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ORIGINAL ARTICLE

Assessment of microbiological cleanness of selected medicinal herbs in relations to the level of resource fragmentation

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Abstract:
Herbs are commonly used in the food and pharmaceutical industries. Their vast use is connected with their antibacterial or antioxidising properties, as well as numerous pro-health properties. The aim of the presented research was assessment of the quantitative and qualitative composition of moulds which contaminate samples of dried herbs: Sage (*Salvia officinalis* L.), Camomile (*Matricaria chamomilla* L.) and Melissa (*Mellisa officinalis* L.) with different degrees of resource fragmentation. The dried herbs investigated had a characteristic mould content below $1 \cdot 10^6$ CFU/g according to the recommendations of the European Herbal Infusions Association (EHIA). The most contaminated resource turned out to be Camomile, the least – Melissa. The most often isolated moulds were: *Aspergillus*, *Penicillium*, *Ulocladium, Alternaria*. Moreover, it was observed that more fragmented dried herbs were characteristic of lower – by approx. 40–55% microbiological contamination -depending on the type of tested herb, which might be connected with the time of dried herbs' processing, higher aeration, moisture changes or mechanical damaging of fungi's fragments in the case of a resource with higher fragmentation. High contamination of a herbal resource might be harmful for a consumer, and moulds and their metabolites in the form of mitotoxins might constitute a threat for human health. To keep all the sensory features and activity of herbs' active substances, it is extremely important to secure their high microbiological quality.

Key words
herbs, moulds, microbiological cleanness, food products.

INTRODUCTION

Herbs are commonly used both as spices, medicinal products, and additions to cosmetics. Herbs are often used because of their antibacterial properties: germicidal and fungicidal [1], despite their vulnerability to microbiological contamination. The antioxidising properties of herbs [2] are used in preserving food products, cosmetics or pharmaceutics [3]. Preservation of food products is important to maintain their sensory attractiveness and for the safety of consumers. This also relates to spices which allow varying and improving the taste and aroma of prepared dishes. Herbs are also used because of the following properties: dying, stimulating appetite, allowing the limitation of sugar, salt or fat intake, especially in products for special purposes [1].

Important aspects for herbal production are both the amount of resource crop, and its quality, which is made of the content of active substances and mineral elements, and microbiological cleanness [4].

The microbiological cleanness of spices is diversified when it comes to the quantity and types of microbes [4]. The characteristic microflora of herbs used as spices is based on bacteria types: *Bacillus* and *Clostridium*, and mould types: *Penicillium*, *Aspergillus*, sometimes *Rhizopus* or *Fusarium* [1].

Research results often indicate strong microbiological contamination of herbs, both fresh and dried [1, 5, 6]. The contamination of a resource – herbs, might be the reason for its decay, changes of sensory and organoleptic properties, and also might constitute a health threat because of the possible occurrence of pathogenic flora or mitotoxins [7]. As a result, the utilisation of highly contaminated herbs used as spices will negatively influence the quality of food produced with their use. Herbs, like many other food products, are vulnerable to microbiological contamination while growing, harvesting, processing, and during distribution [8]. Drying herbs after harvest (especially traditional) is thought as a 'critical point', which exposes this resource to different types of contamination. Dried herbs might be contaminated with a number of microorganisms [9], including pathogenic bacteria [10] or toxic fungi and their metabolites [5].

Objectives: The aim of the presented study was assessment of the microbiological cleanness of medicinal herbs in relation to the level of fragmentation of a plant resource.

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MATERIALS AND METHODS

The material for the research constituted three batches of dried medicinal herbs: Sage (*Salvia officinalis* L.), Camomile (*Matricaria chamomilla* L.) and Melissa (*Mellisa officinalis* L.). Each herb occurred in two different levels of fragmentation. (Tab. 1).

Table 1. Tested levels of fragmentation of selected dried herbs.

Tested herb	Sage	Camomile	Melissa
Tested level of fragmentation	Below 100 µm Above 100 µm	Below 250 µm Above 250 µm	Below 250 µm Above 250 µm

A test of the quantitative and qualitative composition of moulds in dried herbs was conducted. This test was carried out according to a classic method of dilutions in physiological solution of sodium chloride, and the method of surface inoculation.

Aliquots of the herbal resource were placed in bulbs with saline solution which was shaken for one hour. When the material fell to the bottom, from the liquid above the sediment, consecutive dilutions were made within the range 10^{-2} – 10^{-5}. Two growth media were used for the growth of fungi: PDA (Potato Dextrose Agar) and MEA (Malt Extract Agar) with an addition of chloramphenicol (5 mg/100ml of growth medium). An inoculation from each dilution was made in three repetitions. Petri dishes with PDA growth medium were incubated at the temperature of 24 °C for 72 hours, and with MEA growth medium at the temperature of 30 °C for 72 hours. During the following 96 hours, the dishes were incubated at room temperature. On the basis of macro- and microscopic properties, the analysis of quantitative composition of mould fungi was made with the use of selected items from the field of toxicology literature [11, 12, 13, 14]. According to the PN-ISO 7945:1999 [15] norm the quantity of mould fungi for each of the tested herbs at particular levels of fragmentation was calculated and expressed in Colony Forming Units for 1 g of the resource (CFU/g).

RESULTS

In total, 293 strains of mould fungi were isolated, which belonged to 17 types. For each of the tested medicinal herbs the occurrence of particular types and species of mould was defined, which is presented in Table 2.

Among the types of fungi isolated from Sage (*Salvia officinalis* L.) the most frequently identified type was *Penicillium* (58.5% of all moulds isolated from Sage), and *Aspergillus* (25%). The most frequently isolated species were *Penicillium expansum*, as well as *Aspargillus versicolor* and *A. candidus*. The remaining species of fungi were less numerous. Yeast also rarely occurred.

Among the most often isolated moulds which contaminated dried Camomile (*Matricaria chamomilla* L.), *Ulocladium botrytis* was identified, which constituted 55% of all moulds isolated from this herb. Fungi from *Phoma* type (35%) and 'pink yeast' also occurred in large quantities.

The most numerous types of mould fungi which contaminate Melissa (*Mellisa officinalis* L.) were: *Aspergillus* (46%), *Trichophyton* (11.5%), and *Mucor* (9%). Colonies of yeast were also quite abundant.

Table 2. Types and species of mould fungi isolated from the tested dried medicinal herbs.

Tested herb	*Filamentous Fungi*
Sage	*Absidia spp.* *Acremonium (A. charticola, A. spp.)* *Aspergillus (A. candidus, A. clavatus, A. flavus, A. fumigatus, A. sydowii, A. versicolor, A. spp.)* *Aureobasidium spp.* *Chaetomium (C. atrobrunneum)* *Curvularia (C. lunata)* *Fusarium (F. oxysporum, F. spp.)* *Mucor (M. racemosus, M. circinelloides, M. spp.)* *Penicillium (P. citrinum, P. expansum, P. glabrum, P. griseofulvum, P. purpurogenum, P. spp.)* *Rhizomucor spp.* *Trichophyton spp.* *Ulocladium (U. botrytis)*
Camomile	*Aspergillus (A. clavatus, A. parasiticus, A. spp.)* *Epicoccum spp.* *Mucor (M. hiemalis, M. racemosus, M. spp.)* *Penicillium (P. phialosporum, P. spp.)* *Phoma spp.* *Trichophyton spp.* *Ulocladium (U. botrytis, U. chartarum)*
Melissa	*Absidia (A. corymbifera, A.spp.)* *Acremonium (A. falciforme, A. spp.)* *Alternaria (A. alternata)* *Aspergillus (A. candidus, A. clavatus, A. flavus, A. fumigatus, A. niger, A. oryzae, A. sydowii, A. versicolor, A.* spp.*)* *Cladosporium (C. herbarum, C.* spp.*)* *Mucor (M. hiemalis, M. racemosus, M. spp.)* *Penicillium (P. expansum, P. spp.)* *Rhizopus (R. oryzae, R.* spp.*)* *Trichophyton spp.*

The results, regarding the quantity of fungi in dried herbs of the tested medicinal herbs in relation to the level of sample fragmentation, are presented in Table 3.

Table 3. Total quantities of mould fungi (CFU/g) isolated from tested herbs in relation to the level of fragmentation of a dried herbs sample.

Tested herb	Total No. of fungi (CFU/g)		Difference between lowest and highest level of sample fragmentation	
	Higher level of sample fragmentation (below 100/250 µm)	Lower level of sample fragmentation (above 100/250 µm)	(CFU/g)	(%)
Sage	$1.1 \cdot 10^4$	$2.6 \cdot 10^4$	$1.5 \cdot 10^4$	≈58
Camomile	$1.9 \cdot 10^4$	$3.0 \cdot 10^4$	$1.1 \cdot 10^4$	≈37
Melissa	$0.5 \cdot 10^4$	$1.1 \cdot 10^4$	$0.6 \cdot 10^4$	≈55

The highest total quantity of fungi was found in dried Camomile with a lower level of sample fragmentation ($3.0 \cdot 10^4$), and the lowest in dried Melissa with a higher level of sample fragmentation ($0.5 \cdot 10^4$). The results allow observing significant differences in the quantity of microorganisms isolated from dried herbs in relation to the size of dried herbs' fragments after grinding. We observed, 37% in case of Camomile, 55% in case of Melissa and 58% in case of Sage, lower contamination by mould fungi in samples with higher level of sample fragmentation.

DISCUSSION

According to the recommendations of the European Herbal Infusions Association (EHIA), the permissible quantity of mould in dried herbs should not exceed the value of $1 \cdot 10^6$ CFU/g [16]. In the presented study, in none of the dried herbs observed were there higher quantities of mould fungi than recommended.

In order to eliminate the threat originating from the excessive microbiological contamination of herbs, a number of methods are used for sterilizing dried plants. The most common method of sterilising the resource consists in steam treatment at the temperature of 100–200 °C, drying with hot air and quick cooling. All the methods aim at achieving a product of high microbiological cleanness and adequate sensory properties.

It is also important to preserve the biologically-active substances which are responsible for the properties of each medicinal herb. The achieved level of microorganisms reduction is usually assessed at the level of 10^2 – 10^4 CFU/g; unfortunately, this is often connected with a loss of active substances or ethereal oils [17]. The conditions of growing, drying, and the human factor all play a vital role in the microbiological quality of herbal products. Microbiological cleanness also depends on the part of a plant which is dried, as each part differs when it comes to the availability of water for microorganisms; also, each part has different contact with the external environment during its growth [18]. Research by Dutkiewicz J. *et al.* conducted in herbs processing plants, identified that they are characteristic of very high air pollution with bacteria and fungi. The highest congestion of microorganism was recorded at the first stages of herbs processing while cleaning and fragmentation. Part of the identified fungi was classified as moulds with allergenic and toxic properties [19, 20].

In the presented research, dried Camomile was characteristic of the highest levels of contamination, whereas dried Melissa the lowest. In research by Steinka I. *et al.* the highest microbiological contamination was observed in dried Camomile and Melissa [18], which is partly proved by the results of the presented study. In literature, there are also presented data from which it results that dried Camomile and Melissa usually do not contain more than 10^5 of microbes, and Camomile belongs to herbs with the highest level of contamination [21].

The most often isolated species from herbal products belong to types *Aspergillus* and *Penicillium* [5]. Moreover, species *Mucor, Rhizopus, Alternaria* have also been isolated [22]. Among species of fungi often isolated from herbal preparations are toxin-producing: *Aspergillus flavus, Aspergillus versicolor, Penicillium expansum, Fusarium moniliforme* and *Alternaria alternata* [23]. It should be remembered that fungi from types: *Absidia, Rhizopus, Mucor, Alternaria, Cladosporium* are among the highly allergenic fungi [24], whereas moulds *Aspergillus, Penicillium* or *Fusarium,* apart from their allergenic properties, are also toxin-producing. Fungi from the type *Trichophyton* might cause infections [25].

The contamination of resources with fungi *Aspergillus, Penicillium* and *Fusarium* is highly significant because of secondary metabolites – mycotoxines produced by the fungi. Mycotoxines are created in food products contaminated with fungi while storing these products under inadequate conditions. They are harmful for human beings due to their carcinogenic, teratogenic, estrogenic, and toxic properties [5, 26]. In the herbs tested in the presented study, high quantities of moulds of *Penicillium* types were found in the case of Sage, or *Aspergillus* in the case of Sage and Melissa. Among moulds contaminating Sage there were also identified species belonging to the type *Fusarium*. The presence of these toxic fungi carries a serious risk of contamination of dried medicinal herbs with mycotoxines.

The results of the conducted research draw attention to significant differences in the quantity of microorganisms isolated from the same herbs in relation to the size of dried herbs' particles after their previous grinding. 40 – 55% lower contamination of samples with mould fungi was observed in samples with a higher level of fragmentation, in relation to the type of a tested herb. These differences might be related to the extended time of dried herbs' processing, higher aeration, different humidity, or mechanical damage of parts of fungi in the case of dried herbs with a higher level of fragmentation.

CONCLUSIONS

The participation of particular species and types of filamentous fungi depends on the type of a tested herb and the level of its fragmentation. The most contaminated herb proved to be Camomile, the least – Melissa. It was observed that the more fragmented dried herbs were characteristic of lower microbiological contamination. The results seems to be interesting and worth further research. Herbs which undergo the processes of drying and sterilisation, as well as grinding, would be characteristic of higher cleanness and could be used as additions to food products.

High microbiological contamination of a herbal resource causes poor quality and poor medicinal properties, as well as constituting a potential threat for consumers' health. This is why it is important to provide the highest possible microbiological quality of resources.

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