The Use of Natural Biopolymer Derived from *Gleditsia triacanthos* in Reducing the Cracking Process of Cherries

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The experiment was carried out to study the effect of mineral nutrients foliar treatment (1% Ca as calcium chloride ($CaCl_2$), 1% Zinc as zinc sulfate ($ZnSO_4$), 0.1% polyphenolic extract derived from seeds of Vitis vinifera, and 0.1% humic acid extracted from lignite) mixed with 1% galactomannan extracted from seeds of Gleditsia triacanthos on the phenomenon of cracking in cherries. As research material we used four varieties of sweet cherry fruit (Kordia, Simone, Regina, Summit) treated 2 weeks before harvest. Highlighting the treatment effect was achieved by determining the maximum time of water absorption, the maximum quantity of water absorbed and fruit cracking index. The maximum water absorption time showed an increase of 25.7% for Simone, 36.8% for Kordia, 36.8% for Summit and 52.6% Regina compared to control samples. The amount of water absorbed decreased by 19.75% for Kordia, 44.4% for Regina and 50% for Summit and Simone Regina compared to the control samples. Fruit cracking Index values dipped in T1 were lower by 21.7% for Kordia, 26.7% for Simone, 27.3 % for Summit and 41.3% for Regina. Fruits dipped in T2 have values lower than the control sample by 47.2% for Kordia, 48.5% for Summit, 52.4% for Simone and 65.1% for Regina, and compared to fruits dipped in T1 by 29.7% for Summit, 32% for Kordia and Regina and 34.6% for Simone.

Key words: sweet cherry, fruit cracking, galactomannan

The need to ensure a significant production of cherry fruit varieties for use in Vâlcea county zoning conditions the cherry fruit varieties were chosen for their ability to adapt to the eco-climatic conditions in the county.

The study on the behavior in culture in Vâlcea county of cherry varieties on little rootstocks has been done in Copăceni area located at latitude 45, longitude 23.983 45°0'0" North, 23°58'59" East with an area of 6.137 ha, altitude 329 m and temperate oceanic climate.

As research material we used four varieties of sweet cherry fruit (Kordia, Simone, Regina, Summit) to which was determined the degree of cracking of the fruit during ripening period.

The cracking of cherry fruit (*Prunus avium* L.) caused by rain is a problem in most producing areas of the world causing economic losses [1]. It occurs in the stylar point, vertical side, longitudinal or circular in peduncle cavity and can occur in firstfruits and ripening phenophases of fruit. Fruit cracking is a complex phenomenon and many factors seem to be involved in its production (variety, growing conditions, irrigation management, rootstock, fruit size, osmotic potential of the fruit, skin cuticular characteristics and stage of development of fruit) [2].

The underlying mechanism that causes the cracking of the fruit, although not fully understood, appears to be rapid increase in the water absorption by fruits. This increase in volume of water in the fruit may be the result of direct water absorption through the skin cuticle of the fruit or its absorption through the vascular system [3-8], making more important than rain intensity its duration and the time the fruits are soaked in water drops.

There is a variety of methods currently used to reduce fruit cracking process done in several ways [9-11]: reducing moisture of ripening fruit; reducing the osmotic potential of the fruit during wet periods; protection of fruits with different polymers that form a protective film surface of the fruit cover the crops with polyethene foil the crops; avoid irrigation before harvest etc.

The use of foliar treatments with various polymers that form a film on the surface of the fruit which act as the semi-permeable membrane, were used to culture the cherry fruit for cracking phenomenon. This paper shows the results of the research on cherry fruit concerning their behavior the foliar treatment containing natural polymer (Galactomannan extracted from *Gleditsia triacanthos*) mixed with substances that reduce the cracking process (Ca, Zn, humic acids extracted from lignite and fatty polyphenols extracted from the seeds of *Vitis vinifera*).

Experimental part

As research material we used four varieties of sweet cherry fruit (Kordia, Simone, Regina, Summit) treated 2 weeks before harvest. Three sets samples were formed:

- a control sample (witness);
- a sample treated with solution obtained of 1% calcium chloride, 1% zinc sulfate, 0,1% polyphenols extracted from *Vitis vinifera* seeds and 0.1% humic acid extracted from lignite (T1);
- a sample treated with the solution obtained of galactomannan extracted from seeds of *Gleditsia triacanthos*, 1%; calcium chloride, 1%; zinc sulfate, 1%; polyphenols extracted from *Vitis vinifera* seeds 0.1% and humic acid extracted from lignite 0.1% (T2).

Foliar treatments were applied at intervals of 4 days.

Extraction of humic acids from lignite was carried out using the method of the International Society of humic substances - IHSS.

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Extraction of polyphenols from seeds of Vitis vinifera

The seeds used to obtain the extract were purchased from wine producers in the region. To extract the seeds were subjected to a fry operation [12,13] at 220°C for 15 min in a Rombat type toaster and crushed in a Viacenza 200 machine. Extraction was performed in a spherical reactor having a capacity of 50 L using water as a solvent at a temperature of 80°C. The resultant extract was filtered through a fabric filter and subject to a film evaporation process.

Extraction of Galactomannan from Gleditsia triacanthos seeds

The polysaccharide extraction of Gleditsia triacanthos, was performed with ethanol and distilled water. In this process, the seeds were removed from the pods, cleaned and placed in a blender, where they were mechanically broken. Following this operation, the endosperm was manually separated from the germ and the hull, suspended in ethanol (purity 99.8%) in a proportion 1:3 (seeds:ethanol) at 70°C during 15 min to inactivate the enzymes and eliminate low-molecular-weight compounds [14,15]. The ethanol was decanted and distilled water was added in a proportion of 1:5 (endosperm:water), the suspension was left to rest for approximately 24 h. Then water, in a proportion of 1:10, (suspension:water) was added and mixed in a blender for 5 min. [16].

The endosperm mixed in the blender was filtered through a nylon net followed by a centrifugation step at 3.800 rpm during 20 min at 20°C. The precipitation of the galactomannan was achieved by adding the supernatant to ethanol (purity 99.8%) a ratio of 1:2. The ethanol was decanted and the precipitated galactomannan was lyophilized and kept in a dry place until further use [16].

Kinetics of water absorption on cherry fruits

Cherry fruit water absorption was determined by mass change measured during fruit dipped in solutions used for treatment until the time of the first signs of cracking.

Determination of cracking index in sweet cherry fruits

50 uniform and well developed cherries of each variety were hervested. They were immersed in 2 L of solution used in the treatment at a temperature of about 20°C. Observations were made at 2; 4 and 6 h after immersion [17,18]. Cracking index was determined by the formula:

$$IP = \frac{(5a + 3b + c).100}{250} \quad (1)$$

where:

a - number of cracked fruits after 2 h;

b - number of cracked fruits after 4 h;

c - number of cracked fruits after 6 h.

Results and discussions

Determinations to the process of water absorption in cherry fruits from Kordia, Simone, Regina and Summit cultivars using the solutions for treatment highlight significant changes in treated samples compared to the control sample as shown in figure 1.

Samples treated with T1 and dipped in the treatment solution showed an increase in the water absorption time to the control samples of 13.75% for Kordia, 20% for Summit, 25.7% for Regina and 25.7% for Simon.

Samples treated with T2 and immersed in the treatment solution showed an increase in water absorption time to the control sample of 25.7% for the Simon cultivation, 36.8% for Kordia, 36.8% for Summit and to 52.6 % for Regina.

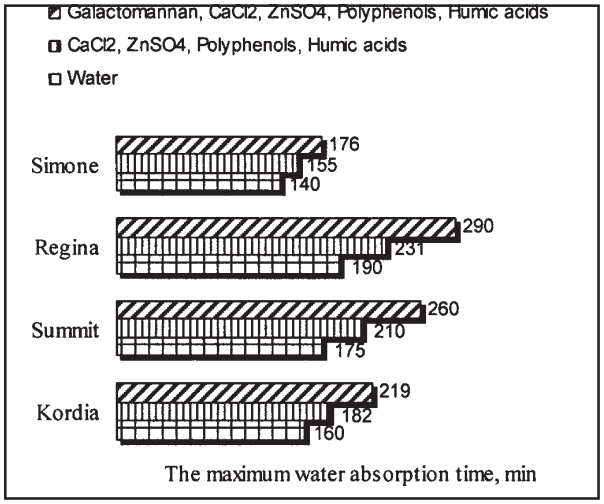

Fig. 1. The maximum water absorption time

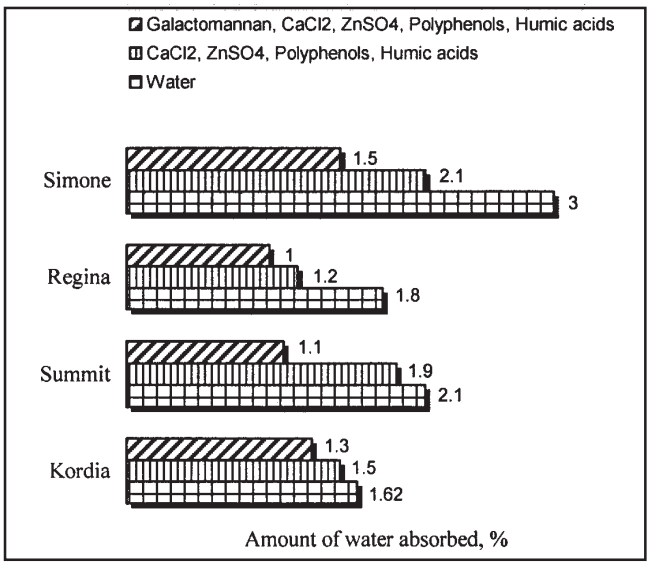

Fig. 2. Amount of water absorbed

Although the experimental data obtained revealed an increase of the duration process of water absorption in the samples treated compared to the control sample that does not adversely affect the cracking process because the absorption is slower and the maximum amount of water absorbed does not require highly the cuticle of the fruit. The amount of water absorbed is lower than the control sample as shown in figure 2.

Cultivars treated with T1 had a decrease in water consumption compared to control samples of 7.5% for Kordia, of 9.52% for Summit, 30% for Simone and 33.3% for Regina.

Cultivars treated with T2 had a decrease in water consumption compared to control samples of 19.75% for Kordia, 44.4% for Regina and 50% for Summit and Simone and compared to fruits dipped in T1 of 13.4% for Kordia, 16.7% for Regina, 28.6% for Simone and 42.2% for Summit.

The changes produced by the treatment on the maximum quantity of water absorbed and the time of water absorption have significantly influenced the cracking process of fruit. This effect is highlighted by the cracking index (IP) of fruits as shown in table 1.

Cracking Index values of fruit dipped in T1 were lower by 21.7% for Kordia, 26.7% for Simone, 27.3% for Summit and 41.3% for Regina. Fruits dipped in T2 had values lower compared to control samples by 47.2% for Kordia, 48.5% for Summit, 52.4% for Simone and 65.1% for Regina, and compared to fruits dipped in T1 by 29.7% for Summit, 32% for Kordia and Regina and 34.6% for Simone.

Cracking index (IP), %			
Cultivar	(Control)	Treatment T1	Treatment T2
Kordia	21.2	16.6	11.2
Summit	26.4	19.2	13.6
Regina	25.2	14.8	8.8
Simone	30.0	22.0	14.4

Table 1
CRACKING INDEX VALUES IN CHERRIES

The experimental results highlight the role of the composition of the treatments used which even if they are hydrophilic solutions, still penetraded the cuticle acting on the physical and chemical properties of the fruit. Researches in this area shows, that unlike classical theories that considered the cuticle that covered cells, as a barrier to the penetration of substances within the plant, on the contrary they are the main way that facilitates their transition into the plant [19]. Plasmoderma and ectoderma is a path for the transport of substances within the cells to the surface. These protoplasm routes linking between protoplasmic of neighboring cells and outer facilitate the contact with the outside and penetrate different substances. The penetration depends on the hydration state of the cell, on its turgidity, its age, the nature of the product, accompanying ion, etc.

Fruit surface penetration is a passive process conducted by the concentration difference between the surface and the interior of the fruit.

The presence of zinc in the treatment helps to maintain the integrity of biomembranes. It binds to phospholipids and membrane sulfhydryl groups and form complexes with cysteine residues in polypeptide chains and thus protecting the membrane lipids and proteins by the degradative oxidation action. Zinc controls the generation of toxic oxygen free radicals by interfering with NAPDH, and capture of oxygen radical. In agreement with this it contributes to increased cell membrane permeability [20]. It maintains the structural orientation of macromolecules maintaining ion transport [21-23].

Humic substances in the treatment act on cherry fruit membranes due to the presence of some molecular "areas" with hydrophobic and hydrophilic nature. They act with membrane phospholipids and act as carriers of nutrients in the cell [24]. Plant metabolism is enhanced immediately after application with a direct effect on the cell membrane. Thus, the humic acid molecules affects cell membrane permeability resulting in the intensification of electron transport and exchange of minerals needed in specific metabolic processes. They stabilize the cell walls by regulating cell permeability and adjust osmotic pressure.

The extract from the seeds of Vitis vinifera by high polyphenol content of 5-8% (gallic acid, flavan monomers 3-ol: catechin, epicatechin, galocatechina, epicatechin 3-O gallate, dimers, trimers and polymers procyanidins), and work 20 times more effectiveley as antioxidants than vitamin C and 50 times stronger than vitamin E [25,26], helps protect fruit biochemical components from oxidative stress occurring due to excess water absorbed and reacts against toxic oxygen free radicals that arise.

They stabilize the cell walls and plasma membranes, reduces the incidence of apical rot, regulates permeability of membrane, ensures cell extension, acid-base balance and regulates osmotic pressure of the fruit.

The presence of calcium in the solution treatment contributes to the increasing of the stability and extensibility of the cell membrane along with the other components directly involved in reducing fruit-cracking process [27-29] and in reducing leakage of ions and the cracking process [30].

Galactomannan role in the treatment composition is evidenced by reducing the cracking of cherries with values between 47.2% and 65.1% compared to the control samples on cultivars fruits dipped in T2 and 29.7% - 34.6% compared to samples immersed in T1. This difference is because besides the benefits of other components of treatment, galactomannans extracted from Gleditsiaia tricanthos seeds form a micro film with role of "fixing" it, giving it an elasticity that will oppose cracking phenomenon due to changes in fruit volume after water absorption. Micro film formed helps to protect the integrity of the cell wall acting on osmotic potential and hence on the reduction of water absorption through the cuticle. They have been used by many authors as sources of food films on the protection of food from harmful external factors [31-33]. Biopolymers films which have the advantage of providing transparency, flexibility and resistance, are transparent [34] and the polyphenol content is a source of natural phenolic and anti-oxidants compounds [16]. The experimental results are similar to data obtained by other authors [1], which considers the cultivars as tolerant to the cracking process.

Conclusions

Results of measurements after treatment with the composition of galactomannans extracted from the seeds of Gleditsiaia tricanthos with Zn, Ca, polyphenolic extract from the seeds of Vitis vinifera and humic acids recommends successful in reducing the cracking process of cherry fruit.

Micro film formed by galactomannan on the fruit surface gives it flexibility and strength capable of reducing the cracking process, and Vitis vinifera seed extract as a potent antioxidant protects the fruit from the damaging effects of oxidative stress as a result of the cracking process

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