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MICROBIOLOGICAL CORRECTNESS OF SPICES ON SALE IN HEALTH FOOD STORES AND SUPERMARKETS IN NIS

SUMMARY

The paper presents the results of microbiological analysis of 101 samples of spices (black pepper in grain/ground, white pepper in grain/ground, cayenne pepper, caraway, ginger, cinnamon, mustard, curry, nutmeg, chilli, oregano, clove, sweet basil, thyme, bay leaf, rosemary and sesame).

Control of microbiological correctness was performed pursuant to the Rulebook of Methods of Performing Microbiological Analyses and Superanalyses of Biotic Victuals, Official Bulletin of Socialist Federative Republic of Yugoslavia No. 25/80 and Rulebook of Microbiological Correctness of Victuals on Sale, Official Bulletin of Federative Republic of Yugoslavia No. 26/93, 53/95, and 46/2002, pursuant to article 30.

It was determined that of 101 samples of spices, 55 (54.45%) samples were not acceptable according to microbiological finding. The most frequent reason was the finding of total score of microorganisms in uncommitted quantity (48 or 47.52%). According to frequency, the finding of moulds in uncommitted quantity (23 or 22.77%) comes next. Pathogenic bacteria were isolated in a relatively small number of samples: 5 (4.95%) sulphate-reducing clostridia, 4 (3.96%) *E. coli*, 1 (0.99%) *Proteus* species. *Salmonella* species. Coagulase-positive staphylococci and yeasts were not found.

Samples: ginger, black pepper in grain and ground, chilli, cinnamon, dill, and sweet basil were most often microbiologically unacceptable, while clove, bay leaf, sesame, white pepper in grain, rosemary, and caraway showed regular findings.

Increased total number of microorganisms was the most frequent cause of incorrectness due to the habitat of the spice plant (soil and feces of insects, birds or rodents), and due to unsuitable storage conditions. Moulds were isolated in such great number because examined spices were inadequately grown or stored in increased humidity with appropriate climate. Many of the isolated moulds were of soil species. Sulphate-reducing clostridia are present in spices because they are frequent residents of soil and, as spores, they can survive unfavourable conditions for a long time. Finding of *E. coli* and *Proteus* species in spices is explained by inadequate maintenance of machines and devices for processing and packaging of spices.

Microbiological correctness of clove, caraway, rosemary and sesame is probably provided by the presence of ethereal oils (eugenol, cinnamic aldehyde), which possess strong antifungal and antibacterial effect. White pepper in grain and bay leaf preserve their correctness thanks to morphological characteristics (presence of pericarp, leathery leaves) and ethereal oils (in bay leaf: cineole and alpha-pinene).

High rate of microbiological correctness may be achieved by introducing a rigorous control into the cycle of processing and storage of spices, starting from plant growing, over harvesting, processing, storage, to sale. This control is achieved by introducing the HACCP plan and discovering the critical control points in the cycle of processing and storage of spices.

Key words: spices, microbiological correctness, HACCP plan

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INTRODUCTION

Spices are different parts of plants, mostly dried, characterized by aromatic scents and strong tastes. We classify them into the large group of supplements to meals and drinks used to achieve certain aroma and taste.

Using of spices is nowadays quite wide-spread. They are used in the world cuisines to improve the taste of food. Likewise, our cuisine uses spices as a useful food supplement (1). Many of the spices have strong antibacterial and antifungal effect (2). Garlic and onion are on the first place followed by cinnamon and caraway in over 80%, while various species of peppers are somewhere in the middle. Black and white peppers have very weak antimicrobial effect, around 25% (3-5). Therefore, these spices are added in the process of meat processing (6). Some of the spices have curative properties, so they are used in the pharmaceutical industry and in official medicine (7).

However, spices as plants may be harmed even in the field and contaminated by bacteria and moulds before the beginning of drying and treatment. Later, due to the bad conditions of storage and ventilation and high percent of humidity, contamination of the stored amounts by pathogenic microorganisms frequently occurs (8). The greatest problem in that sense is growth of some species of moulds that may be producers of mycotoxins (ochratoxin, aflatoxin, zearalenon), which can manifest its toxic and carcinogenic effect. Neither is negligible the presence of bacteria like *E. coli, Salmonella* spp. or sporogenous anaerobes (*Cl. perfringens*), which are possible causes of infections and poisoning in humans.

MATERIAL AND METHODS

The research was done in the Public Health Institute in Nis, in the Department of Sanitary Microbiology. Sampling was conducted by qualified personnel of the Institute and by the author of the research personally, applying the basic rules of sampling.

The samples were provided from the health food stores (6) in bulk and from supermarkets (4) packed in paper. Microbiological examinations were performed on 101 samples of spices. There were the following spices: ground black pepper (9), black pepper in grain (10), ground white pepper (7), white pepper in grain (6), coriander (4), cayenne pepper (6), caraway (3), ginger (7), cinnamon (9), mustard (4) nutmeg (3), chilli (4), oregano (8), clove (4), sweet basil (2), thyme (2), bay leaf (3), rosemary (1), dill (1), and sesame (1).

The following samples were in bulk: ginger (6), coriander (4), cinnamon (5), ground white pepper (6), ground black pepper (5), mustard (3), white pepper in grain (6), cayenne pepper (1), black pepper in grain (7), curry (6), oregano (4), clove (3), nutmeg (1), chilli (2), caraway (1), dill (1), sweet basil (1).

The following samples were in paper packaging: ginger (1), cinnamon (4), white pepper ground (1), black pepper ground (4), mustard (1), cayenne pepper (5), black pepper in grain (4), curry (1), oregano (4), clove (1), nutmeg (2), chilli (2), caraway (2), sweet basil (1), thyme (2), sesame (1), bay leaf (3), rosemary (1).

Examinations were performed according to the Rulebook of Methods of Performing Microbiological Analyses and Superanalyses of Biotic Victuals, Official Bulletin of Socialist Federative Republic of Yugoslavia No. 25/80 (9) and Rule of Microbiological Correctness of Victuals on Sale, Official Bulletin of Federative Republic of Yugoslavia No. 26/93, 53/95, and 46/2002, article 30(10).

The Rule prescribes that spices should be prepared, placed on appropriate culture media and incubated for a regular time in order to determine (or not to determine) the presence of:

- bacteria of *Salmonella* species in 25 g of the sample
- coagulase-positive staphylococci in 0,1 g of the sample
- sulphate-reducing clostridia in 0,1 g of the sample
- *Proteus* species in 0,1 g of the sample
- *Escherichia coli* in 0,1g of the sample
- no more than 100 yeasts and moulds per 1g of the sample
- total number of microorganisms must not be greater than 50000 per 1g of the sample

RESULTS

Spices are very significant because of their antioxidant qualities, toxic effect or antimicrobial action on microorganisms (e.g., garlic and cinnamon), content of ethereal oils (e.g., eugenol from clove), and other qualities.

During this research, 101 samples were treated. Microbiological incorrectness manifested in

55 (55.45%) samples, or 32 (31.68%) from bulk and 23 (22.77%) from paper packaging, *Figure 1.*

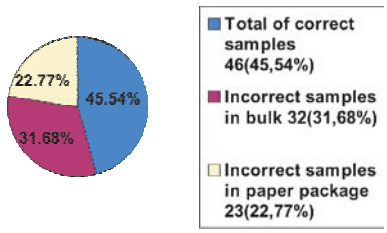

Figure 1. Overview of correct and incorrect samples caused by packing method

The most frequent cause of incorrectness was the finding of total number of microorganisms in uncommitted quantity in 48 (47.52 %) samples, then finding of moulds in 23 (22.77 %) spices.

Finding of pathogenic bacteria was discovered in a small number of samples: as sulphate-reducing clostridia (5/4.95 %), *E. coli* (4/3.96%), and *Proteus* species (1/0.99 %). *Salmonella* species, coagulase-positive staphylococci and yeasts were not found, *Table 1.*

Table 1. Percentual overview of cause of spice incorrectness

Causes of spice incorrectness	Percentage
Salmonella species	0
Coagulase positive staphylococci	0
Escherichia coli	4 (3.96%),
Proteus species	1 (0.99 %).
Sulphato-reducing clostridia	5 (4.95 %),
Total number of microorganisms	48 (47.52 %)
Moulds	23 (22.77 %)
Yeasts	0

Analysis of spices was done on every species separately, when the following results were obtained: ginger (7) - all samples microbiologically incorrect; ground black pepper (9) - all samples microbiologically incorrect; black pepper in grain (10) out of 10 samples, 9 had positive finding, chilli (4) - all samples had positive finding; dill (1) one sample, one incorrect; sweet basil (2) both samples microbiologically incorrect; cinnamon (9) out of 9 samples, 6 showed microbiological incorrectness, *Table 2.*

Table 2.Spices that show maximal microbiological incorrectness

Spice	Total	Positive finding	Negative finding
Ginger	7	7	0
Black pepper ground	9	9	0
Black pepper in grain	10	9	1
Chilli	4	4	0
Dill	1	1	0
Sweet basil	2	2	0
Cinnamon	9	6	3

On the other hand, certain spices showed microbiological correctness, such as white pepper in grain (5/6), clove (4/4), caraway (3/3), bay leaf (3/3), sesame (1/1), rosemary (1/1), *Table 3.*

Table 3. Spices that show maximal microbiological correctness

Spice	Total	Positive finding	Negative finding
White pepper in grain	6	1	5
Clove	4	0	4
Caraway	3	0	3
Bay leaf	3	0	3
Sesame	1	0	1
Rosemary	1	0	1

Total number of all microorganisms as the most frequent cause of incorrectness was found in the following spices: ginger, coriander, cinnamon, ground white pepper, ground black pepper, mustard, cayenne pepper, black pepper in grain, curry, nutmeg, chilli, dill, sweet basil and thyme.

Moulds were found in the following spices: ginger, cinnamon, ground white pepper, black pepper ground, white pepper in grain, cayenne pepper, oregano, nutmeg, chilli, dill, and sweet basil, *Table 4.*

Table 4. Overview of spices in which the presence of total score of microorganisms and moulds in uncommitted quantity was determined

Spice	Total score of microorganisms in uncommitted quantity	Moulds in uncommitted quantity
Ginger	+	+
Coriander	+	-
Cinnamon	+	+
White pepper ground	+	+
Black pepper ground	+	+
Mustard	+	-
Black pepper in grain	+	-
Curry	+	-
Nutmeg	+	+
Chilli	+	+
Dill	+	+
Sweet basil	+	+
Thyme	+	+
Cayenne pepper	+	+
White pepper in grain	-	+
Oregano	-	+
Thyme	+	-

Sulphate-reducing clostridia were found in the following samples: ginger, cinnamon, black pepper ground, and sweet basil.

Escherichia coli were found in the following spices: ginger, cayenne pepper, black pepper in grain, and curry. *Proteus* species was found in only one sample of cayenne pepper, *Table 5.*

Table 5. Overview of spices in which the presence of sulphate-reducing clostridia, E.coli and Proteus species in uncommitted quantity was determined

Spice	Sulphate-reducing clostridia in uncommitted quantity	*E.coli* in uncommitted quantity	*Proteus* species in uncommitted quantity
Ginger	+	+	-
Cinnamon	+	-	-
Black pepper ground	+	-	-
Sweet basil	+	-	-
Cayenne pepper	-	+	+
Black pepper in grain	-	+	-
Curry	-	+	-

DISCUSSION

The most frequent cause of microbiological incorrectness of spices was the finding of total number of microorganisms in uncommitted quantity. Such finding was expected considering that most of the species were plants that may be contaminated from the ground by different microorganisms during its growth in the field, or by animal feces, and later by inadequate storage.

Finding of moulds in uncommitted quantity as the second cause of incorrectness is the outcome of inadequate drying and storage in storehouses, where due to the increased humidity, moulds developed. Different species were found (e.g., different species of genera *Aspergillus, Penicillium*), many of which originate from the soil.

Finding of pathogenic bacteria (sulphate-reducing clostridia, *E. coli* and *Proteus* species) is the most frequent consequence of irregular maintenance of machines and devices used for preparation and packaging of spices, while the finding of sulphate-reducing clostridia may also be the consequence of the presence of soil traces in spices.

Difference in correctness between spices in bulk and packed spices was not determined. Spices like ginger, cinnamon, black and white pepper, oregano and chilli showed similar incorrectness, both in packed and unpacked spices. It indicates that spices were contaminated by microorganisms before the packaging process itself.

Our results are in correlation with researches of many authors. In the researches of Indian microbiologists on a sample of 154 spices, the presence of total number of microorganisms in uncommitted quantity was determined in 51%, while in our research that number was 47.52% (11).

The presence of moulds in spices was the subject of the author's investigation due to its significance for humans. Group of our researchers carried out an investigation on several kinds of spices: black and white pepper (ground and in grain), garlic and onion, ground clove, caraway, and cayenne pepper. Moulds were present in every sample (12). In our investigation, black and white peppers were among the leading samples according to the number of moulds. Exception was the negative finding of moulds in clove and caraway, probably due to the presence of ethereal oils (eugenol in clove and cinnamic aldehyde in caraway), which completely inhibits the growth of some moulds of genera *Aspergillus*, *Penicillium* and *Fusarium* , as are the species *Aspergillus parasiticus, Aspergillus flavus,* and *Aspergillus versicolor* and *Fusarium moniliforme.* All this leads to the assumption that ethereal oils had influence on growth of moulds in other spices, too. Investigations concerning the influence of ethereal oils have been conducted worldwide, too. Those studies also confirm that eugenol from the clove inhibits the growth of *A. parasiticus* and *F. moniliforme*. Inhibitory effect showed cinnamic aldehyde from caraway, then thymol and carvacrol from oregano and myristic acid from nutmeg. Cineole from eucalyptus did not show inhibitory effect on these fungi (13).

Indian microbiologists determined the presence of *Bacillus cereus* and *Clostridium perfringens* and their enterotoxins in some spices, as for example in ground caraway. In the same investigation, they noticed that the number of bacteria and quantities of enterotoxins rose with time of spice reposing, and drastically descended after warming in water bath (14). Mexican microbiologists examined the presence of *Cl. perfringens* in 380 samples of caraway, black pepper, oregano, garlic powder, and bay leaf, as well as the presence of enterotoxins. The presence of the enterotoxin gene was determined in 8 of 188 isolates of *Cl. perfringens* using dot-blot technique by DNA probe for isolating (15).

In our investigations, sulphate-reducing clostridia were found in ginger, cinnamon, black pepper ground, and sweet basil, which was in accord with previous investigations, although it was evident that investigations on larger number of these spices were not performed.

In one investigation of American microbiologists, which was done on samples of black and white pepper, coriander, and dill, the presence of it *E. coli* in four samples of black pepper, and in one sample of white pepper and coriander, was determined, respectively (16). In our investigation, the presence of *E. coli* in ginger, cayenne pepper, black pepper in grain and curry was determined, which confirmed that the finding of this bacteria was the consequence of inadequate and unhygienic maintenance of devices for packaging of spices and that it could be found in different kinds of spices.

We did not find some more comprehensive researches in domestic and foreign literature that treat the presence of *Proteus* species. Our results show only one sample (cayenne pepper) with *Proteus* species in it. Considering that this bacteria is an indicator of fecal pollution, and that it is about cayenne pepper, we may say that the finding of this bacteria, as well as the finding of *E. coli*, is the consequence of unsatisfactory hygienic treatment procedure and the used devices.

CONCLUSION

Spices may manifest microbiological incorrectness. The most frequent cause was the total number of microorganisms in uncommitted quantity and findings of moulds, while sulphate-reducing clostridia, *E. coli*, and *Proteus* species were isolated in a relatively small number.

According to microbiological incorrectness, we can make the following list: ginger, black pepper (ground and in grain), chilli, cinnamon, dill, and sweet basil, while microbiological correctness was registered in clove, bay leaf, sesame, white pepper in grain, rosemary and caraway.

Uncommitted total score of microorganisms was expected as the most frequent cause of incorrectness, due to the habitat of the spice plants (soil and feces of insects, birds, rodents), and later due to the conditions of treatment and storage. Moulds were isolated in such a great number because of inadequate storage conditions and increased humidity which make the favourable climate for their development. In addition, many of the isolated moulds are soil species. Sulphate-reducing clostridia are present in spices because they are soil residents and can survive unfavourable conditions for a long time by spore forming. Finding of *E. coli* and *Proteus* species in spices was the consequence of inadequate maintenance of machines and devices for processing and packaging of spices.

The most probable cause of microbiological correctness of clove, caraway, rosemary and sesame is the presence of ethereal oils (eugenol, cinnamic aldehyde) which have strong antifungal and antibacterial effect. White pepper in grain and bay leaf stay sound because of their morphological characteristics (presence of pericarp, leathery leaves). Bay leaf also contains ethereal oils (cineol, alpha-pinene).

Improving of quality of spices can be done by introducing the quality control into the whole cycle of spice processing "from the field to the table". It is necessary to determine critical control points even before harvesting and proceed all through the sale, with a special accent on storage. Such control is achieved by introducing the HACCP system (Hazard Analysis by Critical Control Points) into the treatment process. Nowadays, this system is in use with all manufacturers that wish to have and keep their customers because of the quality of their products.

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MIKROBIOLOŠKA ISPRAVNOST ZAČINA KOJI SE NALAZE U PROMETU U PRODAVNICAMA ZDRAVE HRANE I SUPERMARKETIMA U NIŠU

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SAŽETAK

U radu su prikazani rezultati mikrobiološkog ispitivanja 101 uzorka začina (crni biber u zrnu/mleveni, beli biber u zrnu / mleveni, aleva paprika, kim, đumbir, cimet, slačica, kari, muskatni orah, čili, origano, karanfilić, bosiljak, majčina dušica, lovor, ruzmarin, susam).

Kontrola mikrobiološke ispravnosti izvršena je na osnovu Pravilnika o metodama obavljanja mikrobioloških analiza i superanaliza životnih namirnica Sl. list SFRJ br. 25/80 i Pravilnika o mikrobiološkoj ispravnosti namirnica u prometu Sl. list SRJ br.26/93, 53/95 i 46/2002 po članu 30.

Utvrđeno je da od 101 uzorka začina, 55 uzoraka je bilo mikrobiološki neispravno (54,45%). Najčešći razlog neispravnosti je nalaz ukupnog broja mikroorganizama u nedozvoljenom broju (48 ili 47,52%). Po učestalosti, sledi nalaz plesni u nedozvoljenom broju (23 22,77%). Patogene bakterije su izolovane u relativno malom broju uzoraka: 5 (4,95%) sulfitoredukujuće klostridije, 4 (3,96%) *E.coli*, 1 (0,99%) *Proteus* vrsta. *Salmonella* vrste, koagulaza pozitivne stafilokoke i kvasci nisu nađeni.

Uzorci: đumbir, crni biber, mleveni i u zrnu, čili, cimet, mirođija i bosiljak najčešće su bili mikrobiološki neispravni, dok su karanfilić, lovor, susam, beli biber u zrnu, ruzmarin i kim pokazali uredan nalaz.

Povećan ukupan broj mikroorganizama je najčešći uzrok neispravnosti zbog samog habitata začinskih biljaka (zemljište i feces insekata, ptica ili glodara), i zbog neodgovarajućih uslova skladištenja. Plesni su izolovane u tako velikom broju jer su istraživani začini porasli u neadekvatnim uslovima ili skladišteni u povećanoj vlažnosti uz pogodnu klimu. Mnoge od izolovanih plesni su zemljišne vrste. Sulfitoredukujuće klostridije su prisutne u začinima jer su česti stanovnici zemljišta i mogu dugo da prežive nepogodne uslove kao spore. Nalaz *E. coli* i *Proteus* vrsta u začinima objašnjava se neadekvatnim održavanjem aparata i uređaja za preradu i pakovanje začina.

Mikrobiološka ispravnost karanfilića, kima, ruzmarina i susama je verovatno obezbeđena prisustvom eteričnih ulja (eugenol, cinamic aldehid) koja imaju jako antifungalno i antibakterijsko dejstvo. Beli biber u zrnu i lovor svoju ispravnost održavaju zahvaljujući morfološkim karakteristikama (prisustvo perikarpa, kožasti listovi) i etarskim uljima (u lovoru: cineol i alfa-pinen).

Visok stepen mikrobiološke ispravnosti može se postići uvođenjem rigorozne kontrole u proces prerade i skladištenja začina počevši od gajenja biljaka, branja, prerade, skladištenja do prodaje. Ova kontrola se postiže uvođenjem HACCP plana i pronalaženjem kritičnih kontrolnih tačaka u procesu prerade i skladištenja začina.

Ključne reči: **začini, mikrobiološka ispravnost, HACCP plan**