

SPROUTING AND BIOACTIVE COMPOUNDS OF THREE OCA (*Oxalis tuberosa*) VARIETIES DURING POSTHARVEST STORAGE

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ABSTRACT

During postharvest storage oca tubers can pass for different stages, in which bioactive compounds can change their content; and specifically the sprouting stage, can alter significantly these components. Therefore, this study evaluated the sprout length, loss weight, reducing sugar content, total phenolics content, and antioxidant capacity of three oca tuber varieties (yellow, purple, and orange) during postharvest storage. Oca tuber varieties were stored at 19 °C ±1 °C and relative humidity of 85% ±1%. A factorial design was used to evaluate the effect of oca variety and postharvest storage time (0, 15, 30, 45, 60, 75, and 90 days). The sprout length of the three oca varieties begin to growth at 15-30 days of postharvest storage, which indicated that dormancy period was broken, moreover, the sprout length was increased until 90 days of storage. Yellow oca had the longest sprout length (26.4 cm) and the highest loss weight (22.55 %) at 90 days of storage, which indicated an effect of oca variety and postharvest storage time (p value ≤ 0.05). The reducing sugar content, total phenolics content, and antioxidant capacity of the three oca varieties exhibited variable behaviors during storage time and initially oca tubers showed a reduction in their values, which were presumably related to the stabilization period. The postharvest storage time and oca variety produced different physiological changes in the oca tubers, which affect the sprout length, weight loss, reducing sugars, total phenolics content, and antioxidant capacity.

Additional Keywords: Antioxidant capacity, reducing sugar content, sprout length, total phenol content

RESUMEN

Brotamiento y compuestos bioactivos de tres variedades de oca (*Oxalis tuberosa*) en almacenamiento postcosecha

Durante el almacenamiento postcosecha los tubérculos de oca pueden pasar por diferentes etapas, en las cuales los compuestos bioactivos pueden cambiar su contenido y específicamente la etapa de brotación puede alterar significativamente estos componentes. Por lo tanto, este estudio evaluó la longitud de los brotes, la pérdida de peso, el contenido de azúcares reductores, el contenido de fenólicos totales y la capacidad antioxidante de tres variedades de tubérculos de oca (amarilla, morada y naranja) durante el almacenamiento poscosecha. Las variedades de tubérculo de oca se almacenaron a 19 °C ±1 °C y una humedad relativa de 85% ±1%. Se utilizó un diseño factorial para evaluar el efecto de la variedad de oca y el tiempo de almacenamiento poscosecha (0, 15, 30, 45, 60, 75 y 90 días). La longitud de los brotes de las tres variedades de oca comenzó a crecer a los 15-30 días de almacenamiento poscosecha, lo que indicó que se rompió el período de latencia, además, la longitud de los brotes se incrementó hasta los 90 días de almacenamiento. La oca amarilla tuvo la longitud de brote más larga (26,4 cm) y la mayor pérdida de peso (22,55 %) a los 90 días de almacenamiento, lo que indicó un efecto de la variedad de oca y el tiempo de almacenamiento poscosecha (p valor ≤ 0,05). El contenido de azúcares reductores, el contenido de fenólicos totales y la capacidad antioxidante de las tres variedades de oca exhibieron comportamientos variables durante el tiempo de almacenamiento e inicialmente los tubérculos de oca mostraron una reducción en sus valores, los cuales presumiblemente estuvieron relacionados con el período de estabilización. El tiempo de almacenamiento poscosecha y la variedad de oca produjeron diferentes cambios fisiológicos en los tubérculos de oca, que afectan la longitud de los brotes, la pérdida de peso, los azúcares reductores, el contenido de fenólicos totales y la capacidad antioxidante.

Palabras clave adicionales: Capacidad antioxidante, contenido de azúcares reductores, contenido de fenoles totales, longitud del brote

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INTRODUCTION

The trend of food consumption is oriented towards to the consume fresh or minimally

processed foods, which present adequate nutritional and functional properties to optimize the life quality of consumers. This kind of foods presented bioactive compounds such as

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antioxidants, enzymes, minerals, pigments, vitamins, etc., which can prevent the damaging effects of free radicals on tissues and cells. The benefits of antioxidants in human health have been evidenced, such as improving eyesight, preventing heart disease, and anticancer effects (Coronado *et al.*, 2015).

Currently, it is important to find new sources of natural bioactive compounds such as antioxidants because these can help to prevent some diseases. Therefore, conducting studies to identify new sources of antioxidant compounds should be done. In the last years, some studies to identify and determine bioactive compounds of Andean tubers were done. These Andean tubers as potato (*Solanum tuberosum*), mashua (*Tropaeolum tuberosum*), olluco (*Ullucus tuberosum*), and oca (*Oxalis tuberosa*) of different varieties presented bioactive compounds as carotenoids, anthocyanins, phenolic acids, and flavonoids, which are potential antioxidant to prevent some disease (Campos *et al.*, 2006; Campos *et al.*, 2018). Within these tubers, oca tubers are consumed in fresh or processed by the communities that cultivate it, however do not reported industrial use of these tubers, moreover, it has been reported a variable content of secondary bioactive metabolites in these tubers, which could be influenced by the harvest stage and environmental factors (Barrera *et al.*, 2004).

During postharvest storage, tubers as potato (*S. tuberosum*), mashua (*T. tuberosum*), and olluco (*U. tuberosum*) showed different storage period. The first period has relation with tuber stabilization, where the tuber has to adequate their physiology to function without nutrient supply by the plant. The second period include to the first period and has relation with the dormancy period, in which the intensity respiration of the tuber is constant and loss weight is constant too (do not appear sprouts). The third period has relation with the breaking of dormancy period, in which tuber sprouting begin. It can be observed an increase in loss weight and reducing sugar content and a variable content of some bioactive compounds during postharvest storage time (Kays *et al.*, 1979; Lill *et al.*, 1989; Aliaga *et al.*, 2011; Külen *et al.*, 2013; Velásquez *et al.*, 2013; Gonzales *et al.*, 2020). But, a few studies of changes in physicochemical characteristics and bioactive compounds in other tubers as oca has been studied.

Some communities from South America Andean highlands that cultivate oca tubers have some problems with the management of postharvest storage due to the lack knowledge of changes in physicochemical characteristics and bioactive compounds that occur during stabilization period, dormancy period and during sprouting of oca tubers. For that, it is important to evaluate the change in these compounds during postharvest storage time and recommend the storage period, in which the oca tubers maintain their quality characteristics. Therefore, the purpose of this study was evaluated the effect of postharvest storage time and oca variety (yellow, purple, and orange) on sprout length, loss weight, reducing sugar content, total phenolics content, and antioxidant capacity.

MATERIALS AND METHODS

Samples and experimental design. Tubers of three varieties of oca (purple, yellow and orange) were collected after harvest (eight months) from the community of Condorpuyana (6°30'39.1" S, altitude 3019 m a.s.l.), district and province of Chota (Perú). Tubers with the same size without damages were selected, then were cleaned with water and dried at room temperature (19 ± 1 °C). Subsequently, 2 kg (33 units of tuber with average weight per tuber of 60 g) of each variety was weighed (six replicates for each variety) and placed in 15 x 22 x 10 cm cardboard boxes. The tubers were stored in darkness at an average temperature of 19 ± 1 °C and a relative humidity from 80 ± 1 %. Sprout Length, loss weight, reducing sugars content, total phenolics content, and antioxidant capacity of oca tuber varieties were measurement at 0, 15, 30, 45, 60, 75 and 90 days. A factorial design of two factors was used to determine the significant effect of the factors, where a first factor was oca variety with three levels (purple, yellow and orange), the second factor was the postharvest storage time with seven levels (0, 15, 30, 45, 60, 75, and 90 days). The measurement was done in triplicate.

Determination of sprout length and weight loss. The sprout length was determined according to the methodology detailed by Velásquez *et al.* (2013). Briefly, sprout length was measurement with a vernier caliper beginning in the meristems of until to the final part of the sprout. Loss weight

was determined in percentage from the difference in mass among the initial and final weight of the tuber.

Determination of reduced sugar content.

The procedures proposed by Miller (1959) and Silva *et al.* (2003) with certain modifications were employed. Oca tubers were placed in a mortar and were crushed and ground by pestle, then 10 g of pulp was placed in a volumetric flask (100 mL) and distilled water was added until to complete the flask volume. 1 mL of this solution was mixed with 2.0 mL of dinitro salicylic acid (DNS) and 1.2 mL of distilled water in a test tube. A blank was prepared with 2.2 mL of distilled water and 2.0 mL of DNS. The absorbance of the solutions was recorded using a spectrophotometer UV/VIS (Pg Instruments, T80+, United Kingdom), at 540 nm. For quantification, a glucose calibration curve in concentrations from 20 to 100 mg/mL was used.

Determination of total phenolics content.

The Folin-Ciocalteu method was used to determine the total phenolics content of oca tubers with some modifications (Hidayat *et al.*, 2017; Singleton *et al.*, 1999). The sample preparation and dilution were the same of reducing sugars determination, except that instead distiller water, a methanol solution at 70% was added to complete the level of volumetric flask. 100 μ L of diluted oca tuber extracts were placed in a test tube (20 mL) and 250 μ L of Folin-Ciocalteu reagent was added, then the solution was left stand for 6 min. Subsequently, 750 μ L sodium carbonate solution (6%) and 3.9 mL of distilled water were added. The mixture was vortexed for 10 s and left to stand for 30 min in the dark at room temperature. The absorbance of the solution was measured at 765 nm using a spectrophotometer UV/VIS (Pg Instruments, T80+, United Kingdom). Quantification of total phenolics content was performed using a gallic acid calibration curve from 20 μ g·mL⁻¹ to 500 μ g·mL⁻¹. The analyses were performed in triplicate and expressed as mg of gallic acid/g of dry matter.

Determination of antioxidant capacity.

It was carried out following the methodology proposed by Brand *et al.* (1995) and Gonzales *et al.* (2020). The preparation of the oca tubers sample and dilution were the same of total phenolics content. For this, 200 μ L of diluted extract were placed in a 10 mL tube and 3.8 mL of 2,2-diphenyl-1-picrilhidrazalo (DPPH) was added.

The solution was left to stand in the dark for 30 min, and then the absorbance was read and recorded at 515 nm using a spectrophotometer UV/VIS (Pg Instruments, T80+, United Kingdom). To determine the antioxidant capacity, a calibration curve prepared with a 0.2 mM standard solution of Trolox (PM=250.29 g·mol⁻¹) was used, and solutions of 0.02 to 0.2 mM were obtained. Absorbance of solutions were read and recorded at 515 nm. The analyses were performed in triplicate and expressed as μ mol Trolox Equivalent (TE)/g of dry matter.

Statistical analysis. Variance analysis of data was performed using a significance level of 0.05. Tukey's test was performed to compare means among treatments. To determine both analyses Minitab 17.0 software was used.

RESULTS AND DISCUSSIONS

Sprout length. Figure 1 shows the sprout length of the purple, yellow, and orange oca tubers, during the first 15-30 storage days presented a dormancy period, but at 30 days the sprout length increased in the three oca varieties during the storage time (Figs. 1 and 2). However, the tendency to increase the size of the sprouts was greater in yellow and orange oca tubers. Yellow oca tubers had the largest sprout size (26.4 cm) at 90 days of storage, followed by orange oca (19.8 cm) and the purple oca tubers (4.3 cm). These results indicate that oca varieties have influenced the sprout length (Figure 1, p value \leq 0.05), since oca tubers of different varieties stored under the same conditions presented a variability in the sprout length.

Studies on postharvest storage of olluco, potato, and mashua tubers of different varieties reported an increase in sprout length during postharvest storage time (Aliaga *et al.*, 2011; Velásquez *et al.*, 2013; Gonzales *et al.*, 2020). This behavior was observed in our study with oca tubers. To clarify and understand the differences in the behavior of sprout development of different tubers and varieties, studies should be done. Moreover, sprout growth behavior of the tuber has relation with the genetic and environmental conditions, which affect the breaking tuber dormancy period and indicate the initiation of different physiological activities (Sonnewald and Sonnewald, 2014).

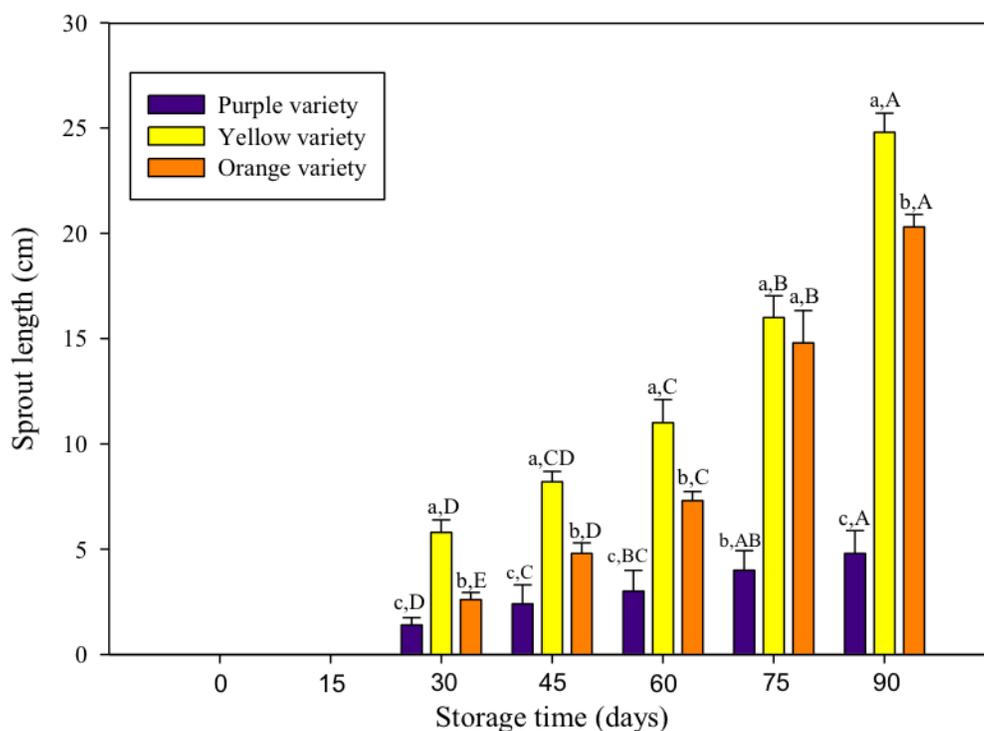


Figure 1. Influence of postharvest storage time and oca (*O. tuberosa*) variety on the sprout length of oca tubers. Different lowercase letters between oca varieties at the same storage time indicate a significant difference ($p \leq 0.05$) in sprout length. Different capital letters for the same oca variety through storage time indicate a significant difference ($p \leq 0.05$) in sprout length

Weight loss. The weight loss of the purple, yellow, and orange oca tubers (Figure 3) increased through the postharvest storage time. Yellow oca tubers exhibited the highest weight loss (22.55 %) at 90 d of storage, followed by orange oca (16.33 %), and purple oca tubers (14.23 %). These differences in weight loss during storage show that oca varieties have a significant effect ($p \leq 0.05$) on this variable. The same behavior was observed in sprout length (Figure 1) and indicates that the breaking in dormancy period affect the weight loss during storage of oca tubers. Postharvest storage studies of other tubers, such as mashua, olluco, and potato, showed that weight loss depends on the tuber type, storage conditions, and tuber variety (Aliaga *et al.*, 2011; Velásquez *et al.*, 2013; Gonzales *et al.*, 2020). Likewise, it has been reported that loss weight occurs because the tubers release free water to the surface due to the vapor pressure deficit (García *et al.*, 2014). This weight loss is uncontrollable and has relation with the respiration intensity and tuber sprouting (Velásquez *et al.*, 2013). In addition, the weight

loss of oca tubers depends on the synthesis and degradation of starch (reserve polymer of tubers), which provides the necessary energy to carry out metabolic activities (Tofiño *et al.*, 2006).

Reducing sugar content. Figure 4 shows the reducing sugars content of oca tuber varieties during the postharvest storage time. After 15 days of storage, a slight increase in the reducing sugar content was observed in the three oca varieties and after 30 days of storage, the reducing sugar content decreased slightly. This behavior indicates that after harvest, oca tubers need a stabilization period (first few weeks) because tubers were separated from the plant and need a stabilize period to adequate physiological changes that occurred without nutrients provided from the plant (Wencomo *et al.*, 2017). At 45 days of storage, oca tubers of the three varieties slightly increased the reducing sugar content and almost kept constant this content until 90 days of storage. This increases in reducing sugar content after 45 days of storage is related to the increase the respiration intensity and it has relation with the loss weight of

oca tubers, because at 45 days of storage was observed that the loss weight presented a more pronounced increase (Figure 4). During the sprouting process, the reserve starch is hydrolyzed and converted into simple sugars, which increases the reducing sugar content (Matsuura *et al.*, 2004).

Therefore, the increase in the sprout length of oca tubers at prolonged storage times (Figures 1 and 2) may affect the physiological processes that govern starch hydrolysis and the reducing sugars production.



Figure 2. Growth of the sprout of the three oca (*O. tuberosa*) varieties during postharvest storage time. The numbers 0, 15, 30, 45, 60, 75 and 90 are the days of storage. For each storage time, the variety sequence from left to right is purple oca, orange oca, and yellow oca

Oca tubers of the purple and orange varieties had a higher content of reducing sugars initially and during postharvest storage time. Differences in reducing sugar content of tubers are due to cultural practice, nutrients provided, genetic, and harvest conditions, similar contents of reducing sugars have been reported in tubers (Morales *et al.*, 2018). According to the data obtained, it was observed that oca variety and the postharvest storage time had an effect on the content of

reducing sugars ($p \leq 0.05$) maybe because oca tubers are affected by stress caused by environmental factors (Morales *et al.*, 2018)

Total phenolics content. The total phenolics content of three oca varieties showed a different behavior during postharvest storage time (Figure 5). The content of phenolic compounds decreased after 15 days of storage in the three oca varieties, however, after 30 days this content of phenolic compounds began to increase, reaching the

maximum content at 45 days of storage (Figure 5), and at 60 days of storage, the total phenolics content decreased abruptly and finally this content increased slightly. Reduction in total phenolics content at 30 days of storage is related to the stabilization period (first weeks) when tubers were separated from the plant and need to adequate their physiology system to continue living without nutrients supply by the plant (similar behavior was observed by reducing sugar content). On the other hand, the increase in the total phenolics content of oca tubers after 30 days of storage coincides with the beginning of the sprouting of tubers (breaking dormancy period), which indicates that

physiological changes of oca tubers alter the total phenolics content. Physiological activity has relation with the biosynthesis of phenolic compounds and depends the presence and activity of enzymes as polyphenoloxidase or peroxidase (Balois *et al.*, 2008). Therefore, differences in the concentration and activity of the enzymes during the postharvest storage time could explain the changes in the total phenolics content. Similar results in the behavior of content of phenolic compounds during the postharvest storage time were reported in potato tubers stored at 15 °C (Yamdeu *et al.*, 2017).

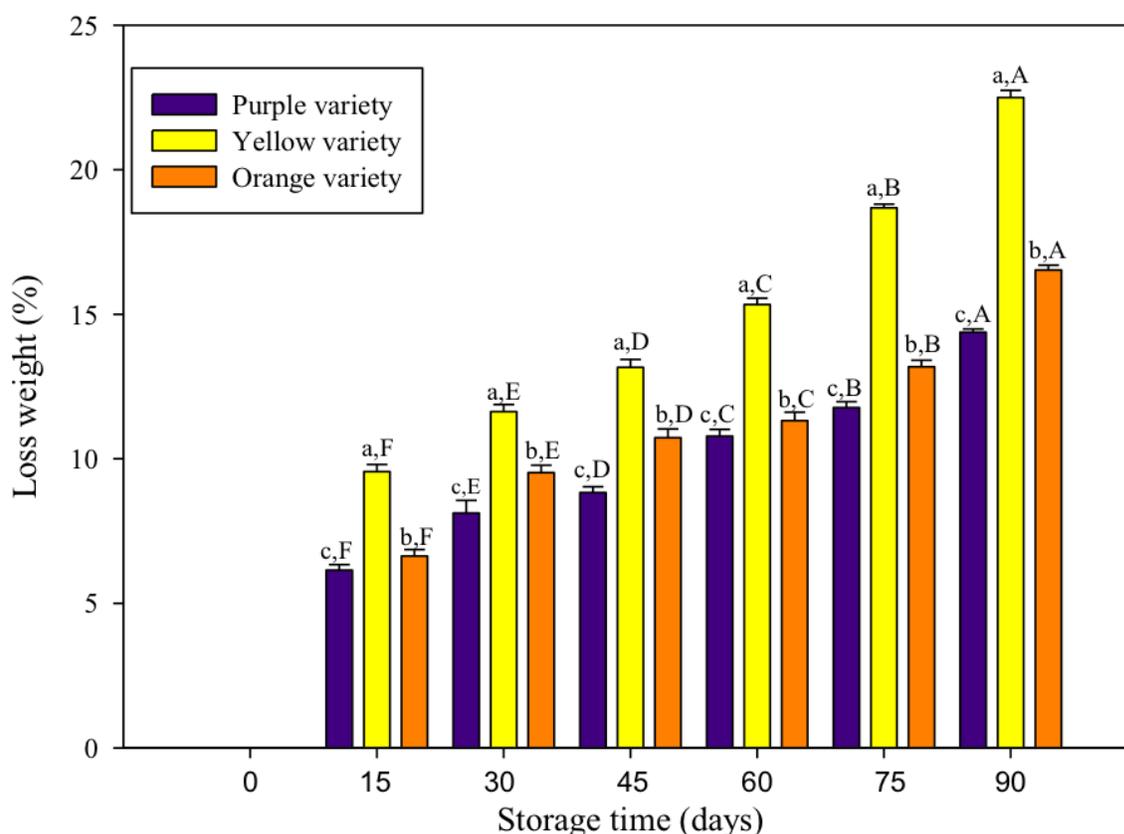


Figure 3. Influence of postharvest storage time and oca (*O. tuberosa*) variety in the loss weight of oca tubers. Different lowercase letters between oca varieties at the same postharvest storage time indicate significant difference ($p \leq 0.05$) in loss weight. Different capital letters for the same oca variety through postharvest storage time indicate significant difference ($p \leq 0.05$) in loss weight

Orange and purple oca tubers had the highest total phenolics content during the postharvest storage time. This behavior indicates an effect of oca variety and the postharvest storage time on the total phenolics content ($p \leq 0.05$; Figure 5). The

differences in the varieties may be due to the effects of the synthesis and degradation of phenolic compounds. Postharvest storage studies of various genotypes of potato tubers during storage time showed that the total phenolics

content increases or decreases depending on the potato genotype. Likewise, these changes in the total phenolics content are attributed to the presence and activity of the enzyme superoxide dismutase and the enzyme ascorbate peroxidase (Kulen *et al.*, 2013; Yamdeu *et al.*, 2017), which

may be different in oca tuber varieties during postharvest storage time. Future study could be carried out in order to determine which specific phenolic compounds could change their concentration in oca tuber varieties during postharvest storage

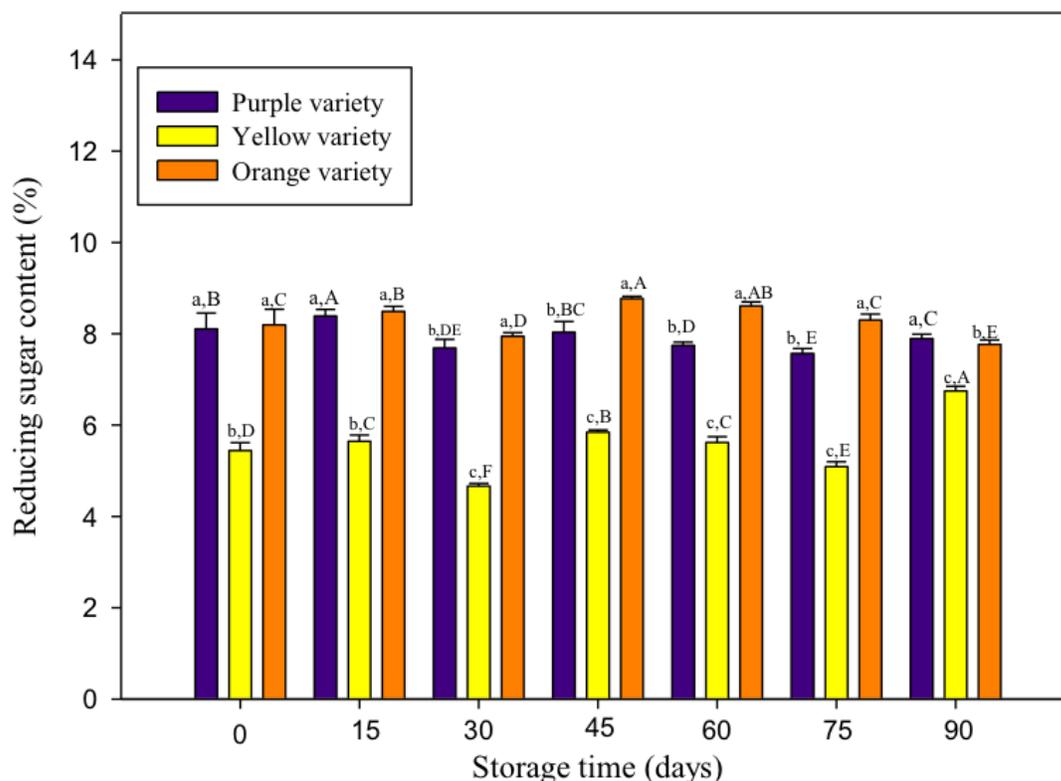


Figure 4. Influence of postharvest storage time and oca (*O. tuberosa*) variety on the reducing sugar content of oca tubers. Different lowercase letters between oca varieties at the same postharvest storage time indicate significant difference ($p \leq 0.05$) in the reducing sugars content. Different capital letters for the same oca variety through postharvest storage time indicate significant difference ($p \leq 0.05$) in the reducing sugars content

Antioxidant capacity. The antioxidant capacity content decreased after 15 days of postharvest storage time in three oca tuber varieties, however, at 60 days the antioxidant capacity in purple and orange oca tubers began to increase (Figure 6), on the contrary, the yellow oca showed low antioxidant capacity, at 75 days of storage. Three oca tuber varieties tended to reduce their antioxidant capacity compared to the initial values, at 90 days of storage time the antioxidant capacity of yellow oca tuber increased slightly (Figure 6). This change in values (increasing and decreasing) was observed for

sprout length, loss weight, and total phenolics content, but the behavior was different for each variable, maybe it has relationship with the stabilization period (first weeks), in which each variable had different behavior and depending of physiological changes. Moreover, during the first 30 days before the sprouting begin, the tendency of the antioxidant capacity and the total phenolics content were different, maybe because during stabilization period, in which different antioxidants were synthesized and increased the antioxidant capacity. In addition, soluble vitamins can increase the antioxidant capacity of stored

products (Gonzales *et al.*, 2020). However, a high correlation between the antioxidant capacity and total phenolic content has been found in potato, mashua, and fruits during postharvest storage

(Chirinos *et al.*, 2007; Chirinos *et al.*, 2008; Madiwale *et al.*, 2011), but in our study the correlation was lower.

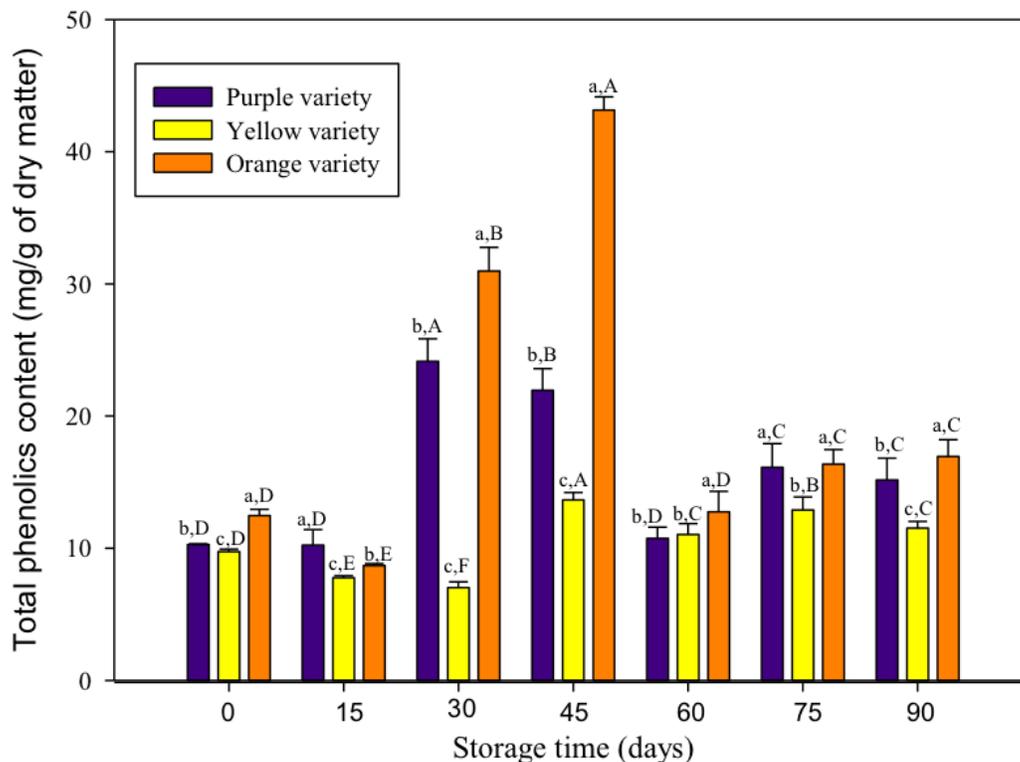


Figure 5. Influence of postharvest storage time and oca (*O. tuberosa*) variety on the total phenolics content of oca tubers. Different lowercase letters between oca varieties at the same storage time indicate significant difference ($p \leq 0.05$) in the total phenolics content. Different capital letters for the same oca variety through storage time indicate a significant difference ($p \leq 0.05$) in total phenolics content.

In general, orange and yellow oca tubers presented the highest content of antioxidant capacity during the postharvest storage time than purple oca tubers. This indicates an effect of variety and the postharvest storage time on the antioxidant capacity ($p \leq 0.05$; Figure 6). Differences in the antioxidant capacity among oca tuber varieties maybe is due to effects of the synthesis and degradation of antioxidants. Studies on postharvest storage of oca tubers of different varieties have reported a variability of antioxidant capacity content during storage time (Gonzales *et al.*, 2020).

Chirinos *et al.*, (2008) pointed out that the

levels of these compounds can vary widely within the same plant species, and even between cultivars, due to genetic and environmental factors that determine germination, growth, development, and crop quality. On the other hand, stress of tuber caused by environmental factor can produce oxidative stress, which can show changes in the quantity of antioxidant compounds and composition, resulting in changes in antioxidant capacity (Liu *et al.*, 2017). Therefore, future studies could be carried out in order to determine which specific antioxidant increase or decrease and affect the level of antioxidant capacity in oca tuber varieties during postharvest storage.

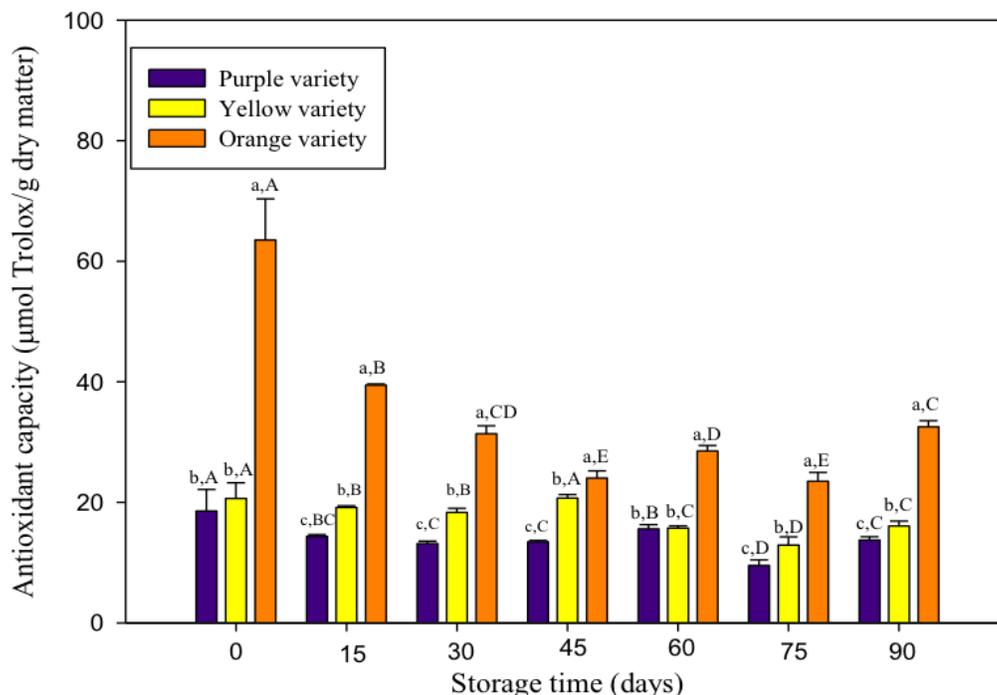


Figure 6. Influence of postharvest storage time and oca (*O. tuberosa*) variety on the antioxidant capacity of oca tubers. Different lowercase letters between oca varieties at the same storage time indicate significant difference ($p \leq 0.05$) in antioxidant capacity. Different capital letters for the same oca variety through storage time indicates significant difference ($p \leq 0.05$) in antioxidant capacity.

This research evaluated the changes that occur during postharvest storage of bioactive compounds (and antioxidant capacity, reducing sugars, and total phenolics), allows us to determine the optimal conditions for their consumption and processing, maximizing their nutritional and functional value. These results benefit producers, food industry, and consumers, by ensuring a higher-quality product with potential applications as functional foods and nutraceuticals.

CONCLUSIONS

The postharvest storage time and oca variety affect the sprout length, weight loss, reducing sugars, total phenolics content, and antioxidant capacity of oca tubers due to different physiological changes. Dormancy period was observed in oca tuber varieties and was broken at 30 days with the growth of sprouts. Period stabilization was observed in oca tuber varieties during the first 15 days, which affected in

different way sprout length, weight loss, reducing sugars, total phenolics content, and antioxidant capacity. After 30 days (dormancy period broken), sprout length and weight loss of oca tubers increased abruptly, which have relation with increase in respiration intensity. Total phenolics content and antioxidant capacity increase and decrease during time for three oca varieties. These findings provide information for optimizing storage time and conservation conditions of oca tubers. Future studies could be carried out in order to determine which specific antioxidant of oca tuber varieties increase or decrease during postharvest storage. Moreover, some inhibitors of sprouting could be applied to understand the effect on oca tuber physiology.

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