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Article

Physicochemical preservation of blueberries treated with chitosan and salicylic acid in postharvest

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Abstract

The blueberry (*Vaccinium corymbosum* L.) fruit is characterized by its antioxidant properties due to its content of phenolic compounds, anthocyanins, and other compounds. However, it is susceptible to deterioration, loss of its quality and shelf life. In order to preserve its physicochemical properties and quality, the use of the combined treatment of chitosan and salicylic acid is proposed as the main objective. The research was carried out during 2022, in which the preservation of blueberry fruits in the postharvest stage was evaluated through the application of a combined treatment of chitosan and salicylic acid. The evaluation of the quality parameters showed that the application of the combined treatment maintained the firmness of the fruits for longer and reduced physiological weight loss by up to 11%. Changes in blueberry total soluble solids, pH, titratable acidity, and color were delayed for more days, but postharvest fruit quality was maintained. The respiration rate of the blueberries decreased by the application of chitosan plus salicylic acid and there was an induction of the phenylalanine ammonia lyase enzyme during the first 24 h of storage of the blueberries due to the effect of chitosan combined with salicylic acid. Through this research, it was concluded that chitosan and salicylic acid as a combined treatment can be a sustainable alternative to the use of fungicides to preserve blueberry fruits in the postharvest stage.

Keywords:

Vaccinium corymbosum, alternative treatments, quality.

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Introduction

The appearance of a fruit is one of the important aspects when selected by consumers, considering its firmness, color, and smell at first sight (Liu *et al*., 2018). That is why maintaining these quality parameters in a fresh fruit is decisive, and even more so in susceptible fruits such as blueberries. Blueberries (*Vaccinium corymbosum* L.) are fruits with very thin skin and are therefore more likely to deteriorate more quickly than other fruits (Ramos-Bell *et al*., 2021).

This fruit has shown that it can represent a health benefit when consumed on a regular basis as it contains a significant amount of phenolic compounds, anthocyanins and vitamins that are related to the prevention of chronic-degenerative diseases (Chiabrando and Giacalone, 2017). In this sense, it is important to preserve this fruit during its postharvest stage by applying an adequate, clean treatment that does not include synthetic fungicides.

According to the final destination of the fruits, different regulatory requirements are established; however, in order to apply the different agricultural practices of chemical and biological control, etc., there is the Mexican Standard NOM-022-SAG/FITO-2016, which establishes the specific requirements that must be met. Chitosan is one of the compounds approved as non-toxic by the FDA and has been shown to have a positive effect on preserving quality and antifungal power on postharvest crops such as mango and tomato (Moreno-Hernández *et al*., 2022; Rodríguez-Guzmán *et al*., 2022). Li *et al*. (2021) state that chitosan can form a physical barrier on blueberry fruit, decreasing respiration, dehydration, and senescence.

On the other hand, it was found that this compound is easily accessible and inexpensive, that can protect fruits from attacks by phytopathogens since it has an antifungal effect and can induce certain enzymes related to the defense of the fruit (Herrera-González *et al*., 2021). Fruit ripening, respiration rate and water loss are reduced thanks to the chitosan effect of forming a semi-permeable film, which controls gas exchange and reduces transpiration loss.

It was also shown that the application of chitosan activates the resistance of the fruit by increasing the activity of some enzymes related to defense, such as chitinase, glucanase, phenylalanine ammonia lyase, peroxidase and polyphenol oxidase (Berumen Varela *et al*., 2015). To achieve a greater antifungal effect when using chitosan and reduce concentrations, it is advisable to combine it with another compound of a similar nature (Ramos-Bell *et al*., 2022).

Salicylic acid is a component of natural origin whose application in crops such as grapes maintained stable physicochemical parameters such as firmness and soluble solids (Qin *et al*., 2015); likewise, its application in apples preserved their quality and shelf life (da Rocha-Neto *et al*., 2016).

Salicylic acid (SA) is a natural phenolic compound present in many plants, it is derived from the phenylalanine amino acid and is a molecule that activates defense responses against attack by various pathogens. The application of exogenous SA induces the synthesis of PR proteins, an increase in the concentration of reactive oxygen species, and the production of antimicrobial phytoalexins in fruits (Shi *et al*., 2018).

In this sense, the objective of this study was to evaluate the combined effect of chitosan and salicylic acid on the preservation of blueberry fruits in the postharvest stage, evaluating important quality parameters such as firmness, color, weight, soluble solids content and others, as well as to determine the enzymatic activity as a possible mechanism of action of defense of the fruits.

Materials and methods

Plant material

Blueberries in the physiological maturity stage were collected during the months of February and March 2022 from a commercial orchard in the locality of San Luis de Lozada in the state of Nayarit. The blue color of the fruits was used as a criterion for harvesting. These were previously washed and disinfected with a 2% sodium hypochlorite solution before their respective analyses.

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Solution preparation

Commercial chitosan (47.5 kDa, 90% deacetylation, Golden-Shell Co., China) and salicylic acid (Sigma Aldrich, USA) were used. For the combined treatment of salicylic acid and chitosan, concentrations of 0.07 and 1.5%, respectively, were used, according to a previous study (Ramos-Bell *et al*., 2022).

The concentration of salicylic acid was obtained from a stock solution at 50 mM, subsequently diluted in distilled water and 5% glycerin, its pH was adjusted to 5.5 in 10% KOH solution (w/v). For chitosan, its concentration was obtained in sterile distilled water with the addition of 1% acetic acid, its pH was adjusted to 5.6 with 1N NaOH, and the solution was kept in constant stirring for 24 h (Ramos-Guerrero *et al*., 2018).

Both compounds were mixed to form the combined treatment, the treatment was applied to the fruits by immersion for 2 min, then they were left to dry and stored at room temperature (25 °C) and refrigeration temperature (4 °C) under a relative humidity of 90-95%. Their storage period was 9 days and samples were taken every third day for physicochemical analyses.

Physicochemical evaluation

Firmness

Firmness was recorded as the shear strength using a texture meter (Stable Micro Systems, TA-XT Plus, UK) equipped with a 2 mm diameter punch. The results were expressed in Newton (Montalvo-González *et al*., 2021).

Physiological weight loss

The percentage of physiological weight loss was determined by considering the initial and final weight of the treated fruits and the control fruits. A digital scale (Ohaus Corporation, USA) was used; the results were expressed as a percentage of fresh weight lost based on the initial weight of the fruit (Liu *et al*., 2018).

Color

A colorimeter (High-Quality Colorimeter, Shanghai, China) was used to evaluate the coordinates L^*, a^*, b^*, where: L^*, luminosity; a^*, red (positive values) or green (negative values) and b^*, yellow (positive values) or blue (negative values).

Total soluble solids, pH, and titratable acidity

The soluble solids content was expressed as °Brix, for which 5 g of sample was homogenized and a few drops of juice were placed in a digital refractometer (Hanna Instruments, HI 96801, USA), previously calibrated with distilled water. For pH detection, 5 g of fruit sample was homogenized and analyzed in a potentiometer (Sension TM, Barcelona, Spain), previously calibrated with standard buffers. Titratable acidity was determined by titration with a titrated solution of 0.1 N sodium hydroxide, using 5 g of blueberry pulp homogenized with 25 ml of distilled water and three drops of phenolphthalein as an indicator. These analyses were governed by the method of (AOAC, 2005).

Ripeness index

The ripeness index was calculated as the ratio between the value of total soluble solids and titratable acidity (Rokayya *et al*., 2021).

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Respiration rate

Respiration rate was determined using the method proposed by Tovar *et al*. (2001) with slight modifications, such as the time the fruits remained in the container, as well as the amount of sample. The fruits (60 g) were placed in airtight containers of known volume. These were closed for 1 h at room temperature (25 °C) or refrigeration temperature (4 °C).

From the free headspace, 0.4 ml of sample was taken and injected into a gas chromatograph (HP model 6890, USA) with an HP-PlotQ column (15 m x 0.53 mm and 40 µm film thickness), a flame ionization detector (FID) and a thermal conductivity detector (TCD). The injector and detector were used at 250 °C, the flow of air and hydrogen was 400 ml min^{-1} and 30 ml min^{-1}, respectively. Respiration rate was reported as ml CO_2 kg^{-1} h^{-1}.

Enzymatic activity

It was based on an enzymatic crude extract, crushing 2 g of blueberry with and without treatment, according to Herrera-González *et al*. (2022), it was homogenized with 10 ml of 100 mM sodium phosphate buffer solution (pH 6.4) and 0.2 g of polyvinylpolypyrrolidone (PVPP) at 4 °C. It was centrifuged at 6 000 rpm for 30 min at 4 °C and the supernatant was taken and recentrifuged under the same conditions.

Zero point one milliliters of crude extract and 0.9 ml of L-phenylalanine 1 mg ml^{-1} were taken and incubated at 40 °C for 30 min. The reaction was stopped by adding 0.25 ml of 5 N hydrochloric acid. Absorbances were determined at 290 nm in a spectrophotometer (Thermo scientific, Genesys 10S UV-Vis, Wisconsin, USA). It was defined as a unit of enzymatic activity equivalent to 1 mmol of trans-cinnamic acid produced per minute per mg of protein.

Statistical analysis

A completely randomized design was carried out using an analysis of variance (Anova). Differences in means were analyzed using the LSD Fisher test ($p< 0.05$). For all trials, 10 fruits were used per treatment, 3 repetitions were performed for each treatment, and trials were performed in duplicate. Statistical analysis was performed using the program of Statistica v12.0 (StatSoft Inc., 2013).

Results and discussion

Firmness

Firmness was not significantly different ($p> 0.05$) between the combined treatment and the control at the end of storage at 25 °C; however, the firmness in the combined treatment of chitosan and salicylic acid was 6% lower than the control (Figure 1). On the other hand, firmness in blueberry fruits stored at 4 °C remained stable with small variations between treatments.

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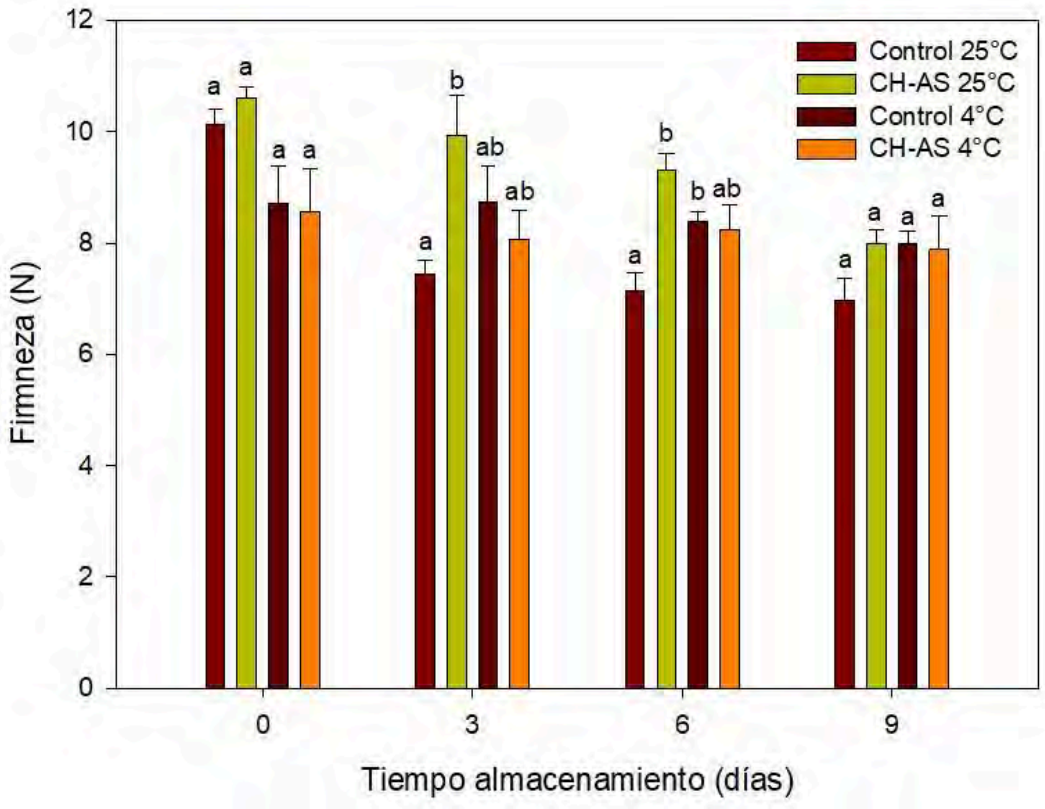
Figure 1. Firmness of blueberry fruits stored at 25 °C and 4 °C under the application of the chitosan-salicylic acid (CH-AS) combination. The values are expressed as mean ± standard error (n= 10).



A similar result was obtained by Li *et al*. (2021), indicating that chitosan effectively prevented increased loss of firmness in blueberries during cold storage. Fruit firmness is an important quality parameter, which is affected by carbohydrate hydrolysis and pectin degradation in the fruit cell wall (Jiang *et al*., 2016).

The refrigeration temperature keeps the firmness of the fruits stable for longer, and this can be due to the decrease at low temperatures in the activity of hydrolytic enzymes such as polygalacturonase, galactosidases, pectin methylesterase and β-1,4-glucanases (Montalvo-González *et al*., 2021).

Physiological weight loss

Weight loss in fruits stored at 25 °C showed significant differences ($p< 0.05$) between treatments (Figure 2), with a smaller weight reduction when applying CH-AS, with an 11% weight loss compared to a 22% weight reduction for the control. On the other hand, storage at 4 °C in treated and untreated fruits kept the weight loss rate lower and without significant differences ($p> 0.05$), with values ranging from 5.25-6.45%. This behavior is since refrigeration slows the ripening of the fruit since the respiration processes are dependent on temperature (Shao *et al*., 2019).

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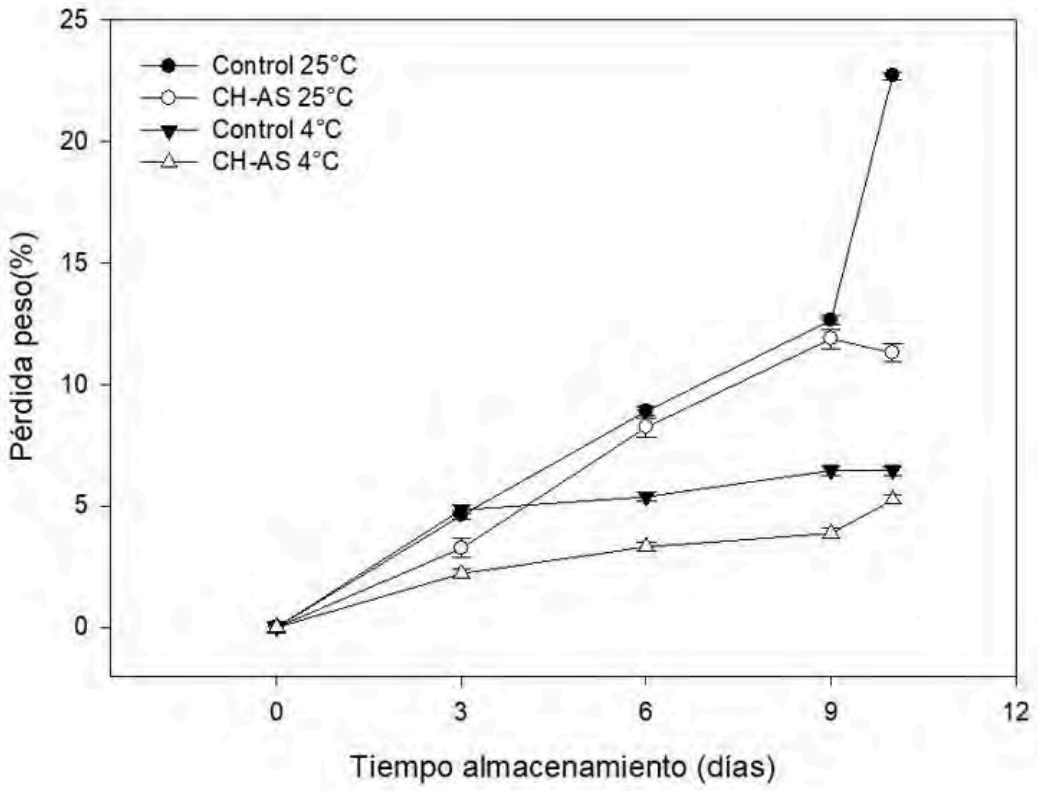
Figure 2. Weight loss of blueberry fruits stored at 25 °C and 4 °C under the application of the chitosan-salicylic acid (CH-AS) combination. Values are expressed as mean ± standard error (n= 10).



It has been mentioned that chitosan can act as a physical barrier on the surface of fruits, causing a reduction in gas exchange as well as fruit respiration, leading to the extension of the shelf life of fruits (Duan *et al*., 2019). The effect of salicylic acid is attributed to the fact that as it is an inhibitor of mitochondrial electron transport, it is likely to decrease the availability of substrate for catabolic reactions, contributing to the maintenance of the weight content of the fruit (da Rocha-Neto *et al*., 2015).

Color

Color results for the L^* parameter tended to decrease during storage at 25 °C and with no difference (p> 0.05) between treatments (Table 1). The luminosity (L^*) shows a decreasing trend due to the ripening process of the fruits (Chiabrando *et al*., 2017). Regarding parameters a^* and b^*, the treatments showed an increase in both storage temperatures, indicating a more intense blue color in the fruits. However, the values in the control fruit were higher than in the fruits with CH-AS, although with a slight significant difference.

Table 1. Color parameters in blueberries treated with chitosan-salicylic acid (CH-AS) combination during storage at 25 and 4 °C.

	Treatments	Storage 25 °C		Storage 4 °C	
		Day 0	Day 9	Day 0	Day 9
L	Control	35.01 ±0.65 a	32.54 ±0.63 a	35.24 ±0.69 a	32.07 ±0.7 a

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	Treatments	Storage 25 °C		Storage 4 °C	
		Day 0	Day 9	Day 0	Day 9
	CH-AS	34.27 ±0.72 a	33.36 ±0.59 a	32.58 ±0.91 b	33.67 ±0.6 a
a	Control	-3.89 ±0.12 a	-4.7 ±0.07 a	-3.79 ±0.11 a	-5.62 ±0.18 a
	CH-AS	-3.2 ±0.2 b	-4.23 ±0.18 b	-4.54 ±0.11 b	-4.63 ±0.13 b
b	Control	-7.04 ±0.23 a	-5.77 ±0.21 a	-6.67 ±0.23 a	-6.63 ±0.23 a
	CH-AS	-6.02 ±0.22 b	-5.1 ±0.18 a	-6.8 ±0.35 b	-6.3 ±0.25 a

Values are expressed as mean ± standard error (n= 10). Different letters indicate significant differences between treatments at ($p< 0.05$).

These results indicate that the combined treatment of CH-AS does not affect the color parameters in the blueberry fruit. This supports what has been reported in previous studies in blueberry fruits treated with edible coatings (Eldib *et al*., 2020). The color change in blueberries is the result of the biochemical processes that occur naturally in the fruits; the synthesis of anthocyanins, which are the compounds that give the blue pigmentation to the fruit, occurs during the ripening stage in the blueberry (Díaz-Rodríguez *et al*., 2021).

Considering that anthocyanins are phenolic compounds with great antioxidant power that contribute to the maintenance of human health, it is important that these compounds are maintained in the fruit and that oxidation does not occur so that the fruit does not lose its biological value. An increase or variation in the color of blueberries can lead to the loss of the commercial value of the fruit (Xu *et al*., 2016), hence the importance of keeping these parameters stable during a certain period of fruit storage.

Total soluble solids, pH, and titratable acidity

Regarding the content of total soluble solids at 25 °C, there were significant differences ($p< 0.05$) between the treatments, with values of 16.6 and 13.53 °Brix for the control and CH-AS, respectively. Fruits with and without treatments stored at 4 °C did not show significant differences; however, on the last day of storage, the control had a higher content of total soluble solids (Figure 3a).

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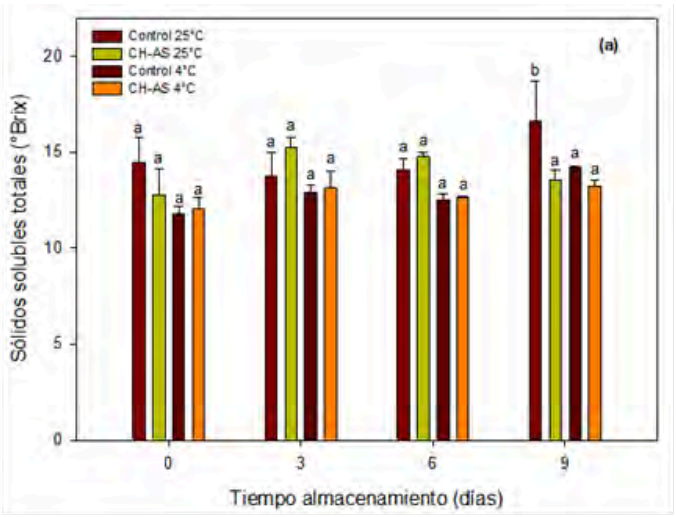
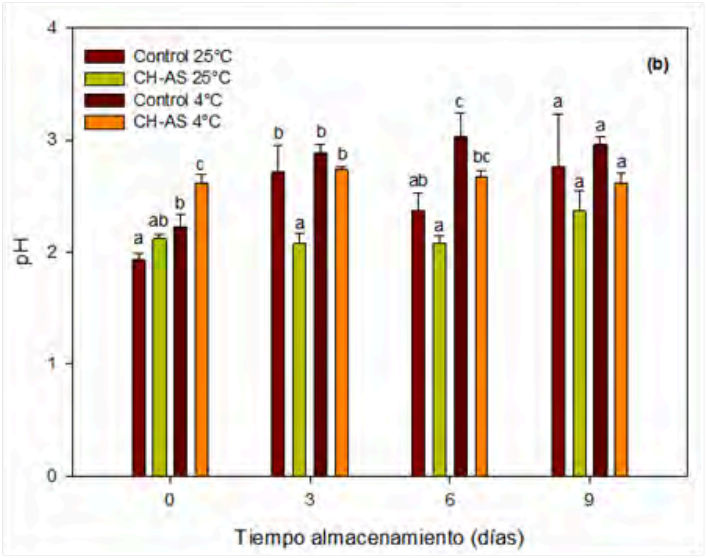
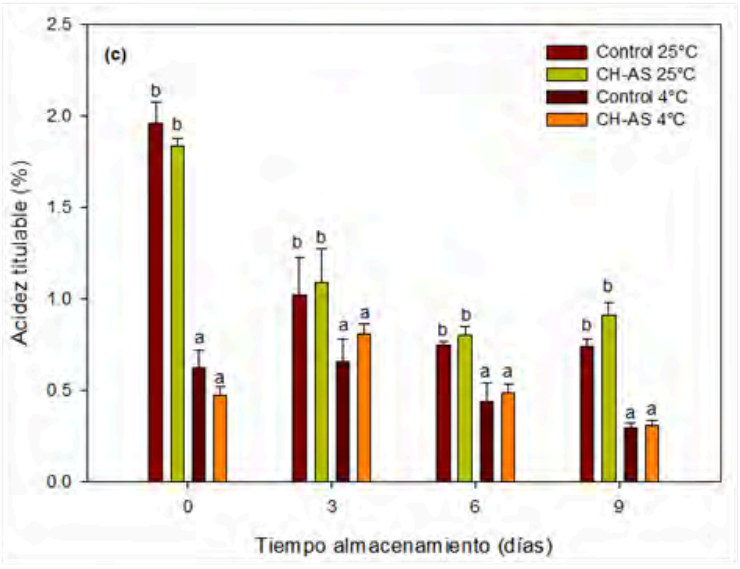
Figure 3. Total soluble solids, pH, and titratable acidity of blueberries stored at 25 °C and 4 °C under the application of the chitosan and salicylic acid combination. Values are expressed as mean ± standard error (n= 10).







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The contents of sugars, organic acids, and vitamins are the main substrates of respiration (Li *et al*., 2021); in a fruit at the physiological maturity stage there is a greater demand for these substrates, therefore, an increase in the content of total soluble solids is to be expected. With the application of chitosan in combination with salicylic acid, a lower soluble solids content was obtained compared to the control.

A chitosan coating applied to blueberry fruits decreased the content of soluble solids compared to the control, as chitosan can decrease fruit metabolism by modifying the atmosphere surrounding the fruit (Eldib *et al*., 2020). Salicylic acid, on the other hand, can maintain the content of soluble solids, as well as other physicochemical characteristics, preventing the degradation of the fruits over time (da Rocha-Neto *et al*., 2016).

The pH value in control fruits stored at 25 and 4 °C was higher than in fruits with CH-AS (Figure 3b). Similar results were reported by Mannozzi *et al*. (2017), who applied edible coatings and the pH values of the blueberry decreased, due to the reduction in the speed of the metabolic processes that convert the starch and acids of the fruit into sugar.

The titratable acidity content of the fruits at 25 °C (Figure 3c), did not show significant differences ($p > 0.05$). Fruits at 4°C showed a lower rate of decrease in acidity content, with the control showing the lowest value (0.3%) and the treatment with CH-AS preserved a higher titratable acidity content during storage (0.51%). Vieira *et al*. (2016) mentions that, during the respiratory process of fruits, they consume organic acids associated with a reduction in titratable acidity values. According to what was reported and obtained in this study, it can be stated that the combined treatment of CH-AS applied to blueberry fruit reduces the consumption of organic acids such as citric acid, which is found in a greater proportion in this fruit.

Ripeness index

The ripeness index evaluated for treated blueberry fruits was lower under the application of the CH-AS combination, with significant differences compared to the control (Table 2). Considering that the optimal value of the ripeness index of fresh blueberries at 25 °C is 35 (Rokayya *et al*., 2021), the combined application of chitosan and salicylic acid was effective in delaying ripening. Similar results were reported by Rokayya *et al*. (2021), a study in which a reduction in the ripening rate was obtained when chitosan was applied to blueberry fruits under controlled storage at room and refrigeration temperatures.

Table 2. Ripeness index of blueberries treated with CH-AS at 25 and 4 °C.

Treatments	Ripeness index
Control (25 °C) CH-AS (25 °C) Control (4°C) CH-AS (4 °C)	54.41 ±5.33 b 27.42 ±5.47 a 19.23 ±0.42 a 15.74 ±0.91 a

Values are expressed as mean ± standard error (n= 10). Different letters indicate significant differences between treatments at ($p < 0.05$).

In these results, the effect that temperature has on the ripening process is evident, as it slows down the development of the total soluble solids of the fruits. Previously, a reduction in the TSS/TA ratio of blueberries under application of chitosan coatings compared to the control was also reported, indicating the delay of the fruit ripening process (Eldib *et al*., 2020).

Respiration rate

The respiration rate of blueberries stored at 25 °C (Figure 4a) and 4 °C (Figure 4b), gradually increased over time. Untreated fruits reached their peak CO_2 production on day 5 (5.19 ml CO_2 kg^{-1} h^{-1}) and day 8 (2.32 ml CO_2 kg^{-1} h^{-1}) for temperatures of 25 °C and 4 °C, respectively. In fruits stored at 25 °C, the CH-AS treatment, in addition to delaying the onset of the climacteric peak by one more day compared to the control, decreased the value of respiration rate.

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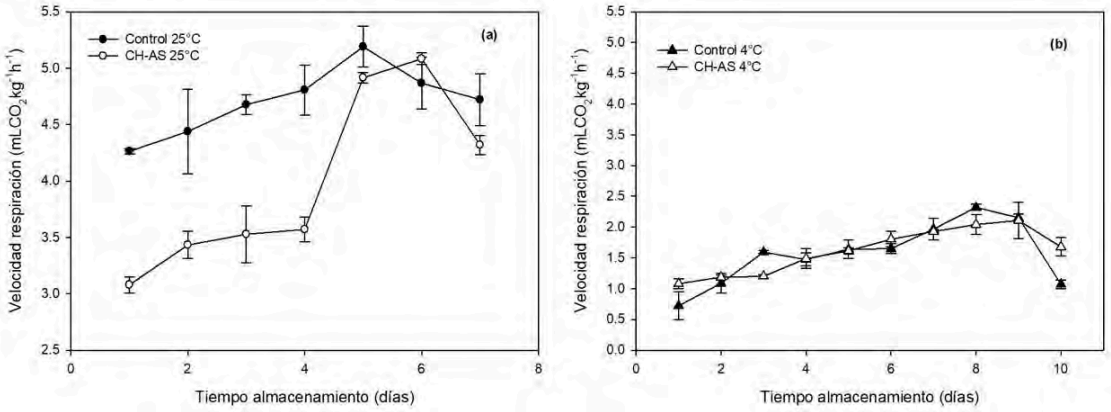
Figure 4. Respiration rate of fruits treated with chitosan-salicylic acid (CH-AS) at storage temperatures of 25 °C (a) and 4 °C (b).



Chitosan can slow down the respiratory process involving water loss and CO_2 production thanks to its ability to form a semipermeable barrier on the surface of the fruit, decreasing the available oxygen and therefore CO_2 production (Ortiz-Duarte *et al*., 2019). On the other hand, in fruits stored at 4 °C, the combination of CH-AS was efficient as it delayed the onset of the climacteric peak compared to the control.

Temperature undoubtedly plays a very important role in the respiratory process of fruits, since at lower temperatures (4 °C), the respiratory rate decreases (ml CO_2 kg^{-1} h^{-1}). Low temperatures decrease the enzymatic activities involved in the respiratory process (Montalvo-González *et al*., 2021), and that is why the respiration rate of blueberries is lower when stored at 4 °C compared to those stored at room temperature.

Enzymatic activity of the phenylalanine ammonia lyase enzyme

The activity of the phenylalanine ammonia lyase (PAL) enzyme was high in fruits with CH-AS in the first 12 h of storage at 4 °C (Figure 5). On the other hand, maximum activity during storage at 25 °C occurred at 24 h for blueberries with the combined treatment of CH-AS, with a value of 2.29 U mg^{-1} protein. The trend of PAL activity in fruits stored at 25 °C and 4 °C was similar; however, after 12 h of storage, the activity at 4 °C was lower.

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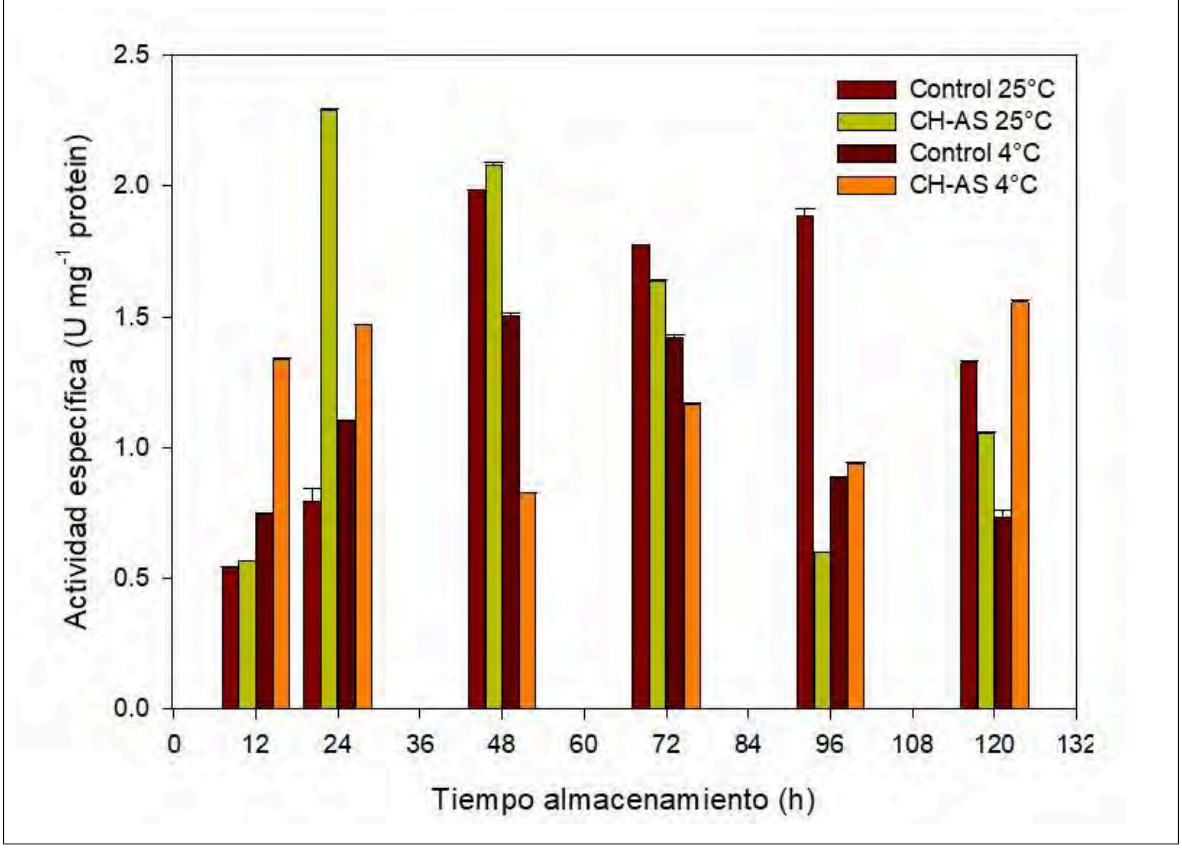
Figure 5. Enzymatic activity of blueberries treated with chitosan-salicylic acid (CH-AS) at 25 and 4 °C. Values are expressed as mean ± standard error (n= 10).



Chitosan has been described as a compound that can induce the different defense responses of the fruit by activating certain enzymes related to defense, such as PAL (Herrera-González *et al*., 2021). Likewise, salicylic acid is an inducer of fruit resistance, this being its main mechanism of action with important results in this regard (Serna-Escolano *et al*., 2021).

PAL is considered a key enzyme in the fruit's defensive arsenal against biotic and abiotic agents, as it is responsible for synthesizing important secondary metabolites, such as phenols, phytoalexins, and lignins, through the phenylpropanoid pathway (Zhao *et al*., 2022). According to these results, we can report that the combined treatment of CH-AS acts additively, according to previous studies (Ramos-Bell *et al*., 2022), inducing the activity of the PAL enzyme in blueberry fruits in the postharvest stage.

Conclusions

Flavor is one of the main indicators of a fruit's quality, and taste involves the soluble solids content, titratable acidity, and pH of the fruit. In this sense, the combined treatment of CH-AS kept the levels of soluble solids and pH below the control and there was a smaller decrease in the titratable acidity content compared to the control. The treated blueberries stayed firm longer, and their physiological weight loss was lower than the control.

The application of CH-AS decreased the respiration rate of the blueberries and caused the rapid activation of the phenylalanine ammonia lyase enzyme. According to these results, we can assert that the compounds of chitosan and salicylic acid combined in concentrations of 1.5 and 0.07%, respectively, are an efficient alternative to preserve the physicochemical quality of blueberry fruits in the postharvest stage.

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