THREE MAIN PATHOGENS THAT AFFECT APPLES DURING STORAGE AND THEIR INFLUENCE ON FRUITS QUALITY

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ABSTRACT

The postharvest pathogens that infect apples during storage period affect fruits quality and lead to important economic losses worldwide every year. Three appreciated apple varieties (Golden Delicious, Jonathan, Starkimson) have been evaluated for the incidence of main storage pathogens that operate in fruits (Penicillium expansum, Botrytis cinerea and Monilinia fructigena) and their impact on fruits quality. The results emphasized negative correlation between water content and dry matter for all apple varieties affected by storage pathogens. Despite the fact that were not significant differences between apple varieties, Golden Delicious has been recorded the lowest water content (74,62% for fruits affected by Penicillium expansum, 77,87% for fruits affected by Botrytis cinerea and 74,68% for fruits affected by Monilinia fructigena) and highest dry matter (24,63% for fruits affected by Penicillium expansum, 25,38% for fruits affected by Botrytis cinerea and 21,86% for fruits affected by Monilinia fructigena). Potassium and Phosphorus content decreased, while Iron content increased for all apple varieties affected by pathogens. The sugar content decreased with almost 20% for apple fruits, the lowest values for all varieties have been recorded in case of Botrytis cinerea and Monilinia fructigena attack. The highest raw protein values have been determined to all apple varieties affected by Monilinia fructigena (Golden Delicious 0,69g, Jonathan 0,89g and Starkimson 0,52g). The study revealed that apple quality during storage depends on the variety susceptibility, postharvest fruit health, fruit mineral composition, fungal inoculum and storage conditions.

INTRODUCTION

Nowadays apple is an important fruit crop cultivated on large areas especially in European Union, covering 473 500 ha in 2017 (EUROSTAT, 2019). About 34% of this area devoted to apple orchards is found in Poland (160 800 ha), with a further one quarter split between Italy (12% - 55 800 ha) and Romania (12% - 55 100 ha). Together with France (8.1 % of the EU total), Germany (7.2 %), Spain (5.8 %) and Hungary (5.3 %), these seven EU Member States accounted for more than two-fifths (83.7 %) of the total EU area under apple trees.

Belonging to *Rosaceae* family, genus *Malus*, species *Malus domestica*, apples are one of the most studied fruits worldwide, coming in an array of colors, tastes and textures, extremely rich source of phytochemicals associated with human health benefits, antioxidants that protect cells from free radical damage, vitamins that support the immune system and natural fibers that improve gut health (LEE, K.W. et al., 2003; WOLFE, Kelly. et al., 2003; CHARDE, M., et al. 2011; HYSON, D., A. 2011; AHMAD, S. et al. 2020; BONDONNO, C.P., et al. 2020; LI, Y. et al. 2020). For sure this the reason why is wide known the phrase ""an apple a day keeps the doctor away". However, the chemical nutritional composition of apples varies depending of the variety, climate, geographical area, maturity, cultural practices and storage conditions. (LEE, C.Y. and MATTICK, L.R., 1989, ER, F. and ÖZCAN, M.M., 2010).

Despite the fact that they are produced in diverse range of natural environments, climates and farming practices across the world (PARTAL, E. et al. 2010, 2014, 2017; COTUNA, O. et al. 2015; CICHI, M. 2016; BONCIU, E. 2019 a,b,c) reflected in a large array of food and drink products that are made available for human consumption and animal feed, as well as a range of inputs for non-food processes, all cultivated plant species need to be protected during both vegetation and storage periods (POPA, T. et al. 2013; COTUNA, O. et al. 2016; SALCEANU, C. and OLARU, L., 2016, 2017; CRISTEA, S. et al. 2017; BONCIU, E. 2020).

Fruits pathogens affect a large variety of fresh fruits in both orchards and storehouses and influence their water content, chemical and nutrient composition (ABDULLAH, Q. et al., 2016; PARASCHIVU, M., et al., 2020). A report of The Food and Agriculture Organization of United Nations mentioned that 33% of the food delivered worldwide for human consumption is lost after harvest due to postharvest diseases (GASTAVSSON, J. et al., 2011).

Apples are the most common fruits exposed to postharvest diseases and physiological disorders worldwide, resulting in significant economic losses, depending on the year, that ranged from 5% up to 60% on susceptible cultivars (BRACKMANN et al. 2000, 2002; JURICK II, W.M. et al., 2011; MICHALECKA, M. et al., 2016). Thus, postharvest diseases of fruits stored for extended periods of time have become a limiting factor of significant concern despite technological advances in postharvest handling of fresh fruit using synthetic fungicides, physical measures (irradiations, hot air/water application, heat, UV-C, modified atmosphere, ozone treatment, electrolyzed water) biological control (yeast antagonist, bacterial antagonist), natural compounds (chitosan, oligochitosan, salts), essential oils (vervain oil, thyme oil, lemongrass oil, tea tree oil, oregano oil) and nanomaterials, (LAGUNAS-SOLAR, M. et al., 2006; MIKANI, A. et al., 2008; MAXIN, P. et al., 2012, 2014; Sharma, R. R. et al., 2009; WENNEKER, M. et al.,2013; DROBY, S. et al., 2016; MARI, M. et al., 2016; WISNIEWSKI et al.,2016; USALL, J. et al.,2016; JANISIEWICZ, W. J., and JURICK II, W. M., 2017; ROBERTO, S,R. et al., 2019).

Postharvest diseases of apples are caused by a large range of pathogens (e.g.*Phacidiopycnis washingtonensis* – Speak rot; *Sphaeropsis pyriputrescens* - Sphaeropsis rot; *Mucor piriformis* – Mucor rot; *Neofabraea malicorticis* - Bull's eye rot, etc.), but the most common are *Botrytis cinerea* (Grey mould), *Penicillium expansum* (Blue mould) and *Monilinia fructigena* (Brown rot) (SUTTON, T.B., 2014).The present study aims the interaction between different apple varieties and the most important postharvest pathogens in terms of their impact on fruits quality.

MATERIAL AND METHOD

The experiment included apple fruits belonging to Delicious, Jonathan, Starkimson varieties, collected in 2019 from a private orchard near Craiova city, Dolj county and stored during eight months into a storehouse in hermetically chambers under controlled conditions, 0.75 to 1.0 $kPaO_2$, at 1.0°C (oscillation of ±0.1°C). The experiment was carried out in a completely randomized design, with three replicates 10-fruits/each apple variety. The impact of postharvest pathogens on apple fruits quality was performed after eight-month storage plus three weeks of shelf-life at 20°C air-exposure in artificial infection conditions. The quality parameters assessed in both healthy and diseased samples were water content (W%), dry matter (DM g), raw protein (RP g), minerals (mg) (potassium, phosphorus and iron) and total soluble solids (TSS) (mg). Fruits decay was evaluated by counting typical fungal lesions larger than 5 mm in diameter. The isolation and identification of the fungi on apple fruits was done using direct plating technique described by Pitt and Hocking (1985). The resulting fungi (*Botrytis cinerea, Penicillium expansum* and *Monilinia fructigena*) were isolated, purified and identified according to their fruiting bodies characteristics using Motic B2-320 Microscope. Before chemical components analyses, skin and seeds of samples were removed, and crushed using a commercial blender. Water content was determined using microwave heating of minced sample of fruits for 30 minutes using the technique described by Wenceslao Canet (1988). To determine dry matter fine apple slices from each variety were dried in a convection dryer at 60ºC until they reached a constant weight. The mineral contents (Fe, K, P) of samples were analyzed using Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) according to Duxbury method (DUXBURY, M, 2003).The quantitative determination of sugars content in fruits was done using digital refractometer (WYT-J 0–32% Chong Qing, China) and reported as degrees Brix, which is equivalent in percentage (BALL, 2006; DONGARE et al.,2014).Total soluble solids (TSS) values obtained from the digital refractometer have been adjusted using the factor 0,85 which means that sugars are 85% of TSS. All analysis was carried out three times and the results are mean values. The Pearson's r_{calc}. was determined using Microsoft Excel.

RESULTS AND DISCUSSIONS

After harvest, apple fruits are stored for many months under specific conditions, such as dynamic controlled atmosphere, ultralow oxygen concentration and low temperature conditions, which extends the postharvest life of apple fruits by reducing respiration rates, ethylene (C_2H_4) biosynthesis and action (YEARSLEY, C.W. et al. 1997; ZANELLA, A. 2003; BRACKMANN et al. 2008; WEBER, A. et al. 2011; THEWES, F.R. et al. 2015). Also, these conditions try to preserve apples nutrients and to protect fresh fruits against postharvest pathogens that impact fruits quality and marketable value.

Previous studies showed that postharvest diseases have been started in preharvest conditions when contamination occurred, especially because of physical damage or because of increased humidity during long time transportation from a place to another (SNOWDON, A.L.,1990; FATIMA, S. et al., 2012). Pathogens like *Botrytis cinerea, Penicillium expansum* and *Monilinia fructigena* can determine latent symptomless infections even in storage conditions, leading to rapid decay of fruits after they are removed from storehouses -controlled conditions. However, knowledge on epidemiology of the causal agents of latent postharvest diseases is limited.

The findings of this study showed that after storage period apple fruits presented visible decay symptoms during the three weeks of self-life exposed at 20°C air temperature, starting with the 8th day. *Penicillium expansum* was the fungus most frequently isolated on apple samples (F=12,6% Golden Delicious; F =10,4% Jonathan; F=7,2% Starkimson), followed by *Monilinia fructigena* (F=7,4% Jonathan; F=6,6% Golden Delicious; F=5,6% Starkimson) and *Botrytis cinerea* (F=5,2% Golden Delicious; F=4,5% Jonathan; F=3,2% Starkmison). Also, previous studies reported that *Penicillium* appeared to be the most fungal genus infected apples (NARAYANASAMY, P. 2006; DOV PRUSKY et al. 2010).

After several months in controlled conditions storage, symptoms start to appear when physiological and biochemical changes occurred in host. Thus, it was observed that alteration of diseased apples led to changes in appearance, taste, small and chemical composition comparatively with healthy fruits (Fig.1,2,3).

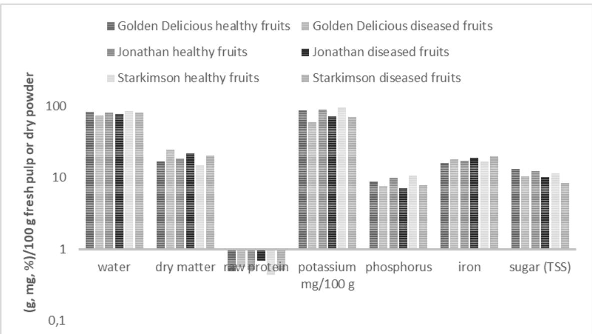

Fig.1.Chemical composition of apple fruits affected by Penicillium expansum (Blue mould) comparatively with healthy fruits

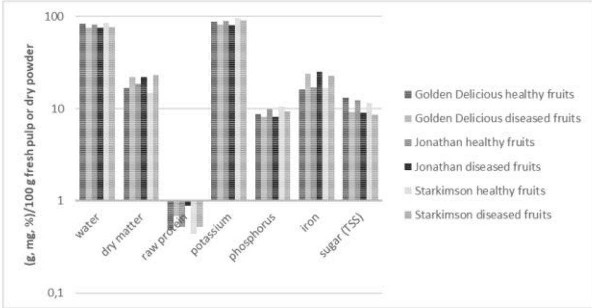

Fig.2.Chemical composition of apple fruits affected by Monilinia fructigena (Brown rot) comparatively with healthy fruits

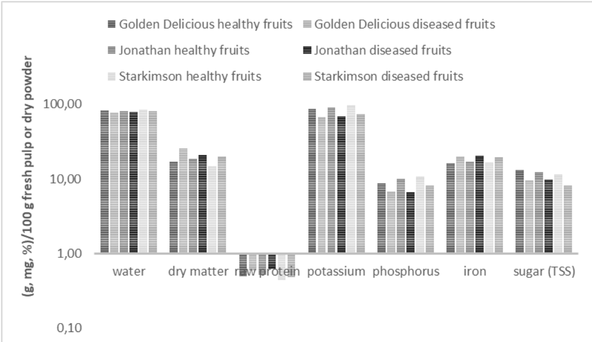

Fig.3.Chemical composition of apple fruits affected by Botrytis cinerea (Grey mould) comparatively with healthy fruits

Apples with skin contain about 84-86% water, the same as pineapple (86%), raspberries (86%) and close to that in oranges (87%), apricots (87%), or blueberries (84%) (GEBHARDT, S.E. et al., 1982; www.mydata.com). The water content in healthy fruits evaluated in the study depends on the variety (Jonathan – 81,44%; Golden delicious – 83,63%; Starkimson - 85,24%). In all diseased fruit samples, the water content decreased for all varieties due to the elevated metabolically activity within the fruits. This may be explained by previous findings as respiration rate may increase few times in diseased fruits affected by *Monilinia frutigena*, comparatively with healthy fruits (van LEEUWEN, G.C.M. et al., 2000). Despite the fact that were not significant differences between apple varieties, Golden Delicious has been recorded the lowest water content (74,62% for fruits affected by *Penicillium expansum*, 77,87% for fruits affected by *Botrytis cinerea* and 74,68% for fruits affected by *Monilinia fructigena*). The water content in diseased apples belonging to Jonathan and Starkimson varieties, was slightly closed.

Dry matter content (DM) increased in all diseased samples for all apple varieties with 11,69-56,70%. The highest dry matter values were recorded in Golden delicious (24,63% for fruits affected by *Penicillium expansum*, 25,38% for fruits affected by *Botrytis cinerea* and 21,86% for fruits affected by *Monilinia fructigena*), which means Golden delicious it was very affected, while de best behavior was noticed in Jonathan, in term of the impact of pathogens on dry matter. Among pathogens *Monilinia fructigena* was the one who produced the highest dry matter content in fruits, as well as the lowest water content. A significant negative correlation (r^2 = -0.842) was noticed between water content and dry matter in all diseased apples (table 1). The same significant negative correlation was observed between water content and raw protein (RP) (r^2 = -0.614).

Table 1

Matrix of the Pearsons' r_{calc} values

Specification	W %	DM (g)	RP (g)	K (mg)	P (mg)	Fe (mg)	TSS (mg)
W (%)	1	**-0.842****	**-0.614***	0.405	0.436	**-0.651***	0.389
DM (g)		1	0.368	**-0.592**	**-0.559**	0.466	-0.435
RP (g)			1	-0.158	-0.380	**0.600**	-0.179
K (mg)				1	**0.852****	-0.116	0.289
P (mg)					1	-0.216	0.252
Fe (mg)						1	**-0.625***
TSS (mg)							1

p 0.1 ≥ 0.604 *; 0.05 ≥ 0.743 **; p 0.005 ≥ 0.854 ***

Apples contain a smaller amount of protein (less than 0.3%) than many of the other fruits: 1.40% in apricots, 1.03% in bananas, 1.00% in cherries, 0.63% in grapefruits, 0.63% in grapes, 0.94% in oranges, 0.70% in peaches, 0.39% in pears, and 0.39% in pineapples (GEBHARDT et al.,1982). The raw protein content is positively correlated with dry matter content and it was noticed that it increased with 10% (Golden delicious) - 70% (Jonathan) in diseased fruits comparatively with healthy fruits. The highest raw protein values have been determined to all apple varieties affected by *Monilinia fructigena* (Golden Delicious 0,69g, Jonathan 0,89g and Starkimson 0,52g), comparatively with the samples affected by the other two pathogens. In case of the attack of *Botrytis cinerea*, all diseased samples had raw protein values closed to the healthy ones for all apple varieties.

Although apples cannot be considered an important source of minerals, they are comparable to other fruits in this aspect. Compiled average data of the USDA Handbook 8-9 show that the mineral content in apples is low (0,26%), including iron, magnesium, manganese, zinc and copper. Potassium constitutes the main portion of the total mineral content of apples and phosphorus and calcium are the next most prevalent minerals. Other studies pointed out the low mineral content of apples (UPSHAW, S.C. et al.,1978).

Potassium (K) and Phosphorus (P) content decreased, while Iron (Fe) content increased for all apple varieties affected by pathogens. The lowest potassium values were determined in apple samples affected especially by Penicillium *expansum* şi *Botrytis cinerea*. It was noticed a slightly decrease in potassium content in samples affected by *Monilinia fructigena*, because is already known the post-infection resistance given by potassium against this pathogen. All pathogens determined increased iron values in diseased apples comparatively with healthy ones (17mg/100 g fruit), because iron is involved in oxidative reactions within pathogens infectious activity. The highest iron content was noticed in apple samples affected by *Monilinia fructigena* (22,8 mg/100 g fruit – Starkimson; 23,9 mg/100 g fruit – Golden delicious; 25,1 mg/100 g fruit-Jonathan). There was a significant positive correlation between potassium and phosphorus content (r^2 = 0.852).

Fresh matured apples contain about 15% total carbohydrates and 10 - 13% total sugars. The most common sugars are fructose (5-7%), sucrose (3-5%), and glucose (1 – 2%) (LEE, C.Y. et al.,1970). Analyzing apple samples, it was noticed that the sugar content decreased with almost 20% for diseased apple fruits, the lowest values for all varieties have been recorded in case of *Botrytis cinerea* and *Monilinia fructigena* attack. Pathogens need energy to continue infectious process within the plants and for this they consume monosaccharides, especially. This can be explained also by the significant negative correlation between iron and sugar (TSS) content (-0.625).

The results indicate that apple varieties had individual reaction on the postharvest pathogens especially when they were exposed to the attack after long time of storage under controlled conditions, maybe because of their antioxidant capacity. TODEA, D. et al., 2014 showed that Jonathan variety has high polyphenols content, especially quercetin, which may explain its good behavior against postharvest pathogens in this experiment. However, further investigations about the implication of apples polyphenols to post-storage pathogens resistance are needed.

CONCLUSIONS

Despite the fact that postharvest decay is a natural process in all fresh fruits, losses due to postharvest pathogens still represent a major concern from economic point of view. The increase of word population requires more food demand and fresh fruits are necessary to be stored for long periods of time without significant alteration in their chemical and nutritional values. The current study showed that postharvest pathogens such as *Penicillium expansum*, *Botrytis cinerea* and *Monilinia fructigena* can affect apples content in water, dry matter, raw protein, minerals and sugar, which lead to faster or lower fruits decay depending of each variety. The findings are useful for a better understanding of host-postharvest pathogen relationship and for thinking more efficient control strategies. Also, this issue is of importance as long as apples are the most targeted fruits worldwide and mostly consumed by economically low-income people. Therefore, it is necessary to control these pathogens in storehouses and supermarkets in order to protect people health and promote food security. The results show that *Penicillium expansum* presents the highest occurrence risk, followed by *Monilinia fructigena* and *Botrytis cinerea*. Among all apple varieties, Starkimson and Jonathan had the best behavior against these fungi. Further investigations are necessary to explain better which is the mechanism involved in resistance of these varieties to postharvest pathogens.

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