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Microbiological quality of selected spices and herbs including the presence of *Cronobacter* spp.

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ABSTRACT

The cultivation of spices and herbs in parts of the world characterized by warm climate and high humidity provides excellent conditions for the development of microorganisms, including the undesirable ones. The aim of this study was to determine the microbiological quality of spices and herbs available on the Polish market, considering the occurrence of *Cronobacter* species bacteria.

Analyses covered 60 samples of commercial spices and herbs, including 38 samples of dried herbs (basil, bay leaves, thyme, oregano, tarragon, marjoram, dill, parsley, rosemary, lovage) and 16 samples of seasoning blends as well as 6 samples of spices seeds and fruits (pimento, black pepper, coriander). All samples were tested for the total count of aerobic mesophilic bacteria (TAMB) and for the presence of *Cronobacter* spp.

In most of the samples of spices and herbs (60.0%), the TAMB did not exceed 10^4 CFU/g, and the level regarded as unacceptable (>10^6 CFU/g) was not identified in any of the samples. The presence of *Cronobacter* spp. was demonstrated in 10 (16.7%) samples of the analyzed products, however these were mainly samples of herbs (basil, tarragon, parsley) and one sample of a seasoning blend (Provence herbs). The highest microbiological contamination (TAMB) was found in samples of herbs (oregano, tarragon, basil) and in ready seasoning blends, in 21.1% and 25.0% of which the total count of aerobic mesophiles was in the range of 10^5–10^6 CFU/g. In all samples of spices seeds and fruits (coriander, black pepper and pimento), the total count of aerobic bacteria reached <10^4 CFU/g.

Results achieved in the study indicate good hygienic conditions in the production process of spices and herbs available on the Polish market. The study demonstrated also that dried spices and herbs may be carriers of *Cronobacter* species bacteria, though their presence in not often detected in products of this type.

1. Introduction

Spices may include various anatomical parts of plants (e.g. roots, rhizomes, leaves, flowers, fruits, seeds, corms, bark) that have no nutritional value but are characterized by specific sensory values and exert beneficial effects on digestive processes. Substances which determine such properties of spices include volatile oils, essential acids, alkaloids, resins, sulfur compounds and flavonoids (Burt, 2004).

The microbiological quality of spices is determined, to a significant extent, by the hygienic status and environmental conditions of the region they originate from and where they are, usually, only pre-treated. Contaminations of spices with undesirable microflora may occur at each stage of their production process, i.e. harvest, processing, as well as during storage, distribution, retail and use by consumers (McKee, 1995).

Most spices are significantly contaminated with spore-forming bacteria of the genus *Bacillus*, bacilli of the family Enterobacteriaceae and fungi (Banerjee and Sarkar, 2004; Garcia et al., 2001; Witkowska et al., 2011). Herbs and spices constitute the main sources of spore-forming bacteria in soups, cooked and steamed foods or sauces that provide beneficial conditions for

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microorganisms proliferation (Banerjee and Sarkar, 2003). In the years 1973–2010, in the United States, Canada, Great Britain, France, Germany, Denmark and Norway, consumption of contaminated spices and herbs caused of 1946 food poisonings cases, 128 hospitalizations and 2 deaths (Van Doren et al., 2013).

The genus *Cronobacter* which belongs to the family Enterobacteriaceae includes seven species: *C. sakazakii*, *C. malonaticus*, *C. turicensis*, *C. muytjensii*, *C. dublinensis*, *C. universalis* and *C. condimenti* (Iversen et al., 2008; Joseph et al., 2012). This genus includes Gram-negative bacteria, facultative anaerobes, and motile bacilli included amongst opportunistic pathogens, which optimal temperature of growth fits within the range of 37–44 °C (Iversen et al., 2004b). All *Cronobacter* species, except *C. condimenti*, are causative agents of severe food poisonings, especially in infants and children as well as adult persons with impaired immunity (Abdesselam and Pagotto, 2014). Signs of poisoning include sepsis, necrotic enteritis and colitis, and meningitis; and the mortality rate in children reaches 80% (Iversen et al., 2008; Lai, 2001; Mullane et al., 2007; Townsend et al., 2008).

It is suggested that bacteria of the *Cronobacter* species are not permanent components of typical intestinal microflora of humans and animals. Their main reservoirs include aquatic habitat, vegetation and soil (Chenu and Cox, 2009; Iversen and Forsythe, 2003). *Cronobacter* species have been isolated from different food products, including dairy and meat products, rice and seeds of other cereals, from vegetables and fermented vegetable products (Edelson-Mammel et al., 2005; Friedemann, 2007; Iversen and Forsythe, 2004; Kandhai et al., 2010; Shaker et al., 2007). *Cronobacter* species are characterized by high survivability rate in products with a low water content (0.30–0.83) (Lin and Beuchat, 2007), hence their presence has often been identified in dried food products, e.g. in milk powder, but also in spices and herbs (Friedemann, 2007; Kandhai et al., 2010).

The aim of this study was to determine the microbiological quality of selected spices and herbs, and the presence of *Cronobacter* species bacteria in these products.

2. Materials and methods

The experimental material included 60 samples of commercial products:

- 38 samples of herbs: bay leaves (*Laurus nobilis*) – 6 samples, thyme (*Thymus vulgaris*) – 6, oregano (*Origanum vulgare*) – 6, tarragon (*Artemisia dracunculus*) – 2, basil (*Ocimum basilicum*) – 4, marjoram (*Origanum majorana*) – 4, dill (*Anethum graveolens*) – 2, parsley (*Petroselinum crispum*) – 4, lovage (*Levisticum officinale*) – 2, and rosemary (*Rosmarinus officinalis*) – 2;
- 16 samples of seasoning blends: Provence herbs – 6 samples, seasoning for pickled cucumbers – 2, seasoning for meat courses – 4, and seasoning for fish courses – 4;
- 6 samples of spices seeds and fruits: pimento (*Pimenta dioica*) – 2 samples, coriander (*Coriandrum sativum*) – 2, and black pepper (*Piper nigrum*) – 2.

The samples were purchased in retail stores in the city of Warsaw (Poland) and originated from 5 different producers. All tested spices and herbs were imported and packaged by Polish producers.

All samples were determined for:

- total count of aerobic mesophilic bacteria (TAMB) on PCA medium (Merck, Poland, incubation 30 °C/72 h, deep inoculation);
- presence of *Cronobacter* spp. bacteria according to below-described methodology:

Ten-gram weighed portions of samples were transferred to 90 mL of buffered peptone water and incubated at a temperature of 37 °C for 18 h for preliminary proliferation of microflora. Next, 0.1 mL portions of the culture were transferred to 10 mL of mLST culture medium with the following composition [g/L]: NaCl 34, enzymatic hydrolyzate of animal and plant tissues 20, $C_{12}H_{22}O_{11}$ 5, KH_2PO_4 2.75, K_2HPO_4 2.75, $C_{12}H_{25}NaO_5S$ 0.1, and vancomycin (Sigma–Aldrich, Poland) 0.01, and incubated for 24 h at 44 °C for selective proliferation. Afterward, 0,1 mL of the culture was surface-inoculated onto selective chromogenic medium HiCrome *Enterobacter sakazakii* Agar (ESIA) (Fluka, Poland). ESIA plates were incubated at 44 °C for 24 h. On the HiCrome medium, the *Cronobacter* spp. bacteria grow in the form of blue or blue-green colonies. The selective, chromogenic factor in the applied ESIA medium was 5-bromo-4-chloro-3-indolyl α-D-glucopyranoside. The selectivity of this medium was based on the activity of α-glucosidase which is displayed by all strains of *Cronobacter* spp. The colonies suspected to belong to *Cronobacter* spp. were streaked onto trypsin-soybean agar TSA (Oxoid, Poland), on which majority of *Cronobacter* strains produce a characteristic yellow pigment. The final confirmation of bacteria affiliation to the *Cronobacter* spp. was carried out using API 20 E tests (BioMerieux, Poland). In order to control the correct evaluation of growth of the analyzed bacteria, parallel inoculations of *C. sakazakii* ATCC 29544 strains were performed onto HiCrome and TSA media.

3. Results and discussion

Table 1 presents results of determinations of the total count of aerobic mesophiles in the analyzed products. According to guidelines elaborated by the International Commission on Microbiological Specifications for Foods (ICMSF, 2005), the total bacteria count in spices below 10^4 CFU/g is indicative of their acceptable quality, the count of 10^4–10^6 CFU/g indicates their permissible quality, whereas bacterial count exceeding 10^6 CFU/g is unacceptable. In

Table 1
Total count of mesophilic aerobes in the analyzed samples of spices and herbs.

Product (number of analyzed samples)	Number of samples			
	Total count of aerobic mesophilic bacteria (TAMB) [CFU/g]			
	$\geq 10^2$–10^3	$>10^3$–10^4	$>10^4$–10^5	$>10^5$–10^6
Bay leaves (6)	4	2	–	–
Thyme (6)	–	4	2	–
Oregano (6)	2	2	–	2
Tarragon (2)	–	–	–	2
Basil (4)	–	–	–	4
Marjoram (4)	–	3	1	–
Dill (2)	1	1	–	–
Parsley (4)	–	3	1	–
Lovage (2)	–	2	–	–
Rosemary (2)	–	–	2	–
Herbs (38)	7	17	6	8
Provence herbs (6)	1	3	2	–
Seasoning for pickled cucumbers (2)	1	1	–	–
Seasoning for meat courses (4)	–	–	1	3
Seasoning for fish sources (4)	–	–	3	1
Seasoning blends (16)	2	4	6	4
Pimento (2)	–	2	–	–
Coriander (2)	2	–	–	–
Black pepper (2)	–	2	–	–
Spice seeds and fruits (6)	2	4	0	0
Total (60)	11	25	12	12