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DEVELOPMENT OF COATINGS WITH QUINOA SAPONIN (*Chenopodium quinoa* WILLD.) FOR CONTROL OF GREEN ROT IN ORANGE (*Citrus sinensis)*

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Abstract: Quinoa (Chenopodium quinoa Willd.) is an Andean grain that has gained great commercial importance in the last 15 years. One of the drawbacks is the presence of an anti-nutritional factor known as saponin. The objective of this research work was to evaluate the inhibitory effect of coatings made from quinoa saponin applied on Penicillium digitatum of isolated strain and coded strain. In vivo tests of the coatings were carried out at four concentrations 15000, 11300, 7500 and 3700 ppm; The quality parameters were evaluated: damage index, firmness and presence of fungi. The loss of firmness was 6.6% in the coated samples at a concentration of 15,000 ppm and the control samples presented 5.96% loss of firmness and the damage index from the isolated strain was 11.49 and from the coded strain was 10.02. On the other hand, the presence of fungi in the samples with coating at a concentration of 15,000 ppm was at 13 days, while the standard sample had the presence of fungi at seven days, thus showing a difference of five days of delay. It is concluded that the coatings developed in this research are a good alternative for the control of green rot since it delays its appearance.
Keywords: Coating, quinoa, saponin, inhibition, Penicillium digitatum.

INTRODUCTION

The main application of quinoa is as food, mainly due to the high protein value of its grains (Koziol, 1992). One of the drawbacks is the presence of an anti-nutritional factor which is saponin (Monje & Raffaillac, 2009), a bitter-tasting substance located mainly in the episperm of the grain, which must be eliminated before human consumption (Lescano, 1994). For its elimination, quinoa processing companies have developed a processing process where the episperm is separated from the grain through two processes: the first is based on friction between grains by mechanical action (scarification), obtaining a powder rich in saponins called " Mojuelo."

The second is a water washing process to remove the remaining episperm. The yield of the "mojuelo" is around 4.5% compared to grain, so tons of this waste are generated every year (León, 2003; Mujica, 2006).

Much research attention has focused on diosgenin, however its precursors, saponins, are themselves compounds that may have great biotechnological interest, since they are involved in the defense of plants against microorganisms, especially fungi (Osbourn, nineteen ninety six).

Fruit crops are attacked by many diseases and pests, one of the main agents being phytopathogenic fungi, one of the main causes of postharvest losses in citrus fruits (Mathews, 2008).

In the postharvest of citrus, the main diseases are caused by the pathogenic fungi Penicillium digitatum (green rot) and Penicillium italicum (blue rot) (Brito et al., 2012), which have a higher incidence, producing close to 80% of postharvest losses in citrus fruits. Fruit infection takes place through wounds or micro wounds produced in the bark before, during or after harvest, resulting in irreversible infections within a period of 48 hours at 20 - 25°C (Ochoa et al, 2007); The objective of this work was to evaluate the inhibitory effect of coatings made from quinoa saponin (Chenopodium quinoa Willd.) applied on Penicillium digitatum of isolated strain and coded strain.

MATERIALS AND METHODS

ISOLATION AND IDENTIFICATION OF PENICILLIUM DIGITATUM STRAINS

a) Preparation of the coded Penicillium digitatum strain

Coded strain ATCC 36038: Penicillium

digitatum was obtained from the strain bank of the Oswaldo Fiocruz Institute – Brazil. The active strain was reactivated on Mueller Hilton agar and kept refrigerated, for the experimental part it was reactivated on Potato Dextrose agar (PDA). The spore concentration was adjusted to 1 x 105 using the Mc Farland scale and they were incubated at 25°C for seven days.

b) Isolation of the natural strain of Penicillium digitatum

To obtain the strains of Penicillium digitatum, the methodology for isolating microorganisms from infected tissue proposed by Mondino (2012) was used, where spores were obtained from the surface of oranges with the presence of fungi, and they were sown on PDA plates. at 25 °C for seven days. Subsequently, the identification and purification of the Penicillium digitatum microorganism was carried out according to the methodology proposed by the ICMSF, 2006.

Evaluation of antifungal activity in in vitro tests of saponin

The evaluation of the antifungal activity was carried out through the agar diffusion methodology proposed by Viuda et al., (2008) in which discs are inoculated with saponin at different concentrations (15000, 11300, 7500 and 3700 ppm) in the PDA culture medium. Follow-up was done for 10 days.

PREPARATION OF THE COATING

The methodology of Tongdeesoontorn et al., (2011) was followed where a base coating was formulated, to which different concentrations of quinoa saponin were added (15000, 11300, 7500 and 3700 ppm).

For the application of the coating, the oranges were immersed for three minutes in each formulation, dried at room temperature for 20 min, three incisions were made in the equatorial area of the oranges, and 10 µL of the suspension was sown in each incision. which is equivalent to 105 spores/mL according to the Mc Farland scale, they were stored at 25 °C for 2 weeks. Subsequently, the damage index, presence of fungi and firmness were evaluated.

DAMAGE INDEX EVALUATION

The damage index was evaluated using a hedonic scale for each damage taking into account the range from 0 to 4 for the assessment (where 0 = zero damage index and 4 = severe damage index) (López, 2012). The following equations were used:

The damage symptom (SD) was determined using the following equation:

$$Index\ of\ SD = \frac{Damage\ level\ x\ Number\ of\ fruits\ in\ the\ damaged\ level}{\#Total\ fruits\ evaluated} \quad (1)$$

The damage index (DI) was calculated using the following equation:

$$ID = \frac{\Sigma Index\ of\ each\ damage\ symptom}{2} \quad (2)$$

The damages considered are two: firmness and presence of fungi.

The evaluation of the presence of fungi (Penicillium digitatum) in oranges was done based on a hedonic scale. (Figure 1)

RESULTS AND DISCUSSION

EVALUATION OF THE INHIBITORY EFFECT OF DIFFERENT CONCENTRATIONS OF SAPONIN TO INHIBIT THE GROWTH OF PENICILLIUM DIGITATUM

The disk diffusion methodology demonstrated that the fungus: *Penicillium*

Value 0 value 1 value 2 value 3 Value 4

Figure 1: Hedonic scale for the evaluation of the presence of fungi.

Digitatum, both the encoded strain and the isolated strain are resistant to saponin. In the present investigation, it is observed that quinoa powder did not generate an inhibition zone, unlike the antifungal Tiabendazole that was used as a positive inhibition control; which generated inhibition zones of 16.7 mm on average with a concentration of 15,000 ppm. On the other hand, the discs with diosgenin did not generate a halo of inhibition, this being the majority component (5%) of the saponin molecule, which indicates that both substances (quinoa powder and diosgenin) are not controlling agents of Penicillium digitatum.Corzo, (2012) states that, due to the presence of some secondary metabolites such as alkaloids, steroids, and triterpenes, they may be responsible for the non-inhibitory effect. On the other hand, studies carried out by Bader et al., (2000) demonstrated that the antifungal activity of saponins against different strains of Candida albicans can be influenced by the variation of the carbohydrate units linked in the aglycone. Demonstrating that saponin compounds present little or no antifungal activity.

Stuardo & San Martin, (2008) in their research "Antifungal properties of quinoa saponin (Chenopodium quinoa Willd) treated with alkali against Botrytis cinerea" concludes that quinoa extracts not treated with alkali showed minimal activity against the growth of mycelium of B. cinerea. Furthermore, no effects against conidial germination were observed, even at 7 mg saponins/ml. However, when saponin extracts were treated with alkali, mycelial growth and conidia germination were significantly inhibited. At a dose of 5 mg/ml, 100% inhibition of conidial germination was observed, even after 96 h of incubation. The greater antifungal activity of alkaline-treated saponins is probably due to the formation of more hydrophobic saponin derivatives that may have a greater affinity for sterols present in cell membranes. Therefore, comparing this research with that of Stuardo & San Martin, (2008) it was deduced that saponin with its own compounds does not have an inhibitory effect on some families of fungi, such as the case of Penicillium digitatum.

The results shown in this work, inhibitory effect of quinoa saponin (Chenopodium quinoa Willd) on the natural and induced fungal flora of Penicillium digitatum in oranges (Citrus sinensis), do not coincide with the results previously reported in the literature. Tenorio et al., (2010) where they mention that concentrations of saponin isolated from Chenopodium quinoa Willd were evaluated to reduce the growth rate of phytopathogenic fungi by the plate dilution method. It was shown that saponin can inhibit the growth of up to 42% of Aspergillus flavus, 35% of Ulocladium spp, and 47% of Fusarium at the initial four days of the experiment. Tenorio et al., (2010) concludes that saponins

can be considered as controlling agents of phytopathogenic fungi.

On the other hand, Gómez et al., (2009) studied the antifungal activity of steroidal saponins from Discorea against phytopathogenic fungi. The antifungal activity was evaluated by microdilution in liquid medium, with four fungal strains, three phytopathogens and one saprophyte: Mucor sp., Fusarium sp., Fusarium moniliforme and Trichoderma sp. respectively. There was an antifungal activity of 50 to 200 µg/ml of the two classes of saponin, with the F. moniliforme fungal strain being the most sensitive than the other fungi. According to the same author, the difference in resistance may be related to the presence of specific saponins that allow the fungus to hydrolyze saponins and infect a specific plant species.

It must be noted that these authors did not work with the fungus Penicillium digitatum, which is also considered a phytopathogen. As mentioned by Ochoa et al., (2007) in their research in which phytopathogenic orange fungi (Citrus sinensis L. Osbeck) were isolated and identified that affected the quality of the fruits during their storage, having the fungi A. flavus, F. oxysporum, P. digitatum, P. italicum and P. variabile as the main causal agents of rots and diseases in fruits.

The results obtained in this work are in agreement with the findings of Woldemichael and Wink (2001), in which the total saponin fraction of Chenopodium quinoa Willd showed little antifungal activity against Candida albicans. However, when quinoa saponins were treated with alkali, their antifungal activity against B. cinerea increased significantly. This is probably due to the formation of more hydrophobic saponins that have a higher affinity with the sterols present in fungal cell membranes.

EVALUATION OF THE APPLICATION OF COATINGS WITH SAPONINS

In general, most of the inoculated oranges developed infection after the first week of storage under optimal conditions (oven conditioning). The desired values of the response variables were determined: damage index, firmness to the touch and presence of fungi; It must be noted that this research requires fifteen experimental units (replicas). The results are presented below:

a) Damage index (ID)

In Figure 2, the variation of the ID of the control oranges and oranges treated with the coating during the storage time where the work is carried out is observed with respect to the isolated strain Penicillium digitatum. The symptoms of damage in the oranges, both the control and the evaluated ones, are observed from the third day, in which the oranges show symptoms of loss of firmness and presence of fungi.

On the third day, the ID in the control sample was 4.34; while the oranges coated with the 15,000 ppm saponin treatment presented a damage index of 2.20. On the ninth day, the control sample presented an ID of 13.34, which indicates the loss of commercial quality and affected the useful life of the orange.

On the other hand, the oranges treated with saponin coatings presented a similar trend to the control oranges with respect to deterioration, with the difference that the damage appeared in a lower proportion, giving more emphasis to the oranges treated at 15,000 ppm, which obtained an index damage of 11.49 on the ninth day.

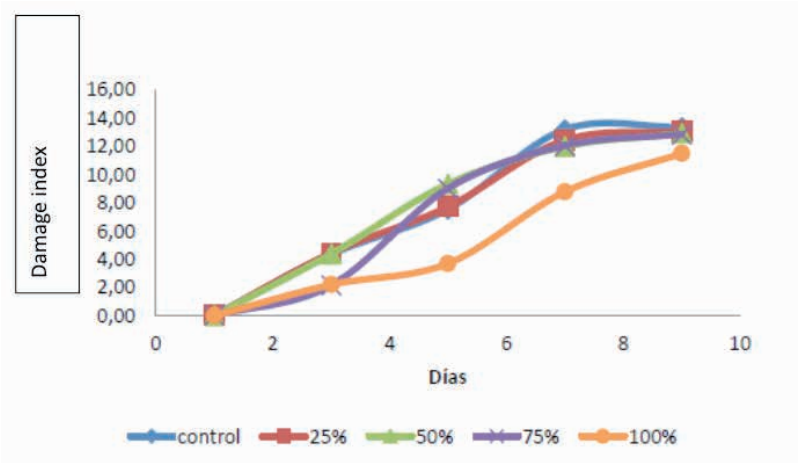

Figure 2: Damage index in control oranges and oranges treated with saponin coatings stored at 25 °C against isolated strain Penicillium digitatum.

However, all these samples present non-commercial characteristics on the ninth day of storage.The results obtained in the quantitative analysis with a significance level of 5% significantly affect the concentration factor, being more predominant between the treatment at 15,000 ppm of saponin compared to the control sample.

In Figure. 3 shows the damage index of control and treated oranges against the coded strain Penicillium digitatum. The control orange that was inoculated with Penicillium digitatum of the coded strain presented an ID of 15.48 on the ninth day of storage, while the orange with treatment at 15,000 ppm obtained an ID of 10.02. The results corresponding to the quantitative analysis indicate that there is a degree of significance at 5% between the treatments at 3700 ppm and 15000 ppm of saponin concentration. It is evident that the control oranges presented an early and greater development of the loss of commercial quality compared to the oranges treated with saponin coating.

b) Firmness

To determine firmness, a texture analyzer was used: a penetrometer with a spherical probe of half an inch in diameter, a deformation of 5 mJ and a load of 10 g at a test speed of 10 mm/s.

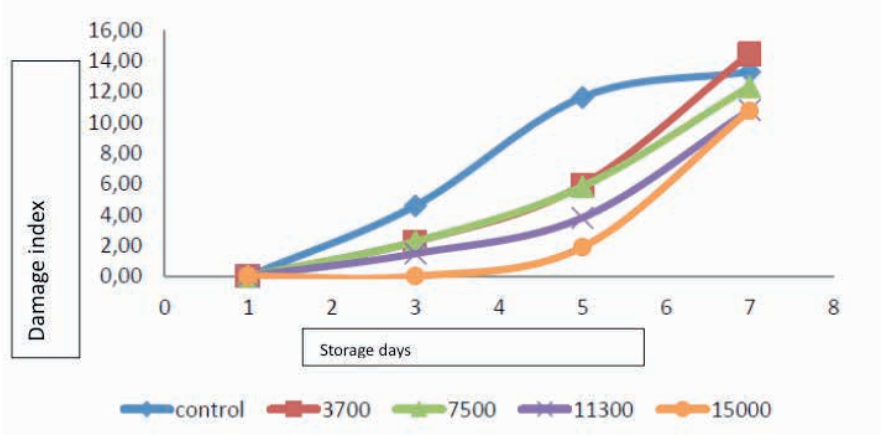

Figure 3: Damage index in control oranges and oranges treated with saponin coatings stored at 25 °C against Penicillium digitatum of coded strain.

The results in Figure 4 and 5 correspond to the quantitative analysis with a significance level of 5%, where the application of saponin coatings did not maintain the firmness of the oranges in any of its concentrations. The control and treated oranges showed a reduction in firmness during storage. For the control oranges on day zero, the average value recorded was 45.31 mJ/g and 25.98 mJ/g on day nine, reaching a percentage of firmness loss of 5.96% at the end of storage. The oranges treated with saponin recorded values similar to those of the control sample with texture values from 45.39 mJ/g to 45.93 mJ/g on average on day zero and from 25.88 mJ/g to 26.40 mJ/g on the ninth day.

The difference between both Figures 4 and 5 show the values obtained from the coatings applied to both the isolated strains (Figure 4) and the coded strain (Figure 5) where both remain relatively close during the storage period, which indicates a percentage 6.6% loss of firmness.

Similar results were observed in mandarins and oranges to which chitosan and shellac-based coatings were applied, which reduced the loss of firmness by 5% after two weeks of storage (Monterde et al, 2003; Cuquerella & Jávega, 2002).

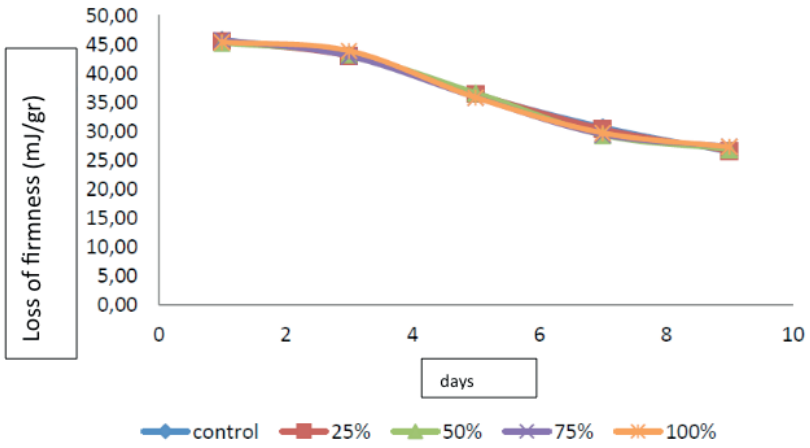

Figure 4: Firmness in control oranges and treated with saponin coatings stored at 25 °C against isolated strain Penicillium digitatum.

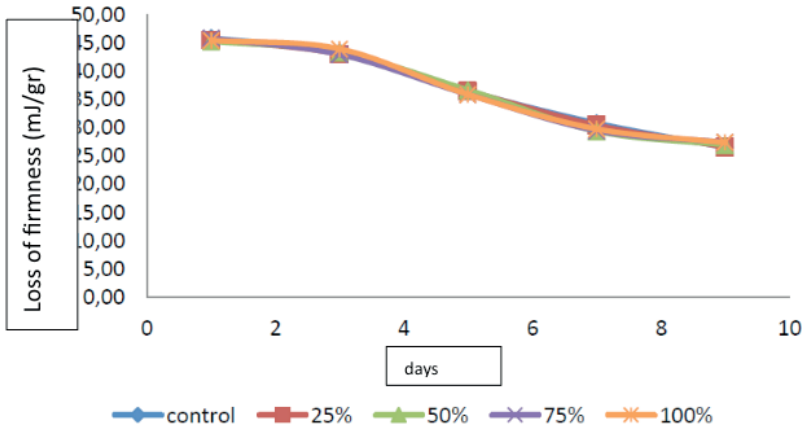

Figure 5: Firmness in control oranges and oranges treated with saponin coatings stored at 25 °C against Penicillium digitatum of coded strain.

c) Presence of fungi

In general, most of the oranges inoculated with the fungus developed the infection after the first week of storage in ambient conditions, because the temperature and humidity were beneficial for the growth of the fungus, in addition, the oranges used as raw material had a degree of commercial maturity which enhances the development of fruit infection.

Finally, it was determined that the coatings used to reduce the development of Penicillium digitatum from the strain isolated and coded in oranges do not present a significant difference (95% confidence level) between any of the treatments and the control sample.

In Figure 6, the increase in growth of the isolated strains of Penicillium digitatum with respect to time is observed.

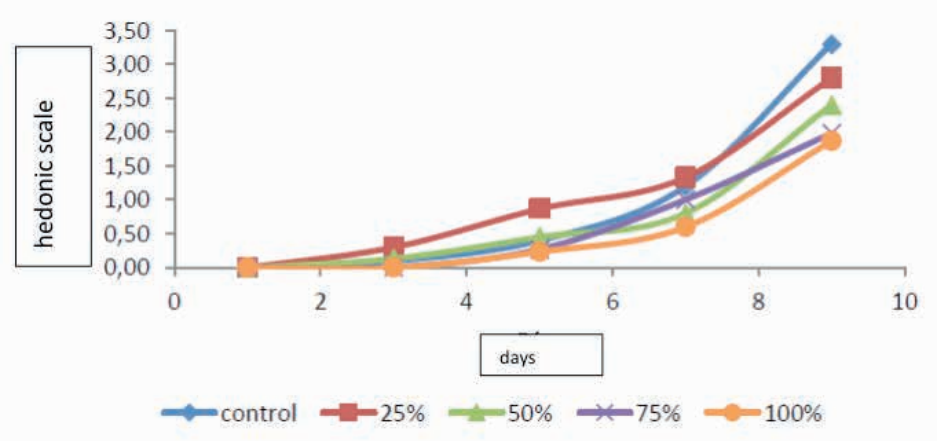

Figure 6: Development of Penicillium digitatum strain isolated in control oranges treated with saponin coatings stored at 25 °C.

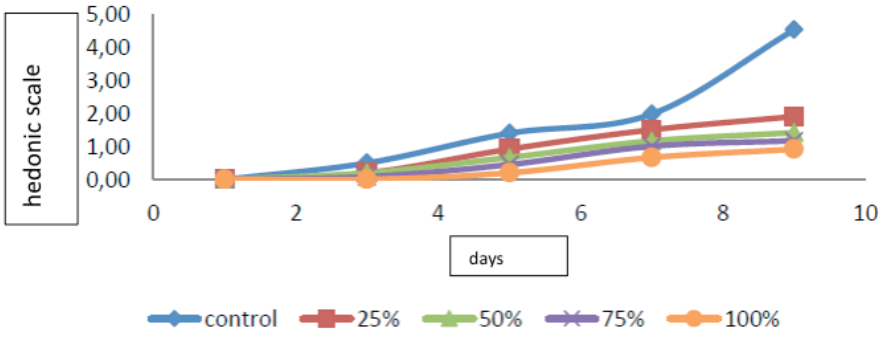

Figure 7: Development of Penicillium digitatum strain coded in control oranges treated with saponin coatings stored at 25 °C.

Likewise, Figure 7 shows the increase in rot of Penicillium digitatum of the coded strain with respect to time.According to the analysis carried out on oranges treated at different concentrations of saponin, it was found that the application of the different coatings did not generate a delay in the appearance of the infection compared to the control samples; all of them began to show signs of fungal deterioration after four days. storage

None of these significantly reduces the development of Penicillium digitatum, both isolated and coded strains, as seen in Figures 6 and 7 with respect to control oranges. This result agrees with those obtained by Valencia et al. (2008) where no significant differences were observed in the growth of the fungus.

Brito et al., 2012 states that the application of coatings based on chitosan and lemon essential oil in the control of Penicillium italicum of oranges shows little effectiveness in the control of rot caused by this microorganism.

CONCLUSIONS

The coatings made from saponin did not show inhibitory capacity. The orange control samples and the samples with coatings showed significantly similar deterioration values under ambient storage conditions. On the ninth day of storage, the loss of firmness was 6.6% in the coated samples and the control samples presented 5.96% loss of firmness. Finally, with these results we can deduce that there is no difference in the degree of infection coming from an isolated or coded strain of the fungus under study since the damage index was 11.49 and 10.02 respectively, therefore, it is concluded that saponin of quinoa is not an inhibitor of the isolated strain Penicillium digitatum fungus as well as a coded strain.

Quinoa saponin slows the growth or mycelial appearance of the fungus Penicillium digitatum, influencing the delay phase of the fungal growth curve, showing a delay of five days with respect to the appearance of young fungi and a reduction in microbial load.

REFERENCES

Bader, G., Seibold, M., Tintelnot, K., & Hiller, K. (2000). Cytotoxicity of triterpenoid saponins – part 2: relationships between the structures of glycosides of polygalacic acid and their activities against pathogenic Candida species. *Die Pharmazie.* 55, 72 – 74.

Brito, A., Sanchez, L., Gonzalez, Ch., Vargas, M., & Chafer, M. (2012). Aplicación de recubrimientos a base de quitosano y aceite esencial de limón en el control de la poscosecha de la podredumbre azul de naranjas. Tesis de grado para optar el grado de master en ciencia e ingeniería de los alimentos. Universidad Politécnica de Valencia.

CLSI, (2008). Clinical and Laboratory Standards Institute. Reference method for broth dilution antifungal susceptibility testing of filamentous fungi; Approved standard. M38 – A2. 28(16), 1 - 2, 34 – 35.

Corzo, D.C. (2012). Evaluación de la actividad antimicrobiana del extracto etanólico. *Revista Mexicana de Ciencias Farmacéuticas.* 43(3), 81 - 86.

Cuquerella, J. & Jávega, J. (2002). Efectos fisiológicos de diferentes recubrimientos sobre frutas cítricas durante su almacenamiento. *Citriculture*. 2, 735-737.

Gómez, J.A., Alba, J., Cerda, C.M., & Ramos, A.C. (2009). Actividad antifúngica de saponinas esteroidales de Dioscorea contra hongos fitopatógenos. Centro de investigación y estudios avanzados. Universidad Nacional Autónoma de México.

ICMSF. (2006). Microorganisms in foods – microbial Ecology of Food Commodities. Blackie Academic & Professional. Editorial Acribia. Zaragoza – España.

Koziol, M.J. (1992). Chemical Composition and nutritional evaluation of quinoa (*Chenopodium quinoa* Willd). *Food composition and analysis*, 5, 35-68.

León, J. (2003). Hibridación y comparación de la F1 con sus progenitores en tres cultivares de quinua (*Chenopodium quinoa* Willd.) en Puno, Perú. Tesis de grado para optar el título de Ingeniero Agrónomo. Universidad nacional del Altiplano. Perú.

Lescano, J. M. (1994). Genética y mejoramiento de cultivo alto andinos quinua, kañihua, tarwi, kiwicha, papa amarga, olluco, mashua y oca. INADE - PELT –COTESU. Puno – Perú.

López, J. (2012). Aplicación de recubrimientos comestibles en carambola (Averrhoa carambola L.). Tesis de Grado para optar el Título profesional de Ingeniero de Alimentos. Universidad Tecnológica Equinoccial. Ecuador.

Mathews, K.R. (2008). Microbiología de las frutas y verduras frescas. Editorial Acribia. España.

Mondino, P. (2012). Métodos de aislamiento: Aislamiento de hongos y bacterias. *Fitopatología*. 8, 15 – 22.

Monje, C. Y., & Raffaillac J. P. (2009). Determinación de saponina total en quinua (*Chenopodium quinoa* Willd) método Espectrofotométrico. Memoria IV Congreso Nacional de la Asociación Boliviana de Protección Vegetal. - Dpto. Fitotecnia-FCAPV UTO. ABPV. 217-218.

Monterde, A., Salvador, A., Ben-Abda, J., & Jávega, J. (2003). Efecto de la aplicación de recubrimientos de origen natural en la calidad de mandarinas y naranjas en maduración y post-recolección de frutos y hortalizas. *Levante Agrícola*. 365, 203-208.

Mujica, A. (2006). Agroindustria de la quinua (*Chenopodium quinoa* Willd) en los países andino. Proyecto quinua cultivo multipropósito para los países andinos.

Mujica, A., Jacobsen, S.E., Izquierdo, J., & Marathee, J.P. (2001). Quinua (*Chenopodium quinoa* Willd.) Ancestral cultivo andino, alimento del presente y futuro. Cultivos Andinos – FAO.

Ochoa, J.L., Hernández, L.G, Latisnere, H., León, J.L., & Larralde, C.P. (2007). Aislamiento e identificación de hongos patógenos de naranja (*Citrus sinensis L. Osbeck*) cultivada en baja California sur, México. *Revista de Ciencia y Tecnología Alimentaria*, 5(5), 352-359

Osbourn, A. (1996). Saponins and plant defense: a soap story. *Trends in Plant Science.* 1(1), 4-9.

Stuardo, M., & San Martin, R. (2008). Antifungal properties of quinoa (*Chenopodium quinoa* Willd) alkali treated saponins against *Botrytis cinérea. Industrial Crops and Products*, 27, 296-302.

Tenorio. R., Terrazas. E., Álvarez, M.T., Vila, J.L., & Mollinedo, P. (2010). Concentrados de saponina de *Chenopodium quinoa* y de *Caiphora andina*: alternativas como biocontroladores de hongos fitopatógenos. *Revista Boliviana de Química,* 27, 33-40.

Tongdeesoontorn, W., Mauer, L.J., Wongruong, S., Sriburi, P., & Rachtanapun, P. (2011). Effect of carboxymethyl cellulose concentration on physical properties of biodegradable cassava starch-based films. *Chemistry Central*. 5(1), 6.

Valencia, C.S., Pérez, M.B., & Palou. L. (2008). Efecto del recubrimiento con quitosano en el control de las podredumbres verde y azul de los cítricos. IX Simposio Nacional y VI Ibérico sobre Maduración y Postcosecha 2008.

Viuda, M., Ruiz, Y., Fernandez, J., & Perez, J. (2008). Antifungal activity of lemon (*Citrus lemon* L.), mandarin (*Citrus reticulata* L.), grapefruit (*Citrus paradisi* L. and orange (*Citrus sinensis* L.) essential oils. *Food control*. 19, 1130–1138.