EFFECT OF CHITOSAN-BASED SPRAYING ON THE QUALITY OF HIGHBUSH BLUEBERRIES (SUNRISE CULTIVAR)

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Abstract

The consumption of highbush blueberries has been growing rapidly in recent years due to their taste and health-promoting qualities. Various solutions have been sought to obtain the highest quality fruit after harvest. In the era of eco-friendly products, it is important that the methods used are natural and ecological. For this purpose, chitosan (CH) was sprayed five times on highbush blueberry bushes before harvesting. Different molecular weights of CH (5, 12, 21, 50, 125, and 500 kDa) were used in this study. The physical and biochemical characteristics of the fruit were investigated. The antioxidant activity, microbial contaminants, and mycotoxins in fruit were also analysed. Application of CH affected the quality of highbush blueberries after harvest. The molecular weight of CH had a significant effect on the studied traits. The application of high-molecular-weight CH improved physical characteristics such as the average weight of 100 blueberries, firmness, and puncture. Furthermore, the blueberries had a more intense blue colour; were characterised by a higher content of L-ascorbic acid and polyphenols, especially anthocyanins; and did not contain mycotoxins. Spraying with CH can be recommended in the organic cultivation of highbush blueberries to obtain robust fruit with health-promoting qualities.

Keywords**:** *chitosan, highbush blueberry, fruit quality, mycotoxins, polyphenols, fungi*

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1. Introduction

In recent years, there has been marked growth in blueberry acreage and yield [1-5]. Recent global data indicate yearly blueberry production of nearly 1.0 million ton [4]. The largest global producers of highbush blueberries are the United States; Chile; Canada; and European countries such as Spain, Poland, and Germany [2, 4]. Dark blue and sweet blueberries are classified as a so-called 'superfood' due to their health-promoting biological properties [6] and are highly consumed among the public, mainly in dessert form [1, 3, 5].

Blueberries are a rich source of valuable nutrients such as minerals, vitamins, flavones, and polyphenols [7, 8]. Numerous studies indicate that blueberries are a source of natural antioxidants. Many of the health-promoting properties of blueberries are attributed to phenolic acids and various flavonoids, especially anthocyanins [7, 9]. Among fruit, blueberries have the highest anthocyanin content; hence, they are recommended as a functional food as well as a dietary supplement [6].

Consumers judge the quality of fresh fruit based on their appearance and freshness on the store shelf [10]. Various measures and technologies are used to achieve the highest quality fruit. It is important not to reduce natural properties of fruit and their benefits for human health by using chemicals – ecological solutions should be favoured. The optimal solution seems to be environmentally safe chitosan (CH), extracted most often from the shells of shrimp and other crustaceans [11]. CH is the second most abundant biopolymer on earth after cellulose [12]. It forms a transparent, non-toxic, biocompatible, biofunctional, and biodegradable film [13-15]. In addition, it exhibits haemostatic and antimicrobial activity, mucoadhesive properties, and excellent processability (including film-forming capabilities) [13]. CH shows activity against multidrug-resistant bacteria and fungi, which pose a challenge to modern medicine [16]. CH plays an important role in plant pathogen resistance and defence mechanisms [15, 17, 18]. Coating fruit and vegetables with edible CH extends their shelf life by minimising respiration rates and reducing water loss [17].

Physicochemical characteristics such as the degree of deacetylation and molecular weight play a key role in the quality of CH in its diverse applications. CH can be divided into four groups according to the degree of deacetylation: low (55%-70%), medium (70%-85%), high (85%-95%), and ultra-high (95%-100%) [19]. Moreover, there is low-molecular-weight CH (LMC) and high-molecular-weight CH (HMC). Molecular weight affects the biological activity of CH [20]. LMC usually exhibits more significant biological properties than HMC [21]. HMC (500-1,000 kDa) is readily soluble in dilute acids but insoluble in water [20].

This study aimed to investigate the effect of CH of different molecular weights on physicochemical changes in highbush blueberries of the Sunrise cultivar after harvest. The type and amount of microbial contaminants and mycotoxins in the fruit were also analysed.

2. Materials and Methods

2.1. Characteristics of the Research Area and Plant Material

The experiment was conducted in the Department of Horticulture at the West Pomeranian University of Technology in Szczecin from 2018 to 2020. Highbush blueberries of the Sunrise cultivar were harvested from bushes grown on a specialised highbush blueberry farm, located about 25 km east of Szczecin, in the Goleniowska Forest. The plantation contains a 4.9 ha field irrigated by droplets and has mineral soil: loamy sand, organic matter 2.21%, organic carbon 0.56%, electrical conductivity 0.29 mS/m, and pH 4.0-4.2 [22].

2.2. Characteristics of the CH and Treatment Application

Depolymerised CH obtained by controlled free-radical degradation was used in the experiment. Chitosan was produced by the Center for Bioimmobilization and Innovative Packaging Materials of the West Pomeranian University of Technology in Szczecin. Different molecular weights of CH (5, 12, 21, 50, 125, 500 kDa) with a degree of deacetylation of 85% were used in the study.

CH was sprayed five times on highbush blueberry bushes: three times before harvesting, and after the second and third harvests. The control group consisted of bushes sprayed with water.

2.3. General Fruit Parameters

Every year, the blueberry yield and weight were measured (using a WPX 4500 instrument, with precision of ± 0.01 g; RADWAG, Poland). The content of soluble solids was determined by an electronic refractometer (PAL-1, Atago, Japan). Acidity was determined by titration of the aqueous extract with 0.1 N sodium hydroxide (NaOH) to an endpoint of pH 8.1 (Elmetron CX-732, Poland), according to the PN-90/A-75101/04 standard [23]. The L-ascorbic acid and nitrate contents were measured with an RQflex 10 requantometer (Merck, Germany) [22].

2.4. Colour

The CIE L^*, a^*, and b^* parameters defined by the Colour Measurement Committee of the Society of Dyers and Colourists (CMC) were measured using a Konica Minolta CM-700d spectrophotometer (Japan). The colour parameters and indices were averaged over 35 measurements [24].

2.5. Firmness

Firmness and puncture resistance of the blueberry skin was measured with a FirmTech2 apparatus (BioWorks, USA) on 100 randomly selected blueberries from three replicates. Punctures were made using a stamp with a diameter of 3 mm [24]. It is expressed as a gram-force causing the blueberry surface to bend 1 mm.

2.6. Extraction and Identification of Polyphenols

Three replicates of 1000 g of randomly chosen blueberries were kept frozen in polyethylene bags at -65°C until analysis, then prepared according to the methodology described by Lachowicz *et al.* [25]. Compounds were extracted with methanol acidified with 2.0% formic acid. The separation was conducted twice by incubation for 20 min under sonication (Sonic 6D, Polsonic, Poland) followed by shaking from time to time (a few times or rarely). Subsequently, the suspension was centrifuged in an MPW-251 centrifuge (MPW MED. INSTRUMENTS, Poland) at 19000 *g* for 10 min. Prior to analysis, the supernatant was purified with a Hydrophilic PTFE 0.20 µm membrane (Millex Samplicity Filter, Merck).

In blueberry extracts, polyphenols were identified by using an ACQUITY Ultra Performance LC system with a binary solvent manager, a photodiode array detector (Waters Corporation, USA), and a G2 Q-TOF micro mass spectrometer (Waters, UK) equipped with an electrospray ionisation (ESI) source operating in both negative and positive modes (UPLC-PDA/QTof-MS/MS) [25].

2.7. Antioxidant Activity

For the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay, the procedure followed the method of Arnao *et al.* [26]. The ferric ion reducing antioxidant

power (FRAP) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) assays were conducted according to the method of Brand-Williams *et al.* [27]. The antioxidant capacity is expressed as µmol Trolox equivalent (TE) g^{-1} dry weight (dw). Measurements for the $ABTS^{\cdot+}$ and FRAP assay involved the UV-2401 PC spectrophotometer (Shimadzu, Japan). The L-ascorbic acid content was measured with an RQflex 10 quantometer (Merck, Germany) [24].

2.8. Fungal Infestation and Mycotoxin Content in Blueberries

Analysis of the degree of fruit infestation by fungi (yeasts and moulds) was based on the European standard ISO 70 [28]. After cultivation of spore-forming fungal inoculates, samples were subjected to taxonomic evaluation using the traditional method of macroscopic observation of colonies and microscopic observation of spores and filaments.

Mycotoxins were determined by using high-performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS) [29]. The sample was purified on the AflaTest immunological affinity columns from Vicam (USA) for aflatoxins and OchraPrep from R-Biopharm AG (Germany) for ochratoxin A, according to the procedures specified by the manufacturers. Patulin, deoxynivalenol, T2, HT2 toxin, and zearalenone were analysed by HPLC-MS/MS. The samples were purified on Bond Elut® Mycotoxin columns from Agilent (USA). Each sample was run in triplicate.

2.9. Statistical Analysis

All statistical analyses were performed with Statistica 12.5 (StatSoft Polska, Poland). The data were subjected to one-way analysis of variance (ANOVA). Group comparisons were performed using Tukey's least significant difference (LSD) test; significance was set at $p < 0.05$.

3. Results and Discussion

3.1. Blueberry Quality

The mean weight of 100 blueberries, puncture, firmness, soluble solids (SS), titratable acidity (TA), nitrate nitrogen ($N\text{-}NO_3$), and nitrite nitrogen ($N\text{-}NO_2$) were determined in fresh blueberries soon after harvest. Blueberries from bushes sprayed with CH 125 kDa had the highest weight (342 g/100 fruit), while fruit sprayed with CH 5 kDa had the lowest weight (262 g/100 fruit). The average weight of 100 blueberries was much higher than in the study by Ochmian *et al.* [30] on the highbush blueberry Patriot cultivar, where it was 120-140 g on average. The heaviest fruit was also the firmest (428 G mm^{-1}) and had the highest puncture value (142 G mm^{-1}). Fruit that had been sprayed with CH 21 kDa had the lowest $N\text{-}NO_3$ and $N\text{-}NO_2$ contents (30.2 and 0.09 mg 1000 g^{-1}, respectively), while fruit sprayed with CH 125 and 500 kDa had the highest $N\text{-}NO_3$ and $N\text{-}NO_2$ contents ($N\text{-}NO_3$: 55.9 and 0.15 mg 1000 g^{-1}, respectively; $N\text{-}NO_2$: 52.3 and 0.17 mg 1000 g^{-1}, respectively). Ochmian *et al.* [30] obtained lower $N\text{-}NO_3$ (19.3-31.4 mg 1000 g^{-1}) and higher $N\text{-}NO_2$ (0.75-0.95 mg 1000 g^{-1}) (Table 1).

3.2. L-Ascorbic Acid and Antioxidant Activity

The L-ascorbic acid content ranged from 20.7 to 39.6 mg 100 g^{-1}. Ochmian *et al.* [30] reported a vitamin C content in highbush blueberries of 23.4-26.2 mg 100 g^{-1} for the Patriot cultivar, depending on the substrate. Zia and Alibas [31] reported a vitamin C content for mighty fresh blueberry of 40.29 mg 100 g^{-1} dw. Kalt *et al.* [32] reported that the L-ascorbic acid content of fresh blueberries was 7-20 mg 100 g^{-1}. However, López *et al.* [33] reported that the vitamin C content of fresh blueberries was 20.97 mg 100 g^{-1}. Blueberries sprayed with CH 500 kDa had the highest L-ascorbic acid content; it was

almost twice as high as the content in the blueberries sprayed with CH 12 kDa. Compared with the control group, spraying with CH 50, 125, and 500 kDa increased the L-ascorbic acid content, while spraying with CH 5 and 12 kDa decreased the L-ascorbic acid content (Table 1).

The FRAP method was used to determine the antioxidant capacity of the blueberries. The antioxidant capacity varied due to the molecular weight of CH (5.77-10.12 μmol TE g^{-1}). The blueberries treated with CH 125 kDa had the highest value, even higher than in the study by Ochmian *et al.* [5], where the FRAP value was 4.33-7.23 μmol TE g^{-1}. Among the treatments, the blueberries treated with CH 12 kDa had the highest antioxidant activity, denoted by DPPH and $ABTS^{\cdot+}$ radical scavenging. The antioxidant activity, determined by DPPH, was as much as twice as high (31.0 μmol TE g^{-1}) as for the 125 kDa sample (15.5 μmol TE g^{-1}) (Table 1).

Table 1. The quality and antioxidant capacity of highbush blueberries depending on the molecular weight of the applied chitosan.

	Molecular weight of chitosan (kDa)						
	Control*	**5**	**12**	**21**	**50**	**125**	**500**
Weight of 100 blueberries (g)	274ab**	262a	288bc	302c	335d	342d	291bc
Puncture (G mm^{-1})	124bcd	130cde	108a	117ab	121bc	142e	133e
Firmness (G mm^{-1})	358bc	366bc	317a	359bc	352b	428d	375c
Soluble solid (%)	15.3b	15.8c	15.6c	15.2b	14.5a	14.4a	15.1b
Titratable acidity (g 100 g^{-1})	0.88d	0.81bc	0.85cd	0.87d	0.74a	0.77ab	0.80b
L-ascorbic acid (mg 100 g^{-1})	26.5b	22.4a	20.7a	27.8b	35.7c	36.2c	39.6c
$N\text{-}NO_3$ (mg 1000 g^{-1})	37.3bc	41.5c	33.4ab	30.2a	36.1b	55.9d	52.3d
$N\text{-}NO_2$ (mg 1000 g^{-1})	0.11ab	0.13bc	0.14c	0.09a	0.15cd	0.15cd	0.17d
$ABTS^{\cdot+}$ (μmol TE g^{-1})	14.7a	17.8b	20.2c	21.3c	18.4b	17.7b	15.5a
FRAP (μmol TE g^{-1})	8.11bc	7.36b	9.55d	8.78cd	5.77a	10.12e	9.84d
DPPH (μmol TE g^{-1})	22.2b	15.7a	31.0d	28.4c	21.6b	15.5a	16.3a

Note. *Control – not spraying; **Means with the same letter do not differ significantly according to Tukey's test ($p > 0.05$).

3.3. Blueberry Colour

One of the key parameters affecting consumer acceptability of fruit is the surface colour. Highbush blueberries have a blue-black skin but due to the waxy coating, they appear light blue [34]. The colour of the studied blueberries was determined by the L^*, a^*, and b^* parameters. A change in the L^* parameter indicates blueberry darkening [35].

Depending on the molecular weight of the applied CH, the blueberries changed from pink to blue, as indicated by significant changes in the *a** and *b** parameters. The darkest and most intense blue shade was observed in blueberries treated with CH 500 and 125 kDa (Figure 1), which is undoubtedly related to the high anthocyanin content. The darkest blueberries had the highest anthocyanin content (Figure 1 and Table 2). On the other hand, the blueberries treated with CH 50 kDa exhibited the lightest purple-pink colouration. Ochmian *et al.* [35] reported similar *L** (26.2 to 32.6) and *b** (-28.3 to - 25.9) values.

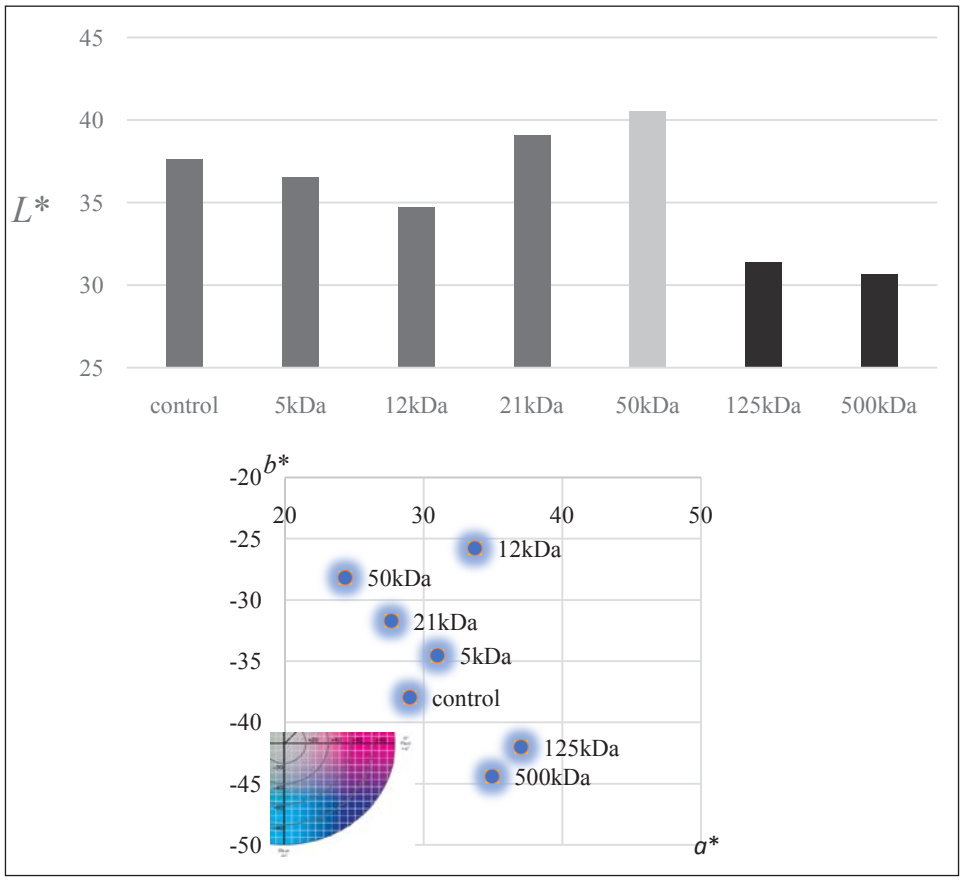

Figure 1. Change in highbush blueberry colour depending on the molecular weight of the applied chitosan.

3.4. Polyphenols

Highbush blueberries are a rich source of polyphenols such as phenolic acids, anthocyanins, and other flavonoids [36]. The studied highbush blueberries contained 36 polyphenols (Table 2). The most abundant were anthocyanins followed by phenolic acids; the least abundant were flavonols and flavan-3-ols. The polyphenol content depended to a large extent on the molecular weight of the applied CH (Table 2). There were nine anthocyanins detected in the blueberries (Table 2). Anthocyanins are the most important polyphenols in blueberries [8] and are responsible for their black, blue, and red pigments. Blueberries treated with CH 500 kDa had the highest anthocyanin content (499.70 mg

100 g^{-1}); it was 2.05 times greater than the anthocyanin content of blueberries treated with CH 12 kDa. Similarly to the studies by Su *et al.* [35] and Ochmian *et al.* [5], chlorogenic acid (a phenolic acid) was the dominant polyphenol. On average, it accounted for between 29.57% and 36.49% of the total polyphenol content in highbush blueberries (Table 2).

Table 2. Polyphenol content in blueberries [mg 100 g^{-1}].

Compounds (mg 100 g^{-1} DW)	Molecular weight of chitosan (kDa)						
	Control	**5**	**12**	**21**	**50**	**125**	**500**
Caf-glu*	1.71	1.13	0.55	6.28	0.57	0.33	0.82
Caf-glu	4.59	5.15	5.71	1.32	7.33	4.63	10.03
Caf-glu	0.60	0.85	1.10	2.54	2.20	2.05	2.35
Neochl acid	6.08	4.38	2.68	3.56	3.72	3.88	3.56
Chlo acid	271.92	242.03	212.14	217.83	238.49	254.21	269.58
Crychlo acid	1.64	1.13	0.62	2.69	2.22	3.07	1.37
Phenolic acid	**286.53d****	**254.67bc**	**222.80a**	**234.21ab**	**254.53bc**	**268.16cd**	**287.70d**
Myr 3-gal	3.79	3.57	3.35	3.80	10.96	9.99	11.92
Que diglu	1.16	0.78	0.40	0.99	0.85	0.96	0.74
Que 3-rha-hex	6.27	5.46	4.66	4.38	1.44	1.16	1.72
Que 3-rut	3.44	3.29	3.15	2.72	1.67	0.70	2.63
Que 3-gal	4.62	7.63	10.65	5.59	16.22	13.48	18.97
Que 3-methex	3.01	4.48	5.94	3.41	4.22	4.12	4.33
Que 3-glu	1.03	1.45	1.87	2.42	1.10	0.73	1.47
Que 3-ara	4.11	5.52	6.93	2.55	2.99	2.38	3.60
Que 3-cafgal	1.19	1.13	1.06	0.80	0.49	0.32	0.67
Que 3-cafglu	0.68	0.61	0.54	0.45	0.28	0.25	0.32
Que 3-oxapen	6.18	10.79	15.41	4.23	1.85	0.13	3.57
Que 3-rha	0.55	0.89	1.23	1.18	1.40	2.19	0.61
Que 3-dimethoxyrha	1.04	2.53	4.02	0.21	0.35	0.63	0.07
Que 3-(6'-acetyl) gal	0.08	0.32	0.55	0.51	0.15	0.15	0.14
Que 3-(6'-acetyl) gal	0.40	0.51	0.62	0.26	0.03	0.02	0.04
Flavonols	**37.55a**	**48.96bc**	**60.37d**	**33.50a**	**43.99b**	**37.19a**	**50.79c**
Procyanidin dim	5.84	6.70	7.56	9.63	6.11	6.19	6.03
Procyanidin dim	5.34	6.01	6.68	6.71	5.60	4.59	6.61
Procyanidin dim	13.06	40.34	67.63	19.80	23.27	30.50	16.03
Cat	13.01	9.80	6.60	9.22	17.99	32.00	3.99
(-)Epicat	4.25	7.94	11.64	8.45	8.77	6.38	11.16
Procyanidin trim - B3	0.89	2.63	4.38	2.23	3.76	3.03	4.49
Flavan-3-ols	**42.38a**	**73.43de**	**104.48f**	**56.04bc**	**65.50cd**	**82.68e**	**48.31ab**
Del-3-*O*-glu	91.75	71.34	50.92	61.18	87.16	55.54	118.78
Del 3-ara	2.28	2.11	1.95	1.27	2.39	2.36	2.41
Pet-3-*O*-glu	68.61	53.66	38.72	39.34	69.79	103.94	91.21
Cya-3-*O*-glu	47.04	43.43	39.82	44.49	51.96	71.58	66.38
Cya 3-ara	8.33	11.96	15.58	10.45	20.98	26.39	15.57
Pet 3-ara	27.19	24.99	22.79	20.47	34.34	49.14	42.24
Mal 3-gal	79.98	63.51	47.04	49.97	59.56	86.48	98.63

Compounds (mg 100 g^{-1} DW)	Molecular weight of chitosan (kDa)						
	Control	**5**	**12**	**21**	**50**	**125**	**500**
Mal 3-ara	4.75	5.08	5.41	8.40	7.70	8.53	6.87
Mal-3-*O*-glu	32.77	26.99	21.20	40.42	49.06	40.51	57.62
Anthocyanins	**362.69c**	**303.07b**	**243.44a**	**275.99ab**	**382.93c**	**444.46d**	**499.70e**
TOTAL	**745.20c**	**693.25b**	**641.30a**	**611.28a**	**765.48c**	**853.25d**	**911.72e**

*Caf-glu - Caffeoyl-glucose; Neochl acid - Neochlorogenic acid; Chlo acid - Chlorogenic acid; Crychlo acid - Cryptochlorogenic acid; Myr 3-gal - Myricetin 3-galactoside; Que diglu - Quercetin diglucoside; Que 3-rha-hex - Quercetin 3-rhamno-hexoside; Que 3-rut - Quercetin 3-rutinoside; Que 3-gal - Quercetin 3-galactoside; Que 3-methex - Quercetin 3-methoxyhexoside; Que 3-glu - Quercetin 3-glucoside; Que 3-ara - Quercetin 3-arabinoside; Que 3-cafgal - Quercetin 3-caffeoylgalactoside; Que 3-cafglu - Quercetin 3-caffeoylglucoside; Que 3-oxapen - Quercetin 3-oxalylpentoside; Que 3-rha - Quercetin 3-rhamnoside; Que 3-dimethoxyrha - Quercetin 3-dimethoxyrhamnoside; Que 3-(6'-acetyl)gal - Quercetin 3-(6'-acetyl)galactoside; Procyanidin dim - Procyanidin dimer; Cat - Catechin; (-)Epicat - (-)Epicatechin; Procyanidin trim - B3 - Procyanidin trimer - B3; Del-3-O-glu - Delphinidin-3-O-glucoside; Del 3-ara - Delphinidin 3-arabinoside; Pet-3-O-glu - Petunidin-3-O-glucoside; Cya-3-O-glu - Cyanidin-3-O-glucoside; Cya 3-ara - Cyanidin 3-arabinoside; Pet 3-ara - Petunidin 3-arabinoside; Mal 3-gal - Malvidin 3-galactoside; Mal 3-ara - Malvidin 3-arabinoside; Mal-3-O-glu - Malvidin-3-O-glucoside.

**_Note._ Means with the same letter are not significantly different according to Tukey's test ($p > 0.05$).

3.5. Fungi and Mycotoxins

Filamentous fungi are widely distributed throughout the world [37, 38]. They are found in soil, water, and materials of organic origin, and their spores are found in the air and on the surfaces of all kinds of materials. Some species of *Aspergillus*, *Penicillium*, *Alternaria*, and *Fusarium* produce toxic secondary metabolites called mycotoxins, which threaten human and animal health [39]. Fresh fruit, including blueberries, are susceptible to fungal infections that occur both on plantations and at harvest [40]. Toxigenic species of *Fusarium* and *Alternaria* are often classified as field fungi because they require very high substrate moisture for growth and mycotoxin synthesis [41]. Fungi belonging to seven different genera (*Acremonium*, *Alternaria*, *Aspergillus*, *Aureobasidium*, *Cladosporium*, *Fusarium*, and *Penicillium*) were isolated from blueberries after harvest. *Fusarium* spp. were dominant in the samples (44%-56%) (Table 3). *Penicillium* spp. (16%-80%) and *Aspergillus* spp. (35%-37%) were also very abundant. *Acremonium* spp. were the least abundant: they appeared only in the control group and blueberries that had been sprayed with CH 5 kDa (5% and 7%, respectively). *Penicillium* spp. were highly abundant (80%) in blueberries that had been sprayed with CH 125 kDa, and *Cladosporium* spp. (73%) were highly abundant in blueberries that had been sprayed with CH 21 kDa.

Four different types of mycotoxins were detected in the tested samples: patulin, aflatoxin, deoxynivalenol, and zearalenone. Mycotoxins are secondary metabolites of filamentous fungi and therefore occur naturally in food [37]. One fungus can produce different mycotoxins, and one mycotoxin can be produced by several different fungi [42]. The mycotoxins were found at low levels (Table 3). The patulin content (1.03-3.04 μg kg^{-1}) (Table 3) was significantly lower than the maximum allowable patulin concentration in apple juice (50 μg kg^{-1}) [43] set by the World Health Organization. Mycotoxins appeared only in the control group and the two groups that had been sprayed with the lowest molecular weights of CH (5 and 12 kDa). The absence of mycotoxins in the samples may have been related to the occurrence of *Aureobasidium* spp. in the samples. They can degrade mycotoxins through microbial pathways [44]. They show strong antagonism

to fruit-contaminating moulds and can provide effective bioprotection [45]. It can also be speculated that HMC inhibited the growth of mycotoxins. According to Gutierrez-Martinez *et al.* [40], CH could be an environmentally friendly alternative to the use of chemical fungicides in controlling postharvest diseases of fruit.

Table 3. Fungal genera and mycotoxins identified in the blueberries

Chitosan molecular weight (kDa)	Percentage of fungal genera (%)		Mycotoxins (µg kg^{-1})	
Control	*Fusarium*	44	Patulin	1.03
	Aspergillus	35	Deoxynivalenol	0.88
	Penicillium	16		
	Acremonium	5		
5 kDa	*Aspergillus*	36	Aflatoxin	1.79
	Penicillium	32	Patulin	0.65
	Alternaria	22		
	Acremonium	7		
	Cladosporium	3		
12 kDa	*Fusarium*	54	Patulin	3.04
	Aspergillus	37	Deoxynivalenol	0.59
	Cladosporium	9	Zearalenone	0.10
21 kDa	*Cladosporium*	73		
	Penicillium	19		
	Aureobasidium	8		
50 kDa	*Penicillium*	56		
	Aureobasidium	27		
	Alternaria	13		
	Cladosporium	4		
125 kDa	*Penicillium*	80		
	Aureobasidium	13		
	Cladosporium	7		
500 kDa	*Fusarium*	51		
	Penicillium	44		
	Alternaria	5		

4. Conclusion

The results of the study indicate that spraying highbush blueberries with CH improved their quality after harvest. The molecular weight and high deacetylation degree of CH have a decisive influence on its physical, chemical, and biological properties. The application of HMC (125 and 500 kDa) improved blueberry physical parameters such as mean weight of 100 fruit, puncture, and firmness, and they had the highest L-ascorbic acid, N-NO3, and FRAP content relative to the control group. These blueberries also had the most intense blue colour and high polyphenol contents, especially anthocyanins, and were not contaminated with mycotoxins. Based on the trend of improvement of the studied traits,

it can be assumed that using higher molecular weight CH than that used in the experiment would produce even better results.

5. References

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