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Review
POTENTIALITIES OF CHITOSAN FOR STRAWBERRY. USES IN THE IMPROVEMENT AND CONSERVATION OF THE FRUITS

Revisión bibliográfica
Potencialidades del quitosano para la fresa. Usos en la mejora y conservación de los frutos

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ABSTRACT. The fruit of the strawberry (*Fragaria ananassa* Duch.) contains an important amount of antioxidants and micronutrients, being a source rich in vitamin C and folic acid. Compared to other fruits also it has a high water content, less low molecular weight carbohydrates and a higher glucose / fructose ratio. The growth, production and quality of the strawberry fruit depends on the different agricultural treatments that are carried out during its development season. At present, efforts are being made worldwide to minimize the amounts of chemical fertilizers and pesticides applied to vegetables and edible plants to reduce the cost of its production and the pollution of the environment, without a reduction on the performance. In this sense, there is necessary to search alternatives to integrated pest management in strawberry crop. One of the strategies which has been the most studied in recent years is the use of chitosan to regulate the phytopathogen populations, increase the fruits yields and to improve the quality and commercial life of the fruit. Among the oligosaccharines, chitosan is the most studied and applied in the field of pre and postharvest agriculture. For these reasons, the aim of this review is to highlight the main results found in different studies where the action of this biostimulant on strawberry cultivation has been tested.

Key words: strawberry, yield, quality

RESUMEN. El fruto de la fresa (*Fragaria ananassa* Duch.) contiene una cantidad importante de antioxidantes y micronutrientes, siendo una fuente rica en vitamina C y ácido fólico. En comparación con otras frutas posee un alto contenido de agua, menor cantidad de carbohidratos de bajo peso molecular y una mayor relación glucosa/fructosa. El crecimiento, la producción y la calidad de la fruta están en función de los diferentes tratamientos agrícolas que se realicen durante su temporada de desarrollo. Actualmente, a nivel mundial se están realizando esfuerzos para minimizar las cantidades de fertilizantes y plaguicidas químicos que se le aplican a las hortalizas y plantas comestibles con el fin de reducir el costo de producción y la contaminación del medio ambiente, sin que haya una reducción del rendimiento. En este sentido, surge la necesidad de buscar alternativas para el manejo integrado de plagas en el cultivo de la fresa, y una estrategia muy estudiada en los últimos años ha sido el uso de la quitosana con el fin de regular las poblaciones de determinados fitopatógenos, aumentar los rendimientos así como mejorar la calidad y la vida comercial de la fruta. La quitosana es, entre las oligosacarinas, la más estudiada y de mayores aplicaciones en el campo de la agricultura de pre y poscosecha, por lo que el objetivo de ésta revisión está enfocado en resaltar los principales resultados obtenidos en los diferentes estudios donde se ha probado la acción de este bioestimulante en el cultivo de la fresa.

Palabras clave: calidad, fresa, rendimiento

INTRODUCTION

The consumption of foods rich in antioxidants, micronutrients and phytochemicals is a preventive measure to reduce the risk of chronic diseases caused by oxidative stress (1). The fruit of the strawberry contains a significant amount of antioxidants and micronutrients, being a source rich in vitamin C and folic acid. In comparison with other fruits such as: pineapple, banana, apple, pear and grape, it has a high water content, a lower amount of low

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molecular weight carbohydrates and a higher glucose/fructose ratio (2-4).

However, it is a very perishable fruit due to the high speed with which vital metabolic processes take place as demonstrated by its high respiratory rate (50 - 100 ml CO_2 / Kg h at 20 °C). Useful commercial life for this fruit is established in 5-7 days. Another aspect to take into account related to its physical characteristics is that it has a relatively soft pulp, covered with a thin and delicate cover, very susceptible to breakage. These characteristics cause that the strawberry bruises by effect of pressures of relatively low intensity. As a consequence, the fruit presents a great facility to suffer injuries during harvest and subsequent post-harvest handling. These lesions become preferential attack points of different pathogenic microorganisms, among which the fungus *Botrytis cinerea* (5) stands out.

In societies with a high economic level, the main factor of consumer preference is the quality of agrifood products. To obtain quality productions an adequate growth of the plant and fruit development is required during the pre-harvest period (6).

Many authors state that the growth, production and quality of strawberry fruit depends to a large extent on the use of chemical fertilizers and pesticides that are used during their development season to obtain high yields.

In spite of this, at present, efforts are being made to minimize the application of these compounds in vegetables and edible plants, in order to reduce production costs and environmental pollution, without there being a reduction in yield. (7) Simultaneously, the food industry, trying to satisfy the demands of consumers, has promoted the development and design of new methodologies that allow offering fresh and healthy food, in its most natural state and to increase the shelf life (8).

Therefore, there is a tendency to use organic and growth stimulating products to maintain efficiency in agricultural production. Many farmers apply them by foliar spray or in the soil to improve the growth, productivity and quality of the fruits produced. The results of these last two decades allow glimpsing the development of a new generation of compounds that are harmless or less aggressive to the environment and to man. These base their utility on the manipulation of the plants natural responses, against the different stresses and on maximizing the intrinsic potentialities of the crops to increase their yields (7). Strawberry cultivation has the most varied and complex possibilities of cultural management from open-field production, even in controlled environment conditions (9), hence the need for a study on biostimulants that economically improve production in this crop.

Chitosan is, among the oligosaccharins, the most studied and most applicable in the field of pre and post-harvest agriculture. It has essential characteristics in its biological activity that make it desirable in this field: i) they benefit the increase in growth and yields of many tested crops and ii) they cause defensive induction and resistance against pathogens in applied plants (10-12). Together it favors the growth of beneficial or pathogenic biota and, in turn, increases the population and the microbial activity in the soil, which improves the disposition of nutrients and their properties (13). It is recognized that its biological activity is related to the positive free charges, present in the amino group under acidic conditions, which interact with opposite charges of cell wall components and the membranes of microorganisms and plants.

In the cultivation of the strawberry, when this compound has been applied to determine its effect on the response of the plant, very good yields, size and quality of the fruit have been achieved.

At the same time, many authors have referred to foliar sprays from the flowering stage to maturity, as a fungicidal strategy to control postharvest rot. Others, taking into account their biocompatibility characteristics and their antimicrobial properties, have used it as an edible coating (RC) to improve the commercial life of fruits. Hence, the objective of this work is focused on highlighting the main results obtained in the different studies where the action of this biostimulant has been tested, with the purpose of recommending it as a favorable alternative to increase yields and quality of strawberry fruit.

Effects of chitosan on the growth and development of the strawberry

A strawberry plant with quality should have abundant roots, multiple crowns, differentiated buds and high carbohydrate content to settle quickly in the field of cultivation and with it obtain a fast production and high yield. Genetic and environmental factors such as: water availability, day and night temperatures and daylight intensity, influence the growth of plants and fruit productivity and quality (14). It has been proven that high temperatures, under nursery conditions, affect the quality of the daughter plants and in the production, yield and size of the fruit (Table 1).

In turn, the altitude of the sowing ground above sea level of the plantation influences the size and number of crowns of the plants, so that the establishment of strawberry nurseries at high altitudes (above 3 000 m s.n.m) (Table), can cause damages to the plants by the low temperatures that occur, in contrast, at low altitudes the plants come to present lower content of starch, with a low yield of production. On the other hand, more crowns can be obtained in shorter periods, compared to plants produced at high elevations. Soluble sugars play a fundamental role in the growth and development of plant species; when their organs end up developing, said sugars are temporarily stored as starch in the roots and crowns of the strawberry as a result of the shortening of the photoperiod and the decrease in ambient temperature. This aspect is very important, because the starch content is necessary for the propagation of the plant, the long-term storage, the establishment of the plantation, the precocity and the yield of the fruits (14).

It is also stated that to achieve an acceptable production of strawberry requires large amounts of renewable and non-renewable resources such as soil cover that consists of covering the grooves with some plastic material that prevents the fruit from having direct contact with the ground, thus preventing pathological damage and in turn preventing the growth of weeds. Likewise, it needs a considerable amount of water for the establishment of the plantations and depends on frequent applications of fertilizers, to increase its productivity (16).

One of the main challenges in the development of the commercial crop and even in the poscosecha of the fruit is the handling of the diseases that in his great majority are of fungus character; followed by some bacterial problems, of nematodes and occasionally by viruses. The management of these pathologies is based on the use of pesticides such as: Benomil, Captan, Iprodione, Azuflor 90wp (17) and others of chemical synthesis, which generate high risk for human and environmental health, also affect the safety of the fruit and they increase production costs, the situation being so critical nowadays, that developed countries restrict and even nullify the use of certain agrochemicals (10). In this sense, there is a need to look for alternatives for integrated pest management with the use of biofertilizers or natural biostimulants.

One of the bioactive compounds that take much relevance for the control of pathogens in the post-harvest handling of fruits and vegetables is chitin and its derivatives. Many authors claim that the antifungal capacity of chitosan has been demonstrated in different studies such as Mohamed *et al*., 2008, which using tomato as a model, showed that a concentration of 2000 and 4000 mg / L of chitosan completely inhibits growth of the fungus *Botrytis cinera* (gray mold). In turn, 1% proved to be effective in reducing the growth of this same fungus and *Rhizopus rot*, in grapes, strawberries and blackberries (Romanazzi, 2010). Also in citrus fruits when low molecular weight chitosan has been used, there was a significant reduction of infections caused by *Penicillium digitatum, Penicillium italicum, Botrydiplodia lecanidion* and *Botrytis cinerea* after 14 days of storage at 25 °C (Chien *et al*. , 2007). Bhaskara Reddy *et al*. (2000) treated strawberries with the biopolymer before harvest and observed that there was no damage caused by gray mold at temperatures of 3 and 13 °C during storage (5). For the foregoing, one of the most studied strategies in recent years with the aim of regulating the populations of certain phytopathogens, increasing yields and improving the quality and commercial life of strawberry fruits.

All this has resulted in the growing interest of many researchers in continuing to test the effect of chitosan on the growth and development of the

Table 1. Favorable climatic conditions for strawberry cultivation

Altitude	1 300 a 3 000 ms.n.m.
Temperature	• Ideal: daytime between 18 and 25 ° C and night between 8 and 13 ° C • Less than 15 ° C: slow ripening, high T °: early coloration • More than 32 ° C: floral abortions • 12 °C on the ground: root stimulation • 0 °C: severe damage to pollination, deformed fruits, flower necrosis • -8 °C: very serious tissue damage • -10 a -12 °C: death of the plant
Luminosity	3 000 hours of sunshine / year
Relative Humidity	60 a 75 %
Precipitations	Minimum (1 000 a 2 000 mm/año)
Hail	Severe mechanical damage, facilitates the entry of pathogens

strawberry, as well as its behavior in different cultivars. In a study with the application of the 90-95 % bioproduct (2-Amino-2-deoxy-beta-D-glucosamine) at 1 %, that is, 10 g of chitosan dissolved in 0,1 of sodium hydroxide (NaOH)) and completed in one liter of distilled water with a concentration of 5 g L^{-1} an increase in the height of the treated plants, in the fresh and dry weight, in the number of leaves and crowns and in the foliar area with regarding the witness. At the same time, when they estimated the fruit quality parameters, average vitamin C values of 43,83 mg/100 g were observed, while in the control treatments of 31,58 mg/100 g and in terms of anthocyanin content in all the plants treated with chitosan an average value of 88,37 mg/100 g was obtained and in the untreated ones of 80,93 mg/100 g, they obtained significant differences (18).

In another trial they tested two concentrations 2,5 and 5 g L^{-1} of the commercial product Chitosan care composed of (Nitrogen 1000 mg L, P_2 O_5 500 mg L, K_2 O 500 mg L, Fe 100 mg L, Zn 100 mg L, Cu 50 mg L, Mn 50 mg L and B 50 mg L) with several applications at 30, 60 and 90 days after transplanting the stolons and noticed a significant increase in vegetative growth (plant length, number of leaves / plant , foliar area, root growth, fresh and dry weight as well as the yield attributes (fruit weight, early yield and total / plant) with the two concentrations used, and indifferently said authors considered the most effective treatment to be the most effective 5 g L^{-1} (19).

In new research, taking into account that infections by pathogens such as *Botrytis cinerea* and *Rhizopus stolonifer* occur at the stage of crop development and their symptoms generally develop after harvest, they have referred to the sprays of chitosan with a formulation commercial grade of a deacetylation degree of 80-90 %, a viscosity of 0,08-0,12 (1 % w / v solution), the molecular weight of the d-glucosamine hydrochloride monomer of 215,62 g / mol and the molecular weight of the N-acetyl-d-glucosamine hydrochloride monomer is 257,66 g / mol, from the stage of flowering to ripening, as a fungicidal strategy to control post-harvest rot (20).

In this study they used the infection index (Mc Kinney, 1923) that incorporates both the incidence and severity of decomposition of the fruit and with the concentrations of 5 and 10 g L^{-1} of the product, witnessed an increase in the yields of the plant and a significant reduction of 50 and 49 % compared to the control, of the presence of gray mold and Rhizopus in the fruit, as well as a post-harvest decay of 36 and 29 % after 4 days of useful life. In addition, they verified that with 5 g L^{-1} there was a lower incidence of microorganisms and in turn when they evaluated the fruit quality parameters, in one of the cultivars, with both concentrations the firmness and color of the fruit was maintained (20), which are the fundamental parameters for consumer acceptability (21,22).

Therefore it can be considered that the treatments from the pre-harvest with this biostimulant, besides favoring the growth and development of the plant, could supplement the use of conventional fungicides to extend the shelf life of the strawberry. It is important to highlight the results obtained in these investigations where they have applied chitosan since the pre-harvest and have observed that with the lower concentration of the biopolymer the appearance of damages by microorganisms such as *Botrytis* in the post-harvest decreases. However, in other reported trials these damages are reduced as the concentrations increase (23) and only when applied after harvesting the fruits or when mixed with other compounds such as soy lecithin, calcium, organic acids and others. (24)

In this sense, it must be taken into account that chitosan plays a fundamental role in the metabolism of plants, which is the phytoprotection, so that improving and maintaining health will lead to a positive effect on vegetative growth and, at the same time, on the yield of the fruits. It is suggested that its antimicrobial action is mainly due to the polycationic nature of the molecule when it is found in solutions at pH below 6, since the positively charged amino groups can interact with the phospholipids of the cell membranes of microorganisms and alter its permeability. This can cause osmotic imbalances that lead to structural disorganization and can finally culminate with cell lysis (25,26). In the case of oligochitosans, the internalization of these molecules in the microbial cell has been demonstrated and their possible interaction with the DNA of the same is speculated (27). The magnitude of the affectations found can vary, fundamentally, depending on the physico - chemical properties of the polymer and the concentrations that are used (10,25).

Recently, there has been an increase in the evaluation of chitosan under controlled, uncontrolled and greenhouse conditions and even its extension and evaluation as a result of government decisions. At present, this polymer is recognized within biopesticides as a "derivative of crustaceans activating the defense of plants" (7).

This protection found against the attack of pathogens can be due to the antimicrobial activity that these polymers and oligomers exert on the microorganisms or it can be the result of the elevation of the basal resistance of the plant, caused by the activation of induced resistance exerted by these compounds in the vegetable. In many cases both effects can occur simultaneously (28-30).

Effect of chitosan in the improvement and conservation of strawberry fruits. Uses as edible coatings (RC)

Today, the quality of agrifood products becomes the main factor of consumer preference. Both the organoleptic and nutritional quality are a reflection of the chemical composition of the fruit, since it determines the sensorial characteristics that the consumer directly assesses with their senses: color, aroma, flavor and texture and the nutritional value by providing the essential nutrients for health of it, proteins, carbohydrates, vitamins, minerals, etc. To obtain strawberry fruits with quality requires adequate growth and development of the plant during the pre-harvest period. The different factors that control these complex processes determine the quality of the product at the time of harvest and also its behavior and useful commercial life during post-harvest (6).

The strawberry is a non-climacteric fruit, very delicate and has a very short shelf life. Due to its physiological conditions, it is very susceptible to moisture loss and attack by microorganisms, especially the fungus *Botrytis cinerea* as mentioned above, which causes great losses during transport and commercialization because it reduces the attributes of flavor, aroma and texture, affecting its commercial quality and its attractive freshness for the consumer (31).

Cold storage is a common practice to prolong the storage period of fruits (32). In the case of strawberry, it reduces the rate of respiration and loss of moisture and slows microbial growth, allowing extending the shelf life and preserving the quality of the fruit. In the application of packaging technologies for this fruit, the use of modified atmospheres combined with cold has contributed significantly to its conservation, since it reduces respiration due to the low presence of O_2 and the increase of CO_2 (33).

On the other hand, in the market of large surfaces, thermoformed bioriented polystyrene boxes (BOPs) and expanded polystyrene (EPS) trays covered with vinylpel (extendable plastic film), stored in refrigeration, with practical results of up to three days in conditions with acceptable appearance. As an alternative to these traditional conservation methods, the use of edible coatings (CR) is applied at low temperatures, as a means to reduce the rate of deterioration in the quality attributes of the fruits during storage (34).

Edible coatings (RC) are defined as substances that are applied on the outside of food so that the final product is suitable for consumption. They have been used for centuries in the food industry, with the main objective of preventing the loss of moisture in food. These coatings must be legal, safe for consumption, acceptable to consumers and must provide added value to the food (8). In addition, they reduce the mechanical, physical and chemical damages that the environment generates to the product (35).

Over the years, the use of these RCs has gained great importance due to the increase in demand for fresh foods. Its use in combination with other barriers, processing methods, good hygiene practices and adequate storage conditions, can contribute to improving the quality and safety of fresh, minimally processed and processed foods. Currently, researchers and industrialists have focused on testing new components for the development of CR applied to various foods and the incorporation of additives that improve the quality of coated products.

The purpose of CR in fruits is to reduce water loss, retard aging, give shine and preserve color, thus allowing a better quality and price of these products (33,34). The Chitosan-based RC applied are very effective, since this polysaccharide has a high selective permeability against gases and a slight resistance to water vapor, has an enormous potential due to its physicochemical properties, such as biodegradability, biocompatibility with human tissues, zero toxicity and especially for its antibacterial and antifungal properties (8,36).

The application of coatings made with chitosan delays the ripening process of certain fruits such as: orange (37), mango (38), medlar and raspberry, therefore the interest of its application in strawberries.

Taking into account the advantages of this polymer in its combined use with other compounds such as RC, they evaluated it with cinnamon oil (AC) and with different concentrations (1 and 2 g L^{-1} of Q + 0,1 g L^{-1} AC) and managed to prolong the useful life of the strawberry for up to 15 days and eliminated the development of aerial mesophiles (39).

These authors reported that the RCs that showed the greatest reduction in the fungal and yeast population were those composed of (1 g L^{-1} of Q + 0,4 g L^{-1} of starch (A) + 0,03 g L^{-1} of AC and 2 g L^{-1} of Q + 0,1 g L^{-1} of AC). The lowest value of total phenols (140 mg of gallic acid / 100 g of fresh weight) was found in strawberries treated with (1 g L^{-1} of Q + 0,1 g L^{-1} of AC) and this is considered to be it is probably due to the fact that it was the treatment in which the least deterioration was presented, and that perhaps it is related to the effect of the CR of diminishing the activity of PAL (enzyme phenylalanine ammonialase) and to delay the deterioration process, as well as to reduce the accumulation of said phenolic compounds due to the decrease of the metabolic processes of the fruit that bring with it the senescence of the same. The highest antioxidant capacity was observed in the control fruits and strawberries treated with the RC of (1 g L^{-1} of Q + 0,03 g L^{-1} of AC), which was also related to the higher content of phenols (39).

Possibly antifungal activity has been attributed to the activation of the fruit defense mechanism influenced by the presence of chitosan, through the activation of the chitinase enzyme and the synthesis of phytoalexins and other compounds (40). At the same time in many treatments a high accumulation of phenols was found in treated and untreated strawberries, which could be promoted by the activity of the PAL, which could increase its activity to the stress caused by said treatments, by increasing the deterioration process, which could have originated the decomposition of the cellular structure with the end of the senescence and the consequent increase in the content of phenols (41).

Some authors have related the accumulation of phenolic compounds with the increase in the antioxidant capacity in plants and fruits (42,43) and it is worth noting that the strawberry has a high antioxidant capacity and that it is not significantly influenced by the presence of cinnamon oil or RC. However, the antioxidant capacity can come from different phytochemical compounds, for example, a decrease in the content of vitamin C and an increase in the anthocyanin content in strawberries treated with RC of chitosan and proteins of wheat gluten with thymol have been reported. , respectively (44); being phenolic compounds those that provide the greatest antioxidant capacity. It is proposed that the antioxidant capacity of a food depends on the nature and concentration of the great variety of natural antioxidants present in it.

Another of the properties that makes chitosan widely used is that it forms a film on the fruit and as mentioned above, has good antifungal activity against gray mold, which is one of the main causes of deterioration and post-harvest decay in strawberries In an assay where chitosan (Q) was used accompanied with quinoa oil (CH) and sunflower oil (SO) during all days of the analysis, coated strawberries had a significantly lower amount of fungal and yeast growth than strawberries without coat; this reduction being more evident in those who were coated only with chitosan (Q) (45). In addition, when they evaluated the physicochemical properties they observed that the color of the strawberries was not influenced by the coatings, a result that is very satisfactory because in other studies they have shown that the application of film forming solutions emulsified in strawberries can cause color changes and increase the opacity of the coated fruits (46).

The lower effect of (CH - Q and CH - Q - SO) is due to the ionic and hydrophobic interaction between (Q - CH and Q - CH - SO), respectively (46), which reduces the availability of the groups reactive aminos of the Q to the antimicrobial properties. Other authors have shown similar results with the Q coating; because it potentially causes severe cell damage in molds and yeasts by altering the synthesis of fungal enzymes, induction of morphological changes causing structural alterations and molecular disorganization in the cells of the fungus.

In general, many have been the investigations that have been carried out and continue to be developed in the search for efficient, economical and healthy alternatives to improve and preserve the commercial life of the strawberry. Similarly, many trials have been reported in which chitosan is combined with several compounds such as: vanilla, acetic acid, cactus mucilage (47) and together with chitosan from different sizes of *Cheraz quadricarinatus* (48).) and all have reported favorable results on the effect of the product as an edible coating to extend the useful life of the fruit.

CONCLUSIONS

- ♦ Chitosan is a favorable biostimulant for the growth and development of strawberries. The treatments from the pre-harvest, besides improving the yields of the plant can supplement the use of conventional fungicides in the control of the post-harvest loss of the fruit.
- ♦ Its use as an edible coating in the strawberry is a promising alternative to improve the quality of the fruits during processing and conservation, as it is an environmentally friendly technology that responds to the increasing demand by consumers of natural foods, safe, healthy and obtained through a minimum processing.

RECOMMENDATIONS

It is recommended the use of the results obtained by the different authors cited in this review, as a documentary value for the producers of this valuable fruit and in turn for future research where you want to test the action of this biostimulant in the improvement and conservation of the fruit of strawberry.

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