EVALUATION OF THE IMPACT OF MOLECULAR WEIGHT AND CONCENTRATION OF CHITOSAN ON THE QUALITY AND POSTHARVEST LONGEVITY OF RASPBERRY FRUITS

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Abstract

Chitosan is a biopolymer with antimicrobial and biostimulatory properties, widely utilised in plant protection and fruit quality enhancement. This research aimed to determine the effect of chitosan on raspberry fruit firmness and assess the relationship between its concentration and molecular weight with yield quality. The study was conducted on raspberries treated with chitosan, applied as a spray, varying in molecular weights (3.3, 5, 500, and 950 kDa) at two concentrations (0.2% and 0.4%). The results showed that chitosan significantly enhanced fruit firmness, with the highest values observed for high-molecular-weight chitosan (500 and 950 kDa). Moreover, low-molecular-weight chitosan (3.3 and 5 kDa) positively influenced fruit mass, whereas high-molecular-weight forms had a limiting effect. It was also found that a higher chitosan concentration (0.4%) led to increased fruit firmness. Previous studies indicated that raspberry fruit quality was most influenced by low-molecular-weight chitosan (3.3 and 5 kDa), while fruit health was most improved by high-molecular-weight chitosan (500 and 950 kDa). Therefore, these chitosan variants were selected for further investigation to determine whether the fruit's response is primarily driven by molecular weight or if concentration also plays a crucial role. These findings indicate that chitosan may be an effective tool for improving raspberries' quality and postharvest longevity, with its efficacy depending on the appropriate choice of molecular weight and concentration. The application of chitosan may serve as an alternative to conventional fruit protection and preservation methods, contributing to prolonged fruit shelf life and enhanced commercial value.

Keywords: *fruit firmness, chitosan molecular weight, Botrytis cinerea, postharvest raspberry quality, biostimulants in plant protection*

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1. Introduction

The red raspberry (*Rubus idaeus* L.) belongs to the *Rosaceae* family and is widely distributed across temperate regions of Europe, Asia, and North America [1]. Due to its flavour attributes and nutritional value, it is among the most highly valued berry fruits. Poland is one of the leading raspberry producers in Europe, benefiting from favourable climatic and soil conditions [2]. Global raspberry production has increased by approximately 80% over the past decade, reaching 684,000 tonnes in 2019. The largest raspberry-producing countries include Russia, Poland, the United States, Serbia, Mexico, Ukraine, Spain, Chile, the United Kingdom, and Bosnia and Herzegovina. Furthermore, new players such as Morocco and South Africa have entered the global raspberry market, increasing competitiveness in the sector [3, 4].

Raspberries are characterised by their unique taste and high content of bioactive compounds, including vitamin C, quercetin, and anthocyanins [5]. These compounds exhibit strong antioxidant properties, neutralising free radicals and potentially contributing to protection against cardiovascular diseases, cancer, and cellular ageing [6]. Additionally, the antioxidant components of raspberries have demonstrated anti-inflammatory effects, which may lower the risk of developing chronic diseases [7].

However, raspberries are highly perishable fruits, susceptible to fungal and viral infections, including grey mould (*Botrytis cinerea*), powdery mildew, and anthracnose [8–10]. Ensuring optimal cultivation conditions, such as pruning, irrigation, fertilisation, and the application of plant protection products, is crucial for maintaining plant health [11, 12]. Environmental stress factors, including drought, excessive rainfall, frost, and high temperatures, can lead to yield reduction and diminished fruit quality [13, 14]. Key quality indicators for raspberries include fruit size, colour, and firmness, which determine their suitability for harvesting, transport, and storage [15]. A decrease in fruit firmness increases their susceptibility to fungal infections [16].

Growing concerns over the environmental impact of synthetic crop protection agents have led to the search for alternative strategies to improve plant health. One promising biostimulant is chitosan—a non-toxic, biodegradable polysaccharide derived from the deacetylation of chitin, a major component of crustacean and insect exoskeletons and fungal cell walls [17, 18]. Chitosan not only exhibits antibacterial and antifungal properties but also stimulates plant defence mechanisms, making it a versatile agent in sustainable agriculture [19]. Its application in various forms, such as foliar sprays, soil amendments, seed coatings, and hydroponic solutions, has been shown to reduce pathogen incidence while also promoting plant growth and stress tolerance [20].

The effect of chitosan on fruit quality is dependent on its molecular weight and concentration. High-molecular-weight chitosan has been shown to delay fruit ripening and improve postharvest quality (the highest value of titratable acidity, fruit firmness, and also resulting in a reduction of weight loss, ethylene production, and respiration rate). Low-molecular-weight chitosan is more soluble and penetrates plant tissues more effectively, resulting in higher antibacterial activity [21, 22]. The mode of action of chitosan involves electrostatic interactions between its amino groups and negatively charged structural components of microbial cells, which can inhibit their growth [23]. Moreover, chitosan may suppress fungal polygalacturonase production, thereby reducing fungal colonisation of fruit tissues [24]. Due to its multifaceted mode of action, chitosan presents a promising alternative to synthetic plant protection agents, contributing to improved raspberry yield quality and reduced postharvest losses caused by pathogens [25].

In previous studies conducted on several fruit plant species, we demonstrated that the effectiveness of chitosan varies depending on its molecular weight. Therefore, in this study, we selected extreme molecular weights - both low and high - which had shown the most pronounced effects in previous experiments. The objective of this study was to determine whether the primary factor influencing raspberry fruit quality is the molecular weight of chitosan or its concentration and to assess the impact of both parameters on the firmness and size of raspberries. Additionally, the study evaluated whether the application of chitosan in the form of a spray could reduce grey mould (*B. cinerea)* infection after two days of storage under simulated shelf-life conditions.

2. Materials and Methods

2.1. Characteristics of the Area of Research and Plant Material

The experiment was conducted at the Horticulture Department of the West Pomeranian University of Technology in Szczecin.

The experiment was established on a precipitous-gley podzolic soil developed from till with a mechanical composition of light clay (26 - 35 fraction < 0.02 mm) [26] under the climatic conditions of north-western Poland [27].

During the experiment, treatments were carried out for plant care. According to the established rules, they were carried out throughout the growing season. The soil was watered using a permanently mounted T-Tape drip line with an emitter capacity of 1 L/h (5 L of water per hour over a 1-meter section of the system). The doses and intensity of water emitted were modified according to the degree of soil moisture, determined by contact soil tensiometers. The soil in the orchard was an agricultural soil with a natural profile developed from silt-loam with a pH of 6.5. Annually, from the start of vegetation (March/April), nitrogen fertilisation was applied at a rate of 90 kg N, divided into three equal doses.

2.2. Edible Coating Preparation and Application of Treatments

Chitosan samples with varying molecular weights were obtained through radical degradation following the methodology described by Ochmian and Krupa-Małkiewicz [25], and their molecular weight was determined using the HPLC/GPC technique.

Raspberry underwent treatment with chitosan variants of different molecular weights: 3,333 Da (3.3k), 5,000 Da (5k), 500,000 Da (500k), and 950,000 Da (950k). Chitosan was dispersed in 300 g of distilled water, and the solution was titrated to a pH of 7.7 by adding 1 M sodium hydroxide (NaOH). Then, the volume was adjusted to 500 ml with distilled water, and 500 g of 0.2 M acetic acid was added.

Every year, raspberries were sprayed with a 0.2% and 0.4% chitosan solution (0.2 litres per metre of row), prepared from the aforementioned chitosans. The application was conducted to ensure complete wetting of the leaves and, later in the season, both leaves and fruits, without allowing the solution to drip from the plants. Spraying was carried out exclusively in the morning hours using a backpack sprayer. It was performed only on dry plants, and following periods of heavy rainfall, the application was conducted within 24 h once the foliage was dry.

The spraying schedule included three applications at the start of the vegetation period, three during the flowering phase, and three during the fruit-bearing stage. Conversely, the control group of plants received only distilled water sprays. Throughout the study, no additional chemical protections were applied.

From 2021 to 2023, significant weather anomalies were observed compared to this region's historical data. During July and August, temperatures consistently exceeded 30°C for several days and did not drop below 20°C at night, resulting in tropical night conditions. Extended periods of drought were recorded during the summer months: 32 consecutive days without rainfall in 2021, 46 days in 2022, and 37 days in 2023. Following these dry spells, intense rainfall events occurred, with precipitation exceeding 100 mm per day. September, in contrast, exhibited typical weather patterns consistent with multi-year averages (data obtained from the Meteorological Experimental Station in Lipnik, Poland, 53°20′35″ N, 14°58′10″ E).

In the experiment, three plots were designated for each treatment/chitosan. In subsequent years, the raspberry rows became denser. Each year, the excess new plants are reduced; in the spring, using a rototiller, and during the growing season, plants that grow in the row spacings are mowed. Each plot consisted of a raspberry row section one meter in length and approximately 30 cm in width. A solution dosage of 600 litres per hectare was applied, which equates to 0.2 litres of solution per 1 metre of raspberries.

2.3. General Fruit Parameters

The fruits were harvested when fully ripe, based on the assessment of fruit colour, and successively throughout the vegetation season. The weight of 100 fruits was measured in fresh fruits immediately after harvest. Annual measurements were taken for fruit mass using a RADWAG WPX 4500 scale (accuracy of ± 0.01 g).

2.4. Firmness

The firmness and puncture resistance of the skin were assessed using a FirmTech2 device (BioWorks, USA) on 100 berries chosen at random from three replicates. The results were expressed in gram-force required to cause a 1 mm deformation of the fruit surface, as reported by Ochmian *et al.* [28].

2.5. Assessment of Grey Mould (*Botrytis cinerea*) Infection in Raspberries Under Simulated Shelf-life Conditions

Raspberries were harvested at full maturity based on colour and firmness assessment. Subsequently, the fruits were placed in plastic containers with a single layer of fruit, similar to those used for retail sale. The containers with samples were stored for 2 days in climate chambers with controlled temperature (22 ± 1°C) and relative humidity (85 ± 5%) to simulate retail storage conditions. To maintain consistent humidity, trays with containers were lined with moist filter paper.

After the storage period, the fruits were visually assessed to identify the presence of grey mould (*B. cinerea*). The incidence of infection was recorded based on visible symptoms, such as grey fungal growth, necrosis, and rot. The results were expressed as the percentage of infected fruits relative to the total number of fruits in each sample, calculated using the formula:

$$\% \text{ infected fruits} = (\text{number of infected fruits} / \text{total number of fruits}) \times 100 \quad (1)$$

2.6. Statistical Analysis

Statistical analyses were conducted using Statistica 12.5 (StatSoft Polska, Kraków, Poland). The data underwent one-way analysis of variance (ANOVA). Comparisons of means were made using Tukey's least significant difference (LSD) test, with a significance level established at $p < 0.05$. The multivariate analysis was fulfilled by applying principal component analysis (PCA) and agglomerative hierarchical cluster analysis (HCA) by the Ward method (Euclidean distance).

3. Results and Discussion

Previous studies have demonstrated that the quality of raspberry fruit was most significantly influenced by low molecular weight chitosan (3.3 and 5 kDa), whereas fruit health, particularly in reducing *Botrytis cinerea* infections, was most effectively improved by high molecular weight chitosan (500 and 950 kDa) [25]. These findings suggest that the mode of action of chitosan may depend not only on its concentration but primarily on its molecular weight. Therefore, for further research, the extreme molecular weight variants of chitosan - low (3.3 and 5 kDa) and high (500 and 950 kDa) - were selected to determine whether the key factor affecting fruit quality and health is solely its molecular weight or also the applied concentration in foliar treatment.

3.1. Weight of 100 fruits

At the outset, it is important to note that the control treatment (315 g) was shared across all comparisons, serving as a reference point for both the analysis of the main factors and their interaction (Table 1).

Table 1. The effect of chitosan molecular weight and applied concentration on the quality of raspberry fruits.

Chitosan concentration [%]	**Molecular weight of chitosan [kDa]**				
	3.3	**5**	**500**	**950**	mean
	mean weight of 100 fruits [g]				
0.2	411 ± 9^{e}	405 ± 8^{de}	322 ± 7^{c}	295 ± 6^{b}	358^{B}
0.4	397 ± 8^{d}	424 ± 10^{f}	218 ± 9^{a}	300 ± 8^{b}	335^{AB}
mean conc	404^{c}	415^{c}	270^{a}	298^{b}	
control	$315 \pm 9^{\mathrm{b/A/}c}$				
	firmness fruits – with a floral base [$G\ mm^{-1}$]				
0.2	129 ± 12^{b}	130 ± 10^{b}	166 ± 11^{c}	179 ± 9^{d}	151^{A}
0.4	135 ± 11^{b}	117 ± 9^{a}	236 ± 10^{f}	208 ± 12^{e}	174^{B}
mean conc.	132^{a}	124^{a}	201^{c}	194^{c}	
control	$161 \pm 10^{\mathrm{b/AB/}c}$				

Note. The control treatment is shared across both main factors and their interaction. CAPITAL letters indicate statistically significant differences between chitosan concentrations, lowercase letters denote significant differences for the molecular weight of chitosan, and *italicised lowercase letters* indicate significant differences observed within the interaction between these factors.

Averaging the results without considering the molecular weight of the applied chitosans, it was found that the chitosan concentrations had no significant effect on the mass of 100 raspberry fruits. The highest average fruit mass (358 g) was recorded at a 0.2% concentration, and a slightly lower mass (335 g) at 0.4%. However, these differences were not statistically significant. In contrast, the lower chitosan concentration (0.2%) significantly increased fruit mass compared to the control (315 g).

Significant differences were observed among the applied molecular weights of chitosan. The highest average fruit mass (415 g) was recorded for chitosan with a molecular weight of 5 kDa, which significantly exceeded the results obtained for other variants. A similarly positive effect was noted for 3.3 kDa (404 g), while considerably lower values were recorded for high-molecular-weight chitosan: 500 kDa (270 g) and 950 kDa (298 g). These findings align with previous results, which also demonstrated that the application of lower molecular weight chitosans positively influences the size of raspberry fruits [25]. These results indicate that low molecular weight chitosans contributed to improved fruit size, likely due to enhanced tissue penetration and activation of metabolic processes [29]. However, for haskap berry and strawberry, the most pronounced effect on fruit size was observed with chitosan of 125 kDa (chitosan, haskap berry and strawberry).

The highest mass of 100 fruits was obtained with 0.4% chitosan of 5 kDa (424 g) and 0.2% chitosan of 3.3 kDa (411 g). High fruit mass was also observed for the remaining low molecular weight chitosan variants. In contrast, the smallest fruits were recorded for the 0.4% chitosan of 500 kDa (218 g per 100 fruits). However, the application of 0.2% chitosan of 500 kDa resulted in significantly larger fruits - 100 fruits weighed 322 g, slightly higher than the control. These findings highlight the significant interaction effect between chitosan concentration and its molecular weight.

The study results clearly indicate a significant impact of chitosan concentration and molecular weight on the mass of 100 raspberry fruits. It has been demonstrated that optimal effects are achieved using low-molecular-weight chitosan (3.3 kDa and 5 kDa) at concentrations of 0.2 - 0.4%, while high-molecular-weight chitosan (500 and 950 kDa) negatively affects fruit size. Similar observations were made by Ochmian and Krupa-Małkiewicz [25], who reported that chitosan at 21 kDa positively influenced fruit growth, whereas the application of 500 and 950 kDa resulted in fruit size reduction.

The mechanism by which chitosan influences fruit growth appears to be closely associated with its impact on plant metabolism, enhanced nutrient uptake, and the alleviation of abiotic stress. According to Kananont *et al.* [30], the application of chitosan at concentrations ranging from 0.01% to 0.08% (10 - 80 mg/L) significantly improved seed germination and protocorm growth in *Dendrobium formosum*, particularly at lower concentrations (0.01% and 0.02%). This effect was more pronounced in the initial stages of growth, suggesting that low concentrations of chitosan may effectively stimulate early plant development. In contrast, for *D. bigibbum var. compactum*, chitosan concentrations of 0.01% were most effective in promoting protocorm growth, despite having no discernible impact on seed germination. These findings underscore the importance of optimising chitosan dosage for specific plant species and developmental stages, as excessively high concentrations may not necessarily enhance plant growth.

Salachna *et al.* [29], using chitosans with molecular weights ranging from 2.5 kDa to 950 kDa, further highlighted the potential of low-molecular-weight chitosan to prolong plant growth. The authors emphasised that the application of 0.2% chitosan with molecular weights up to 6.5 kDa significantly increased plant biomass, demonstrating that lower molecular weights can effectively enhance plant growth, possibly through improved tissue penetration and activation of metabolic processes. This effect aligns with the results of Kananont *et al.* [30], reinforcing the notion that lower concentrations and molecular weights may be optimal for specific plant responses.

A comparable outcome was observed by Grajkowski and Ochmian [15], who applied a commercial chitosan formulation and reported an increase in raspberry yield and fruit size. The findings suggest that chitosan, irrespective of its source or molecular weight, has the potential to positively influence plant growth and yield, particularly when applied at appropriate concentrations.

Furthermore, Pichyangkura and Chadchawan [31] demonstrated that chitosan not only promotes growth but also activates a range of defence mechanisms in plants. It induces the expression of pathogenesis-related genes, such as glucanase and chitinase, and stimulates the production of enzymes involved in the reactive oxygen species (ROS) scavenging system, including superoxide dismutase, catalase, and peroxidase. Additionally, chitosan initiates a signalling cascade involving hydrogen peroxide and nitric oxide, which can modulate gene expression through interactions with chromatin. These biochemical responses may further explain the growth-promoting effects observed at lower chitosan concentrations, as they potentially enhance plant resilience to environmental stress and improve nutrient uptake efficiency.

This combined evidence underscores the multifaceted role of chitosan in plant development, highlighting both its direct impact on metabolic processes and its capacity to activate defence-related pathways. Consequently, selecting appropriate chitosan concentrations based on plant species and developmental stage is crucial for maximising its beneficial effects.

A crucial factor is also the formation of a chitosan coating on the fruit surface, which may reduce water loss and help maintain fruit mass [32]. This effect may be particularly significant in the case of low-molecular-weight chitosan, which penetrates plant tissues more effectively and activates physiological mechanisms related to growth regulation. In contrast, high-molecular-weight chitosan may limit its effectiveness due to reduced tissue penetration ability, resulting in lower metabolic activation and less efficient nutrient uptake.

3.2. Fruits' Firmness

When considering the effect of chitosan concentration without accounting for molecular weight, chitosan application did not significantly influence the firmness of raspberry fruits compared to the control. The average fruit firmness for the 0.2% concentration was 151 G mm^{-1}, and for the 0.4% concentration, it was 174 G mm^{-1}. Both values were comparable to the control (161 G mm^{-1}).

However, when the chitosan molecular weight was considered, a significant impact on fruit firmness was observed. Regardless of the applied concentration, the highest firmness values (201 - 194 G mm^{-1}) were recorded in fruits treated with high molecular weight chitosan (500 and 950 kDa). These values were higher compared to both the control (161 G mm^{-1}) and the fruits treated with low molecular weight chitosan: 3.3 kDa (132 G mm^{-1}) and 5 kDa (124 G mm^{-1}).

For chitosan with molecular weights of 500 and 950 kDa, the higher concentration (0.4%) had a more pronounced effect on increasing fruit firmness. However, it is noteworthy that the fruits characterised by higher firmness were also significantly smaller, suggesting that the observed effect may be indirectly related to a reduction in fruit size. As previous studies indicate, larger fruits generally exhibit lower firmness, while smaller fruits tend to be more compact. Therefore, it remains unclear whether the increase in firmness was a direct effect of chitosan application or a consequence of limiting fruit growth.

Chien *et al.* [33] demonstrated that the effect of chitosan on fruit firmness is closely related to its molecular weight and concentration. In their study, chitosan with molecular weights of 15.1 kDa and 357 kDa was applied at concentrations of 0.05%, 0.1%, and 0.2%. The most pronounced increase in fruit firmness, exceeding 30% compared to the control and high molecular weight chitosan (357 kDa), was observed for 0.2% chitosan of 15.1 kDa.

Zhang and Quantick [34] reported that the application of a chitosan coating significantly improved the firmness of strawberries and raspberries stored at 4°C. In their study, the most pronounced effects were observed with a 2% chitosan solution, which formed a visible coating on the fruit surface. The coating not only enhanced firmness but also positively affected titratable acidity, vitamin C content, and anthocyanin levels, indicating that chitosan can effectively maintain fruit texture and nutritional quality during storage.

Similarly, Singh *et al.* [35] demonstrated a significant correlation between fruit firmness and chitosan concentration, with the best results also obtained at a 2% chitosan solution. However, in Singh's study, fruits were dipped in the chitosan solution to form a coating, which differs from the spray application method used in the present study.

It is important to note that soft fruits such as raspberries are typically delivered to consumers in a ready-to-eat form and are not washed before consumption. Unlike other fruits and vegetables, they are not suitable for the application of coatings that leave a visible residue on the surface, which may reduce consumer acceptance. Therefore, while the formation of a protective chitosan coating can enhance fruit firmness, it is essential to consider the visual and sensory impact of such treatments, especially for soft fruits intended for immediate consumption.

Interaction analysis revealed that the highest firmness value (236 G mm^{-1}) was achieved with 0.4% chitosan at 500 kDa, whereas the lowest value (117 G mm^{-1}) was recorded for 0.4% chitosan at 5 kDa. This indicates that selecting an appropriate molecular weight is crucial for improving fruit texture, with higher concentrations enhancing this effect in specific cases.

Firmness is a key physical parameter in assessing fruit quality during ripening, storage, and distribution [36]. It has been demonstrated that firmness values vary between cultivars, potentially due to differences in lignin content [37]. During storage, fruit firmness gradually decreased in both uncoated and chitosan-coated fruits, with the rate of decline being cultivar-dependent. In studies by Petriccione *et al.* [37], a 2% chitosan coating maintained strawberry firmness more effectively than a 1% coating, although significant differences were only observed after six days of storage. Research by Perkins-Veazie [38] suggests that chitosan can inhibit the degradation of the middle lamella in cortical parenchyma cells, thereby preventing fruit softening.

Firmness is a critical quality attribute for consumer acceptance, as rapid firmness loss during ageing shortens postharvest shelf life and increases susceptibility to fungal infections. The present findings align with Ali *et al.* [39], who demonstrated that a 2% chitosan coating significantly improved papaya firmness compared to other postharvest treatments in cold storage. Similar effects were observed in mangoes and tomatoes treated with chitosan [40].

Previous studies have confirmed the positive impact of various protective coatings on fruit texture, including cactus mucilage [41], chitosan with oleic acid [42], chitosan combined with calcium dips [43], and chitosan with beeswax [44]. Integrating chitosan with other protective coatings and optimising its formulation may enhance fruit quality and extend postharvest shelf life.

The findings indicate that chitosan significantly influences the firmness of raspberry fruits, with its effectiveness depending on molecular weight and concentration. The highest firmness was observed for high-molecular-weight chitosan (500 - 950 kDa), suggesting its role in stabilising fruit structure.

3.3. Percent of Fruits Infected by Grey Mould (*Botrytis cinerea*)

The application of chitosan significantly reduced *Botrytis cinerea* infection in raspberry fruits (Table 2). The average percentage of infected fruits in the chitosan-treated groups was 15 - 16% for both 0.2% and 0.4% concentrations, representing a substantial reduction compared to the control (33%).

Table 2. The effect of chitosan molecular weight and concentration on grey mould (*B. cinerea*) incidence in raspberry fruits after 2 days of storage.

Chitosan concentration [%]	Molecular weight of chitosan [kDa]				
	3.3	**5**	**500**	**950**	mean
	amount of fruits infected by grey mould (*B. cinerea*) [%]				
0.2	27 ± 6^{cd}	22 ± 3^{b}	6 ± 1^{a}	3 ± 1^{a}	15^{A}
0.4	25 ± 5^{bc}	30 ± 6^{de}	4 ± 1^{a}	4 ± 1^{a}	16^{A}
mean conc	26^{b}	27^{b}	5^{a}	4^{a}	
control	$315 \pm 9^{b/A/c}$				

Note. The control treatment is shared across both main factors and their interaction. CAPITAL letters indicate statistically significant differences between chitosan concentrations, lowercase letters denote significant differences for the molecular weight of chitosan, and *italicised lowercase letters* indicate significant differences observed within the interaction between these factors.

The highest efficacy in reducing *B. cinerea* infection was observed for high-molecular-weight chitosan (500 and 950 kDa), where the percentage of infected fruits was 5% and 4%, respectively. In contrast, low-molecular-weight chitosan (3.3 and 5 kDa) was less effective, with 27% and 30% infection rates, respectively. These findings confirm previous reports indicating that chitosan's effectiveness in plant protection is closely related to its molecular weight [45].

The mode of action of chitosan may involve direct damage to fungal cell walls, leading to growth inhibition and developmental disruption [46]. Additionally, chitosan can alter environmental pH, limiting pathogen survival [33]. Zheng *et al.* [47] demonstrated that chitosan with a molecular weight of 350 kDa at a concentration of 0.5% effectively inhibited the development of *B. cinerea* and *Penicillium expansum* in fruit stored at 25°C and 4°C, aligning with the results obtained for raspberries. Chitosan not only suppressed fungal growth but also induced the expression of catalase, superoxide dismutase, and ascorbate peroxidase - key enzymes involved in neutralising reactive oxygen species (ROS). The increased activity of these enzymes confirms the role of chitosan in stimulating plant defence mechanisms.

Chitosan may also act as a physical barrier limiting pathogen access to plant tissues [33]. This is particularly relevant for raspberries, which have a short postharvest shelf life due to firmness loss and susceptibility to decay [48]. Bhaskara Reddy *et al.* [49] demonstrated the efficacy of chitosan in controlling postharvest pathogen development in strawberries, highlighting its potential application in raspberry protection. The authors applied chitosan sprays at concentrations of 0.2%, 0.4%, and 0.6%, observing an apparent dose-dependent effect on pathogen suppression. The incidence of decay decreased with increasing chitosan concentration, but the protective effect diminished over time as the storage period extended.

The most pronounced effect was noted for the 0.6% chitosan concentration, applied twice at a 10-day interval. This treatment effectively reduced fruit decay and maintained fruit quality at an acceptable level throughout the entire 4-week storage period at 3°C. These findings suggest that higher chitosan concentrations may be particularly beneficial in extending the postharvest shelf life of perishable fruits, especially when applied as a pre-harvest treatment.

The study by Bhaskara Reddy *et al.* [49] aligns with the present findings, emphasising the role of chitosan as a protective agent against fungal infections in soft fruits. Moreover, their results suggest that the timing and frequency of chitosan application, in addition to concentration, are critical parameters for maximising its protective effects. Integrating these factors into pre-harvest and post-harvest management strategies could potentially enhance the efficacy of chitosan in reducing pathogen incidence in raspberries and other susceptible fruits.

These findings confirm that chitosan effectively reduces *B. cinerea* infection in raspberries, particularly in its high-molecular-weight forms (500 - 950 kDa). Its protective action may stem from both antifungal properties and its ability to form a protective coating on fruit surfaces. Furthermore, future studies should consider the application of nanotechnology, which could enhance chitosan's efficacy by improving coating stability and increasing the bioavailability of active compounds. Integrating chitosan with nanotechnology presents new opportunities for plant disease biocontrol and postharvest quality improvement, offering a promising alternative to conventional plant protection methods.

3.4. HCA of Raspberry Quality and Health Under Chitosan Treatment

Agglomerative hierarchical clustering analysis (HCA) using Ward's method was employed to assess the similarity of the analysed raspberry fruit samples depending on the applied chitosan. The dendrogram (Figure 1) reveals distinct sample groupings, indicating significant differences in fruit quality and health status as a function of chitosan molecular weight and concentration.

The most significant similarity was observed in the samples treated with high molecular weight chitosan (500 and 950 kDa) at both concentrations (0.2% and 0.4%), which formed a separate cluster in the hierarchical classification. Fruits treated with low molecular weight chitosan (3.3 and 5 kDa) also exhibited mutual similarity, but their distance from the control group was more pronounced. The control samples, which were not treated with chitosan, formed a separate branch, highlighting their significant differences compared to chitosan-treated fruits.

The results of the analysis indicate that the application of high molecular weight chitosan (500 and 950 kDa) strongly differentiated the samples, likely due to its greater efficacy in limiting fruit infections by pathogens as well as its distinct influence on fruit quality parameters compared to low molecular weight chitosan. The clear separation of groups suggests that both the molecular weight and concentration of chitosan influence raspberry quality and health status, and their optimal combination may maximise the benefits of this biopolymer application.

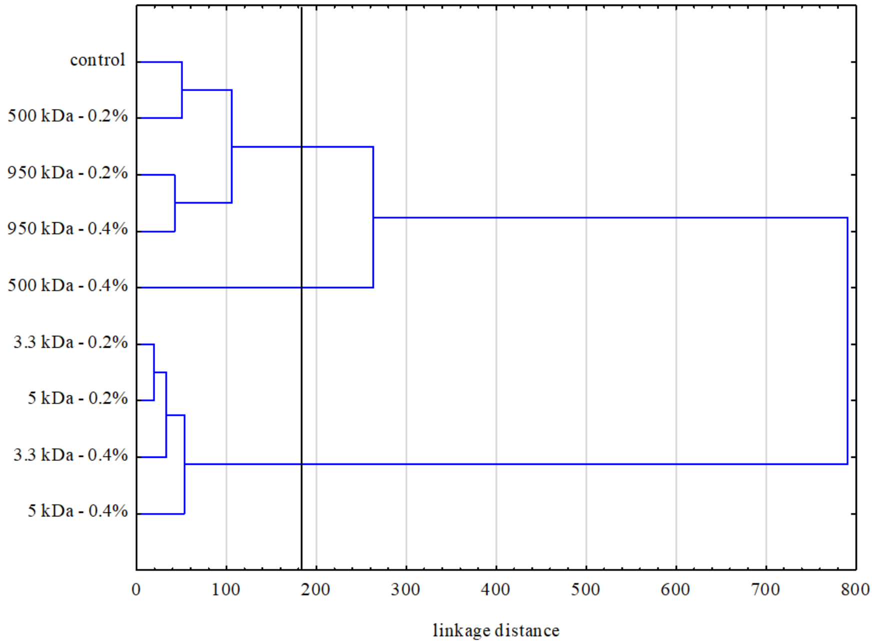

Figure 1. Agglomerative Hierarchical Clustering Analysis (HCA) (Ward's method) showing the distances between the analysed raspberry fruit traits depending on the applied chitosan.

These findings corroborate our previous research, which demonstrated that the effect of chitosan is dependent on its molecular weight. Low molecular weight chitosan exhibits different effects than solutions based on high molecular weight chitosan [25].

4. Conclusions

1. Low-molecular-weight chitosan (3.3 kDa and 5 kDa) applied at concentrations of 0.2 and 0.4% increased the fruit mass of raspberries, while 500 kDa chitosan at a concentration of 0.4% reduced fruit mass by over 30% compared to the control.
2. High-molecular-weight chitosan (500 kDa and 950 kDa) applied at a concentration of 0.4% increased the firmness of raspberry fruits.
3. Regardless of concentration, high-molecular-weight chitosan (500 kDa and 950 kDa) reduced grey mould (*B. cinerea*) incidence on raspberries to 4 - 5%, compared with 33% in the control.
4. The effects of chitosan depend primarily on its molecular weight and concentration: low-molecular-weight forms enhance fruit growth, whereas high-molecular-weight forms are more effective in pathogen control, confirming the polymer’s utility as an eco-friendly alternative to conventional plant-protection agents and a tool for improving post-harvest quality.

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