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Study on fungal load of pome fruits, hazardous factor for quality maintaining and ensuring the consumer safety

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Abstract Three varieties of apples and pears from indigenous and external sources have been studied, in regards the mesophilic and psyhrophilic fungal load. Czapek medium isolation method based on the principle of dilution allowed us to determine the number of fungi after 5 days of incubation. The biggest load of mesophilic fungi we determined for Idared apples from Romania. After the macroscopic and microscopic examination of isolated fungi we were identified six species of filamentous fungi *Botrytis cinerea, Penicillium expansum, Aspergillus restrictus, Penicillium chrysogenum, Penicillium solitum,* and *Fusarium equiseti.* In the category of strict mesophilic fungi we have *Aspergillus restrictus, Penicilium chrysogenum and Fusarium equiseti.* The *Penicilium expansum, Botritis cinerea and Penicillium solitum ,* we find in both classes of mesophilic and psyhrophilic fungi. Calculation of frequency of occurrences on the plates for each fungal species showed that the most bigger frequency was determined for *P. solitum (100%)* for the Golden apples from Romania and *B. cinerea (80*%) for the Cure pears from Romania. Application of postharvest measures of conditioning and storage has results in reducing and limiting the spread of fungi, their importance was established on the William pears from Chile.

Key words

fungi spoilage, mesophilic and psyhrophilic fungus, apples, pears

Horticultural products for immediate use in food consumption are a valuable food for vitamins, minerals, simple sugars and fibbers that covers the needs of everyday people [5, 10]. Before harvesting the plants have defense mechanisms to fight against the invading ability of microorganisms that induce alterations. Thus plant tissues by high water content, pH neutral or acid fail to prevent colonization by opportunistic organisms compared with other types of food. The degree of colonization and invasion depends on the competitive ability of plant tissue and evolution of plant – parasite relationship, but in postharvest the evolution of relationship host-parasite is determined by physical factors [2, 7]. In this case it is essential to maintain the vital properties of horticultural products based on the biosis principle. Based on this principle to ensuring the product viability and the keeping of immunity against pathogens invasion the first horticultural products are also called "living foods ". Microbial degradation of living foods, as fresh fruits or fresh vegetables can be ensured through chemical methods, research in this area presenting a special attention to ensuring consumer protection[1, 5]. The use of inadequate chemical methods can seriously affect the health of the consumer rather than the presence of spoilage unwanted microorganisms.

Another safety interest connected with living foods is linked to mycotoxins [1, 6]. These are secondary metabolites of filamentous fungi; the most responsible and unsafe are species of the *Penicillium* and *Aspergillus* genera [9]. They fall into the category of opportunistic microflora, with high possibilities of colonization since the harvesting period of horticultural products, when the pH of the tissue increased, the skin layers became soften and soluble carbohydrates build up and defence barriers is weaken. The storage of fresh fruit postharvest need to balance maturity against storage life and transportability, ripening against over ripening, balanced maturation against breakdown of desirable qualities, all with the ever present problems of controlling fungal invasion and spoilage as well [l0].

The objective of present study is the estimation of fungal load of fruit known as mycotoxin producers.

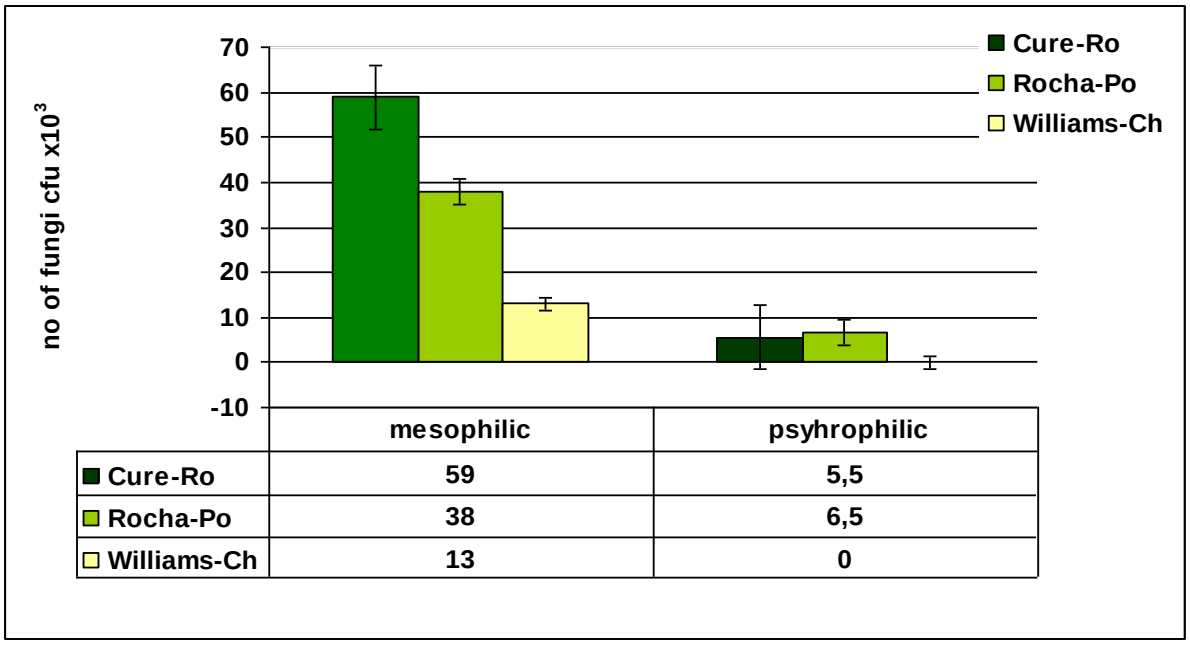
Material and Methods

In order to accomplish the objective we have determined the microbial loads of two categories of fruit, apples and pears from the open market of indigenous and strangers provenience. Were studied two segments of fungal microbiota: mesophilic and

psihrophilic. In the experience we have used three varieties of pears: Cure, indigenous provenience (Cu-Ro), Rocha, provenience Portugal (Ro-Po) and Williams, provenience Chile (W-Ch). Regarding the apple varieties were studied Golden, provenience Italy (Go-I), Golden, provenience Romania (Go-Ro) and Idared, provenience Romania (Id-Ro). For isolation of fungi and their estimation number we used Czapek medium with a supplement of chloramphenicol and streptomycin to inhibit bacteria growth [5]. We used the successive dilutions technique in which the surface of fruits product was washed with 0,85% Na Cl medium dilutions. From the first dilution were also prepared two additional, and from the last we have made isolation on the Czapek medium by surface plating technique. We worked in four repetitions for each samples, two of them were incubated in thermostat at 25^0C and two at 5^0C. The colonies counting were made after 5-7 days, followed by macroscopic and microscopic examination to identify the species [5, 8]. The frequency of occurrence for the each fungus species identified it was made by reporting between colony number of fungi identified and total number of fungus colonies on the plates replicates

Results

Results of fungus load of pears. The biggest fungus load was estimated for the category of mesophilic fungi. As we can see in the Figure 1the highest load was determined for pear Cure the average it was 59 000 CFU/pear, the second load it was determined from the Rocha pear with 38 000 CFU/pear. The smallest fungal load was determined for the variety Williams pears, provenience Chile. Regarding psyhrophilic fungus their number is lower than mesophilic. Rocha Pears had the highest psyhrophilic fungal load with the average of 6 500 CFU / pear. Williams's pears variety didn't have psyhrophilic mycobiota.



	mesophilic	psyhrophilic
Cure-Ro	59	5,5
Rocha-Po	38	6,5
Williams-Ch	13	0

Fig 1. The average number of spoilage fungi for pears

Results of fungus load of apples. The determining the number of opportunistic fungus on apple underlined that these apples are more colonized than pears. As can see in the figure 2 Idared variety apples have registered an average of mesophilic fungi about 190 000 CFU / apple.
It is follow Golden variety, provenience Romania, with average 110 000 cfu/apple and the least Golden variety, provenience Italy, with 10000 CFU/apple fungus load. The psychrophilic fungus loads an apple were smaller than mesophilic fungus loads. On the Golden variety apples, from Romania, it was counted an average of 30 000 cfu/apple and for Idared the fungus load average it was about 20000 CFU/apple. The Golden apples from Italy don't have any fungus load.

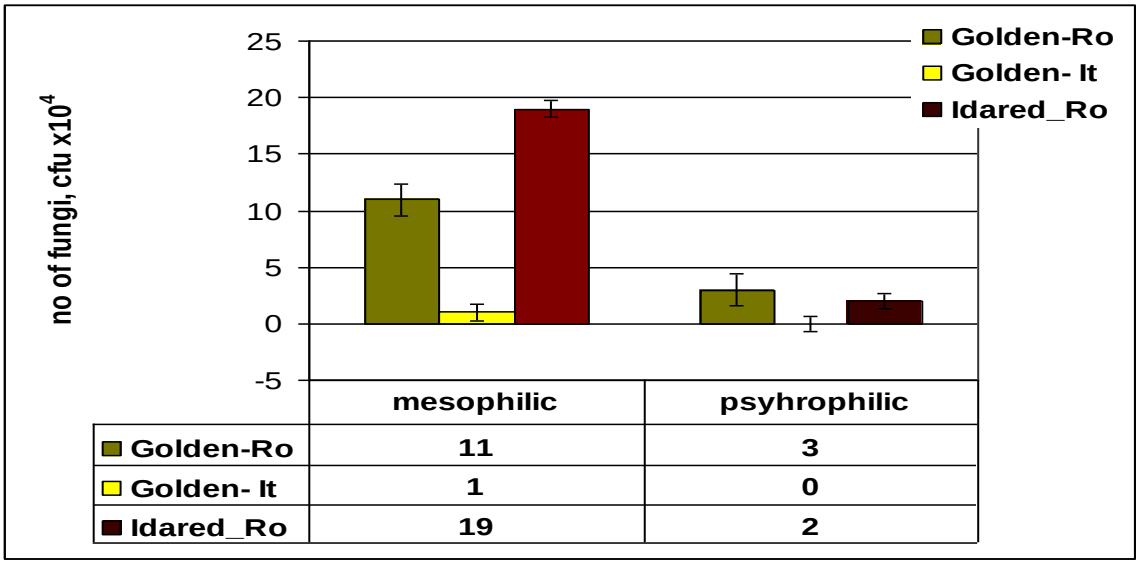


Figure 2. The average number of spoilage fungi for apples

Result of colonization the fruits with micotoxin fungi producers The fungus examination we made in 7 th day from initial incubation period. The macroscopic examination allowed us to observe the colony aspects, size, colour of mycelium and fructifications, reverse colour of colony and the fructifications aspects.

Table 1

Macroscopic and microscopic aspects of fungi isolated on the fruits

Fungus species	**Macroscopic aspects**	**Microscopic aspects**
Botrytis cinerea, non-micotoxin producer	Colonies covering the whole Petri dish, floccose, mycelium white, becoming grey to dark grey, reverse grey. ∅ of colonies up to 5 mm.	Conidiophores borne from aerial hyphae, each bearing terminally an irregular cluster of short branches with swollen spherical apices.
Penicillium expansum- patulin and citrinin producer	Colonies with 30-40 mm, typically tufted in one or more annular bands, with adjacent areas floccose; mycelium white, presence of orange brown to brown exudates, soluble pigment and reverse pale to brown	Conidiophores borne from surface or subsurface hyphae, singly or in definite coremia, green conidia
Aspergillus restrictus non-micotoxin producer	Colonies on 5-10mm, dense and velutinous; mycelium inconspicuous, white; conidial heads often poorly formed, green colour, reverse pale to very dark green.	Conidiophores borne from surface hyphae, with colourless, phialides crowded, 8–10 mm long; conidia borne as cylinders and adhering in long columns
Penicillium chrysogenum non-micotoxin producer	∅35–45 mm, velutinous; mycelium white to yellowish; conidial production light to moderate, greyish turquoise to dull green, yellow brown exudates, reverse usually brilliant yellow or yellow brown, radially surface, with white area in the colony edge.	Conidiophores borne from surface or subsurface hyphae, penicilli typically triverticillate, conidia ellipsoidal or subspheroidal
Penicillium solitum non micotoxin producer	∅ of 25-30 mm with velutinous surface or lightly floccose in central. areas; mycelium white, exudates absent , reverse greyish orange to brownish	Conidia very abundant, conidiophores single born from surface hyphae, biverticilate, with dark green colour
Fusarium equiseti trichothecene producer	Colonies on CZK with white, light rose mycelium, floccose and reverse coloured pale salmon	Macroconidia curved with 5 septa, apical cell elongated and curved

There were identified four genus of filamentous fungi *Botritis, Penicillium, Aspergillus and Fusarium.* By the microscopic examination (100 x) we could see the origin of conidiophores, their morphology and appearance. In the table 1 it's presenting the fungus species that were isolated on the fruits. Of them the fungus *Penicillium expansum* it is known as patulin and citrinin producers [3, 6] and *Fusarium equiseti* which synthesizes the trichotecene micotoxin [5]

The biodiversity of fungus witch colonize the fruits it is variable (table 2). In the case of Cure and Rocha pears it was determine a high frequency of occurrences on the plates for *Botritis cinerea*, fungus was present in both of incubation temperatures. This aspect and the fact that the fungus can give phytopathogenic diseases during the growing season can tell us more about the missing of chemical treatment in the preharvest period [2]. In the case of Williams pears the fungal load and their biodiversity is lower.

For the apples *Penicillium solitum* has a higher frequency than other fungus on all samples that we used in experiences. On the Idared apples we can observe the highest frequency of occurrences about 50% on replicates. The second frequency of occurrences is for *Fusarium equiseti,* from the Golden variety from Romania (22%). The most dangerous contaminant *P. expansum*, micotoxin producers it was determined with 35% frequency of occurrences on the Idared variety, provenience Romania and also it is present in psyhrophilic mycobiota with 65% frequency of occurrences.

Table 2

The fungi with the highest frequency of occurrences on fruits

Samples	**Variety**	**Fungus species**	**Frequency of occurrences %**	
			Mesophilic	**Psyhrophilic**
Pears	Cure -Ro	*Botrytis cinerea*	40	80
		Penicillium solitum	60	20
	Rocha-Po	*Botrytis cinerea*	35	35
		Aspergillus restrictus	30	-
		Penicillium solitum	35	65
	Williams- Ch	*Saccharomyces sp*	15	-
		Penicillium solitum	10	-
Apples	Golden -It	*Penicillium solitum*	15	85
		Fusarium equiseti	15	-
		Aspergillus restrictus	10	-
	Golden -Ro	*Fusarium equiseti*	22	-
		Penicillium solitum	10	100
		Aspergillus restrictus	22	-
	Idared- Ro	*Penicillium expansum*	35	65
		Penicillium solitum	50	35
		Penicillium chrysogenum	15	-

Conclusions

Fungal load of pome fruits is depending of technological methods, harvesting and storage methods they we can apply and use. Idared apples from Romania have the highest fungal load while imported apples have the lower load. This shows us the existence of some measures to prevent or decrease of the microbial attack by the chemical or physical treatments [4], for the pomes fruits with external provenience.

Penicillium solitum has a large range of distribution and adaptation and it is the one that appears in mesophilic and psyrophilic fungal microbiota. Fungus appears in both, apples and pears microbiota. Taking into consideration the fact that the fungus does not produce mycotoxins, it can only affect the losses of fruits harvest even if the fruit is storage at low temperature [2].

Penicilium expansum, the most dangerous fungal contaminant was determined on Idared apples provenience Romania. It seems that the household system of fruit obtaining where chemical pesticide treatments is limited allows colonization of fruit by fungi at a high level. The fact that the fungus produces mycotoxins, its presence will affect both the quantity and quality of fruit but also consumer safety [9]. In this case we can put the question: the organic products are able to ensure food security really [4]. To this question the advanced research at multidisciplinary level could find a response in the future.

The packaging of fruit is a measure to prevent degradation and spread of microbial contamination. In the case of Williams's pear we have determination the lowest load of fungus this because the fruits were waxed and wrapped in paper.

References

1.Cotuna Otilia, Popescu Gh., 2009, Securitatea si calitatea produselor vegetale, siguranț a vieții, Ed. Mirton, pg 108 Timisoara;

2.Hall, E.G. and Scott, K.J., 1989, Pome Fruit. In: Beattie, B.B., McGlasson, W.B., and Wade,N.L. (eds.) Postharvest Diseases of Horticultural Produce. Vol. 1. Temperate Fruit. CSIRO, Australia pg 69;

3.Morales, H., Marın S., Rovira, A., and Sanchis, V. 2006, .Patulin accumulation in apples by Penicillium expansum during postharvest stages. Lett. Appl. Microbiol. 44:pg30;

4.Morales, H., Sanchis, V., Rovira, A., Ramos, A.J. and Marın, S., 2007b, Patulin accumulation in apples during postharvest: Effects of controlled atmosphere storage and fungicide treatments. Food Control 18: pg 1443;

5.Pitt J. I., Hocking Ailsa D., 2009, Fungi and Food Spoilage, Springer Science+Business Media, LLC, pg 383, 423;

6.Roshni Amalaradjou Mary Anne and Venkitanarayanan Kumar, 2008, Detection of Penicillium,Aspergillus and Alternaria Species Fruits and Vegetables in ”Mycotoxins in Fruits and Vegetables, Academic Press, USA, pg 225.

7.Savu C-tin, Georgescu Narcisa, 2004, Siguranț a alimentelor, riscuri si beneficii. Ed. SemnE, pg 135;

8.Snowdon, A.L. 1990. A Colour Atlas of Post-harvest Diseases and Disorders of Fruits and Vegetables. 1. General Introduction and Fruits. London: Wolfe Scientific, pg 34-36, 114;

9.Sanderson, P.G. and Spotts, R.A. 1995. Postharvest decay of winter pear and apple fruit caused by species of Penicillium. Phytopathology 85: 103–110.

10.Sapers Gerald M., Gorny James R., Yousef Ahmed E., 2006, Microbiology of Fruits and Vegetables, Ed Taylor & Francis Group, LLC, pg 3, 95, 543.