THE EFFECTS OF APPLYING CHITOSAN OF DIFFERENT MOLECULAR WEIGHTS ON THE QUALITY OF KAMCHATKA BERRIES (*LONICERA CAERULEA* L.): PART 2

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Abstract

We evaluated the effects of chitosan of different molecular weights on the quality of Kamchatka berries after harvest and storage. Chitosan with lower molecular weights (from 3 to 21 kDa) positively influenced the soluble solid content after harvest. However, chitosan had no significant effect on titratable acidity after harvest. Regardless of the chitosan molecular weight, the berries contained from 7.4% to 30% more total polyphenols compared with the control (162 mg per 100 g) after harvest. After storage, the total phenolic content decreased slightly and depended on the storage conditions. The L-ascorbic acid content was the highest after harvest and storage after treatment with the highest molecular weight of chitosan (950k kDa). Chitosan 50 kDa positively influenced the firmness and puncture resistance of the berries after harvest (146% of control) and storage (135% of control).

Keywords: *blue honeysuckle, total polyphenol, firmness, fruits storage, puncture resistance*

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1. Introduction

The storage of fruit and vegetables is a widely discussed topic in the scientific literature. There are a lot of references concerning the possibility of applying substances and how they are applied to effectively protect the fruit against loss of properties during long-term storage or/and transport. Recently, there have been increased efforts to discover new preservatives derived from natural sources without harmful effects on human health. It is thought that coating fruit with preservatives would reduce the rate of transpiration, thus delaying ripening and ageing [1]. According to several studies [2–4], chitosan (CH) has a broad spectrum of antimicrobial activity. Indeed, CH treatment can control or delay post-harvest rotting of fruits and vegetables. In addition, as an exogenous elicitor CH can influence the activity of defence-related enzymes and induce the accumulation of special substances in certain plants that are known to participate in defence mechanisms and the prevention of biotic and abiotic stress factors [5, 6]. Due to its structure, CH appears to be an ideal preservative coating for fresh fruit because of its excellent film-forming and biochemical properties [7].

The biological activity of a biopolymer undoubtedly depends on its molecular weight [4]. According to Bof *et al.* [8], the lower the CH molecular weight, the greater the colour differences and film solubility of treated fruit. High-molecular-weight CH (360 kDa) produces a layer that delays ripening and maintains high post-harvest fruit quality more effectively compared with low-molecular-weight CH (40 kDa). Most studies have sprayed or soaked the fruit in a CH solution. In contrast, there are no reports in the literature on the quality of Kamchatka berries (*Lonicera caerulea* L.) harvested directly from bushes sprayed with a CH solution. Kamchatka berries are soft, lose their firmness relatively quickly, and are therefore not very resistant to storage and are susceptible to damage during transport [9]. In addition, the berry bushes have a short vegetation season: they begin and end fruiting quickly. Hence, the results presented in this study continue the study of the effects of foliar-applied with different molecular weights of CH on Kamchatka berry bush growth, yield, and quality [10].

2. Materials and Methods

2.1. Characteristics of the Research Area and Plant Material

The experiment was conducted at the orchard of the West Pomeranian University of Technology in Szczecin. Blue honeysuckle bushes, cultivar Zielona, were planted at a spacing of 3 × 1 m in clay soil classified as Class III, with pH 6.2–6.5.

2.2. Production of CH and Film Preparation

Chitin was obtained by demineralising, deproteinising, and deodourising shrimp (*Farfantepenaeus brasiliensis*) waste.

Kamchatka berry bushes were treated with CH with molecular weights of 3,000 (3k), 5,000 (5k), 12,000 (12k), 21,000 (21k), 50,000 (50k), 125,000 (125k), 500,000 (500k), and 950,000 (950k) kDa. The degree of deacetylation was 85%. The plants were sprayed once after the start of vegetation and three times per week after the end of flowering. The control plants were sprayed with distilled water only. No other chemical plant protection was applied during the experiment. The berries were harvested several times (5–7 times) in June as they ripened.

2.3. Storage

Cold-shocked berries (temperature drop to 3–4°C) and berries not submitted to cold shock were stored in a cold room with a normal atmosphere. The berries were stored for 2 weeks at 2 ± 0.2°C and relative air humidity of 95%. The experiment was performed in five replicates, each with 0.25 kg of berries.

2.4. General Fruit Parameters

The total soluble solid content (SSC; °Bx) in samples was measured at 20°C with a digital refractometer (PAL-1, Atago, Japan). Titratable acidity was determined by titration of the aqueous extract with 0.1 N sodium hydroxide (NaOH) to pH 8.1 (Elmetron CX-732, Zabrze, Poland), according to the PN-90/A-75101/04 standard.

The total phenolic content was determined with a ultra-performance liquid chromatography (UPLC) coupled to photodiode array detection (PDA) and mass spectrometry (MS) Waters ACQUITY system (Waters, USA) consisting of a binary pump manager, a sample manager, a column manager, a photodiode array (PDA) detector, and a tandem quadrupole mass spectrometer (TQD) with electrospray ionisation (ESI) [11].

The *L*–ascorbic acid content was measured with a RQflex 10 requantometer (Merck, Germany) [11].

Firmness and puncture resistance of the skin were measured with a FirmTech2 apparatus (BioWorks, USA) on 100 randomly selected berries from three replicates. The values are expressed as a gram-force causing the fruit surface to bend 1 mm [12].

2.5. Statistical Analysis

All statistical analyses were performed with Statistica 12.5 (StatSoft Polska, Poland). The data were subjected to one-way variance analysis (ANOVA). The Tukey least significant difference (LSD) test was used to compare the means between the groups. Significance was set at $p < 0.05$.

3. Results and Discussion

Among natural elicitors, CH offers great potential as a biodegradable substance that has antimicrobial activity and elicitation activity. CH coatings for storing strawberries [4], tomatoes and grapes [13], and blueberries [7] have been developed. The effect of the foliar-applied CH solutions of different molecular weights on the quality of Kamchatka berries is shown in Table 1.

CH affected the chemical composition of the berries. The quality properties in berries are the SSC (consisting mostly of mono- and disaccharides) and titratable acidity, which contribute to the sweetness and acidity of berries and their products [14]. From the consumer's point of view, the taste of berries is important and is significantly influenced by the ratio of sugars to acids. The SSC in Kamchatka berries after harvest was lower compared with stored berries. Foliar spraying with CH 50k produced the most significant differences in the SSC and titratable acidity (8% and 4% higher compared with berries after harvesting). Storage also influenced the SSC of the berries. The results obtained in this study are comparable to a previous report from our group: we found that CH 5k and 12k increased the SSC in strawberries compared with the control, while CH 125k and 500k significantly decreased the SSC [4]. Our group also confirmed the positive influence of Biochikol 020PC, the active ingredient of which is CH, on the SSC in the fruit of several raspberry cultivars [15].

Foliar spraying with CH had no effect on the titratable acidity content of the berries before harvest and depending on the storage method (Table 1). However, spraying with

CH 50k increased titratable acidity by 11% compared with the pre-harvest control and by 6% and 11% (not submitted to cold shock and cold shocked, respectively) in stored berries.

Table 1. The soluble solid content and titratable acidity (TT) of Kamchatka berries treated with different molecular weights of chitosan immediately after harvest and after 2 weeks of cold storage.

Chitosan	**Soluble solid content [%]**			**Titratable acidity [g citric acid 100 g^{-1}]**		
	After harvest	**After storage**		**After harvest**	**After storage**	
		Not cold shocked	**Cold shocked**		**Not cold shocked**	**Cold shocked**
Control	$10.3^{a/BC}$	$10.7^{b/BC}$	$10.5^{ab/ABC}$	$3.7^{a/C}$	$3.4^{b/BC}$	$3.5^{b/B}$
3k	$10.5^{a/DE}$	$10.8^{b/CD}$	$10.7^{b/CD}$	$3.6^{a/C}$	$3.4^{a/BC}$	$3.5^{a/B}$
5k	$10.6^{a/E}$	$10.9^{b/D}$	$10.8^{ab/D}$	$3.4^{b/A}$	$3.0^{a/A}$	$3.3^{b/A}$
12k	$10.2^{a/B}$	$10.5^{b/D}$	$10.4^{b/AB}$	$3.6^{a/BC}$	$3.5^{a/BCD}$	$3.4^{a/AB}$
21k	$10.6^{a/E}$	$10.8^{a/CD}$	$10.7^{a/CD}$	$3.5^{a/AB}$	$3.3^{a/B}$	$3.4^{a/AB}$
50k	$9.9^{a/A}$	$10.7^{c/CD}$	$10.3^{b/A}$	$4.1^{b/D}$	$3.6^{a/CD}$	$3.9^{b/C}$
125k	$10.4^{a/CD}$	$10.9^{b/D}$	$10.6^{a/BCD}$	$3.7^{b/C}$	$3.3^{a/A}$	$3.4^{a/AB}$
500k	$10.5^{a/DE}$	$10.8^{b/CD}$	$10.6^{a/BCD}$	$3.5^{a/AB}$	$3.7^{a/D}$	$3.5^{a/B}$
950k	$10.2^{a/B}$	$10.6^{b/AB}$	$10.4^{ab/AB}$	$3.4^{a/A}$	$3.7^{b/D}$	$3.5^{ab/B}$
Mean	**10.36^{a}**	**10.74^{b}**	**10.56^{ab}**	**3.61^{a}**	**3.43^{a}**	**3.49^{a}**

Note. The molecular weights of chitosan: 3,000 (3k), 5,000 (5k), 12,000 (12k), 21,000 (21k), 50,000 (50k), 125,000 (125k), 500,000 (500k), and 950,000 (950k) kDa. Mean values with the same letter do not differ significantly ($p > 0.05$) according to the Tukey test. The lowercase letters indicate comparisons between the rows; the uppercase letters indicate comparisons between the columns.

According to many authors [16, 17], Kamchatka berries are a valuable source of vitamins, minerals, and secondary metabolites with properties that are important for maintaining proper human health. These berries are characterised by their high total phenolic content and antioxidant activity. However, the chemical composition of the berries is dependent on genetic factors, climate, and agronomic treatments [16, 18]. Regardless of the CH molecular weight applied, the berries contained from 7.4% (CH 50k) to 30% (CH 3k) more total polyphenols compared with the control (162 mg 100 g^{-1}) after harvest (Table 2). The exception was berries treated with CH 50k, whose total phenolic content was 2.5% lower than the control. Furthermore, after storage the total phenolic content decreased slightly. It was slightly lower in berries not submitted to cold shock compared with cold-shocked berries. However, these differences were not significant (Table 2). Based on the Folin-Ciocalteu method, the total phenolic content ranged from 140.5 to 1142 mg gallic acid equivalents (GAE) per 100 g fresh weight [18]. These values are lower compared with other fruit, such as strawberries (238.0 mg GAE 100 g fresh weight) [19], red raspberries (455.5 mg GAE 100 g fresh weight) [20], and blueberries *(Vaccinium virgatum*) from southeastern North America (330.0 mg GAE 100 g fresh weight) [21].

Kamchatka berries are a source of vitamin C and contain from 30.5 to 186.6 mg of ascorbic acid per 100 g fresh weight [22], which is comparable to the content of vitamin C in redcurrant fruit (25.6–40.0 mg 100 g fresh weight) [23], oranges (54.0 mg 100 g fresh weight) [24], and kiwi fruit (29.0–80.0 mg 100 g fresh weight) [25], recognised as rich sources of vitamin C. The content of *L*–ascorbic acid in Kamchatka berries before storage ranged from 330 mg 1000 g fresh weight (after treatment with CH 50k) to 671 mg 1000 g fresh weight (after treatment with CH 950k). The *L*–ascorbic acid content increased with the applied CH concentration. The exceptions were berries harvested from bushes sprayed with CH 50k, which had the lowest *L*–ascorbic acid content (Table 2). In addition, there was an unknown decrease in the *L*–ascorbic acid content of the fruit after storage. Although the method of storage did not statistically affect the *L*–ascorbic acid content, there was a non-significant increase in the *L*–ascorbic acid content in cold-shocked berries.

Table 2. The total phenolic and *L*-ascorbic acid contents of Kamchatka berries treated with different molecular weights of chitosan immediately after harvest and after 2 weeks of cold storage.

Chitosan	**Total phenolic content [mg 100 g]**			***L*–ascorbic acid content [mg 1000 g]**		
	After harvest	**After storage**		**After harvest**	**After storage**	
		Not cold shocked	**Cold shocked**		**Not cold shocked**	**Cold shocked**
Control	$162^{a/A}$	$147^{a/AB}$	$151^{a/A}$	$452^{a/DE}$	$427^{a/C}$	$439^{a/CD}$
3k	$211^{b/D}$	$178^{a/CD}$	$190^{ab/BC}$	$395^{b/BC}$	$366^{a/B}$	$378^{b/B}$
5k	$187^{a/BCD}$	$195^{a/DE}$	$202^{a/C}$	$388^{b/B}$	$324^{a/AB}$	$367^{b/B}$
12k	$203^{a/CD}$	$207^{a/E}$	$199^{a/BC}$	$415^{b/BCD}$	$382^{a/B}$	$400^{ab/BC}$
21k	$180^{a/ABC}$	$174^{a/BCD}$	$183^{a/BC}$	$573^{b/E}$	$545^{b/D}$	$463^{a/D}$
50k	$158^{b/A}$	$133^{a/A}$	$147^{ab/A}$	$330^{a/A}$	$301^{a/A}$	$317^{a/A}$
125k	$174^{b/AB}$	$153^{a/ABC}$	$170^{b/AB}$	$439^{b/CDE}$	$384^{a/BC}$	$406^{a/BC}$
500k	$193^{ab/BCD}$	$179^{a/CDE}$	$195^{b/BC}$	$582^{c/E}$	$512^{a/D}$	$544^{b/E}$
950k	$204^{b/CD}$	$186^{a/DE}$	$199^{b/BC}$	$671^{a/F}$	$655^{a/E}$	$659^{a/F}$
Mean	$\mathbf{186}^{a}$	$\mathbf{172}^{a}$	$\mathbf{182}^{a}$	$\mathbf{472}^{b}$	$\mathbf{433}^{a}$	$\mathbf{441}^{ab}$

Note. The molecular weights of chitosan: 3,000 (3k), 5,000 (5k), 12,000 (12k), 21,000 (21k), 50,000 (50k), 125,000 (125k), 500,000 (500k), and 950,000 (950k) kDa. Mean values with the same letter do not differ significantly ($p > 0.05$) according to the Tukey test. The lowercase letters indicate comparisons between the rows; the uppercase letters indicate comparisons between the columns.

Firmness is an indicator of freshness and also determines the resistance of fruit to damage during transport [26]. We measured the firmness of the berries immediately after harvest and after 2 weeks of storage. Foliar application of all CH, except CH 21k, positively influenced the firmness of the Kamchatka berries (Figure 1). The berries harvested from bushes sprayed with CH 50k and CH 3k were the firmest (146% and 141% of the control, respectively). In addition, the puncture resistance of the berries harvested from CH-treated bushes was higher than the control by 3% (CH 500k) to 35% (CH 50k). The exception was berries harvested from bushes treated with CH 21k and 125k, whose puncture resistance was lower than the control by 5% and 12%, respectively (Figure 1). Furthermore, there were changes in firmness after storage for berries submitted and not submitted to cold shock. Cold shocking the berries after harvesting initially increased their firmness compared with the berries not submitted to cold shock (Figure 2). The cold-shocked berries harvested from bushes sprayed with CH 12k were the firmest, while the cold-shocked berries harvested from bushed sprayed with CH 125k were the least firm (Figure 2). Regarding both methods of storage, cold shocking showed the best ability to preserve the firmness and puncture resistance of the berries. In these conditions, the loss of firmness was several percent only compared with the control group; the exception was berries harvested from bushes sprayed with CH 12k and 125k. However, Ochmian *et al.* [27] showed that the fruit of blue honeysuckle (*L. caerulea* var. *kamtschatica*) 'Wojtek' and 'Zielona' (180–220 G mm^{-1}) showed a decrease in firmness of 14%–16% already after several days of cold storage.

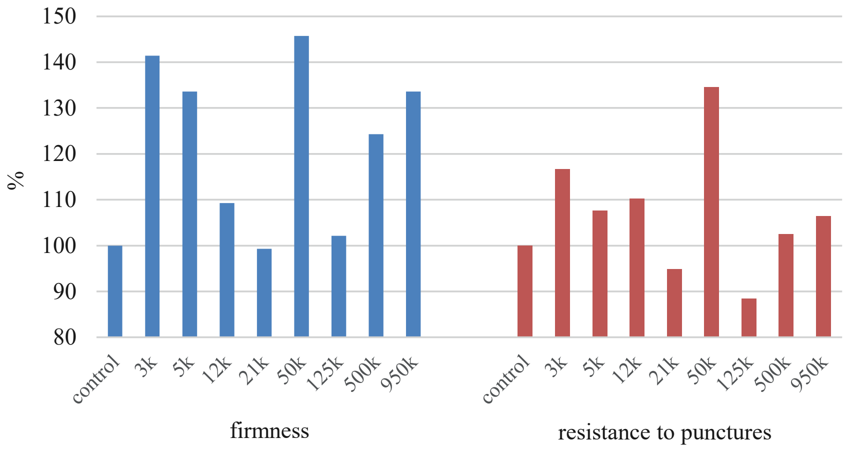

Figure 1. The percent change in firmness and puncture resistance of fresh Kamchatka berries treated with different molecular weights of chitosan compared with the control (control = 100%). The molecular weights of chitosan: 3,000 (3k), 5,000 (5k), 12,000 (12k), 21,000 (21k), 50,000 (50k), 125,000 (125k), 500,000 (500k), and 950,000 (950k) kDa.

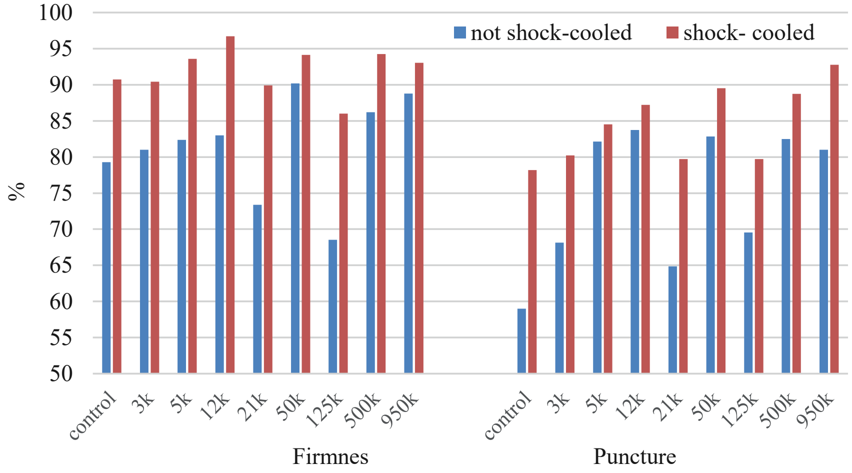

Figure 2. The percent change in firmness and puncture resistance of Kamchatka berries treated with different molecular weights of chitosan and stored for 2 weeks in the cold compared with fresh fruit (fresh fruit = 100%). The molecular weights of chitosan: 3,000 (3k), 5,000 (5k), 12,000 (12k), 21,000 (21k), 50,000 (50k), 125,000 (125k), 500,000 (500k), and 950,000 (950k) kDa.

4. Conclusions

The results of this study indicate that spraying Kamchatka berry bushes with CH influences the fruit quality after harvest and after 2 weeks of cold storage. We recommend higher molecular weight CH (500k and 950k) to increase the total phenolic and *L*–ascorbic acid contents, and lower molecular weight CH to improve the SSC, firmness, and puncture resistance. The cold storage conditions we tested influenced the quality of the berries. The Kamchatka berries that were not cold shocked had a higher SSC but lower titratable acidity and total phenolic and *L*–ascorbic acid contents. In addition, the berries that were not cold shocked had a significant loss of firmness and puncture resistance.

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