New non-chemical postharvest technologies reducing berry contamination

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Abstract

One of the most important postharvest diseases of strawberry and raspberry is grey mould, caused by Botrytis cinerea Pers.: Fr. New non-chemical food safety technologies reducing berry rots and extending storage time were tested. Photosensitization is an innovative method for inhibition of pathogens in berry and is based on the interaction of light (λ = 400 nm, 20 mW cm⁻²) and photoactive compound (chlorophyllin derivative). The aim of this study was to evaluate the effect of the chlorophyllin-based photosensitization on the reduction of grey mould (B. cinerea) in postharvest strawberry and raspberry fruits. The berries were soaked in a treatment solution for 1 hour and then exposed to prototype (light) for 30 minutes. The obtained data indicated that chlorophyllin derivative inhibited grey mould infection on fruits. The infection of B. cinerea in strawberries after a 6-day storage decreased by 8% and in raspberries after 1-day storage it decreased by 3%. The chlorophyllin had no impact on the visual quality and nutritional properties of strawberries and raspberries.

Key words: Botrytis cinerea, chlorophyllin derivative, photosensitization, raspberry, strawberry.

Introduction

Raspberries and strawberries, which are consumed fresh and processed, are the most popular soft fruits in the world and Lithuania (Uselis et al., 2008). Berries contain many vitamins, minerals, and a high content of phenolic compounds, other antioxidants and bioactive compounds (Viskelis et al., 2010; Bobinaitė et al., 2013). Phenolic compounds and anthocyanins are regarded as healthy, natural and functional components in human nutrition (Bobinaitė et al., 2012; Lukšiene et al., 2013).

Fungal and bacterial infections are the main reason for 50% or greater yield losses of soft fruits during postharvest storage (Botrytis..., 2007). The initial strategy for reducing postharvest losses is appropriate agrotechnical handling and chemical pesticides (Droby, Lichter, 2007; Droby et al., 2009).

One of the most important postharvest diseases of soft fruits is grey mould caused by Botrytis cinerea Pers.: Fr., which results in a yield reduction ranging from 15% to 50% (Staats et al., 2005; Droby, Lichter, 2007). The B. cinerea infects more than 200 host plants worldwide (Staats et al., 2005). Symptoms of grey mould vary depending on the host, infection starts through flowers, various injuries, cracks and cuts. The pathogen has the capability to remain quiescent in unripe tissue and disease symptoms develop in ripe fruit. In berries, grey mould causes preharvest and postharvest fruit losses (Staats et al., 2005; Botrytis..., 2007; Droby et al., 2009).

Conventionally grey mould (B. cinerea) in strawberries is controlled by routine applications of fungicides once a week during flowering. Significant amounts of pesticides against diseases are used during the growing season of strawberries (Staats et al., 2005; Botrytis..., 2007). This type of application has become unacceptable because the inadequate usage of pesticides leads to the pathogen resistance (Botrytis..., 2007; Droby et al., 2009). Using forecasting models, applications of fungicides are made only when the model shows favourable conditions for B. cinerea development (Valiuškaitė et al., 2010; Rasiukevičiūtė et al., 2013). The cultivation of strawberries on plastic mulch had a positive effect on reducing fruit rot (Uselis et al., 2008). It is very expensive to use chemical fungicides and they have a negative impact on human health and environment (Botrytis..., 2007; Lukšiene, Zukauskas, 2009).

Photosensitization is an innovative method for eliminating fruit pathogens based on simultaneous use of light and a photosensitizer. Chlorophyllin-photoactive compound is known as a photosensitizer, it is a water-soluble food additive (E140), and chlorophyllin derivative is safe as well (Lukšiene, 2005; Lukšiene, Zukauskas, 2009; Lukšiene et al., 2010; Lukšiene, Brovkov, 2013; Lukšiene, 2014). The data presented in Lukšiene and Paskevičiute (2011) research clearly indicated that food pathogens and moulds in lab-scale experiments were
effectively removed from the surface of strawberries. The aim of this study was to evaluate the effect of the chlorophyllin-based photosensitization on the reduction of grey mould (*B. cinerea*) in postharvest strawberry and raspberry fruits.

**Materials and methods**

**Experiments.** The experimental protocol for chlorophyllin-based photosensitization decontamination of berries was elaborated in Vilnius University, Institute of Applied Research. The effect of the photosensitization of chlorophyllin derivative (Child) (*‘Alfa Aesar’, Germany*) on decontamination of berries was investigated at the Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry in 2012 and 2013. In the experiments, we evaluated the effect of postharvest photosensitization on strawberry (*Fragaria × ananassa Duch.*) cv. ‘Darselect’ and raspberry (*Rubus idaeus L.*) cv. ‘Polka’. The illumination experiments and assessment of diseased fruit were conducted in the Laboratory of Plant Protection. The quality tests were done in the Laboratory of Biochemistry and Technology. The raspberry (August 2012) and strawberry (June 2013) fruit samples were collected in the experimental field of Institute of Horticulture. The experimental design of treatments is provided in Table 1.

**Table 1.** The experimental design of postharvest treatment of strawberries and raspberries

<table>
<thead>
<tr>
<th>No.</th>
<th>Treatment</th>
<th>Treatment abbreviation</th>
<th>Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Untreated and not illuminated</td>
<td>control</td>
<td>–</td>
</tr>
<tr>
<td>2.</td>
<td>Treated with water solution of chlorophyllin derivative + illuminated by prototype</td>
<td>Chld 1.5 × 10⁻³ M</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Treated with sterile distilled water + illuminated by prototype</td>
<td>water</td>
<td>–</td>
</tr>
</tbody>
</table>

**Illumination.** The LED-based light source prototype for photosensitization was constructed in the Institute of Applied Research of Vilnius University (Fig. 1 B). The InGaN light emitting diode (LED) array Lz1-00U/A00 (LED Engine Inc., USA) was used as a source of the light. The source of the light consisted of an illumination chamber and a supply unit (Luksiene, Zukauskas, 2009; Luksiene, 2014). The berries used in the experiments were of the same maturity, size and free of physical damage. Visually healthy fruits were soaked in chlorophyllin derivative (Chld) or water solution for 1 hour. Dehumidified fruits were placed on a sterile tray in a chamber of light source and were exposed for 30 minutes (15 min one side and 15 min – the other side). LED-based prototype emitted light in the λ = 400 nm with the intensity of 20 mW cm⁻² on the surface of fruits. The InGaN light emitting diodes in the prototype ranged from the top of the illumination chamber. The control treatment was not soaked in Chld or water and not illuminated. After treatment, fruits were stored in a climate chamber KBF720 (‘Binder’, German) at a temperature of 5–7°C.

**Figure 1.** Strawberries and raspberries infected by *Botrytis cinerea* (control – untreated) (A, B), light source (illumination chamber) (C), strawberries after the application of chlorophyllin derivative and illumination (D)
to photosensitization (Luksiene, 2005; Luksiene, Paskeviciute, 2011). Benzimidazoles fungicides were introduced for plant disease control in the 1960’s, but within a few years resistance emerged (Kretschmer, Hahn, 2008; Debieu et al., 2013; Rodriguez et al., 2014). Our research was carried out to test the new non-chemical measures reducing B. cinerea on berry fruits. The photosensitization or volatile compound-based plant protection does not cause pathogen resistance (Neri et al., 2014; Rodriguez et al., 2014).

According to our analysis, treatment with photosensitization can reduce the contamination of berries with B. cinerea and prolong the disease-free period of fruits (Figs. 2–3). In our investigations, B. cinerea was determined as only one causal agent of fruit rots. Experimental data revealed that 6 days after the Chld treatment, the number of Botrytis infected strawberries reduced by 8% and the number of disease-free strawberries increased by 6% compared with the control (Fig. 2). After 6 days of storage, in the Chld treatment there were 15% less Botrytis-infected strawberries compared with water treatment. After an 8-day storage, in the Chld treatment there were 20% and 5% more healthy fruits compared with the control and water treatment. In water treatment, the incidence of B. cinerea after a 6-day and 8-day storage was 17% and 16% higher compared to the Chld treatment (Fig. 2). The experimental results revealed that in Chld treatment there were less infected fruits (8% and 13%) compared to the control.

Experimental data showed that after a 6-day and 8-day storage in water treatment, the number of Botrytis infected strawberries increased by 9% and 3% in comparison with the control (Fig. 2). After a 6-day and 8-day storage, the B. cinerea frequency of occurrence in Chld treatment was slightly lower (2% and 3.25%) compared with the control. In Chld treatment, after a 6-day and 8-day storage, B. cinerea frequency of occurrence was 4.25% and 2.5% lower compared with the water treatment. According to our experiments, we can conclude that in Chld treatment, after 6 days of storage, B. cinerea was reduced by 8%.

Raspberries are highly perishable fruit. During storage, they lose firmness and change colour. The nutrition value of raspberries is very high, phenolic compounds are a major group of phytochemicals found in berries (Verde et al., 2013).

The experiments on raspberries revealed, that Chld treatment reduced B. cinerea by 3% (after 1-day storage) and by 4% (after 3-day storage), respectively, compared with the control (Fig. 3). There were 6% (after 1-day storage) and 54% (after 3-day storage) less infected fruits in Chld treatment, compared with water treatment. The comparison of Chld treatment with the control and water treatments revealed, that there were 3% and 5% more healthy fruits after 1-day of storage. Our experimental data indicated that after a 3-day of storage, in Chld treatment, there were 7% and 55% more healthy fruits, compared with the control and water treatment. After 1-day and 3-day storage B. cinerea frequency of occurrence in Chld treatment was lower (1% and 2%) compared with the control. After 1 and 3 days, in Chld treatment B. cinerea frequency of occurrence was 2% and 13% lower compared with water treatment (Fig. 3). The analysis of both experiments showed the same tendency for raspberries and strawberries, – the Chld treatment reduced B. cinerea and water treatment stimulated it.

The amount of anthocyanins in berries was determined by a pH differential method and expressed as cyanidin-3-glucoside equivalents (µmol 1 g−1 berry).

Anthocyanin concentration was calculated according to the formula:

\[
\frac{(A_{600 \text{ nm}} - A_{700 \text{ nm}})}{\varepsilon \cdot L} \cdot MW \cdot k \cdot e,
\]

where \( \varepsilon \) – molar absorption (the coefficient of molar extinction), \( L \) – thickness of the test solution (cm), \( MW \) – molecular weight of cyanidin-3-glucoside, \( k \) – dilution factor, \( A \) – absorption. All measurements were carried out three times and the results were presented as mean.

Statistical analysis. The pathogens were identified according to the morphological traits, which were typical of the colonies (Vidhyasekaran, 2004; Botrytis..., 2007). The data were analysed with a module ANOVA of the software STATISTICA 7.0. The standard deviation was calculated and marked in a figure as bars.

Results and discussion

Postharvest diseases cause a great loss of fruits during the process of transportation and storage. Chemical fungicides are the main strategy for controlling postharvest losses (Sharma et al., 2009). Various studies of non-chemical treatments, including modified atmosphere with high CO2, volatile compounds, edible coatings, UV-C and others, were conducted in order to prolong the postharvest period of soft fruits. Nigro et al. (2000) found that UV-C 254 nm reduces postharvest decay on strawberries. Neri et al. (2014) ran a test on thirty-five strawberry volatile organic compounds on B. cinerea in vitro and found that 14 compounds significantly influenced the conidial germination of B. cinerea. Tezotto-Uliana et al. (2014) ran a test on pre and post-harvest chitosan coating in order to prolong the shelf life of raspberries.

The data obtained in our previous study revealed that chlorophyllin-based treatment reduced food pathogen Listeria monocytogenes by 7-log in vitro (Luksiene et al., 2010). Luksiene and Brovko (2013) found that chlorophyllin reduced Listeria and Bacillus populations by 4.5-log and 5-log cycles, respectively. The data obtained in previous experiments clearly indicate that food pathogens can be easily destroyed by photosensitization on the surfaces of food products, also on strawberry fruits (Luksiene, 2005; Luksiene et al., 2010; Luksiene, Paskeviciute, 2011; Luksiene, Brovko, 2013). It is known that B. cinerea can rapidly adapt to the environment and develop resistance, but it is susceptible to photosensitization (Luksiene, 2005; Luksiene, Paskeviciute, 2011). Benzimidazoles fungicides were introduced for plant disease control in the 1960’s, but within a few years resistance emerged (Kretschmer, Hahn, 2008; Debieu et al., 2013; Rodriguez et al., 2014). Our research was carried out to test the new non-chemical measures reducing B. cinerea on berry fruits. The photosensitization or volatile compound-based plant protection does not cause pathogen resistance (Neri et al., 2014; Rodriguez et al., 2014).

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Figure 2. The inactivation of *Botrytis cinerea* by non-chemical chlorophyllin derivative (Chld) treatment in strawberries

Figure 3. The inactivation of *Botrytis cinerea* by non-chemical chlorophyllin derivative (Chld) treatment in raspberries

The control of postharvest infections caused by *B. cinerea* relies on the preharvest applications. Nowadays chemical pesticides are major products used for applications against *B. cinerea* (Droby, Lichter, 2007; Droby et al., 2009). Due to the nontoxic properties of photosensitization, this treatment can be applied for microbial control as well (Luksiene et al., 2010). Recent studies of Tezotto-Uliana et al. (2014) showed that different chitosan concentrations inhibit the growth of pathogens by up to 88.9%.

The amount of soluble solids, vitamin C, total phenolic, anthocyanins content, antioxidant activity and firmness in strawberries were evaluated as well (Table 2). The results presented in Table 2 suggest that the chemical composition and firmness of strawberries among the treatments varied a little, but the treatment itself did not have a statistically significant effect. The total phenolic compounds in the control strawberries was 273.7 ± 8.8 mg 100 g⁻¹, whereas in the Chld-treated it was 270.0 ± 12.5 mg 100 g⁻¹. The level of anthocyanins in strawberries after the Chld treatment was 158.8 ± 10.48 mg 100 g⁻¹ and similarly in the control 153.8 ± 8.28 mg 100 g⁻¹. The data presented in Table 2 indicated that the average firmness of Child-treated strawberries was 16.02 ± 0.33 N cm⁻².

The firmness of the control strawberries was 16.66 ± 0.55 N cm⁻². The colour coordinates of strawberries, compared with the control treatment, showed a very

Table 2. The influence of non-chemical treatment on the colour and chemical composition of strawberries

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fruit firmness</th>
<th>Ascorbic acid</th>
<th>Anthocyanins</th>
<th>Soluble solids</th>
<th>Total phenolic compounds</th>
<th>DPPH µmol 1 g⁻¹ berries</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N cm⁻²</td>
<td>mg %</td>
<td>mg 100 g</td>
<td>%</td>
<td>mg 100 g⁻¹</td>
<td>1 g⁻¹ berries</td>
</tr>
<tr>
<td>Control</td>
<td>16.66 ± 0.55</td>
<td>70.3 ± 1.2</td>
<td>153.83 ± 8.28</td>
<td>10.9 ± 0.2</td>
<td>273.7 ± 8.8</td>
<td>14.85 ± 0.14</td>
</tr>
<tr>
<td>Chlorophyllin derivative (Chld)</td>
<td>16.02 ± 0.33</td>
<td>70.7 ± 2.5</td>
<td>158.83 ± 10.48</td>
<td>10.7 ± 0.2</td>
<td>270.0 ± 12.5</td>
<td>14.74 ± 0.06</td>
</tr>
</tbody>
</table>

Colour parameters

<table>
<thead>
<tr>
<th></th>
<th>L*</th>
<th>C*</th>
<th>h°</th>
<th>a*</th>
<th>b*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(lightness)</td>
<td>(chroma)</td>
<td>(hue angle)</td>
<td>(+ redness)</td>
<td>(+ yellowness)</td>
</tr>
<tr>
<td>Control</td>
<td>36.27 ± 3.98</td>
<td>38.58 ± 3.00</td>
<td>33.76 ± 4.12</td>
<td>31.93 ± 1.86</td>
<td>21.50 ± 3.62</td>
</tr>
<tr>
<td>Chlorophyllin derivative (Chld)</td>
<td>32.47 ± 3.03</td>
<td>38.01 ± 4.22</td>
<td>30.91 ± 3.99</td>
<td>32.44 ± 2.84</td>
<td>19.65 ± 4.07</td>
</tr>
</tbody>
</table>

Note. The results are expressed as mean ± standard deviation.
slight, statistically insignificant difference as well, which led to the conclusion that the use of Chld did not have any influence on the colour of strawberries. Colour is the main indicator of fruit quality, which has an influence on the appearance and price (Luksiene et al., 2013).

In order to estimate specific changes of nutritional quality of raspberries, we evaluated the amount of ascorbic acid, total phenolic compounds, soluble solids, DPPH and flavonoids anthocyanins (Table 3). The raspberry fruit firmness depends on the variety, berry maturity, environmental conditions, processing, transportation and others factors (Verde et al., 2013). It was difficult to evaluate the influence of treatments on the disease incidence because raspberries are very fast perishable fruits (data are not shown).

Table 3. The influence of non-chemical treatment on the chemical composition of raspberries

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ascorbic acid mg %</th>
<th>Anthocyanins mg 100 g</th>
<th>Soluble solids %</th>
<th>Total phenolic compounds mg 100 g⁻¹</th>
<th>DPPH µmol 1 g⁻¹ berries</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>27.5 ± 1.8</td>
<td>81.4 ± 5.8</td>
<td>9.8 ± 0.3</td>
<td>311.4 ± 9.8</td>
<td>18.8 ± 2.2</td>
</tr>
<tr>
<td>Chlorophyllin derivative (Chld)</td>
<td>27.7 ± 1.7</td>
<td>81.0 ± 4.7</td>
<td>9.6 ± 0.4</td>
<td>314.6 ± 11.5</td>
<td>18.1 ± 2.6</td>
</tr>
</tbody>
</table>

Note. The results are expressed as mean ± standard deviation.

Concerning ascorbic acid content, total anthocyanin and soluble solids, the photosensitization treatment had no impact on their amount in treated raspberries. The amount of total phenolic compounds in raspberries in the control treatment was 311.4 ± 9.8 mg 100 g⁻¹, similar results were obtained in the Chld-treated it was 314.6 ± 11.5 mg 100 g⁻¹. The soluble solids content in the Chld-treated was 9.8 ± 0.3%, which is similar to the control 9.6 ± 0.4%. According to our investigation, no significant colour changes were detected in the treated raspberries (data not shown) in comparison with the control. Based on our experiments, we can conclude that Chld did not affect the appearance and nutritional value of strawberries and raspberries.

Conclusions

1. The chlorophyllin derivative-based photosensitization is an innovative postharvest treatment for the inhibition of Botrytis cinerea on strawberries and raspberries without any negative influence on the colour, antioxidant activity and phenolic compounds.
2. The chlorophyllin derivative-based photosensitization reduced the contamination of strawberries with B. cinerea by 8% (after a 6-day storage) and that of raspberries by 3% (after 1-day storage).
3. Photosensitization has potential as a non-chemical plant protection tool, which could be used for the development of new plant protection technologies in agriculture.

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Naujos necheminės technologijos, mažinančios uogų užkrėstumą jų laikymo metu

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Santrauka
Viena pagrindinių braškių ir aviečių uogų nuostolius sukeliantių ligų yra keckerinis pvinys (Botrytis cinerea Pers.: Fr.). Tirtos naujos necheminės maisto saugos technologijos, mažinančios uogų užkrėstumą ir išsaugojusios jų vartojimo trukmę. Fotosensibilizacija yra novatoriškas metodas, taikomas sprogti uoginių patogenams uogose, pagristas matomos šviesos (λ = 400 nm, 20 mW cm⁻²) ir fotoautorejs mediciną (chlorofilino darinio) šviesa. Tyrimo tikslas – taikant fotosensibilizacijos metodą įvertinti chlorofilino darinio įtaką mažinant aviečių ir braškių uogų užkrėstumą kekeriniu puviniu. Tyrimo metu uogos buvo išsaugotos maisto įtakos. Naujos necheminės technologijos, mažinančios uogų užkrėstumą ir išsaugojusios jų vartojimo trukmę.