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*Arkadiusz TELESIŃSKI, Grzegorz MIKICIUK[1], Małgorzata MIKICIUK,
Michał STRĘK, Maciej PŁATKOWSKI, Małgorzata STATKIEWICZ[1]*

# EFFECT OF PREHARVEST USE OF ANTI-CRACKING PREPARATIONS ON CHANGES IN SELECTED PARAMETERS OF SWEET CHERRY FRUITS DURING FROZEN STORAGE

# WPŁYW STOSOWANIA PRZED ZBIOREM PREPARATÓW ZAPOBIEGAJĄCYCH PĘKANIU NA ZMIANY WYBRANYCH PARAMETRÓW OWOCÓW CZEREŚNI W TRAKCIE ZAMRAŻALNICZEGO PRZECHOWYWANIA

Department of Plant Physiology and Biochemistry, West Pomeranian University of Technology Szczecin, Poland
[1]Department of Horticulture, West Pomeranian University of Technology, Szczecin, Poland

**Streszczenie.** Celem pracy było porównanie podczas zamrażalniczego przechowywania zmian zawartości suchej masy, polifenoli ogółem, flawonoidów ogółem oraz aktywności antyoksydacyjnej w owocach czereśni, w zależności od zastosowanych przed zbiorem preparatów zapobiegających pękaniu. Materiał do badań stanowiły owoce czereśni odmiany ‘Burlat’, na których zastosowano preparaty zapobiegające pękaniu owoców. W świeżych owocach oraz w trakcie ich zamrażalniczego przechowywania w temperaturze –25°C (po 60, 120 i 180 dniach) oznaczono wymienione parametry. Po zastosowaniu wszystkich preparatów zapobiegających pękaniu w świeżych owocach czereśni wystąpiło zwiększenie zawartości flawonoidów ogółem oraz aktywności antyoksydacyjnej. W trakcie zamrażalniczego przechowywania owoców, na których nie zastosowano preparatów zapobiegających pękaniu, zaobserwowano systematyczny spadek oznaczanych parametrów antyoksydacyjnych. Zastosowanie przed zbiorem osmoregulatorów i antytranspirantów w czasie zamrażalniczego przechowywania zwiększyło spadek zawartości flawonoidów ogółem i aktywności antyoksydacyjnej, ograniczyło natomiast zmiany zawartości suchej masy oraz polifenoli ogółem. Analiza wartości parametrów kinetycznych degradacji podczas przechowywania mrożonych czereśni wykazała, że zawartość flawonoidów ogółem charakteryzowała się większą stabilnością w owocach, na których nie zastosowano preparatów zapobiegających pękaniu. Odwrotną tendencję stwierdzono w przypadku polifenoli ogółem.

**Key words:** sweet cherries, freezing, anti-transpirants, glycine betaine, antioxidants.
**Słowa kluczowe:** czereśnie, mrożenie, antytranspiranty, betaina glicynowa, antyoksydanty.

## INTRODUCTION

Markets have increased the requirements for sweet cherry fruits. Fruit cracking constitutes a serious problem in sweet cherry cultivation. Thus, many studies have been conducted to explain the reasons and the mechanism of cracking that would limit the arising losses (Chełpiński et al. 2007). Currently, the producers are forced to do operations, that maintain

Corresponding author – Adres do korespondencji: Arkadiusz Telesiński, Department of Plant Physiology and Biochemistry, West Pomeranian University of Technology, Szczecin, Juliusza Słowackiego 17, 71-434 Szczecin, Poland, e-mail: arkadiusz.telesinski@zut.edu.pl

their competitiveness in the national and global markets (Szot et al. 2013). To limit fruit cracking, the use of anti-transpirants and osmotic regulators has gained popularity (Mikiciuk et al. 2013).

One of the most commonly used food (including fruits) preservation methods is freezing (Białas et al. 2004). Apart from the low temperature, the preserving factor of the process is also an increase of water activity due to phase transition of water into ice, which occurs during freezing (Pham 2008). Despite the fact that sweet cherries are the fruit of extremely delicacy, intended for direct consumption, many people want to extend their enjoyment of food by freezing.

However, the frequent situation that occurs during defrosting, especially of soft fruits, is the decrease in the firmness and juice leakage. Therefore, it accompanies the factors such as loss of soluble nutrients and flavors and fragrances. This phenomenon occurs due to the damage of fruit tissue structure caused by ice crystals, which are formed during freezing. The level of tissue damage primarily depends on the freezing rate, amount of available water, and storage temperature (Białas et al. 2004; Gomez and Sjoholm 2004).

The preharvest use of fruit anti-cracking preparations, including osmotic regulators and anti-transpirants, aims at decreasing the cell turgor pressure. Glycine betaine is a natural cell product with an osmotic protective effect (Hoffmann et al. 2013). Vapor Gard, containing pinolene, forms thin, elastic film protecting the sprayed fruits against the influence of unfavorable external factors. It has also been demonstrated that the preparation with Vapor Gard can improve storage properties of stone fruits. Calcium is one of the main elements responsible for firmness, and it also influences the storage life of fruits (Ochmian 2012).

The objective of the study was to compare the changes in dry weight content, total polyphenols content, total flavonoids content, and antioxidant activity in sweet cherry fruits of the 'Burlat' cultivar, depending on the used preharvest anti-cracking preparations.

## MATERIAL AND METHODS

The study material was sweet cherry fruits of the 'Burlat' cultivar, harvested from 16-year improved trees on the root stock PHL-A, in the span of 4 × 3 m in Karwowo near Szczecin, Poland. The experiment was established in a split-block design and in five replications. During the experiment, the following anti-cracking preparations were used: Greenstim (physioactivator-osmotic regulator containing 97% glycine betaine) at a dosage of 4 kg · $ha^{-1}$ (with foliar application three weeks prior harvest), $CaCl_2$ at a concentration of 0.3% (with foliar application three weeks prior harvest), and anti-transpirant Vapor Gard (96% pinolene obtained from resin of Canadian pine) at a concentration of 0.75% (administered in the fruit coloring phase); these were used in the following combinations: control (sprayed with distilled water), Greenstim, Greenstim + $CaCl_2$, Greenstim + Vapor Gard, Greenstim + $CaCl_2$ + Vapor Gard. The fruits were collected on June 24, 2013.

The fruits were initially washed, cleaned, and then preserved by freezing in a freezer room at temperature –25°C. Portions of 10 fruits intended for freezing were placed in polyethylene bags, then frozen and stored at temperature –25°C. Prior determinations, the fruits were defrosted at room temperature.

Dry weight content, total polyphenols content, total flavonoids content, and antioxidant activity were determined in fresh fruits and during their frozen storage (after 60, 120, and 180 days). Dry weight was determined using the method of drying till a constant weight was obtained at temperature 105°C. Total polyphenol content was determined according to Yu et al. (1995) method with Folin–Ciocalteu reagent, the total flavonoid content was determined using the Kumaran and Karunakaran (2007) method with $AlCl_3$. Antioxidant activity, consisting in scavenging of the DPPH radical, was determined using the procedure described by Yen and Chen (1995).

The obtained results were statistically elaborated using a two-factor variance analysis. The least significant differences (LSD) were calculated following Tukey's procedure at the significance level α = 0.05. The Statistica 10.0 software by StatSoft and Excel 2013 spreadsheet were used for statistical analyses.

Because there was a drop in the determined total polyphenol and total flavonoid content during the frozen storage, kinetic parameter values of these antioxidant components were also calculated using formulas provided by Pukszta (2013):

- constant rate of first-order changes:

$$k = \frac{1}{t}\ln\frac{C_0}{C}$$

where:

$t$ – time [days],

$C_0$ – initial antioxidant content,

$C$ – antioxidant content after time $t$;

- half-life time for first-order changes:

$$g = \frac{0.693}{k}$$

## RESULTS AND DISCUSSION

After the use of all anti-cracking preparations in fresh sweet cherry fruits, an increase in the total flavonoids content, and antioxidant activity was determined (Tables 1 and 2). Following the literature data, the use of anti-transpirant Vapor Gard did not have an effect on the micro nutrient concentration in the fruits of different sweet cherry cultivars, but led to an increase of manganese, copper, zinc, nickel, and cadmium content in the leaves (Jiménez et al. 2004; Mikiciuk et al. 2013). On the contrary, in our study, an increase of dry weight content after administering the combination with anti-transpirant Vapor Gard was determined (Table 1), whereas total polyphenol content was at its highest level in fresh leaves after the use of Greenstim only (Table 2). Karjalainen et al. (2002) determined the highest catechin, gallic acid, quercetin, and kaempferol concentration in strawberry fruits, which were treated with osmotic regulators: glycine betaine and benzothiadiazole. As Ochmian (2012) states, in the majority of cases, after the foliar application of calcium fertilizers, the polyphenol and ascorbic acid content was decreased in the fruits of the northern highbush blueberry.

Table 1. Changes of dry weight in sweet cherry fruits induced anti-cracking preparation and frozen storage
Tabela 1. Zmiany zawartości suchej masy w owocach czereśni w zależności od preparatów zapobiegających pękaniu oraz czasu zamrażalniczego przechowywania

| Combination of preparations<br>Kombinacje preparatów<br>(B) | Time of frozen storage [days]<br>Czas zamrażalniczego przechowywania [dni]<br>(A) | | | | |
|---|---|---|---|---|---|
| | 0 | 60 | 120 | 180 | mean<br>średnia |
| Control – Kontrola | 13,02 | 12,42 | 12,34 | 12,03 | 12.45 |
| Greenstim | 12,23 | 12,25 | 12,22 | 11,43 | 12.03 |
| Greenstim + $CaCl_2$ | 12,16 | 12,08 | 12,02 | 11,91 | 12.04 |
| Greenstim + Vapor Gard | 14,07 | 14,05 | 13,47 | 13,65 | 13.81 |
| Greenstim + $CaCl_2$ + Vapor Gard | 13,91 | 13,51 | 13,31 | 12,98 | 13.43 |
| Mean – Średnia | 13.08 | 12.86 | 12.67 | 12.40 | 12.75 |
| $LSD_{0.05}$ – $NIR_{0,05}$ | A = 0.161<br>A × B = 0.199 | | B = 0.113<br>B × A = 0.179 | | |

Table 2. Changes of antioxidant parameters in sweet cherry fruits induced anti-cracking preparation and frozen storage
Tabela 2. Zmiany parametrów antyoksydacyjnych w owocach czereśni w zależności od preparatów zapobiegających pękaniu oraz czasu zamrażalniczego przechowywania

| Combination of preparations<br>Kombinacje preparatów<br>(B) | Time of frozen storage [days]<br>Czas zamrażalniczego przechowywania [dni]<br>(A) | | | | |
|---|---|---|---|---|---|
| | 0 | 60 | 120 | 180 | mean<br>średnia |
| Total polyphenols [mg gallic acid · $kg^{-1}$ dm]<br>Polifenole ogółem [mg kwasu galusowego · $kg^{-1}$ s.m.] | | | | | |
| Control – Kontrola | 787.55 | 654.45 | 624.02 | 585.55 | 737.89 |
| Greenstim | 908.19 | 904.59 | 803.55 | 671.05 | 821.85 |
| Greenstim + $CaCl_2$ | 838.28 | 778.17 | 626.14 | 554.83 | 699.36 |
| Greenstim + Vapor Gard | 769.86 | 739.27 | 626.43 | 626.82 | 690.60 |
| Greenstim + $CaCl_2$ + Vapor Gard | 764.86 | 677.77 | 624.53 | 644.12 | 677.82 |
| Mean – Średnia | 813.75 | 750.85 | 680.93 | 656.47 | 725.50 |
| $LSD_{0.05}$ – $NIR_{0,05}$ | A = 1.928<br>A × B = 8.325 | | B = 6.285<br>B × A = 9.563 | | |
| Total flavonoids [mg quercetin · $kg^{-1}$ dm]<br>Flawonoidy ogółem [mg kwercetyny · $kg^{-1}$ s.m.] | | | | | |
| Control – Kontrola | 129.78 | 102.68 | 103.61 | 91.54 | 106.90 |
| Greenstim | 180.01 | 110.80 | 102.56 | 97.18 | 122.64 |
| Greenstim + $CaCl_2$ | 167.65 | 104.24 | 101.60 | 68.19 | 110.42 |
| Greenstim + Vapor Gard | 142.57 | 108.49 | 108.44 | 73.18 | 108.17 |
| Greenstim + $CaCl_2$ + Vapor Gard | 153.82 | 132.80 | 123.10 | 82.44 | 123.04 |
| Mean – Średnia | 154.77 | 111.80 | 107.80 | 82.51 | 114.23 |
| $LSD_{0.05}$ – $NIR_{0,05}$ | A = 6.325<br>A × B = 9.632 | | B = 7.083<br>B × A = 9.832 | | |
| Antioxidant activity [% DPPH]<br>Aktywność antyoksydacyjna [% DPPH] | | | | | |
| Control – Kontrola | 33.37 | 32.58 | 31.28 | 26.33 | 30.89 |
| Greenstim | 38.29 | 34.99 | 31.61 | 30.54 | 33.86 |
| Greenstim + $CaCl_2$ | 38.56 | 35.54 | 33.32 | 30.63 | 34.51 |
| Greenstim + Vapor Gard | 39.38 | 38.97 | 34.67 | 30.27 | 35.85 |
| Greenstim + $CaCl_2$ + Vapor Gard | 40.41 | 38.42 | 36.00 | 34.46 | 37.32 |
| Mean – Średnia | 38.00 | 36.10 | 33.71 | 31.11 | 34.73 |
| $LSD_{0.05}$ – $NIR_{0,05}$ | A = 1.583<br>A × B =1.832 | | B = 1.320<br>B × A = 1.924 | | |

During frozen storage of sweet cherry fruits, which were not treated with anti-cracking preparations, a systematic decrease of dry weight content, total polyphenols, total flavonoids, and antioxidant activity was observed, which after 180 days of storage amounted to 8%, 26%, 29%, and 11%, respectively. It corroborates with the studies of other scientists on the changes of the biological value of different species vegetables (Daood et al. 1996; Lisiewska et al. 2009), fruits (Ścibisz et Mitek 2007; Pukszta 2013), or herbs (Telesiński et al. 2013) under the influence of frozen storage. A certain group of authors state that the processes that take place during freezing always lead to an inevitable quality loss of frozen products (Buggenhout et al. 2006; Pukszta 2013), while other authors believe that the freezing process, compared to other food processing methods, allows for maintaining high nutritional value of fruits and vegetables (Fik et al. 2008; Lisiewska et al. 2009; Ellong et al. 2015).

By comparing the changes in the determined parameters, compared to values obtained from fruits directly after harvest, it was determined that the preharvest use of osmotic regulators and anti-transpirants during frozen storage deepened the flavonoid content and antioxidant activity drop but limited the changes of dry weight content and total polyphenols content.

By determining the order of changes of the determined sweet cherry fruit parameters during frozen storage, it was found that only total polyphenol content, total flavonoid content, and antioxidant activity were subjected to changes following the first-order reaction. For the changes of total polyphenols content and total flavonoids content, the absolute constant ($k$) and half-life ($g$) for the first-order rate were calculated. As stated by Pukszta (2013), basing on the values of constant ($k$) for the first-order rate, the intensity of changes occurring in a product can be determined. The analysis of $k$ value has demonstrated that during storage of frozen sweet cherries, the total flavonoids content was characterized by higher stability in the fruits, which were not treated with anti-cracking preparations. An opposite tendency was determined in the case of total polyphenols content (Table 3).

Table 3. Values of degradation kinetic parameters chosen antioxidant compounds in frozen sweet cherries treated with anti-cracking preparations
Tabela 3. Wartości parametrów kinetycznych degradacji wybranych substancji antyoksydacyjnych w mrożonych czereśniach opryskanych przed zbiorem preparatami zapobiegającymi pękaniu

| Combination of preparations<br>Kombinacje preparatów | Total polyphenols<br>Polifenole ogółem | | Total flavonoids<br>Flawonoidy ogółem | |
|---|---|---|---|---|
| | $k \cdot 10^{-3}$ | $g$<br>[days – dni] | $k \cdot 10^{-3}$ | $g$<br>[days – dni] |
| Control – Kontrola | 2.22 | 311.6 | 2.57 | 269.3 |
| Greenstim | 0.92 | 751.2 | 5.40 | 128.3 |
| Greenstim + $CaCl_2$ | 1.99 | 348.0 | 5.70 | 121.6 |
| Greenstim + Vapor Gard | 1.18 | 588.0 | 3.51 | 197.3 |
| Greenstim + $CaCl_2$ + Vapor Gard | 1.55 | 446.3 | 2.59 | 267.5 |

The definition of half-life time ($g$) for the first-order rate states that it is inversely proportional value to the intensity of the reaction and constant rate (Szot et al. 2013), which is corroborated by own study results. After the analysis of $g$ value, it was determined that the preharvest use of anti-cracking preparations increased the $g$ value for total polyphenols content and decreased for total flavonoids content.

The research topic reveals some reports on limiting the quality drop in fruits during frozen storage. Białas (2004) demonstrated that coating strawberries with maltodextrin film prior to the freezing process limited the juice leakage during defrosting, positively influencing their quality. On the contrary, frozen storage of strawberries with an addition of cryoprotective substances (ascorbic acid and pectins) caused an increase in total polyphenols content and antioxidant activity (Kolniak 2008).

## CONCLUSIONS

1. The preharvest use of glycine betaine (Greenstim), $CaCl_2$, and anti-transpirant Vapor Gard (containing pinolene) significantly influenced the changes of dry weight content, total polyphenols content, total flavonoids content, and antioxidant activity of sweet cherry fruits of the 'Burlat' cultivar.
2. After the use of all anti-cracking preparations in fresh fruits of sweet cherry, an increase in total flavonoids content, and antioxidant activity was occurred.
3. During frozen storage of the sweet cherry fruits, which were not treated with the anti--cracking preparations, a systematic decrease in the determined parameters was observed. The preharvest use of osmotic regulators and anti-transpirants during frozen storage deepened the decrease of total flavonoids content and antioxidant activity and limited the changes of dry weight content and total polyphenols content.
4. The analysis of the kinetic degradation parameters during frozen storage of sweet cherries demonstrated that total flavonoids content was characterized by higher stability in the fruits, which were not treated with anti-cracking preparations. An opposite trend was determined for total polyphenols content.

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**Abstract.** This paper presents the study on comparison of the changes of dry weight content, total polyphenols content, total flavonoids content, and antioxidant activity in sweet cherry fruit in frozen storage, depending on the used preharvest anti-cracking preparations. The study material was sweet cherry fruits of the 'Burlat' cultivar, that were treated with anti-cracking preparations. In fresh fruits and during their frozen storage at temperature –25°C (after 60, 120, and 180 days), the mentioned parameters were determined. The preharvest use of anti--cracking preparations caused an increase in total flavonoids content, and antioxidant activity was observed. In plant untreated anti-cracking preparations, decrease in antioxidant parameters were observed. The preharvest use of during frozen storage deepened the decrease of total flavonoids content and antioxidant activity but limited the changes in the content of dry weight and total polyphenols. The analysis of the kinetic degradation demonstrated that total flavonoid content was characterized by higher stability in the fruits, which were not treated with anti--cracking preparations. An opposite trend was determined for total polyphenols content.