Chitosan, Nisin, Silicon Dioxide Nanoparticles Coating Films Effects on Blueberry (Vaccinium myrtillus) Quality

Rok Eldib 1,*, Ebtihal Khojah 1, Abeer Elhakem 2, Nada Benajiba 3 and Mahmoud Helal 4,5

1 Department of Food Science and Nutrition, College of Sciences, Taif University, P.O. 11099, Taif 21944, Saudi Arabia; eykhojah@tu.edu.sa
2 Department of Biology, College of Science and Humanities in Al-Kharj, Prince Sattam Bin Abdulaziz University, Al-Kharj 11942, Saudi Arabia; a.elhakem@psau.edu.sa
3 Department of Basic Health Sciences, Deanship of Preparatory Year, Princess Nourah Bint Abdulrahman University, P.O. Box 84428, Riyadh 11671, Saudi Arabia; nabenajiba@pnu.edu.sa
4 Production and Mechanical Design Dept., Faculty of Engineering, Mansoura University, Mansoura 35516, Egypt; mo.helal@tu.edu.sa
5 Department of Mechanical Engineering, Faculty of Engineering, Taif University, P.O. 11099, Taif 21944, Saudi Arabia
* Correspondence: rokayya.d@tu.edu.sa

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Abstract: Chitosan coating plus silicon dioxide nanoparticles and nisin were applied on fresh blueberry samples in order to find out safety packaging assay during the post-harvest process. Studies were performed in-vitro for fruit quality as physicochemical parameters and oxidation, while microbiological analyses as molds/yeast and mesophils populations were examined in-vivo. The selected silicon dioxide nanoparticles 1% and nisin 1%, were added into a chitosan solution, which resulted in four groups of coated blueberries. After storage at ambient temperature, fruits were examined for two, four, six, and eight days. It was noticed that the hardness, chewiness, and cohesiveness of all blueberry samples were increased during the storage. Chitosan-nano-silicon dioxide (CHN-Nano) and (CHN-N-Nano) with the addition of nisin helped to control shrinking (38.52%) and decay rates (8.61%). Moreover, (CHN-N-Nano) reported the lowest L* values (10.54) for the color index, and inhibited the microbial populations (3.60 and 2.73 log CFU/g) for molds/yeast and mesophils, respectively. (CHN-Nano) reported the lowest value for pH (2.61) and the highest for anthocyanin content (75.19 cyanidin-3-glucoside mg/100 g). The chitosan coating substantially maintained Vitamin C (7.34 mg/100 g) and polyphenoloxidase (PPO) (558.03 U min⁻¹·g⁻¹). The results suggest that nano-material with chitosan film coatings that contained nisin were effective for fresh blueberry preservation under ambient temperature.

Keywords: nanoparticles; chitosan; blueberry; films; coating; shelf-life; antimicrobial activity

1. Introduction

Safe fruit development is one of the main drivers of the food industry. The common fruit losses can occur in-between harvesting and the consumption periods in quantitative and qualitative rates [1]. Blueberry (Vaccinium spp.) is one of the most popular berries that can be sold in fresh, dried, frozen, or even processed forms for different industrial applications. Berries are rich in vitamin C, flavonols, tannins, catechins, anthocyanins, and polyphenolic acids, which can prevent several diseases civilization [2]. The storage condition is very essential for the stability of some quality parameters, such as weight loss, shrinking, texture change, color change, gray mold, and ripe rot [3].
Blueberry decay usually occurs due to fungi, such as *Colletotrichum acutatum*, *Salmonella*, *E. coli*, *L. monocytogenes*, *Alternaria* spp., *Botrytis cinerea*, and *S. aureus* [4]. Several preservation technologies, including UV irradiation, cold storage, modified atmosphere packaging, fumigation with sulfur dioxide, and ozonation, have been used to preserve the nutritional quality and prolong blueberries shelf life [5,6]. One such technique is using edible coatings as thin layers on the surface of the blueberries. These coatings are intended to control the decay, titratable acidity, gas exchange, firmness, reduce moisture loss, fungal growth, slowing down the metabolic processes, and maintaining the lightness of the blueberry surface. The common edible coatings include chitosan, pullulan, starch, gellan, pectin, alginate calcium, caseinate, essential oils, and nano-coatings [7–9]. Kou et al., 2019 [10] used the chitosan and nano-silica combination with jujube fruit, while Rok, 2020 [11] applied chitosan-nano-silicon dioxide films to improve cantaloupe pieces qualities. Shi et al., 2013 [12] mentioned nano-silica dioxide application with the addition of chitosan to inhibit the browning of longan fruits. Nano-silicon dioxide with low concentrations is non-toxic according to FDA recommendation and could be used with human foods stuff and food contact materials [13]. Nisin is acting as an antimicrobial peptide that has been approved as safe food additives by (FAO/WHO) for various kinds of foods [14].

Thus, the present study focused on the effects of chitosan, nisin, and silicon dioxide nanoparticles coating films on the quality of blueberry during storage at ambient temperature for future industrial development.

2. Materials and Methods

2.1. Materials

Chitosan (85% deacetylation), nano-silicon dioxide (Purity: >99 wet%—15 nm), and glacial acetic acid nisin were supplied by Sigma (Louis, MO, USA).

2.2. Fruit material and Treatments

Blueberries (*Vaccinium myrtillus*) were procured from a commercial grocery store that was located in Taif City, Saudi Arabia and then transported to the laboratory of the college of science. Blueberries were in uniform size, weight, color, maturity in-between 80% to 90%, and without any mechanical injury. Subsequently, one-quarter of the berry fruits were dipped in deionized water (B-Control), dried, and then stored at ambient temperature (28 ± 0.5 °C) under about 75% relative humidity for up to (eight days) as the untreated samples. The remaining fruits were treated, dipped into various coating solution films, examined for two, four, six, and eight days, and also stored at ambient temperature. Three replicates were assessed for various coatings and treated in the same way. Every replicate was composed of 2 kg of berry fruits, which were divided into small boxes.

2.3. Films Preparations

Chitosan 1% (CHN) film was prepared by blending (glycerol 0.5% and acetic acid 1%) as a plasticizer. The film was well-stirred overnight at 200 rpm and then centrifuged at 4 °C. Aliquant 1% of nano-silicon dioxide was added in 1 L container and named with (CHN-Nano). Nisin 1% containing 0.02 mol/L hydrochloric acid was added to (CHN-Nano) as an antimicrobial agent and then named with (CHN-N-Nano), as in Figure 1.
were blended (Seward Stomacher 400), and then suspended in 90 mL of sterile peptone water as
mesophilics population counts. The results were calculated as log CFU/g (colony-forming units) of
fresh blueberries.

2.4. Measurement of Fruit Quality

Fruit quality was measured after two, four, six, and eight days at ambient temperature storage
shelf life determination. The evaluation of berry texture-profile analysis was measured by TA-XT2 (Plus
Connect, Nottingham, UK) and expressed as newton/meter (N·m⁻¹) by detecting twenty pieces/replicate
at 2-mm diameter flat P/2 probe, speed of (2 mm/s), and (1 mm/s) for pretest, and the test with a trigger
(five grams) [15]. The weight loss was examined by weighing the untreated and the coated berry
samples every other day by a sensitive balance with an accuracy of 0.01 g. The blueberry samples
were calculated as accumulated weight loss percentage during the storage period. Blueberry decay
and shrinking rates were expressed as the percentage (%) of showing decay symptoms and moisture
decrease, respectively [3]. The color parameters of the blueberries were detected by reading at three
points of every sample by using a Minolta 450 color-meter and observation illuminant (D65, 2°) with
an 8 mm aperture for (L*, a* and b*) as a color space index [16]. The pH reading was directly examined
on the berry juice by using a digital pH meter (320, Toledo, Shanghai, China). The vitamin C content
(Vit. c) was examined by the oxalic acid titration method reported by Sun et al. [17]. The (Vit. c) was
calculated as mg/100 g of berry fresh weight. The total soluble sugar content (°Brix) in berry samples
was detected by a digital refractometer (Model 30PX, Mettler Toledo, Worthington, OH, USA). To detect
the titratable acidity (TAA), 10 mL of berry juice was blended with 90 mL distilled water and titrated
with sodium hydroxide solution (0.1 N) until the pH equals (8.1) as a turning point. The SSC/TAA
ratio was achieved throughout the quotient between both of the two variables [18].

2.5. Measurement of Fruit Oxidation

Polyphenol oxidase activities (PPO) were detected by blending 5 g of berry samples with phosphate
buffer (pH 7), filtering, adding to 2.2 mol/L phosphate buffer, and (0.5 mL) of 0.175 mol/L catechol
solution with detection at (410 nm) [10]. Peroxidase activities (POD) were detected by blending
5 g of berry samples with (3 mL) 0.1 mol/L pyrocatechol solution, centrifuged, and then filtrated
with detection at (460 nm) [19]. The total anthocyanin content (TAC) in berry samples was detected
according to Nunes et al. [20], with (0.5% 18 mL HCl)-methanol as the control. The absorbance was
detected at (520 nm).

2.6. In-Vivo Microbiological Analyses

Microbiological analyses were achieved in-vivo, according to Abugoch et al. [9], 10 g berry samples
were blended (Seward Stomacher 400), and then suspended in 90 mL of sterile peptone water as
a rate of (0.1% v/v). Serial dilutions were made (10⁻¹, 10⁻², and 10⁻³), plated on the surface of the
selective media (3M 212 petrifilms), and incubated at (37 °C) for 48 h for molds/yeast, and mesophilics
population counts. The results were calculated as log CFU/g (colony-forming units) of fresh blueberries.
2.7. Statistical Analyses

All of the experiments results were expressed as standard deviation (mean ± SD) and then replicated three times/each value. The data were analyzed using IBM-SPSS version 17.00 software, while the mean separation treatments was significantly detected (p < 0.05) by the Duncan’s multiple tests.

3. Results and Discussion

3.1. Physicochemical Parameters

3.1.1. Texture Profile Analysis (TPA).

Blueberries are frequently loss of texture during postharvest processes, which consequently lean to decrease the shelf life and quality due to the cell wall enzymatic hydrolysis and cell turgor loss [8,21]. Figure 2 instrumentally shows the obtained data for various coating treatments. It was noticed that the hardness (N), chewiness (N), and cohesiveness of all blueberry samples were increased during the storage. On the other hand, springiness was decreased in descending order. In terms of (TPA), hardness is expressed as the maximum force that is applied during the first berry compression, respectively. The most pronounced hardness value of 9.96 N was reported in (CHN-N-Nano) coating treatment, as in Figure 2a. This fact gave a possible proportional relationship between the parameter value and the various coating treatments. (CHN-N-Nano) blueberry samples that were established the lowest value in hardness 17.72 N, which was very similar to the B-Control berry samples values 18.09 N. Mannozzi et al. [8] reported the linked results of chitosan-coated blue berried due to the rigidity of berry skin.

![Figure 2](https://example.com/figure2.png)

Figure 2. Effects of coating on texture profile analysis parameters for blueberry samples under ambient temperature, (a) Hardness; (b) Chewiness; (c) Springness and (d) Cohesiveness.
Chewiness (N) is described as a parameter that can provide information regarding the required work for sample chewing, mechanical properties, and the possible behavior at consumption [22]. According to the interpretation of obtained data, it noticed that (CHN-Nano) berry samples require the most energy for chewing 7.72 N, as in Figure 2b. The followed highest chewiness values were obtained for (CHN) 7.71 N and 7.70 N (CHN-N-Nano) coating treatments, respectively.

Springiness is one of the vital (TPA) components that aim to measure the ability of berry samples to restore their original height after the deformation during the first compression, respectively. It varies from (0 to 1) and it can be expressed as a ratio. (CHN) coating treatment established the highest value followed by (CHN-Nano) and (CHN-N-Nano) coating treatments that released the next high values 0.58 and 0.58, respectively, Figure 2c. That thickness might be influenced by the two various coating treatments. The moderate increase in springiness values leads to an increase in the chewing during berry consumption that has an advantage for coating treatments to obtain the sensorial overall acceptability by the typical “popping” sound. Furthermore, Yaman et al. [23] explained the coating effects to maintain the firmness values of cherries at cold storage.

Cohesiveness is an indicator for measuring the second compression and external stress against the first compression with values from (0 to 1), respectively [22]. The cohesiveness values of blueberry samples were influenced by the various coating treatments, as in Figure 2d, as well as the highest cohesiveness values that were obtained in (CHN) and (CHN-N-Nano) coating treatment samples. It gave the high ground to declare that the addition of chitosan can lead to parameter values increase due to the thickness of blueberry’s skin. Gaining of too low berry cohesiveness values is an indicator of changed fruit structure that was noised in (CHN-Nano) coating treatment.

3.1.2. Weight Loss, Shrinking and Decay Rates

Weight loss was nominal in this research and it ranged from (0.18 to 2.64 %) within eight days of storage at ambient temperature. The weight loss values were observed among various coating treatments or storage time, as in Figure 3. Forney [24] established that maintaining (95% or above) for relative humidity and low temperatures (0–1 °C) storage can minimize the shrivel of blueberries. (CHN-N-Nano) coating treatment was efficiently controlled the weight loss over time by 3.69% when compared to the other treatments. According to Figure 3a, (CHN-Nano) 4.69% treated berries delayed the weight loss, as compared to the B-Control due to the high turgor and the ability to reduce hemicellulosic depolymerization [25]. Thus, nano-material produced more dense structure of the enriched coating formulations as compared to the chitosan only.

![Figure 3. Cont.](image-url)
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Figure 3. Effects of coating treatments on weight loss (a) and (b,c) shrinking and decay rates.

(CHN-Nano) and (CHN-N-Nano) coating treatments helped to control shrinking (38.52%) and decay rates (8.61%) over time at room temperature, as seen in Figure 3b,c. The nano-layers were responsible for decreasing rates due to the surface coating, which make the shrinking and decaying more complex against respiration. Krasniewska et al. [3] reported similar results for Highbush blueberry.

3.1.3. Color Index

Pigments as anthocyanins are in charge of the color of blueberries [8]. Figure 4 presents ($L^*$, $a^*$, and $b^*$) values of B-Control and other coated samples during eight days of the storage at ambient temperature. Immediately after coating (0 days), (CHN-N-Nano) coating treatment reported lower $L^*$ values (10.54) than (CHN) and (CHN-Nano) ones. The established lower lightness was perhaps due to the presence of nano and nisin coating combination, which can cause some surface properties changes. Nevertheless, that performance has not been detected in (CHN) coating treatment almost certainly due to the presence of procyanidins [8]. In all coating treatments, decreases of $a^*$ values until the second day were observed, and then the values were increased again. Both (CHN) and (CHN-Nano) coated blueberry samples displayed higher values against the B-Control during the whole storage period for $b^*$ values. (CHN-N-Nano) coating treatment (2.43) exhibited higher $b^*$ values beginning from the sixth day of the storage period. Roque et al., [26] reported that the decline of the color that might be due to the oxidation reactions of polyphenol compounds occurred, which influenced anthocyanin values, while the color increase can be due to the co-pigmentation phenomenon that promoted the anthocyanins synthesis and polymers formation during blueberry ripening. The color index results were in agreement with those that were established by detecting various edible coatings on blueberries [27].

Figure 4. Cont.
The decreases in pH values for all coating treatments were in charge of blueberry preservation during the storage period. It was declared that (CHN-Nano) as 2.62 coating treatment maintained the lowest acidity values during the storage period, avoiding fungal and bacterial growth as the previous report for edible film coatings [28]. The results detected for (CHN) as 3.17 due to fruit respiration and forming alkaline compounds as nitrogenous [16]. The highest values were detected for (CHN-Nano) and (CHN-N-Nano) coating treatments. In a link to storage, pH values were differentiated among various coating treatments in-between (2.62–3.43), where the highest values were detected for (CHN-Nano) coating treatments as 3.43 due to fruit respiration and forming alkaline compounds as nitrogenous [16]. The decreases in pH values for all coating treatments were in charge of blueberry preservation and avoiding fungal and bacterial growth as the previous report for edible film coatings [28]. The results declared that (CHN-Nano) as 2.62 coating treatment maintained the lowest acidity values during the storage period.

**Figure 4.** Effects of coating treatments on color index $L^*$ (a), $a^*$ (b) and $b^*$ (c).

### 3.2. pH, Vit. C, SSC, TAA and SSC/TAA Ratio

The pH values were differentiated among various coating treatments in-between (2.62–3.43), where the highest values were detected for (CHN-Nano) and (CHN-N-Nano) coating treatments. In a link to B-Control, a difference was only established for (CHN-Nano) coating treatment after the whole storage, as in Figure 5a. From the zero to fourth day of the storage period, enhances in the pH values were detected for (CHN) as 3.17 due to fruit respiration and forming alkaline compounds as nitrogenous [16]. The decreases in pH values for all coating treatments were in charge of blueberry preservation and avoiding fungal and bacterial growth as the previous report for edible film coatings [28]. The results declared that (CHN-Nano) as 2.62 coating treatment maintained the lowest acidity values during the storage period.

**Figure 5.** Cont.
Although, TAA values of B-Control samples decreased TAA abruptly (0.52%–0.24%) due to the fungal (7.34 mg).
The results found for (CHN) coating treatment were superior to those that were obtained in the
correlation between SSC and the maturity degree of blueberries due to the metabolic reactions that enhance
The SSC of B-Control samples was higher than that of other coated
due to the fungal concentration, which might clarify the oxidation-reduction. The activity of the (POD) was the highest
blueberry fruits were detected in (Figure 6). The result of (PPO) was the highest (558.03 U min)
increases in the ratio, which result in a sugary taste to the berry samples. During the whole storage
the sweetness during the storage [3]. The SSC of B-Control samples was higher than that of other coated
treatments were obviously different from the B-Control samples, as shown in (Figure 5e).

Vis-à-vis the SSC/TAA ratio, (CHN-Nano) and (CHN-N-Nano) coating treatments distinguished
the highest ratio on eighth days of storage time. That behavior was justified true belief by the negative
correlation between SSC/TAA ratio with TAA, where any reductions in TAA values could generate
increases in the ratio, which result in a sugary taste to the berry samples. During the whole storage
time, all of the coating treatments were obviously different from the B-Control samples, as shown in
(Figure 5e). Therefore, nano-material with chitosan film coatings were successful in the ripening delay
of blueberry fruits.

3.3. Oxidation Browning Enzyme (PPO, POD) and TAC

The browning enzymatic activities of polyphenoloxidase (PPO) peroxidase (POD) for coated
blueberry fruits were detected in (Figure 6). The result of (PPO) was the highest (558.03 U min
in the (CHN) when compared to B-Control blueberries that reported (400.87 U min), respectively. The results found for (CHN) coating treatment were superior to those that were obtained in the
the (CHN-N-Nano), while the B-Control samples reported (24.67 U min), as shown in Figure 6b, respectively. These results are similar to those that were detected by using some
edible coatings to extend the shelf life [29]. Qiao et al. [13] reported that the activity reductions could be due to the abiotic stress on the fruits.

Blueberry fruits contain several types of anthocyanin components, such as petunidin, cyanidin, malvidin delphinidin, and peonidin [17]. Anthocyanin contents were reduced during the whole storage period, due to phenolics catalyzed oxidation by (PPO, POD) [31]. At the end of the whole storage, the TAC of treated blueberries recorded the highest value as (75.19 cyanidin-3-glucoside mg/100 g) in (CHN-Nano), while that of (CHN) coating treatment was (50.33 cyanidin-3-glucoside mg/100 g), as shown in Figure 6c, respectively.

3.4. Antimicrobial Effect of Chitosan, Nisin, Silicon Dioxide Nanoparticles Coatings on Blueberries

In-vivo study, molds/yeast, and mesophilics populations were examined on the coated blueberries sample. Chitosan component alone was capable to inhibit the mesophilics and molds/yeast growth of blueberry samples stored at ambient temperature during an extended period of eight days, as shown in Figure 7a,b. Figure 8 presents the optical evaluation for (uncoated and coated) fruits at the end of the whole storage period. Although, all coating treatments effectively inhibited the microbial growth, as (CHN) coating treatment demonstrated some growth of both molds/yeast and mesophilics populations when compared to B-Control samples (4.10 and 3.87 log CFU/g) and (4.57 and 4.17 log CFU/g), respectively. In general, a 2 or less log CFU/g reduction was detected by (CHN-Nano) and (CHN-N-Nano) coating treatments, although there was little additional effect after nisin addition as an antimicrobial agent to be (3.60 and 2.73 log CFU/g) for molds/yeast and mesophilics populations, respectively. These results suggested (CHN-N-Nano) coating treatment to extend blueberries shelf...
life. Elsabee and Abdou [15] reported that the chitosan antimicrobial effect was dependent on the microorganisms types as molds, fungi, and bacteria.

![Graph](image1)

**Figure 7.** Antimicrobial effect of chitosan, nisin, silicon dioxide nanoparticles coatings on blueberries, (a) Molds/yeast population and (b) Mesophilics population counts.

![Image](image2)

**Figure 8.** Optical evaluation of (uncoated and coated) blueberry fruits.

4. Conclusions

In summary, this research reported that nano-material with chitosan film coatings that were incorporated with nisin can be an effective substitutional for fresh blueberry preservation under ambient temperature over eight days. The nano-coating films maintained fruit texture and acted as an antimicrobial agent during the whole storage. Nano-materials films might affect the common color of the coated samples, but it was efficiently used for extending the blueberries shelf life and other consumable vegetables and fruits.

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