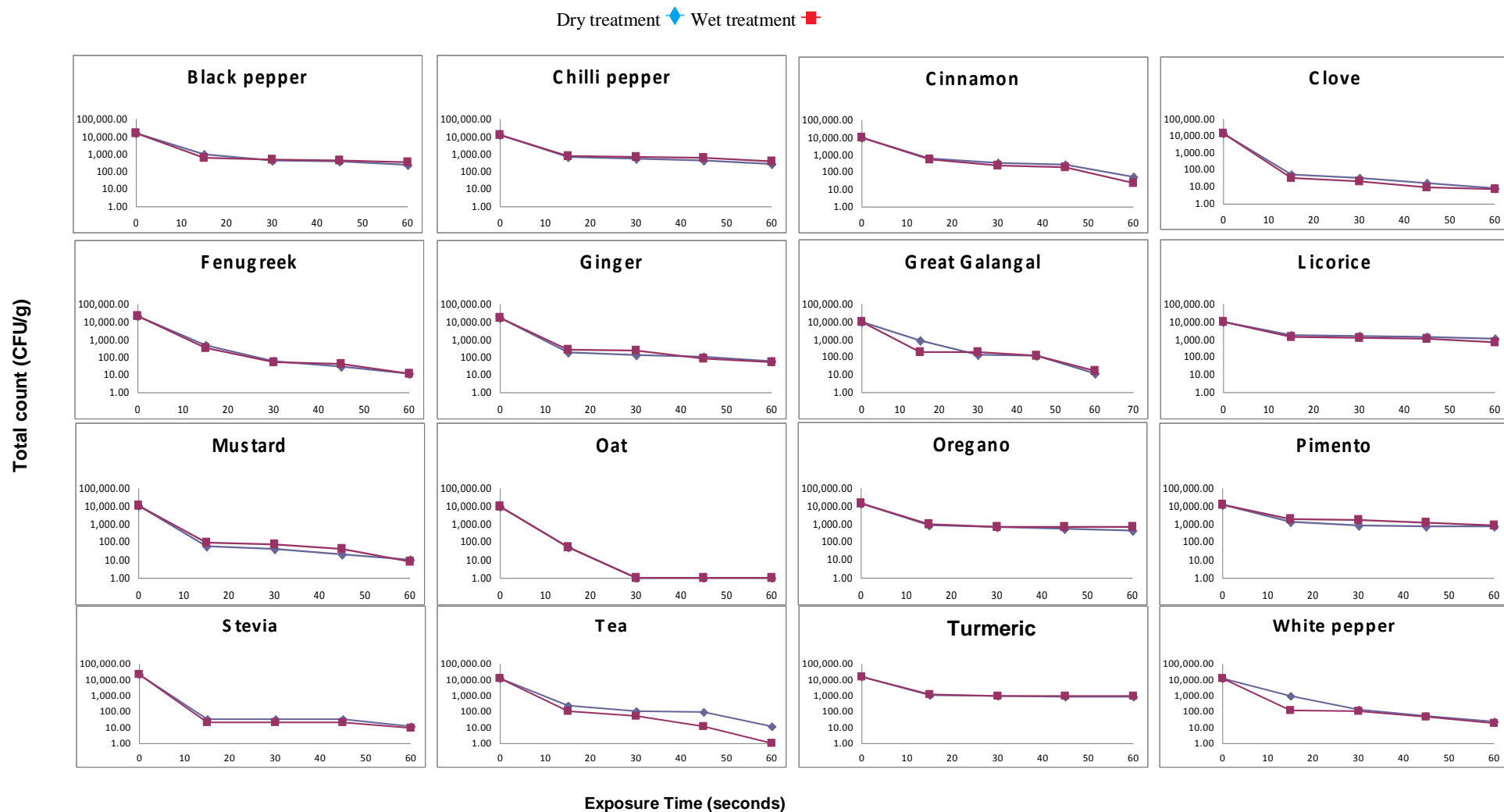


Figure 1. Microwave inactivation curves of fungi in spices and herbs.

The analysis from herbs showed that the percentage of contamination was 26% in both microbiological values. Pathogenic microorganisms like *Staphylococcus aureus*, *Yersinia intermedia*,

*Shigella* spp., *Enterobacter* spp., *Acinetobacter calcoaceticus* and *Hafni alvei* were also isolated (Sospedra et al., 2010). *Pichia* sp., *P. guilliermondii* and *Rhodo-torula* sp. *Bacillus cereus*, *Sal-*

*monella typhi-murium* and *Staphylococcus aureus* were detected in cashew and brazil nut kernels (Freire and Offord, 2002). The most predominant fungal gene-ra seen in seventeen spices samples

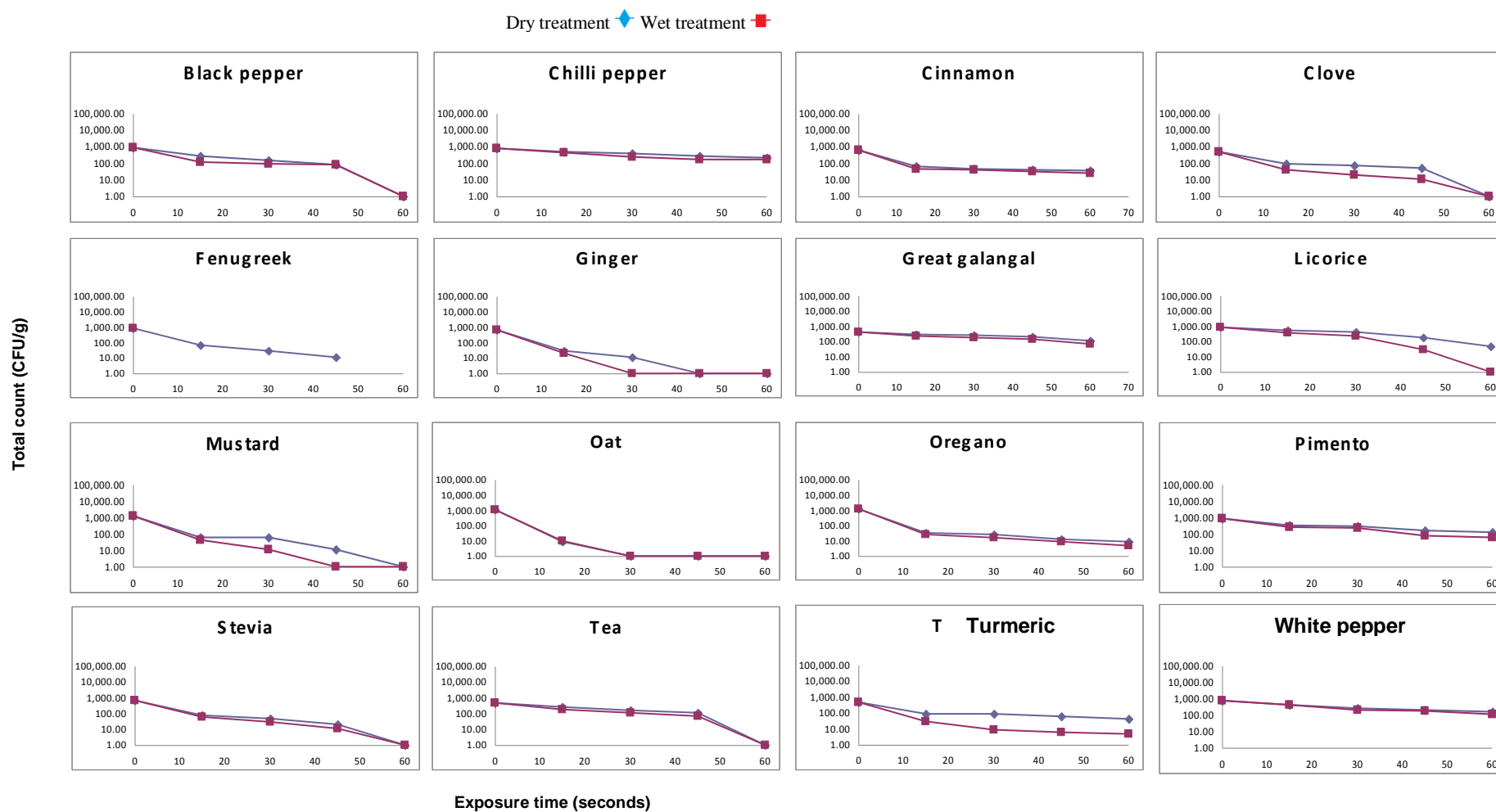


Figure 2. Microwave inactivation curves of thermophilic spore former bacteria in spices and herbs.

in Bahrain were *Aspergillus*, *Penicillium*, *Rhizopus*, *Cladosporium* and *Trichoderma*. Relative occurrence values of taxa disclosed ranged between 36.4% for *A. flavus* and 0.6% for *Aspergillus parasiticus* and *Absidia corymbifera* (Mandeel,

2005). Similar findings were obtained from the study of contamination of common spices in Saudi Arabia markets with potential mycotoxin-producing fungi (Hashem and Alamri, 2010). Furthermore, the FDA published a review of spice recalls

that occurred in the U.S. from 1970 to 2003 (Vij et al., 2006). Twenty-one recalls due to bacterial contamination were reported during this time period. The recalls involved twelve spice types, with paprika implicated in the highest number of

**Table 2.** Destruction effect of microwave on the survival of fungi and thermophilic spore former bacteria in spices and herbs at dry and wet treatment.

Spice and Herb	D value (minute)		D value (minute)	
	Fungi		Thermophilic spore former bacteria	
	Dry	Wet	Dry	Wet
Black pepper	0.258	0.258	0.168	0.203
Chilli pepper	0.360	0.366	0.165	0.159
Cinnamon	0.443	0.437	0.304	0.332
Clove	0.296	0.296	0.335	0.357
Fenugreek	0.218	0.188	0.210	0.210
Ginger	0.228	0.228	0.286	0.286
Great galangal	0.402	0.408	0.340	0.354
Licorice	0.468	0.450	0.165	0.179
Mustard	0.378	0.378	0.189	0.162
Oat	0.402	0.402	0.194	0.187
Oregano	0.324	0.270	0.201	0.196
Pimento	0.336	0.366	0.174	0.180
Stevia	0.188	0.188	0.271	0.274
Tea	0.321	0.320	0.286	0.306
Turmeric	0.288	0.288	0.357	0.351
White pepper	0.336	0.336	0.173	0.170

T = 0.930 for D values of fungi; t = -1.393 for D values of thermophilic spore former bacteria.

four recalls. Origins of recalled spices included India, Spain, Turkey, Egypt, Jamaica, Mexico, Taiwan and the U.S. In all but one instance, the recalled spices contained *Salmonella*.

There are no microbiological standards for dried spices and herbs in Jordan as well as in EC legislation, however, the Codex Code of Hygienic Practice specifies that dried spices and herbs should be free of pathogenic microorganisms at levels that may represent hazard to health and further requires that *Salmonella* should be absent in treated ready-to-eat spices (Codex Alimentarius Commission, 1995). The Commission of the European Union (EU) recommends the enumeration of *B. cereus* and *C. perfringens* as well as to verify the absence of *Salmonella* in 25 g of sample. In addition, the European Spice Association (ESA) also specified that *Salmonella* should be absent in 25 g of spice, *Escherichia coli* to be present at less than 10<sup>2</sup> CFU /g, and other bacteria requirements to be agreed between buyer and seller (Peter, 2001). The International Commission on Microbiological Specifications for Foods (ICMSF) allows maximum limits of 10<sup>6</sup> CFU of total aerobic mesophilic bacteria; 10<sup>4</sup> CFU of yeasts, molds and coliforms; and 10<sup>3</sup> CFU of *E. coli* and *C. perfringens* per g of spice (EC, 2004; ICMSF, 2005).

In contrast to the previous standards, the Spanish specifications for spices set maximum limits of 10<sup>3</sup> CFU/g of sulfite-reducing sporulated anaerobic bacteria and 10 CFU/g of *E. coli*, the absence of *Salmonella* in 25 g of sample and, in general, the absence of microbial pathogens (Anonymous, 2000). The total count of mesophilic bacteria exceeded the level 10<sup>5</sup> CFU/g which did not

agree with Polish standards (Wojcik-Stcponyzska et al., 2009). In spices, bacterial counts above >10<sup>6</sup> CFU/g and fungi counts >10<sup>4</sup> CFU/g were regarded as unacceptable according to Turkish Food Codex (Anonymous, 2000). According to the specification above, the result of this study of the total aerobic mesophilic bacteria considered satisfaction with the regulation of the ICMSF which allows maximum limits of 10<sup>6</sup> CFU/g. On the other hand, 87.5% of the tested samples containing higher than 10<sup>4</sup> CFU/g of fungi were unsatisfactory. No specifications were available to compare the total count of mesophilic and thermophilic spore former.

At the time of this study, it is obvious that there is a lack of microbiological specification and standard of spices and herbs at the national and international level. It requires a great effort to be completed and uniformed. An attempt was made by the European Commission (EC) Recommendation 2004/24/EC required Member States to perform a co-ordinated programme of sampling and testing of dried spices and herbs (EC, 2004). The data reported in this investigation represents a primary evaluation for the microbiological states of spices and herbs that could promote detailed studies leading to set up microbiological standards in Jordan.

### The effect of microwave on microbial load

In this study, the application of microwave treatment on spices and herbs samples was satisfactory in reducing the microbial load of fungi and TSFB within 30 s. The

sharp short time effect could be related to the direct electromagnetic waves on homogeneous killing of the microorganisms. Considerable distractions (1-3 log cycle) were obtained on both fungi and TSFB contaminated spices and herbs with D values ranging from 0.165 to 0.437 min. The variation in D values could be attributed to the initial microflora counts, types of microorganisms, variable active compounds among different spices and herbs, storage conditions and water content. Concerning the effect of microwaves on TFSB and fungi in dry and wet treatments, it is thought that in liquid treatment the water could facilitate extra heat in addition to the microwaves effect. However, the results showed that there were no significance differences between the two treatments at  $\alpha = 0.05$ . This could be related to hard cortex structure of microbial spores (Celandroni et al., 2004). Although, the microbial spores were adapted to the low water activity of the dried samples of spices and herbs, which is more resistant than other microorganisms.

Some related investigations on other types of food showed that when beef samples with an initial bacterial count of  $4.9 \times 10^6$  CFU/g were heated in microwave oven, bacterial counts were reduced by 1 log cycle in 20 s and by 2 log cycles in 30 s exposures (Aziz et al., 2002). This treatment had increased the shelf life of the beef product for two weeks at 5°C and either no change or loss of thiamine levels and free fatty acids greatly enhanced the microbial safety. In another study, Canımır et al. (2002) reported that exposure of *E. coli* to home microwave (2450 MHz) treatments at 900 and 720 W power levels for 60 and 90 s, resulted in a 2-4 logs population reduction of the microbial population in apple juice and no significant differences were found between conventional pasteurization and microwave treatment.

MW were used to inhibit spore-former microorganism, like *B. subtilis* Celandroni et al. (2004) and Giuliani et al. (2010) proposed this approach to reduce spore number of *Alcylobacillus acidoterrestris*, inoculated in asparagus cream, containing olive oil. They reported that a 2 log unit reduction was achieved for a processing time of 5 to 7 min and 80 to 100% of power (2450 MHz to 900 W); moreover, this approach did not affect significantly the oxidation of olive oil and the colour of the cream. In contrast to mold, Basaran and Akhan (2010) studied the effects of microwave treatment on hazelnuts artificially contaminated with aflatoxigenic *A. parasiticus*. Two-log CFU/g reduction was observed after 60 s exposure time, when the surface temperature was increased to nearly 50 to 55°C. For a total residence time of 120 s in the applicator, up to 2.5-log reduction was found for *A. parasiticus* contaminated on hazelnuts. The calculated D-value was 45 s for *A. parasiticus* contaminated on hazelnut. It is well known that high frequency electromagnetic MWs are not direct sources of heat, rather with a frequency of 2.45 GHz; the waves agitate the water molecules, which in turn raises the temperature, causing energy to dissipate in the form of heat. However, whether the sterilization eff-

ect is solely due to thermal heating or to the non-thermal 'microwave effect' is still a matter of controversy (Hong et al., 2004). Some researchers attributed the killing effect exerted by microwaves to the heat the waves generate (Yeo et al., 1999; Canımır et al., 2002); otherwise, Barnes and Ho (1977) and Salvatorelli et al. (1996) proposed a non thermal effect due to microwave energy. Kozempel et al. (1997) reported non-thermal effects of MWs using *Pediococcus freudeareichii* at different exposure times. Shazman et al. (2007) examined the possibility of a thermal effects due to MW radiation in a number of chemical, biochemical and microbial systems and no a thermal effects were detected. Microwaves induced no non-thermal lethal effects in the *A. parasiticus* with the selected power source; heating induced by microwave must be the primary factor causing the lethal effects in *A. parasiticus* (Basaran and Akhan, 2010). Celandroni et al. (2004) reported that the analysis of DPA released from *B. subtilis* spores and the electron microscopy revealed that spores damage induced by microwaves was significantly different from that observed in heat treated spores: in fact, while spores exposed to conventional heating presented an evident swelling of the cortex, no modification of this layer was observed in microwave irradiated spores, underlying that the second hypothesis could have a scientific evidence. However, it has to be confirmed in the future by new assays (Bevilacqua et al., 2008). Finally, this investigation proved that microwave treatment duration of 60 s was found to be capable of reducing the microbial load. This simple effective and innovative process could be suitable for use at home level before seasoning and or cooking the food to inactivate and prevent the harmful contamination effect on human.

## Conclusion

The potential public health risk of using spices and herbs as ready-to-eat foods that potentially undergo no further processing is highlighted in this study. Prevention of microbial contamination in dried herbs and spices lies in the application of good hygiene practices and HACCP at all stages of food chain production. This research provide primary data base for contamination of spices and herbs which could trigger and enhance the authorities to set up a microbiological standard and a complete safety program at national level. Based on our experimental results, this research confirmed the suitability of microwave for decontamination of spices and herbs in less than 60 s and it could be used at home and at industrial levels. Further investigations are required to validate these data in other products and against a larger number of spore-former microorganisms.

## ACKNOWLEDGEMENT

This project was supported by Al-Balqa' Applied University (BAU) during sabbatical leave in 2011 at Mutah Uni-



versity, Jordan. Special thanks to Prof. Dhia S. Hassawi for insightful discussion and revision of the manuscript.

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