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INFLUENCIA DEL RECUBRIMIENTO CON QUITOSANO Y LA TEMPERATURA DE ALMACENAMIENTO EN LA CALIDAD POSTCOSECHA Y NIVELES DE INFECCIÓN EN LA CIRUELA MEXICANA

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Palabras clave: *Spondias purpurea L., jocote, comportamiento postcosecha, pudriciones postcosecha*

RESUMEN

La ciruela mexicana (*Spondias purpurea* L.) es una fruta climatérica, tropical y sensible a daños por frío y por lo tanto con una vida postcosecha limitada. A pesar de su popularidad, se conoce poco acerca del manejo óptimo para extender su almacenamiento. En varios estudios se reporta que el quitosano (derivado de la quitina) es una película semipermeable que alarga la vida de almacenamiento de varios productos hortofrutícolas. Además, este compuesto tiene propiedades fungicidas que reducen las pudriciones postcosecha. En el presente estudio, en frutos de ciruela mexicana se aplicaron concentraciones de quitosano al 0.5, 1.0, 1.5, 2.0 y 2.5 % y se almacenaron a 12, 15 y 27 ± 2 °C por un periodo de tiempo dependiendo de la temperatura. La fruta testigo fue tratada solamente con agua. Los resultados indicaron que el almacenamiento se prolongó 12, 6 y 10 días (d) cuando se almacenaron a 12, 15 y 27 ± 2 °C, respectivamente. En todas las temperaturas de almacenamiento, los sólidos solubles totales (SST) se incrementaron al aumentar el periodo de almacenamiento. La fruta no tratada tuvo los SST más altos comparado con la fruta tratada con quitosano. Durante el almacenamiento, el contenido de ácido cítrico en la fruta fue similar independientemente de la concentración de quitosano. La tasa de pérdida de masa se incrementó con la temperatura y tiempo de almacenamiento, sin embargo, la fruta tratada con quitosano al 2.5 % tuvo la menor pérdida de masa. Los niveles de infección más bajos se observaron en la ciruela tratada con quitosano al 2.0 y 2.5 % a 12 °C (35 %). El principal microorganismo aislados fue *Fusarium* spp, seguido por *Colletotrichum gloeosporioides*, *Penicillium digitatum* y *Rhizopus stolonifer*.

INFLUENCE OF CHITOSAN COATING AND STORAGE TEMPERATURE ON POSTHARVEST LIFE, QUALITY AND INFECTION LEVELS OF CIRUELA FRUIT

Key words: *Spondias purpurea L, red mombin, jocote, hog, postharvest behaviour, postharvest rots.*

ABSTRACT

Ciruela fruit (*Spondias purpurea* L.) is a tropical climacteric fruit, chilling sensitive and therefore with a short postharvest life. In spite of its popularity, little is known about optimum procedures to extend its storage. Several studies report that chitosan (a chitin derivative), a semi-permeable film, extends storage life of several horticultural commodities. Likewise it is also reported that this natural compound has fungicidal properties to reduce postharvest rots. In this study, before storage, ciruela fruit were chitosan-treated at 0.5, 1.0, 1.5, 2.0 and 2.5% concentrations and stored at 12, 15 and 27 ± 2 °C, 90 HR for a period of time. Control fruit were only water treated. Results showed that at 12 °C storage of ciruela was extended for 12 d at 15 °C for 10 days (d) and at 27 °C for 6 d. For all storage temperatures total solid solubles (TSS) increased with storage period. In general, untreated fruit had the highest TSS compared with chitosan-treated ciruela. During storage, content of citric acidity in fruit was very similar during storage period regardless chitosan concentration. Rate of mass loss increased with temperature and storage time, however, chitosan-treated ciruela at 2.5 % concentration showed the lowest percent mass loss. Compared to the untreated ciruela, the lowest infection levels were shown in

chitosan-treated ciruela at 2.0 or 2.5 % at 12 °C (35 %). The main microorganism isolated was *Fusarium* spp, followed by *Colletotrichum gloeosporioides*, *Penicillium digitatum* and *Rhizopus stolonifer*.

INTRODUCTION

Chitosan is a deacetylated form of *N*-acetylchito-oligosaccharides containing poly *D*-glucosamine and is usually prepared from chitin. Chitosan is normally present in the cell walls of crustaceans, insects, yeast and fungi and due to its particular polycationic nature has important industrial applications (Sandford, 1989). Application of chitosan coatings has been shown to improve the storability of various perishable commodities. Ripening of numerous fruits and vegetables-chitosan coated such as tomatoes, strawberries, pears and kiwifruit was slowed down, for example, the loss of firmness was retarded resulting in firmer fruit compared to the untreated fruit (El Ghaouth et al., 1991, 1992; Du et al., 1997). In mangoes and bananas, the content of total solid solubles increased after storage by effect of chitosan dipping. Generally, at the end of the storage period, fruit acidity increased in various chitosan-treated commodities (tomatoes, strawberries and peaches) (El Ghaouth et al., 1992; Li and Yu, 2000; Jiang and Li, 2001). Additional positive effects of chitosan are those related with its antimicrobial activity. For example, lower disease incidence is reported in chitosan-treated fruits such as strawberries, raspberries, papayas, carrots, etc. during or at the end of the storage period (Zhang and Quantick, 1998; Bautista-Baños et al., 2003a; Cheah et al., 1997). In those studies, gray mould, anthracnose and Sclerotinia rot were significantly lower after treated with chitosan compared with the untreated fruit.

During storage, temperature is one of the most important factors affecting the ripening process as well as the occurrence of many postharvest diseases (Barkai-Golan, 2001). In this sense the use of controlled temperature has been largely employed.

Ciruela fruit (*Spondia purpurea* L.), also known as hog, red mombin, jocote, Spanish-plum or purple mombin is a widely distributed tree in Tropical America. Particularly, a wide variety and forms of ciruela

fruit can be found in Mexico and Central America (Popenoe, 1974). Among the great diversity of genotypes of ciruela existing in Mexico, those harvested in September-November are the most consumed. Fruit is about 5cm long, oval to roundish and have a thin skin that can be from green to orange-red colour depending on the ripening stage. Mature fruits are juicy, sweet acid, yellow mesocarp and a large woody endocarp with one seed (Mandujano et al., 1994). Ciruela fruit are mostly consumed fresh and fruit is sometimes processed to obtain candies or desserts. Usually, fruit is harvested at the green ripe stage (surface of the fruit 100 % green) and placed in wooden containers, wrapped in paper and stored at ambient temperature originating irregular ripening (Díaz-Pérez et al., 1999). Ciruela fruit is known to be a highly perishable fruit and sensitive to chilling injury (Díaz-Pérez et al., 1999). During marketing of ciruela, fruit is generally stored at ambient temperature and to the best of our knowledge no recommended temperatures have been reported for this fruit. However, according to previous studies, the high respiration rate of ciruela is directly related to its low storability at room temperature (Canto-Pereira et al., 2000). The objective of this work was to ascertain whether chitosan and temperature treatments in combination may prolong ciruela fruit storage life and control postharvest rots.

MATERIALS AND METHODS

Fruit sampling and storage. Fruit were collected from the ciruela production areas of the state of Morelos at the ripening stage of green-ripe. Fruit were transported to the laboratory and selected discarding damage or diseased fruit. After chitosan-treatment, they were left to dry, kept in plastic containers and stored in controlled-temperature rooms at 12 °C (85 % RH, vapour pressure deficit, VPD, 0.21 kPa), 15 °C (75 % RH, VPD, 0.43 kPa) or at ambient temperature (27±2 °C, 65 % RH, VPD, 1.25 kPa).

Preparation of chitosan. Chitosan (Fluka, Biochemika Sigma Aldrich, Steinham, Switzerland) solutions were prepared weighing 0.5, 1.0, 1.5, 2.0 and 2.5 g of chitosan and then dissolving it in 100 ml of distilled water containing 2 mL of acetic acid. The solutions were heated and constantly agitated and the pH adjusted to 5.5 with NaOH 1 N.

Quality measurements. Juice was squeezed from a slice cut of the fruit and TSS concentration was measured using a hand-held Atago N-20 refractometer (Atago N-1E Japan, brix range from 0-32 %). Juice acidity was titrated with phenolphthalein end point with 0.1 N NaOH and expressed in percent citric acid. Fruit transpiration was measured as a daily or total percent mass loss of the fruit with respect to the initial fruit weight. Individual fruit weight was determined daily on 10 fruit per treatment. Evaluations of TSS and acidity were carried out every two days until fruit showed severe symptoms of wilt.

Phytopathological assessments. Levels of infection were measured every day until the end of the experiment. To identify fungi, small portions of symptomatic tissue were placed on Petri plates containing Potato dextrose agar (PDA) (trade name Bioxon from Becton Dickinson) and incubated at ambient temperature. Conidia were identified by optical microscopy (Burnett and Hunter, 1972; Streets, 1984).

Experimental design. Experiments were arranged in a completely randomized design. Means and standard deviation were calculated for TSS, acidity and mass loss. For acidity and TSS parameters, 10 fruit per treatment were analyzed for each sampling day, while for percentage infection 40 fruit/treatment were evaluated. There were five replicates per treatment.

RESULTS

During storage, life of ciruela was extended with decreasing storage temperature. At 12 °C fruit was able to be stored for 12 d, at 15 °C they were stored for 10 d and at 27 ± 2 °C fruit were kept for only 6 d. Overall, percentage TSS, acidity, mass loss and disease incidence of ciruela fruit varied according to the given combination of temperature and chitosan concentration.

TSS content increased with increasing storage temperatures (Figure 1). Except for the untreated fruit, TSS values of fruit stored at 12 °C, were very similar during storage regardless chitosan concentration; however at higher temperatures (15 °C and 27 °C) TSS decreased with increasing chitosan concentration. At the end of the storage period, for all temperatures the control had the highest TSS values and chitosan-treated fruit at 2.5 % had the lowest (Table 1).

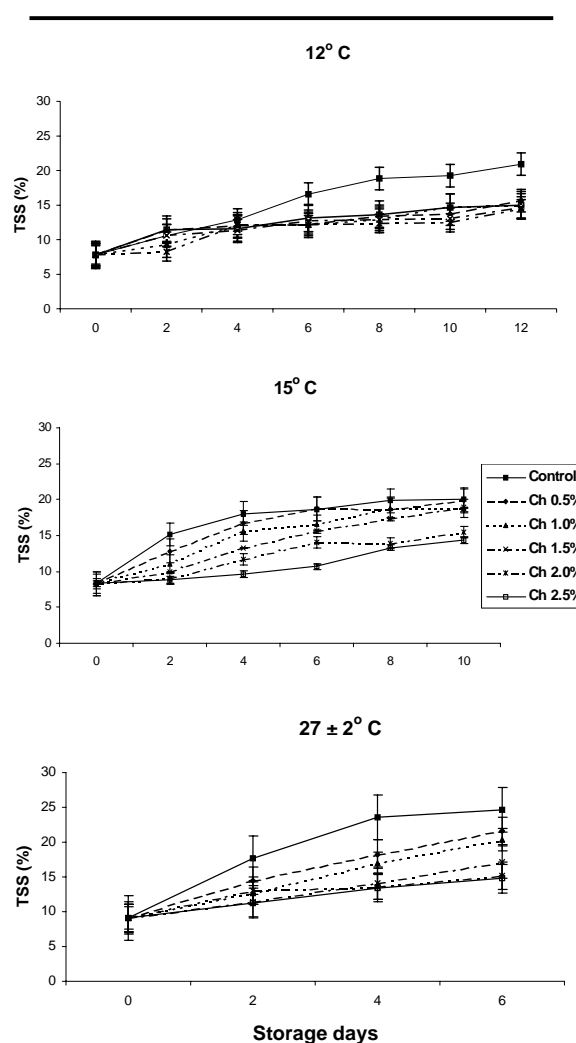


Figure 1. Total solid solubles progress of chitosan (Ch)-treated ciruela held at different temperatures during a storage time.

Table 1. Final mean values of TSS, acidity, mass loss and percentage infection of chitosan-treated ciruela stored at different temperatures.

Treatment	Mean TSS (%)	Mean titratable acidity (%)	Rate of mass loss (%/d ⁻¹)	Infection (%)
12 °C+ Chitosan (%):				
0.5	15.6 (1.8)	0.6 (0.03)	0.55	87
1.0	14.8 (0.8)	0.6 (0.04)	0.54	87
1.5	14.6 (0.9)	0.6 (0.03)	0.51	45
2.0	14.4 (1.2)	0.6 (0.1)	0.48	35
2.5	14.4 (1.9)	0.6 (0.1)	0.45	35
Control*	20.9 (0.9)	0.5 (0.06)	0.56	95
15 °C+ Chitosan (%):				
0.5	19.8 (0.8)	0.6 (0.04)	0.92	90
1.0	18.8 (0.8)	0.6 (0.04)	0.86	72
1.5	18.8 (0.8)	0.7 (0.07)	0.83	70
2.0	15.4 (2.5)	0.7 (0.05)	0.80	63
2.5	14.3 (2.8)	0.8 (0.04)	0.79	55
Control*	20.0 (0.6)	0.6 (0.03)	0.98	95
27 °C+Chitosan (%):				
0.5	21.6 (1.9)	0.8 (0.1)	3.6	-
1.0	20.3 (1.5)	0.9 (0.08)	3.6	-
1.5	17.1 (2.3)	0.9 (0.1)	3.5	-
2.0	15.1 (1.9)	0.9 (0.1)	3.5	-
2.5	14.7 (2.2)	0.9 (0.1)	2.9	-
Control*	24.5 (3.9)	0.8 (0.05)	3.8	-

Values between parentheses indicate standard deviation of the mean.

-No symptoms of disease

* no chitosan added

The titratable acidity values decreased consistently with the storage days for all storage temperatures, showing moderate changes from the initial to the final values (0.9 % to 0.5 % at 12 °C, 1.0 % to 0.6 % at 15 °C and 1.3 % to 0.8 % at ambient temperature) (Figure 2). Apparently, chitosan concentration did not influence acidity values.

Overall, there was more fruit mass loss as temperature (and vapour pressure difference) increased for all treatments (Figure 3). For each temperature, the lowest rate mass loss was in fruit treated with the highest chitosan (2.5 %) concentration (12 °C = 0.45 %, 15 °C = 0.79 % and 27 °C = 2.9 %) and the highest rate was in untreated fruit (12 °C = 0.56 %, 15 °C = 0.98 % and 27 °C = 3.8 %).

At 12 °C and 15 °C, percent infection gradually increased during storage time, however, at ambient temperature fruit shriveled so quickly that no disease infection

was visible (Figure 4). At 12 °C, symptoms of infection in untreated or chitosan-treated at 0.5 % and 1.5% were evident after 9 d storage and, in chitosan-treated fruit at 1.0 % concentration onwards, disease was observed after 11 d storage. At 15 °C infection was observed after 6 d storage. For both 12 °C and 15 °C temperatures, rate of infection decreased as chitosan concentration increased. As compared to the control, the lowest rate of infection was observed at 12 °C in chitosan-treated fruit at 2.0 % and 2.5 % (2.9 %), and at 15 °C, in the chitosan-treated fruit at 2.5 % (5.5 %). In general, the main pathogens isolated and identified from untreated and chitosan-treated fruit stored at 12 °C and 15 °C were *Colletotrichum gloeosporioides*, *Fusarium* spp, *Penicillium digitatum*, *Rhizopus stolonifer* (Table 2). *Fusarium* spp. was the most frequently fungus isolated from all treated and untreated fruit, followed by *C. gloeosporioides* and *R. stolonifer*.

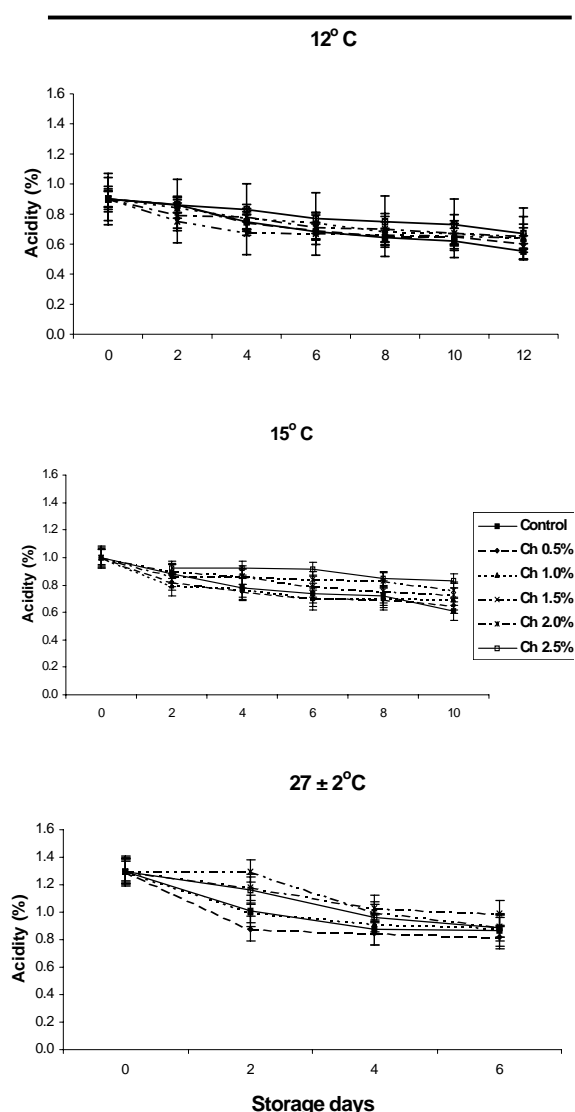


Figure 2. Acidity progress of chitosan (Ch). Treated ciruela held at different temperatures during a storage time.

DISCUSSION

The combination of temperature and chitosan affected fruit TSS development, mass loss and infection levels. We observed that the lowest TSS were in fruit stored at 12 °C regardless chitosan concentration, and in fruit held at both 15 °C and ambient temperature and dipped in chitosan at 2.0 % and 2.5 %. Similarly, at the end of the storage period at this temperature we observed that fruit did not

achieve the characteristic red-orange colour (data not shown). In this study, CO₂ production was not a parameter evaluated however, the lack of an optimum development of TSS and colour can be explained by the ability of chitosan to retard the ripening process of the treated fruit. Generally chitosan has the ability to modify the CO₂ and O₂ production. In tomatoes, chitosan-coated the climacteric trend was inhibited and fruit colour was altered, delaying the development of red colour (El Ghaouth et al., 1992). Zhang and Quantick (1997) mention that litchi chitosan-coated also delayed formation of anthocyanin, flavonoids and total phenolics associated with a normal ripening process.

In line with other reports, we did not observe dramatic changes in acidity content between the treated and the untreated ciruelas. In previous studies, acidity progress of ciruela fruit during storage was little affected by harvesting region, storage temperature and duration, and ripening stage (Díaz-Pérez et al., 1999; Bautista-Baños et al., 2003b). However, Díaz-Pérez et al. (1999) mention acidity ranges from 0.2 % to 0.7 % during storage of 7 d at 20 °C whereas in our studies varied from 1.3 % to 0.7 %. A possible explanation for this might be associated with intrinsic differences in ciruela genotypes.

In our experiments, percent mass loss rates were similar to those reported by Díaz-Pérez et al. (1999). Fruit transpiration was evidently different according to the given temperature and chitosan application and clearly fruit mass loss was dramatically higher in those stored at ambient temperature resulting in higher levels of shriveled fruit and therefore commercially unacceptable. In our study, the efficacy of chitosan was more evident at 12 °C compared to the remaining two temperatures. The pattern of mass loss during storage of the treated ciruela was similar to that of chitosan-treated cucumbers, bell peppers, mandarin and longan fruit among others (El Ghaouth et al., 1991b; Jiang and Li, 2001; Salvador et al., 2003). In those studies as chitosan concentration increased, mass loss regularly decreased.

The highest chitosan concentration of 2.5 % was effective in reducing postharvest rots in this fruit, resulting in lower evidence of

diseased fruit during the storage period of 12 or 10 d at 12 and 15 °C respectively. So far, it has been reported in numerous investigations that reduction of rots increases with chitosan concentration. For example, blue and gray mould of apples and strawberries respectively were controlled at the highest concentration (Bautista-Baños et al., 2004; Zhang and Quantick, 1997). In other studies, Jiang and Li (2001) found that during storage of longan the highest chitosan rate of 2.0% were the most effective to reduce postharvest disease levels. In previous studies carried out at 20 °C *Rhizopus stolonifer* and *Alternaria* spp were the main pathogens identified from ciruela fruit (Bautista-Baños et al., 2003b).

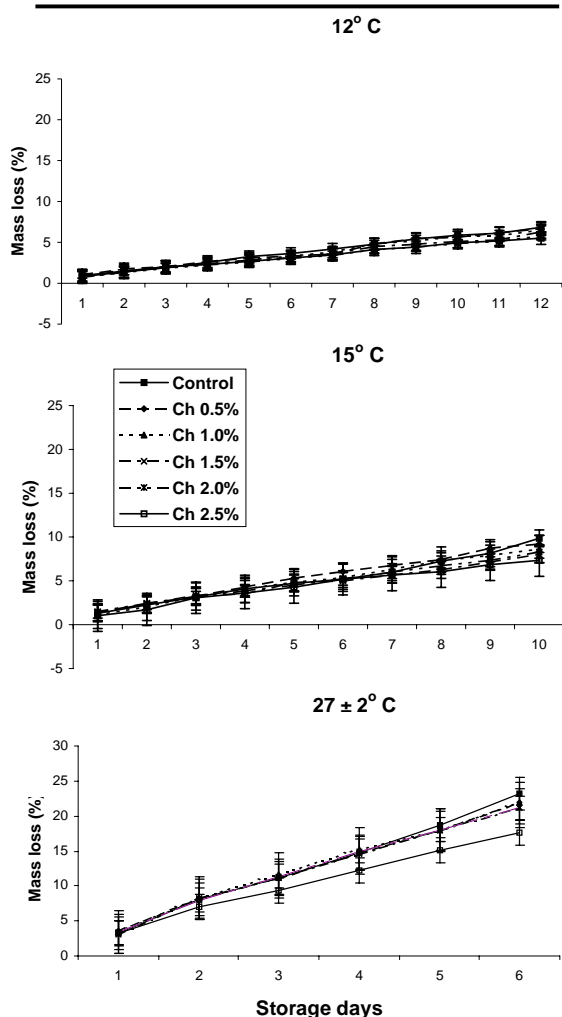


Figure 3. Cumulative mass loss progress of chitosan (Ch). Treated ciruela held at different temperatures during a storage time.

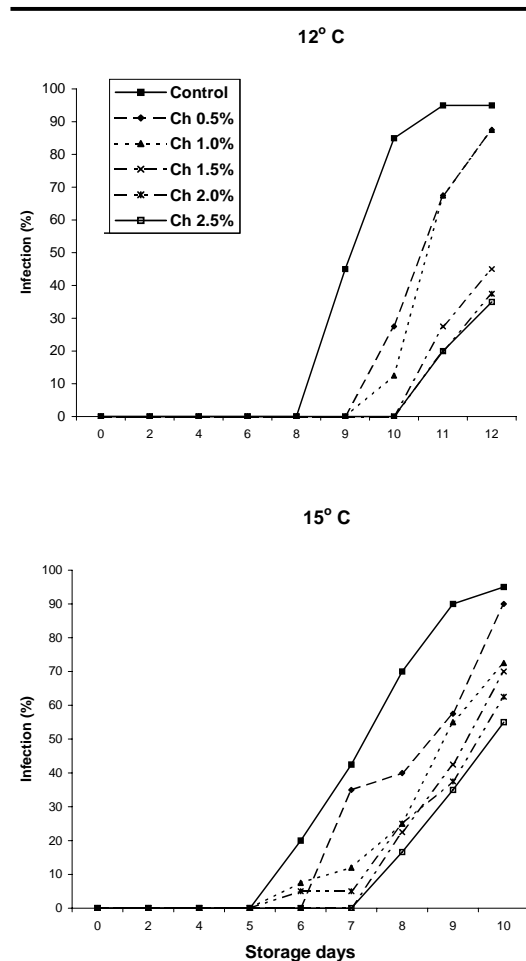


Figure 4. Effect of chitosan (CH) and temperature on the control of decay of ciruela fruit.

CONCLUSIONS

In conclusion, although temperature at 12°C in combination with chitosan concentrations of 2.0 and 2.5 % seemed to be the most effective to avoid excessive mass loss and disease levels, the evidence also showed that its applicability at this temperature will be hampered due to alterations observed in the ripening process therefore, further studies including experiments with temperatures between 12 and 15 °C might open the possibility to apply a controlled temperature. In addition, other edible coatings such as carnauba waxes should be tested.

Table 2. Fungi isolated from chitosan-treated ciruela fruit and stored at 12 °C and 15 °C.

Chitosan treatment (%)	12 °C		15 °C	
	Microorganism	Incidence	Microorganism	Incidence
0.5	<i>Fusarium</i> spp	40	<i>Colletotrichum gloeosporioides</i>	5
	<i>Penicillium digitatum</i>	10	<i>Fusarium</i> spp	33
	saprophyte	10	<i>Penicillium digitatum</i>	10
			<i>Rhizopus stolonifer</i>	15
1.0	<i>Colletotrichum gloeosporioides</i>	15	saprophyte	5
	<i>Fusarium</i> spp	40	<i>Fusarium</i> spp	40
	saprophyte	18	<i>Rhizopus stolonifer</i>	8
			saprophyte	10
1.5			<i>Fusarium</i> spp	30
	<i>Fusarium</i> spp	40	<i>Penicillium digitatum</i>	10
			<i>Rhizopus stolonifer</i>	3
2.0	<i>Fusarium</i> spp	30	<i>Fusarium</i>	32
	saprophyte	17	<i>Penicillium digitatum</i>	12
	<i>Colletotrichum gloeosporioides</i>	30	<i>Fusarium</i> spp	10
2.5	<i>Fusarium</i> spp	12	<i>Aspergillus</i>	2
	<i>Rhizopus stolonifer</i>	10	saprophyte	5
	saprophyte	15		
	<i>Colletotrichum gloeosporioides</i>	40	<i>Colletotrichum gloeosporioides</i>	20
Control	<i>Fusarium</i> spp	40	<i>Fusarium</i> spp	50
	<i>Penicillium digitatum</i>	40	<i>Penicillium digitatum</i>	30
	saprophyte	20	<i>Rhizopus stolonifer</i>	80
			<i>Alternaria alternata</i>	3
			Saprophyte	3

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