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The Bacterial and Fungal Microflora in Seeds of the Hot Pepper, Mild Pepper and Black Pepper

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Abstract: The microbial component can be present in the organism at the first sight seems intact. This presence of the relation microflora-organism (or organ) might be in a symbiotic, parasitic, respectively pathogenic form. But if this biological microflora (in the research of this case the heterotrophic bacteria, general coliforms, yeasts and moulds) is dramatically added might consider as a source of toxins/ bio-toxins. The plants which are used as human food must be toxin-free, respectively without microorganisms especially without pathogenic microorganisms. Many authors have argued that the microbial flora associates the plant whence the seed. Therefore, one can ascertain that seeds respectively seedlings (sprout), which are developed from seeds are in contact with the microorganism whence the food environment. Even the decay of fruits and vegetables is a consequence of the presence of bacteria and fungi in the soil from where they achieve to the vegetative and generative parts of the plant. These microorganisms might be nominated indigenous flora. The environments where these microorganisms are found are: the soil, the water and the air. And if this nutritive environment is pasteurized or sterilized incorrectly, prepared without proper care etc. might be a source of the initial inoculation with microorganisms, which during their growth (germination, blossoming and fruit), through their seed (embryo) will reach again at their new seeds and fruits. It should be noted that microorganisms might reach the organic system of the plant (*e.g.* ploem) even through other paths but the one through soil and water is more argued. In this paper a relatively high density of the bacterial and fungal microflora is registered in the seeds of the "pepperoni" sort, dry hot pepper and black pepper. Measures are made in the weight amount respectively in the surface and inside the seed.

Keywords: *microbial flora, seeds, fruits, heterotrophic bacteria, general coliforms, pathogens, yeasts, moulds, pepper, ground pepper.*

Introduction

In order to dispose a healthy organism - a healthy nutrition intake is required. It is known that the quality of food is influenced by the physical, chemical and microbiological ingredients. The latter reach the food through different paths and are not permanent components of the food. The organism is obliged to intake the food in order to keep itself in a certain stage of energy. Therefore the organism is a "Building" held by the energy. But now the question arises: is there a non-toxic food and what determines the toxicity scale with/or from food? In an indirect way we must look for an answer from Paracelsus, a Swiss-German pharmacist and also a philosopher (XV-XVI century) who asks: "Is there anything that isn't toxic? And there is nothing that isn't toxic. This way, the dosage will determine that an agent is not toxicant". However, it is known that the amount present and the power of the toxicant are crucial to making the effect.

Therefore, the lethal dosage and the virulence of the respective toxicant are discussed (Ottoboni, 1996) and in accordance to this- the allowed amounts of the toxicant in a food are recorded. Therefore, in the technological practice of food preparation it is insisted to respect the amount limits of the toxicants in order to bring those to a minimal level. This entire issue was emphasized because many pathogenic microorganism species are also carriers of organism poisoning by food (Jones et al. 1992).

Food safety is a specific chapter in the science of food. In regard to the question: how does the food infection happen, also as previously mentioned above one might ask: How does the plant get infected by the bacterial and fungal microflora or even the viral one? The answer has to be searched in

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the initial infection (initial inoculation) of the plant from the terrain where the preparation of the sapling from respective seeds is done (14). One must emphasize that if we are completely sure that the food is sterile such as: soil, humidity, air, watering pipes etc. (Wachtel et al. 2002) then the penetration of the microorganism in the plant (not in the greenhouse) might be also searched in other paths such as leaf ramparts, injured parts, (Beuchat, 2002) or in the flower pistil through the pollen channel.

However, potential causes of the infection no less aren't inadequate manipulation, the low level hygiene of the cultivator staff, transportation conditions, storage and distribution conditions (Jones, 1992). At last it should be noted that nowadays, most of the seed plots, plantations etc. in different parts of the world (especially in undeveloped and in developing countries) they grow and cultivate seedlings with the soil mixed with organic manure which is a potential source of pathogens and one must intervene there in the prevention of the spread of pathogens, parasites etc. (Hedberg et.al. 1994).

The purpose of this research paper was the ascertainment of the presence of heterotrophic bacteria, total coliforms, yeasts and moulds in the surface and inside the fruit seeds of some species of "pepperoni" peppers and black pepper. Since the bacterial microflora and likely the fungal microflora together with parasites as residents of rectum especially kokum, often presented as a cause of appendix inflammation, it would be of interest that this microflora situated in the removed appendix to be analyzed and identified the microbial quantitative and qualitative composition. Those data would be compared to the modest data from papers such as this one.

Materials and Method

As a research material one has used the seeds of the ripe fruit (mature fruit) hot pepper and mild pepper known by their popular name "pepperoni". Both of them were preserved, prepared in glass jars for sale in grocery stores. Along with these also the seeds of the fresh hot pepper "pepperoni" have been analyzed untreated with henbane. The contemporary methods of food examination have been applied (Harley et. al. 2002). As a study object were also the seeds of the red hot chili peppers mixed with the powder of the fruit flesh. At the end the seeds of the black pepper have been analysed. Below are the pictures of the study object.

Work performance

Firstly, the amount of the seeds weighting 1 gram has been measures. This amount has served for examination. To each amount of 1 gram an amount of 99 ml cold sterilized water. This way, initially the dilution 10^2 is earned. Such containers/dishes (Erlenmeyer) with samples are placed in the energetic tossing apparatus for 2 hours. The tossing has enabled the microflora to dissociate and to emigrate in the sterile environment with salted sterile water (0.85% NaCl). Afterwards, the samples (from 1 ml) from the dilution 1: 100 have been moved into the verse of Erlenmeyer by 99 ml sterile salted water.

From the tossed Erlenmeyer (first one) the seeds have been removed and rinsed several times and grinded by the grinder? The grinded mass (where the seed hulls has been destroyed and disintegrating within it) it has been filtered and mixed with 10ml water from the second Erlenmeyer and has been tossed for 10 minutes, as the required time to earn the adequate suspension. This mass has been moved to the Erlenmeyer containing 89 ml sterile water- dilution 10^4(10 ml have been initially taken as a suspense of the grinded mass). In this way the cell release has been carried out from the inside of the seeds in the water environment. In the case of red pepper (hot pepper) mixed here and there with some seed, is measured 1 gram of the mass and diluted in the series of sterile salted waters. The seeds of the black pepper weighting 1 gram have been also mixed with 99 ml sterilized salted water, then have been energetically tossed for two hours in order to release the sheltered microflora in the texture of seed surface.

Figure 1. Hot Peppers (in jar)

Figure 1a) Hot Peppers (opened fruits)

Figure 2: Mild Peppers

Figure 2a) Mild Peppers (opened fruits)

Figure 3: Fresh Hot Peppers

Figure 3a): Fresh Hot Peppers

Figure 4. Red Powder Pepper

Figure 5. Black Pepper

During the procedure one has found that 1 gr weight has had averagely 40 seeds of the "pepperoni" pepper studied; one seed had a surface of 30mm ². One gram of the black pepper seeds had approximately 7seeds. The sample planting has been done by the methodology of membrane filters where 10 ml of the sample from the certain dilution have been filtered in the respective apparatus. The filters were from the Gilmans brand with a pore diameter from 0.45 μm. The filters have been moved on the food as it follows:

For heterotrophic bacteria- nutrition agar
For total coliforms- endo agar
For yeasts and moulds- wort agar
All instructions are taken from Merck, URL1. 2017
The incubation has lasted as below:
For heterotrophic bacteria and total coliforms: 48 hours in 37.5 ^{0}C.
For yeasts and moulds: 5-8 days at a temperature of 18-22 ^{0}C.

After the incubation and counting the colonia the number of the microorganisms has been defined according to the formula: (Merck, 1996)

$$No/100 = \frac{Ncx\ Ds \times 100}{VSF}$$ whereas;

No/100: The number of general colonies in 100 ml
Nc: The number of counted colonies (germinated)
Ds: The dilution scale
100: the volume in which the cells are calculated and
VSF: The amount of the sample filtered
It must be emphasized that our calculations are done: number of cells in 1 gram or ml, mm^2 and mm^3.

Results and Discussions

Given the fact that the seeds, be that fresh (ready for sowing and germination) or as fermented, respectively intact or grinded, may be colonized from the bacterial and fungal microflora, the findings of the microbial and fungal microflora density have been carried out in (Lindow et al. 2002). In this paper one has found that the density of the microflora stated in the seeds of pepper fruits "hot chili and mild preserved, fresh hot pepper, red hot pepper" (the flesh part and grinded seeds) and black pepper. The number of the heterotrophic bacteria, total coliforms, yeasts and moulds in gram of the mass (seed, grinded mass, powder) and in mm^2 volume of the seeds was evaluated. The results obtained are presented by the tables below:

Table 1. The density of heterotrophic bacteria, total coliforms, yeasts and moulds in surface and inside the seeds of hot pepper fruit pepperoni".

Examined object	The group of microorganisms	No. of cells/g	Surface/ mm^2	Interior/ mm^3
Preserved hot pepper				
	Heterotrophs	$40x10^4$	333	
Surface	Total coliforms	$28x10^4$	233	
	Yeasts	$6x10^4$	50	
	Moulds	$2x10^4$	16	
Interior	Heterotrophs	$38x10^4$		202
	Total coliforms	$8x10^4$		425

Table 2. The density of heterotrophic bacteria, total coliforms, yeasts and moulds in the surface and interior of mild pepper fruit seeds "pepperoni"

Examined object	The group of microorganisms	No. of cells/g	Surface/ mm^2	Interior/ mm^3
A seed of mild pepper (preserved)				
	Heterotrophs	$10x10^4$	83	
Surface	Total coliforms	$3x10^4$	25	
	Yeasts	$80x10^4$	666	
	Moulds	$75x10^4$	625	
Interior	Heterotrophs	$8x10^4$		425
	Total coliforms	$2x10^4$		106
	Yeasts	$3.5x10^4$		186
	Moulds	$2.5x10^4$		133

After determining the density of the microorganisms studied in the seeds of pepperoni pepper, powder pepper and seeds of black hot pepper also the numbers of the organisms that have colonized the surface in 1 mm^2 and interior mm^3 have been determined. Measures have shown that 1 gram of pepperoni pepper seeds have had averagely 40 seeds. The approximate surface of one seed was 30 mm^2 while the volume of the grinded mass was 4.7 mm^3. Meanwhile the number of the cells at the red hot grinded pepper was expressed in grams, at seeds of black pepper this was the situation: 1 gram

weight was equivalent to the weight of the seeds where one seed had the surface 314 mm^2 while the volume was 523 mm^3.

Table 3. The density of heterotrophic bacteria, total coliforms, yeasts and moulds in the surface and interior of hot pepper fruit seed fresh "pepperoni"

Examined object	The group of microorganisms	No. of cells/g	Surface/ mm^2	Interior/ mm^3
Hot fresh pepper				
	Heterotrophs	$36x10^4$	300	
Surface	Total coliforms	$12x10^4$	100	
Yeasts		$60x10^4$	500	
Moulds		$2x10^4$	16	
Interior	Heterotrophs	$30x10^4$		159
	Total coliforms	$20x10^4$		1063
	Yeasts	$20x10^4$		1063
	Moulds	$4x10^4$		212

Table 4. The density of heterotrophic bacteria, total coliforms, yeasts and moulds in pepper (red pepper) from the fruit flesh and un-milled seeds

Red pepper (powder) Examined object	The group of microorganisms	No. of cells/g
And seed (hot and dry)	Heterotrophs	$9x10^4$
	Total coliforms	$6.8x10^4$
	Yeasts	$88x\ 10^4$
	Moulds	$7.2x10^4$

Table 5. Density of heterotrophic bacteria, total coliforms, yeasts and moulds in seeds of black pepper

Examined object	The group of microorganisms	No. of cells/g	No. of cells / mm^2
Black Pepper matured seeds			
	Heterotrofët	$8.8x10^4$	40
Surface	Total coliforms	$7.2x10^4$	33
	Yeasts	$28x10^4$	127
	Moulds	$22x10^4$	100

It must be emphasized that the values in surface (mm^2) and volume mm^3 at seeds of pepperoni pepper are obtained by dividing the general number of the cells with the number of seeds per gram (e.g. 40) this way was obtained the number of cells in one seed. This number is divided with the value of 30 in order to obtain the number of cells in $1mm^2$ while the same one is divided by 4.7 to obtain the value of the cells in mm^3. The seeds of black pepper were calculated by the same method except that there were 7 seeds with an equivalent weight of 1 gram. Example: if the general number of the cells is 80x10 4 we will have 800.000:40=20.000 cells in one seed. Now 20.000 is divided by 30 where the value of the surface in mm^2=666 is obtained. If the determination of the volume for 1 mm3 is required then the digit 20.000 is divided by 4.7= 4255 cells in 1 mm^3.

Results and Discussion

As it is previously emphasized the bacterial and fungal microflora (and the viral microflora for certain) may reach the surface and interior of the organism through different paths (Beuchat, 1997). If the plant is invaded by microorganism which have penetrated into the seed (inside the seed) then in this examination those microorganisms have been registered as residents sheltered there. But if the pepperoni pepper seeds and black pepper seeds have had a densely microflora in their surface then they also got that microflora from the environment of water, salt, sugar, acetic acid etc.

At the powder hot pepper and seeds of black pepper the contamination with microorganisms could have been done by air, blending machines, during the incorrect manipulation, during packaging etc. (Fields, 1979). Also the vegetative organs such as: seeds, fruits, leafs and so on may serve as ecological niche of many microbial communions. These microfloras might survive even after the chlorine treatment. There are some data that phyto-patogenic bacteria may affect positively in the

survival and development of human pathogens. They may also be detected in seeds and other vegetative materials (Saettler *et al*. 1989).

The data from tables 1-5 demonstrate that the density of heterotrophic bacteria whether or whether not a decay cause of the studied objects ranges from 8.8 $x10^4$ to 40$x10^4$ per gram of the mass. By using the agar plaque it has been found that the bacterial population in seeds of alfalfa sprouts and onion may reach the density to 10^4 of heterotrophic bacteria. (Proctor et al 2001). Similar data have also been found in examined seeds in this paper.

The bacteria of total coliforms (typical and non-typical) in surface studied seeds have had a value of 7.2 x 10^4 to 28x 10^4 cells per gram of the studied mass. In the case of hot pepper seeds it has been found that the interior of the seed has released more coliforms in comparison to the case with the surface of the seeds, respectively the values were $20x10^4$ in the interior and $12x10^4$ in the outside. These data are in accordance to the data of other authors (Wu et al. 2001).

Concerning the yeasts in our research have been registered densities of $6x10^4$ to $80x10^4$ in the surface of studied objects. In comparison to the surface, the interior of the seeds had lower values: from 3.5x 10^4. Certainly the grinded pepper had 88x10 4 yeasts which may have an origin from additives, bread, sugar, air etc. during milling etc.

At the end, moulds were present with a high density value in the case of pepperoni mild pepper: 75$x10^4$ cells per gram of seeds. We are of the opinion that this colonization of the surface comes from the composition of the additive (water, sugar, but also sort of the pepper, the acidic preservants *etc*.) factors which have influenced the growth of the moulds and that with the probability of biofilm.
The data from the table enable the opportunity to interpret the results in the basis of etiological, ecological, physiological and endophilic factors.

Recommendations

In this paper one did not tend to analyse the relation between the taste of the seeds and the flesh parts of the pepper with the presence of microorganism. But, it is known that the burning sensation come from the fruit and the seed too. There are many papers on pepper's capsaicin. The black pepper (*Piper nigrum*) contains constituent carriers of carcinogens (Wrba et al 1992). It must be emphasized that it would be of a great interest for the science of medicine and microbiology the ascertainment of the relation of the presence of bacterial microflora (heterotrophics and coliforms) in the rectum respectively in the appendix and their impact. It is known that the appendix inflammation is usually ad mainly caused by the presence of bacterial microflora in there but one should not skip other factors such as: parasites, hemorrhoids and different conservator. One also knows that this microflora usually has an origin from the fruit seeds of peppers which (be that hot or mild) are greatly consumed. It would be important that the microflora situated in the appendix which can be removed by surgery to be identified quantitatively and qualitatively and to compare it with seeds of pepper fruits which currently are still consumed. It is likely that the microbial cultures obtained by examination as in our paper are identical to the ones isolated from the interior of the appendix.

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