Microbiological contamination of dried culinary herbs

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Summary

The aim of this work was an assessment of a microbiological quality of some dried herbs, offered in retail. A basil (*Ocimum basilicum* L.), marjoram (*Origanum majorana* L.), oregano (*Origanum vulgare* L.) savory (*Satureja hortensis* L.), tarragon (*Artemisia dracunculus* L.) and thyme (*Thymus vulgaris* L.) were investigated. The research material included 28 samples coming from five producers and was purchased in Szczecin in shops of various commercial networks. It was stated that the microbiological contamination was significantly differentiated depending on both: a kind of herbs and a producer. A mean count of microorganisms was the highest in basil while the lowest counts were detected in oregano and savory. A microbiological quality of the majority of the tested herbs attained standards. However, in 25% of the samples an excessive amount of bacteria ($>10^5$cfu·g^{-1}) was detected. Only in individual cases, there was a high contamination by moulds ($>10^3$cfu·g^{-1}) and coliforms (present in 0.01–0.001 g). Nevertheless, there were no *E. coli*, *Salmonella* sp. and coagulase (+) staphylococci in any sample.

Key words: *culinary herbs, bacteria, yeast, moulds, pathogenic organisms*

INTRODUCTION

Culinary herbs are widely used in processing and food preparation thanks to their distinctive flavours, aromas and also biological properties. Herbs are antimicrobiologically active, although, they are susceptible to microbial contamination [1]. As many other agricultural commodities, herbs and spices are exposed to a wide range of environmental microbial contamination during growing, collection,

processing and distribution [2]. Drying of herbs after harvest, especially by traditional methods, is considered as a "critical point" and exposes them to the risk of contamination. Therefore, dried herbs may contain high levels of different groups of microorganisms [3-5] including pathogenic bacteria [6, 7] and toxigenic fungi [8, 9]. The control of current biological quality of herbs is needed. The risk to health is possible, especially when added to "ready to eat" food that undergo no further processing. Considering little current information on the microbiological quality of offered culinary herbs, the objective of this study was to investigate microbiological condition of dried herbs purchased in the market.

MATERIALS AND METHODS

The researches included following dried culinary herbs: basil (*Ocimum basilicum* L.), marjoram (*Origanum majorana* L.), oregano (*Origanum vulgare* L.), savory (*Satureja hortensis* L.), tarragon (*Artemisia dracunculus* L.) and thyme (*Thymus vulgaris* L.). Twenty eight samples of herbs from five producers, were purchased in Szczecin in shops of various commercial nets. Microbiological analysis was carried out in accordance with recommendations of Polish standards [10, 12-16]. The valuation was made on parameters as follows: total count of mesophilic aerobic bacteria and their spores [10], total count of termophilic aerobic bacteria and their spores [11], count of yeasts and moulds [12], titre of coliforms [13] and presence of *Escherichia coli* (in 1 g) [14], *Salmonella* sp. (in 25 g) [15] and coagulase positive staphylococci (in 0.1 g) [16]. Additionally moulds occurring in tested herbs were isolated and identified. Identification was carried out by cultural and morphological characteristics and the taxonomic schemes [17-19] were applied.

The investigations were carried out in three replications. The counts of bacteria and fungi were expressed as colony forming units per gram of sample ($cfu \cdot g^{-1}$) and converted onto $\log_{10} cfu \cdot g^{-1}$. The data were statistically examined by analysis of variance and differences between means were evaluate using the Tukey test at a significance level $\alpha = 0.05$.

RESULTS AND DISCUSSION

The contamination of the tested herbs by aerobic mesophilic and termophilic bacteria is presented in table 1. Total counts of mesophilic bacteria ranged from 2.12 log $cfu \cdot g^{-1}$ in oregano from the 3rd producer, to 6.19 log$cfu \cdot g^{-1}$ in marjoram from the 1st producer. Total counts of termophilic bacteria oscillated from 1.76 to 5.18 log $cfu \cdot g^{-1}$ in savory and basil from the 4th producer, respectively. The highest mean count of bacteria was detected in basil, while the lowest contamination was in oregano and savory. Considerable variations were observed in the number of bacteria even in the samples of the same kind of herbs. It was stated that the

bacterial contamination was significantly differentiated depending both on kind of herbs and producer. Significant differences in the microbial counts among spices and commercial companies were found also by Elshafie et al. [20].

Table 1.

Total counts of mesophilic and termophilic aerobic bacteria (log cfu·g^{-1}) in culinary herbs from different producers

culinary herbs	producer					mean
	1	2	3	4	5	
mesophilic aerobic bacteria						
basil	4.96^{aD}	4.85^{aB}	5.20^{bD}	5.84^{dD}	5.43^{cF}	5.26^{C}
marjoram	6.19^{eE}	4.89^{dB}	4.15^{bC}	4.32^{cC}	2.57^{aB}	4.42^{B}
oregano	3.75^{dB}	3.34^{cA}	2.77^{aA}	3.13^{bB}	4.38^{eE}	3.48^{A}
savory	4.63^{cC}	- *	3.95^{bB}	2.12^{aA}	3.69^{bC}	3.60^{A}
tarragon	-	3.45^{bA}	5.21^{cD}	5.42^{dE}	2.95^{aA}	4.26^{B}
thyme	3.11^{aA}	5.55^{cC}	4.13^{bC}	4.17^{bC}	4.10^{bD}	4.21^{B}
mean	4.53c	4.42c	4.24b	4.16b	3.86a	
termophilic aerobic bacteria						
basil	4.74^{cD}	3.77^{aB}	4.95^{dD}	5.18^{eD}	4.52^{bD}	4.62^{D}
marjoram	4.93^{dD}	4.48^{cD}	3.87^{bB}	3.96^{bC}	2.11^{aA}	3.87^{C}
oregano	2.13^{aA}	2.23^{abA}	2.35^{bA}	2.98^{cB}	3.25^{dC}	2.58^{A}
savory	4.22^{dC}	-	2.26^{bA}	1.76^{aA}	3.42^{cC}	2.91^{A}
tarragon	-	2.33^{aA}	4.32^{dC}	3.83^{cC}	2.91^{bB}	3.35^{B}
thyme	3.13^{aB}	4.21^{cC}	4.10^{cBC}	3.64^{bC}	3.26^{aC}	3.67^{BC}
mean	3.83d	3.40b	3.64cd	3.56bc	3.12 a	

* lack of samples

Similar small letters in the same rows and similar capital letters in the same columns indicate no significant difference, $\alpha=0.05$.

A microbiological analysis of spices and herbs usually includes the evaluation of the amount of mesophilic bacteria. Information on termophilic bacteria is rather rare. However, the results of this work correspond with the observation [4] that a count of termophilic bacteria is lower than a count of mesophilic ones in spices and herbs. In dried herbs, examined by Aguilera et al. [21], a total mesophilic bacteria count was similar to the data of this study and ranged from <1.0 to 6.0 log cfu·g^{-1}. The researches of the other authors [3, 4, 22] indicate that in dried spices and herbs total counts of mesophilic bacteria often had a higher level (10^6-10^8 cfu·g^{-1}). In basil, marjoram and thyme, bought in domestic wholesale, an average number of mesophilic bacteria ranged from 8.2 x 10^6 to 2.1 x 10^8 cfu·g^{-1} [23]. A comparison with International Commission on Microbiological Specification for Foods (ICMSF) standards, which are cited by Banerjee and Sarkar [22] indicates that among herbs tested in this work only one sample (marjoram of 1st producer) had unacceptable

contamination by mesophilic bacteria ($>10^6$ cfu·g^{-1}). However, 25% of the examined samples, in which a total count of mesophilic bacteria exceeded the level 10^5 cfu·g^{-1}, did not agree with Polish standards [24-27].

Mesophilic and termophilic bacteria occurred in the examined herbs mainly as spores. The average percentage participation of mesophilic spores ranged from 56.9% in basil to 73.7% in oregano, while the participation of termophilic spores ranged from 67.1% also in basil to 83.5% in savory (fig. 1). Wieczorkiewicz-Górnik and Piątkiewicz [4] observed that spores accounted for 80–90% of the total aerobic mesophilic bacteria in spices. In various Indian dried spices and herbs mesophilic bacterial spores constituted 0.9–90% of total mesophilic aerobic bacteria population [22]. Spices and herbs are a principal source of spore forming aerobic bacteria (*Bacillus* sp.) and anaerobic bacteria (*Clostridium* sp.) Bacterial spores are potential risk because they may survive cooking temperatures and may multiply to an infective and toxic level [21].

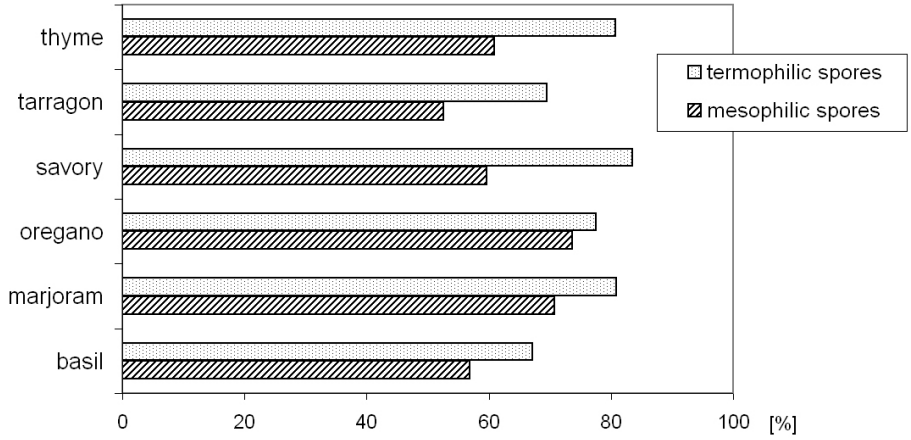

Figure 1. Average percentage share of mesophilic and termophilic bacterial spores in total count of mesophilic and termophilic bacteria in tested herbs

The contamination of the examined herbs by fungi was generally low (tab. 2). Yeasts were absent in most of the samples and in others their counts ranged between <10–55 cfu·g^{-1}. Banerjee and Sarkar [22] stated the presence of yeasts in only one of 154 samples of spices and herbs, while in the samples tested by Mandeel [28] yeasts were frequently recovered. The data presented in table 2 show that in about 30% of the samples moulds did not occur and in others their counts ranged between <10–1300 cfu·g^{-1}. Only in one sample (marjoram of 2nd producer) a number of moulds set up the maximum limit (10^3 cfu·g^{-1}) [24-27]. On the contrary, in all samples of basil, marjoram and thyme, coming from wholesale, counts of moulds ranged between 10^4–10^6 cfu·g^{-1} and did not meet the requirements [23]. In Indian spices and in Brazilian medicinal herbs counts of moulds oscillated in wide range (1.0–6.0 log cfu·g^{-1}) [8, 22]. It was stated that 4.5% of the Indian spices

samples (out of 154), and almost 55% of the Brazilian medicinal herbs samples (out of 91) did not comply with the maximum acceptable limit for fungal contamination [8, 22].

Table 2.

Contamination of examined culinary herbs by moulds and yeasts

culinary herbs	producer				
	1	2	3	4	5
	moulds [cfu·g^{-1}]				
basil	<10	<10	<10	110	120
marjoram	60	1300	50	absent*	absent
oregano	absent	absent	20	<10	absent
savory	40	-	<10	<10	
tarragon	-	<10	<10	absent	absent
thyme	<10	absent	<10	<10	<10
	yeasts [cfu·g^{-1}]				
basil	absent	<10	absent	55	20
marjoram	absent	30	25	15	absent
oregano	absent	absent	absent	absent	absent
savory	absent	-	absent	<10	<10
tarragon	-	absent	absent	<10	absent
thyme	absent	absent	<10	absent	<10

* – absent in 1 g

Among samples tested in this work 79 strains of moulds were isolated. Strains of *Aspergillus* sp. and *Penicillium* sp. were frequently occurred. Their participation in the total number of strains were (40.5%) and (25.3%), respectively. Moreover, *Mucor* sp. (10.2%), *Rhizopus* sp. (7.6%) *Alternaria* sp. (5.1%), *Cladosporium* sp. (5.1%), *Fusarium* sp. (2.5%) *Trichothecium* sp. (2.5%) and *Absidia* sp. (1.2%) were isolated. Among *Aspergillus* sp. mainly *Asp. glaucus* was identified, followed by *A. niger, A. flavus and A. candidus*. The results of this study correspond with previous reports that showed *Aspergillus* and *Penicillium* were the main contaminant of different spices and herbal samples [4, 5, 8, 9]. While investigating spice samples from retail outlets, Mandeel [28] stated that the most predominant fungal genera encountered were *Aspergillus, Penicillium*, *Rhizopus, Cladosporium* and *Trichoderma*. Elshafie et al. [20] isolated 20 fungal species (from 105 samples) in which *A. flavus, A. niger, Penicillium, Rhizopus* and *Syncephalastrum racemosum* were the most dominant. The genera *Aspergillus* and *Penicillium* are extremely important from the mycotoxicological standpoint [8, 9]. The presence of toxigenic moulds causes a serious risk of contamination of spices and herbs by mycotoxins [29, 30].

The objective of this study was also the evaluation of hygienic status of culinary herbs (tab. 3). It was stated that they did not cause a direct risk for human health because *E. coli, Salmonella* sp. and coagulase positive staphylococci were absent in all tested samples. However, in two cases a titre of coliforms was too low (0.01–0.001 g). Dried species and herbs should be free from pathogenic microorganisms [31] and coliforms should be absent in 0.01g of samples [24-27]. The results of this investigation agree with the opinion that *E. coli*, *Salmonella* sp. and *S. aureus* in spices and herbs are apparently rare [22]. An assessment of the microbiological safety of dried spices and herbs in the UK showed that pathogenic bacteria did not occur in 96% of 2833 samples from retail network [6]. The presence of coliforms, *E. coli* and other pathogenic bacteria may be due to insanitary handling methods. The prevention of microbial contamination in dried spices and herbs lies in an application of good hygiene practices during growing, harvesting and processing "from farm to fork" [6]. The improvement of a microbiological quality of dried spices and herbs may also be achieved by decontamination methods [23, 32]. The use of steam is very effective in reducing fungi count as well as increase of hygienic and sanitary parameters but less effective in decreasing total bacteria amount [23]. This may explain the fact that herbs tested in this study had generally good hygienic conditions and slight contamination by yeasts and moulds and, simultaneously, a higher total count of bacteria.

Table 3.

Hygienic parameters of tested culinary herbs

culinary herbs	titre of coliforms					occurrence of pathogenic bacteria
	1	2	3	4	5	
basil	>0.1	>0.1	0.001	0.1	0.1	in all tested herbs did not occur: - *Salmonella* sp. (in 25 g) - *E. coli* (in 1 g) - coagulase (+) staphylococci (in 0.1 g)
marjoram	0.01	>0.1	>0.1	>0.1	>0.1	
oregano	0.1	>0.1	0.1	>0.1	>0.1	
savory	>0.1	–	>0.1	>0.1	0.1	
tarragon	–	>0.1	>0.1	0.1	>0.1	
thyme	>0.1	>0.1	>0.1	0.1	>0.1	

1–5 – producers

CONCLUSIONS

1. In the examined dried culinary herbs the count of meso- and termophilic bacteria oscillated in range 10^1–10^6 cfu·g^{-1} and significantly depended on a type of herb and on a producer.
2. The contamination of most samples by fungi was relatively low (0–120 cfu·g^{-1}). Moulds were mainly represented by potential toxigenic genera *Aspergillus* (*A. glaucus, A. niger, A. flavus)* and *Penicillium*.

3. *Escherichia coli, Salmonella* sp. and staphylococci coagulase (+) did not occur in any of the examined herbs.
4. 28.6% of the tested samples did not meet the standards because they had an excessive count of mesophilic aerobic bacteria ($>10^5$ cfu·g^{-1}) or moulds ($>10^3$ cfu·g^{-1}) and too low titre of coliforms (0.01-0.001).

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MIKROBIOLOGICZNE ZANIECZYSZCZENIE SUSZONYCH ZIÓŁ PRZYPRAWOWYCH

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Streszczenie

Celem pracy była ocena stanu mikrobiologicznego suszonych ziół przyprawowych dostępnych w handlu. Badaniom poddano susz z bazylii (*Ocimum basilicum* L.), cząbru (*Satureja hortensis* L.), estragonu (*Artemisia dracunculus* L.), majeranku (*Origanum majorana* L.), oregano (*Origanum vulgare* L.) oraz tymianku (*Thymus vulgaris* L.). Materiałem badawczym było 28 prób ziół (od pięciu producentów), które zakupiono w Szczecinie, w placówkach różnych sieci handlowych. Stwierdzono, że zanieczyszczenie przez drobnoustroje było zróżnicowane w zależności od gatunku ziół oraz producenta. Najwyższą średnią liczbą drobnoustrojów odznaczała się bazylia, a najniższą oregano i cząber. Mikrobiologiczna jakość większości ocenianych ziół odpowiadała standardom. Jednak w 25% próbek stwierdzono nadmierną w stosunku do wymagań liczbę bakterii ($>10^5$ jtk/g), natomiast w pojedynczych przypadkach wysokie zanieczyszczenie przez pleśnie ($>10^3$ jtk/g) i bakterie z grupy *coli* (miano 0.01–0.001). W żadnej z próbek nie występowały bakterie *E. coli*, pałeczki *Salmonella* sp. oraz gronkowce koagulazododatnie.

Słowa kluczowe: *zioła przyprawowe, bakterie, drożdże, pleśnie, drobnoustroje patogenne*