THE EFFECT OF DIFFERENT MOLECULAR WEIGHTS OF CHITOSAN ON THE YIELD, QUALITY, AND HEALTH-PROMOTING PROPERTIES OF STRAWBERRIES

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Abstract

Chitosan could be an alternative to synthetic plant protection chemicals commonly used in strawberry cultivation. The aim of this study was to determine the effect of chitosan with different molecular weights on the yield, health, and quality of Korona cultivar strawberries. The largest number of strawberries was harvested from plants sprayed with chitosan with a molecular weight of 50k kDa (611 g/plant). On the other hand, the largest (1017 g/100 fruit) and the firmest (225 G mm) strawberries were harvested from plants sprayed with chitosan125k kDa. Compared with the control, chitosan with a molecular weight >50 kDa also increased the contents of L-ascorbic acid and polyphenols and the antioxidant activity and improved fruit colour compared with the control. The plants were also more resistant to grey mould infestation. The experiment has shown that how chitosan interacts with fruit depends on its molecular weight, and chitosan with a higher molecular weight has a better effect on most traits determining fruit quality.

Keywords: *antioxidant activity, colour, grey mould, firmness, fruit size, polyphenols,*

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1. Introduction

Strawberry (*Fragaria x annanasa*) is one of the best sources of bioactive compounds, including anthocyanins, carotenoids, vitamins (A, B_1, B_2 and C), minerals (iron, calcium, phosphorus, and potassium), flavonoids, and phenols (which have high antioxidant activity) [1, 2]. These compounds show significant anticancer [3] and antioxidant [4, 5] activities. Phenolic compounds differ in structure, physicochemical properties, and biological activity. The content of bioactive compounds in strawberries is influenced by soil and climatic conditions, the degree of fruit ripeness, and the way they are handled after harvest.

The widespread use of chemical crop protection products is one of the causes of environmental degradation. The excessive use of synthetic agrochemicals to increase fruit yield and secondary metabolite content has led to serious environmental and health problems. This is especially true for fruit that are consumed fresh, including strawberries [6].

Chitosan (poly-B-(1,4)*N*-acetyl-D-glucosamine) is the second most abundant polysaccharide in nature after cellulose [7]. Chitosan is a natural polysaccharide derived from the shells of crustaceans and other sources such as insects, fish scales, and fungi [8]. It is a non-toxic and biodegradable compound of natural origin, obtained by deacetylation of chitin [9]. When used in horticultural crops, it reduces the development of diseases before harvest but also reduces losses due to microbial infections during transport and postharvest storage [10-12]. It shows good antifungal activity against several pathogens, especially grey mould, which is one of the main causes of strawberry deterioration and postharvest decay [13]. It stimulates plant growth and development and increases yield and biochemical content [14-17]. Chitosan induces the expression of genes responsible for acquired immunity, the plant immune system, and hormone metabolism [18, 19]. Under stress conditions, chitosan enhances many plant defence mechanisms such as lignification, ion flux variations, cytoplasmic acidification, membrane depolarisation, protein phosphorylation, chitinase and glucanase activation, and phytoalexin biosynthesis [20].

As a natural polysaccharide, chitosan is the partially deacetylated product of chitin; there are various forms with different molecular weights and degrees of deacetylation [21, 22]. The biological activity of a biopolymer undoubtedly depends on its molecular weight [23]. It has been reported that the lower the chitosan molecular weight, the greater the colour differences and film solubility of treated fruit [24]. High-molecular-weight chitosan (360 kDa) produces a layer that more effectively delays ripening and maintains high postharvest fruit quality compared with low-molecular-weight chitosan (40 kDa) [25]. This study aimed to determine the effect of chitosan with different molecular weights on the yield, health, and quality of Korona cultivar strawberries.

2. Materials and Methods

2.1. Characteristics of the Research Area and Plant Material

The experiment was conducted at the Horticulture Department of the West Pomeranian University of Technology in Szczecin. Frigo plants of the Korona cultivar were planted on shafts with spacing of 0.3 × 0.8 m, in three replicates, 20 plants per plot, in a randomised sub-block arrangement. Plants were irrigated and fertilised using a T-Tape drip line. A water-soluble, multi-nutrient fertiliser was applied at a rate of 50 kg nitrogen ha^{-1}.

2.2. Production of Chitosan and Film Preparation

Chitin was obtained via demineralisation, deproteinisation, and deodorisation of shrimp (*Farfantepenaeus brasiliensis*) waste.

Strawberries were treated with chitosan with molecular weights of 5, 12, 21, 50, 125, and 500 kDa. The same plants were sprayed annually with a 0.2% solution until the leaves were fully wet. Spraying was conducted at the beginning of vegetation, three times during flowering, and three times during fruiting. Control plants were sprayed with distilled water only. No other chemical plant protection was applied during the experiment.

2.3. General Fruit Parameters

Strawberry yield and weight were measured each year using a WPX 4500 instrument, with precision of ± 0.01 g (RADWAG, Radom, 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 (CX-732, Elmetron, Radom, Poland), according to the PN standard [26]. The nitrate content was measured with an RQflex 10 quantometer (Merck, Germany) [27].

2.4. Colour

Measurements were conducted with the CIE *L*a*b** system [*L** white (100) black (0), *a** green (-100) red (+100), and *b** blue (-100) yellow (+100)], through a 10º observer type and D65 illuminant using a CM-700d spectrophotometer (Konica Minolta, Japan). The colour parameters and indices were averaged over 50 measurements [28].

2.5. Firmness

Firmness and puncture resistance of the skin was measured with a FirmTech2 apparatus (BioWorks, USA) on 100 randomly selected berries from three replicates. The measures are expressed as a gram-force causing the fruit surface to bend 1 mm [29].

2.6. Extraction and Identification of Polyphenols

Three replicates of 1000 g of randomly chosen strawberries were kept frozen in polyethylene bags at -65°C until analysis, then prepared according to the methodology of Lachowicz-Wiśniewska *et al.* [30]. 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 with a 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 strawberry 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 described by Arnao *et al.* [31]. For the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, the procedure was performed according to the method described Brand-Williams *et al.* [32]. The antioxidant capacity is expressed as mmol Trolox equivalent (TE) g^{-1} dry weight (dw). Measurements for the ABTS·+ assay involved the UV-2401 PC spectrophotometer (Shimadzu Corporation, Japan). The L-ascorbic acid content was measured with an RQflex 10 quantometer (Merck, Germany) [28].

2.9. Statistical Analysis

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

3. Results and Discussion

Chitosan with a lower molecular weight – 12, 21, and especially 50 kDa – increased plant yield (Table 1). In contrast, strawberries harvested from plants sprayed with chitosan 125 kDa were the largest (100 strawberries weighed 1017 g), but the yield was the lowest, similar to the control. Despite the large weight/size, strawberries sprayed with chitosan 125 kDa were the firmest (Table 1). Usually, as the fruit size increases, firmness decreases [33]. The findings indicate that chitosan may have a positive effect on this parameter, and its molecular weight may be the decisive factor. Hernández-Muñoz *et al.* [34] also found significantly firmer strawberries after chitosan treatment. Firmness is a characteristic that is responsible for the storability and transportability of fruit and it is important to be able to increase it.

Chitosan also influenced the chemical composition of the strawberries. The fruit flavour is important to the consumer and is mostly determined by the ratio of sugars to acids. There was no significant effect of spraying on titratable acidity content. However, chitosan, 5 and 12 kDa increased the content of soluble solids compared with the control, while chitosan 125 and 500 kDa significantly decreased it. The application of Biochikol 020PC, the active ingredient of which is chitosan, also increased the soluble solids content in fruits of several raspberry cultivars [35].

Strawberries are a low-calorie fruit with a high water content and health-promoting properties. Regardless of the chitosan molecular weight used, the fruit had very low levels of harmful nitrates, up to 39.4 mg 1000 g^{-1} (chitosan 125 kDa). Based on the applicable regulations, these fruit can be considered safe for the consumer. Nitrate content limits are set only for green leafy vegetables in European Union (EU) legislation. Fresh lettuce may contain up to 5000 mg 1000 g^{-1} nitrate, and processed foods for feeding infants and young children should not exceed 200 mg 1000 g^{-1} nitrate [36]. In contrast, nitrite levels should not exceed 0.07 mg per kg body weight per day.

Chitosan affected the L-ascorbic acid content and antioxidant properties of the tested fruits. Strawberries harvested from plants sprayed with chitosan 500 kDa had the highest L-ascorbic acid content and free radical scavenging capacity determined by the DPPH and ABTS assays (Table 1). The free radical scavenging activity of fruit depends on the polyphenol content. The activity is also influenced by their chemical structure, type, number, and location of substituents (-OH and $-OCH_3$ groups) in the molecule, their concentration, and degree of oxidation. Partial oxidation of polyphenols may result in their increased free radical binding capacity compared with non-oxidised polyphenols. The increased free radical binding capacity of partially oxidised polyphenols is due to their greater ability to release the hydrogen atom of the hydroxyl group at the aromatic ring and the increased ability of the aromatic ring to hold unpaired electrons [37].

Chitosan 500 kDa also significantly reduced the number of grey mould–infested fruit (Table 1). Despite not using chemical protection, the number of infected strawberries was 8%. On control plants and plants sprayed with chitosan 5-50 kDa, yield losses exceeded 20%. Strong infection of plants by *Botrytis cinerea* Pers. may cause yield losses of up to 80% [38]. Fruit have many simple sugars and organic acids; therefore, they are a good medium for yeast and mould. Colonisation of blueberry by fungi depends on microhabitat conditions, numerous abiotic and biotic factors, and their interactions [39]. Chaiprasart *et al.* [40] observed less occurrence of grey mould during storage of strawberries treated with chitosan. The use of this polymer to extend shelf life and to improve the quality of food products is due to its antimicrobial and antioxidant activity and its ability to form coatings and films [41].

Table 1. The quality and antioxidant properties of Korona cultivar strawberries depending on the molecular weight of the applied chitosan.

	Molecular weight of chitosan (kDa)						
	Control	**5**	**12**	**21**	**50**	**125**	**500**
Total yield from plant (g)	408a	414a	514c	570d	611e	437a	483b
Mean weight of 100 strawberries (g)	823a	894b	842a	941c	813a	1017d	956c
Firmness (G mm^{-1})	167ab	154a	182bcd	188cd	176bc	225e	201d
Soluble solids (%)	10.7b	11.5d	11.3c	10.8b	10.7b	10.4a	10.2a
Titratable acidity (g 100 g^{-1})	1.18a	1.04a	1.09a	1.11a	1.07a	1.22a	1.27a
$N\text{-}NO_3$ (mg 1000 g^{-1})	33.2c	28.3b	21.5a	26.0b	27.8b	39.4d	35.0c
L-ascorbic acid (mg 1000 g^{-1})	26.5ab	28.3bc	30.6c	30.3c	24.4a	27.1ab	34.7d
DPPH (mmol TE/100 g)	58.3c	55.8bc	51.3b	46.9a	62.2d	57.0c	43.5a
$ABTS^{\cdot+}$ (mmol TE/100 g)	38.9de	41.3e	33.1c	33.0c	36.9d	27.7b	22.3a
% of strawberries infected by grey mould	29d	21b	25cd	28cd	22bc	18b	8a

Note. Means followed by the same letter do not differ significantly according to Tukey's test ($p > 0.05$).

Orange, red, purple, and blue colours are related to the presence of pigments such as anthocyanins, carotenoids, and betalains [42]. Application of chitosan 5-50 kDa had little effect on strawberry colour (Figure 1). Application of chitosan 125 or 500 kDa changed both the a^* and b^* parameters: the strawberries had a more intense red colour. This was also confirmed by the higher content of anthocyanins, which are responsible for the red colour of fruit (Table 2). The application of chitosan 50, 125, or 500 kDa increased the value of parameter a^*. The effect of chitosan on parameter b^* varied.

Leaves sprayed with chitosan 500 kDa were the greenest, while chitosan 50 kDa treatment produced yellow leaves. Leaf colour, especially a^*, is strongly correlated with the SPAD greening index, which indicates the amount of chlorophyll. A high chlorophyll content indicates good plant health and allows the production of a large amount of assimilates, which may affect the better plant health and its ability to protect against pathogens. Plants sprayed with chitosan 500 kDa had the least grey mould infestation and also showed high health-promoting properties (Tables 1 and 2).

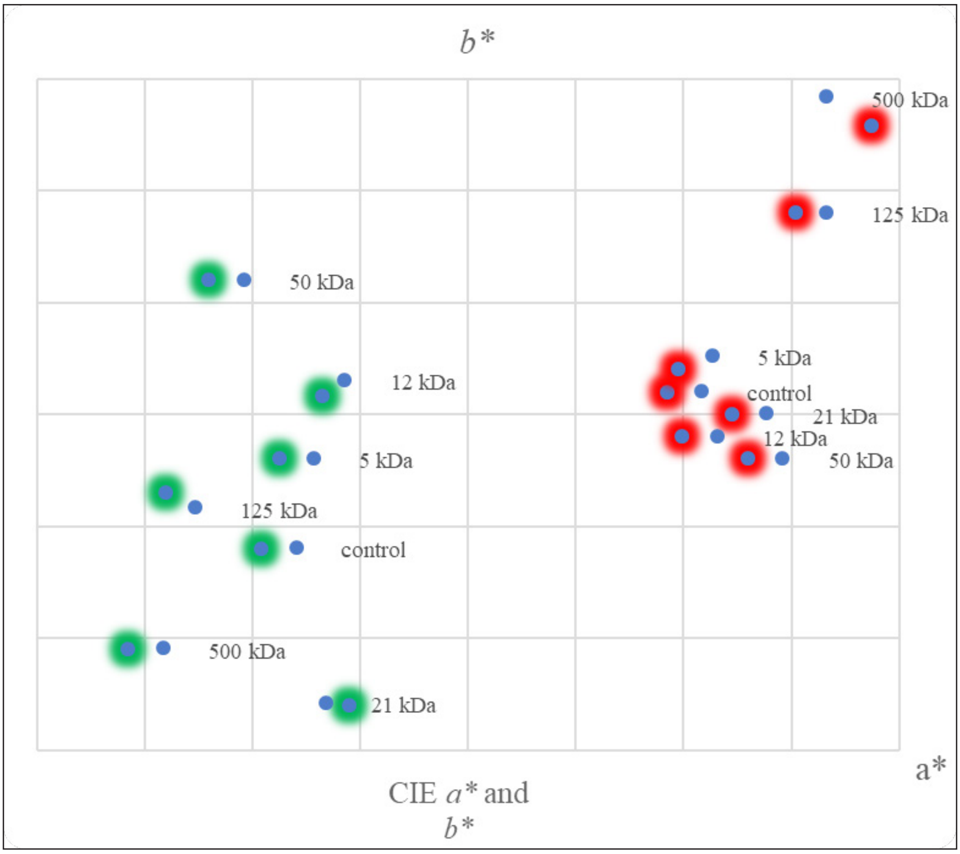

Figure 1. Effect of chitosan on the colour of leaves and fruit of Korona cultivar strawberries. Green = leaves; red = fruit.

Table 2. The phenolic composition of Korona cultivar strawberries (mg 100 g^{-1} fresh weight).

	Molecular weight of chitosan (kDa)						
	Control	**5**	**12**	**21**	**50**	**125**	**500**
Cyanidin-3-*O*-glucoside	1.52	1.85	2.37	2.50	2.43	2.86	3.04
Cyanidin 3-*O*-(6″malonyl) glucoside	0.18	0.16	0.10	0.15	0.21	0.26	0.22
Pelargonidin-3-*O*--glucoside	26.54	21.73	24.30	29.34	35.62	37.30	33.49
Pelargonidin-3-*O*-rutinoside	1.18	1.42	2.46	1.94	1.77	2.28	2.18
Pelargonidin-3--glucoside-malonyl	4.10	4.59	7.40	7.47	8.59	6.84	4.72
Pelargonidin 3-*O*-(6″--malonyl) glucoside	3.63	2.36	1.96	4.11	4.46	5.75	5.21
Anthocyanins	**37.15ab**	**32.11a**	**38.61b**	**45.51c**	**53.08d**	**55.28d**	**48.85c**

	Molecular weight of chitosan (kDa)						
	Control	5	12	21	50	125	500
Kaempferol 3-glucoside	0.50	0.41	0.37	0.47	0.39	0.49	0.44
Quercetin-3-glucoside	5.95	6.23	3.75	5.95	5.54	5.66	4.32
Quercetin-3-glucuronide	0.06	0.13	0.37	0.26	0.24	0.19	0.12
Flavonols	**6.51bc**	**6.78c**	**4.49a**	**6.68c**	**6.17b**	**6.34bc**	**4.88a**
p-coumaric acid glucoside	1.57	1.50	2.67	3.01	1.94	2.76	3.06
Ellagic acid	0.72	0.87	0.92	1.15	0.68	0.77	0.38
Ellagic acid *O*-glucoside	0.71	0.80	1.00	1.14	1.10	1.05	0.84
Ellagic acid *O*-pentoside	4.60	4.51	4.76	4.33	4.91	3.95	3.24
Phenolic acid	**7.59a**	**7.68a**	**9.34c**	**9.64c**	**8.62b**	**8.53b**	**7.52a**
(+)Catechin	5.90	5.67	5.52	5.07	5.11	5.52	5.57
Procyanidin B1	3.68	3.84	4.28	3.42	3.11	3.38	2.81
Procyanidin B3	0.48	0.44	0.39	0.50	0.49	0.75	0.58
Procyanidins and flavan-3-ols	**10.07c**	**9.95c**	**10.19c**	**8.99a**	**8.71a**	**9.65b**	**8.96a**
TOTAL	**61.32b**	**56.52a**	**62.63b**	**70.82c**	**76.59d**	**79.81d**	**70.21c**

Note. Means followed by the same letter do not differ significantly according to Tukey's test ($p > 0.05$).

Due to their high polyphenolic content and antioxidant activity, strawberries are classified as a functional food. Thanks to these properties, the consumption of strawberries contributes to reducing the amount of DNA, protein, and lipid damage, and to lowering the risk of developing cancers, including stomach and oesophageal [43].

Polyphenols, especially anthocyanins, are mainly found in the dark/dark blue fruit peel or directly underneath. [44]. The application of chitosan 21-500 kDa increased the polyphenol content compared with the control (Table 2). In fruit sprayed with higher molecular weight chitosan, total polyphenols ranged from 70.21 to 79.81 mg 100 g^{-1} fresh weight, most of which were anthocyanins (45.51-55.28 mg 100 g^{-1}). This group of polyphenols showed the largest increase. About 70% of the identified polyphenols/anthocyanins was pelargonidin-3-glucoside, with a content from 21.73 mg 100 g^{-1} (chitosan 5 kDa) to 37.30 mg 100 g^{-1} (chitosan 125 kDa). Skupień and Oszmiański [45] found similar amounts of this compound in 'Senga Sengana' strawberries. The effect of chitosan on the content of anthocyanins is reflected by their colour (Figure 1) and higher health-promoting value (Table 1).

Chitosan with a higher molecular weight also decreased in the content of procyanidins and flavan-*3*-ols compared with the control (Table 2). An increase in the content of phenolic compounds, flavonoids, and ascorbic acid, as well as antioxidant activity, was also found in lychee fruits after chitosan treatment [46].

4. Conclusions

1. The yield of Korona cultivar strawberries sprayed with chitosan 12, 21, or 50 kDa was higher than strawberries sprayed with chitosan 125 or 500 kDa. However, the strawberries of plants sprayed with chitosan 125 kDa were the largest and the firmest.
2. Chitosan affected the chemical composition of strawberries. However, the effect depended on the molecular weight. Chitosan with a higher molecular weight resulted higher L-ascorbic acid and polyphenol contents and higher antioxidant activity.
3. Anthocyanins were the largest group of polyphenolic compounds. About 70% of the identified anthocyanins were pelargonidin-3-glucoside. This compound showed the greatest quantitative increase after chitosan treatment.
4. Higher molecular weight chitosan reduced procyanidins and flavan-3-ols in strawberries compared with the control.
5. The use of chitosan with the highest molecular weights, 125 and 500 kDa, resulted in redder strawberries.
6. Plants sprayed with chitosan 125 or 500k kDa were less affected by grey mould.

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