

Scales removal to improve the appearance and quality of onion

Remoción de capas para mejorar la apariencia y calidad de la cebolla

Barrales-Heredia SM¹, Grimaldo-Juárez O^{1*}, Suárez-Hernández AM², Soto-Ortiz R¹, González-Mendoza D¹, Avendaño-Reyes L¹

¹ Institute of Agricultural Sciences, Autonomous University of Baja California. Carretera a Delta/Oaxaca s/n, Ejido Nuevo León, Valle de Mexicali, Baja California, México.

² Faculty of Engineering and Business San Quintin, Autonomous University of Baja California. Carretera Transpeninsular Km. 180.2, Ejido Padre Kino, San Quintín, Baja California, México.

ABSTRACT

Removal of scales in onion is a common practice in markets to improve product appearance and quality during marketing. The objective of this study was to evaluate the effect of scales removal (C0=without removal, C1=without one scale and C2=without two scales) on physical-chemical quality of onion exposed to environmental conditions for periods of 0, 10, 20 and 30 days. Twelve treatments were evaluated in a completely randomized design. Results showed that dry matter, firmness and color saturation (C*) are not affected by the interaction of exposure time and removal scales. Variables hue angle, luminosity (L*), soluble solids, titratable acidity and pH, showed significant variation with the interaction of exposure time and scales removal level. Variation levels of treatments did not show a defined pattern with the exception of onion color tonality (hue). The hue value increased in the treatments with a higher scales removal, manifesting shades towards green color with the elimination of two scales.

Keywords: scales removal, onion, physical-chemical quality.

RESUMEN

La remoción de capas en la cebolla es una práctica común en mercados para mejorar la apariencia y calidad del producto durante el mercadeo. El objetivo de la investigación fue evaluar el efecto de la remoción de capas (C0=sin remoción, C1=sin una capa y C2=sin dos capas) sobre la calidad físico-química de la cebolla expuesta a condiciones ambientales por periodos de 0, 10, 20 y 30 días. Se evaluaron 12 tratamientos en un diseño completamente al azar. Los resultados mostraron que las variables materia seca, firmeza y saturación de color (C*) no son afectadas por la interacción de la remoción de capas y tiempo de exposición. Las variables ángulo hue, luminosidad (L*), sólidos solubles, acidez titulable y pH, mostraron variación significativa con la interacción del tiempo de exposición y nivel de remoción de capas. El nivel de variación de los tratamientos no mostró un patrón definido a excepción de la tonalidad de la coloración de la cebolla (hue). El valor de hue se incrementó en los tratamientos con mayor nivel de remoción de capas, manifestándose tonalidades hacia el color verde con la eliminación de dos capas.

Palabras clave: remoción de capas, cebolla, calidad físico-química.

INTRODUCTION

Poor postharvest handling can cause deterioration of vegetables mainly due to bacteria, viruses or harmful parasites (Ramírez *et al.*, 2021), which generate losses that exceed 20% (Castro *et al.*, 2011). In developed countries, losses are between 5% to 30% and occur at retail, foodservice, and consumer sites, while in developing countries the losses are 50% that occur from production to the points of sale (Kader, 2005). Additionally, environmental factors such as temperature, light, air and to a lesser extent the vapor pressure deficit CO₂ enrichment, affect the organoleptic and functional quality of vegetables (Li *et al.*, 2018). In this situation, postharvest treatments are required to maintain quality and extend products shelf life (Kader, 2002).

Onion is a semi-perishable product, which prior to storage requires curing that consists of drying the external scales close to the neck. This dehydration process is carried out in field environmental conditions or it can be artificial with hot air (Cardoso *et al.*, 2016). Onion bulbs are composed of scales arranged according to their chronological age from younger (inner scales) to older (outer scales) (Galsurker *et al.*, 2017). Mature bulb has one to three dry skins that enclose sequential thin external scales, which in turn enclose several swollen internal fleshy scales (Brewster, 2008; Galsurker *et al.*, 2017).

Curing method and storage conditions affect the bulb life span, and consequently the quality (Petropoulos *et al.*, 2017). Storage generates physiological changes that induce postharvest losses and reduce bulb commercial quality (Sohany *et al.*, 2016). Some studies have shown that at temperature storage conditions of 25±3 °C and relative humidity of 75% reduce red onion shelf life, while a temperature between 2.5 and 6 °C has less effect on storage life and postharvest quality (Sohany *et al.*, 2016).

Under this context, biochemical changes during onion deterioration is manifested in content of dry matter, pungency, concentration of abscisic acid and fructans (Ritsema and Smeekens, 2003; Chandrashekar, 2014). Coloration tonality,

*Autor para correspondencia: Onécimo Grimaldo Juárez
 Correo electrónico: onecimo.grimaldo@uabc.edu.mx

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concentration of soluble solids (TSS), titratable acidity and pH, are also partially modified parameters (Cardoso *et al.*, 2016). Further, onion distribution to commercial centers and regional markets after storage, generates latency break and progressive bulbs deterioration (Gubb and MacTavish, 2002; Benkeblia and Shiomi, 2004). Consequently, onion useful life in the markets depends on handling conditions (Priya *et al.*, 2014).

At the point of sale, the onions are exposed without the outer scales for days or weeks. The scales removal practice is used with the purpose of improving appearance and attract buyers (Hasan and Uddin, 2011). The removal of onion scales is carried out to a greater extent (85% a 100% of farmers) when they present symptoms of aging or undesirable aspects due to the incidence of some pathogen, or remove the loose outer skin from their onions before selling (Adnan *et al.*, 2014).

Physical damage to bulbs and exposure to high temperatures increases respiratory rate (Galsurker *et al.*, 2018). This process demands consumption of organic compounds, generating changes in TSS balance, titratable acidity and pH, in addition to other physical changes such as coloration and dry matter (Chandrashekar, 2014). However, there are not enough studies to determine the effect of scales removal in onion bulbs during market exposure. Therefore, the aim of this research was to evaluate the effect of removing one and two scales on quality of onions exposed to market environmental conditions (temperature of 25 ± 5 °C and relative humidity of 60% to 80%) for a period of 30 days.

MATERIALS AND METHODS

The experiment was carried out in the postharvest physiology laboratory of the institute of Agricultural Sciences of the Autonomous University of Baja California, Mexico, in September 2020. White onion cv "Carta Blanca" (*Allium cepa* L.) has provided by company San Gabriel, Valle de Ojos Negros, Baja California, Mexico. The onion cultivar "Carta Blanca" has a short-day photoperiod with a white bulb, round shape and is tolerant to flowering. Bulbs were harvested in July 2020, dried in the field for one week, trimmed of leaves and roots, and subsequently stored for 2 months. During storage, onions were at 20 ± 5 °C with a 60–70% relative humidity (RH). The onions samples for the study were uniform in appearance and size (7.00 ± 0.50 cm of equatorial diameter and 8.00 ± 0.50 cm of polar diameter).

The evaluation of onion quality was carried out in 240 bulbs under a completely randomized design with a 3×4 factorial arrangement. Twelve treatments were evaluated that resulted from the combination of scales removal levels (C0=without removal, C1=without one scale and C2=without two scales) and exposure periods (0, 10, 20 and 30 days). Twenty repetitions per treatment and one bulb per experimental unit were considered. The bulbs were exposed to environmental conditions with an average temperature of 25 ± 5 °C and RH of $70\pm 10\%$.

The variables evaluated were dry matter (%), firmness

(N), color (L^* , C^* y hue), total soluble solids (°Brix), titratable acidity (%) and hydrogen potential (pH). Dry matter was determined by oven drying method (AOAC, 1998), where a sample of 10 g per bulb was taken. Firmness was quantified with a Chatillon DFE-100 digital force gauge (AETEK Inc, USA), with an 8 mm cylindrical strut that was introduced 3 times in the onions at the equatorial part. An X-Rite SP-62 sphere spectrophotometer (X-Rite Inc., USA) was used to determine the color, in the two opposite parts of the equatorial section of each bulb by treatment. The values expressed were L^* (Luminosity) that defines color clarity, C^* (chroma) indicates color saturation and hue angle (°) that indicates angle tone.

Parameters of pH, titratable acidity (TA) and total soluble solids (TSS) were determined using methodologies proposed by AOAC (1975, 1998). For this, 10 g of fresh plant tissue were used and homogenized in a commercial blender (Osterizer brand) with 50 mL of distilled water (pH=7.0). Subsequently, the extract was filtered through a strainer and the pH was recorded with a multiparametric benchtop meter model H12550-01. For titratable acidity determination, a 10 mL aliquot of extract was taken and 2 to 3 drops of phenolphthalein (Merck brand) were added, adding 0.1 N NaOH solution (Merck brand) until achieving neutralization of acids present in the sample, reporting the result in percent citric acid. The TSS concentration was determined in a drop of extract placed on an Abbe Leika Mark II digital refractometer previously calibrated with distilled water.

Data were analyzed using a factorial arrangement under a completely randomized design, which included the scales removal (C0, C1 y C2) and exposure periods (0, 10, 20 and 30 days) factors. The analysis of variance was performed using the MIXED procedure and the REPEATED option of SAS software ver. 9.1 (SAS Inst. Inc., Cary, NC. 2006). The comparison of means was carried out utilizing the Tukey test at $P \leq 0.05$.

RESULTS AND DISCUSSION

Onion postharvest quality with removal of one (C1) and two (C2) scales and without removal (C0) exposed to environmental conditions (25 ± 5 °C and RH $70\pm 10\%$) for a period of 30 days are presented in table 1. These results show that dry matter was not affected ($p < 0.05$) by scale removal level (C0, C1 and C2), exposure time (0, 10, 20 and 30 d), or both factors interaction. Recorded values varied between 8.72% and 11.17% (Figure 1).

In cv. Sunpower onions with high storage capacity there is a slow reduction of dry matter during storage at 22 ± 1 °C and relative humidity of $70\pm 10\%$ (Sharma *et al.*, 2015). These variations in dry matter content in stored onions are considered to be mainly associated with physiological changes, such as latency braking and internal sprouting, rather than to effect of storage time and temperature (Sharma and Lee, 2016). According to Sharma *et al.* (2014), post-storage deterioration of Sunpower onions (high storage capacity variety) begins at four weeks under ambient conditions (20 to 25 °C

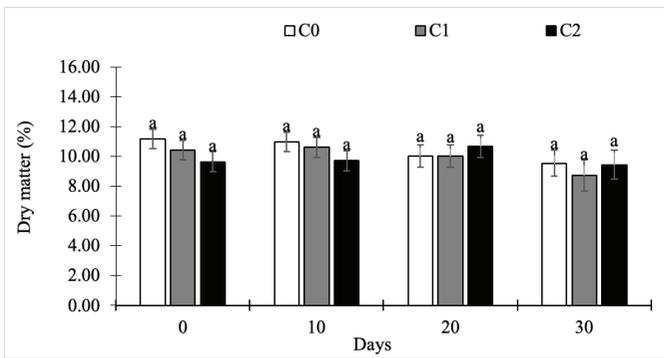


Figure 1. Onion dry matter without removal of scales (C0), without one scale (C1) and without two scales (C2) at 0, 10, 20 and 30 days of exposure to environmental conditions (temperature of 25±5 °C and relative humidity of 70±10%).

Figura 1. Porcentaje de materia seca de cebolla sin remoción de capas (C0), sin una capa (C1) y sin dos capas (C2) durante 0, 10, 20 y 30 días de exposición a condiciones ambientales (temperatura de 25±5 °C y humedad relativa de 70±10%).

Table 1. Analysis of variance of onion quality parameters at different degrees of scales removal and 30 days of exposure to environmental conditions (temperature of 25±5 °C and relative humidity of 70±10%).

Tabla 1. Análisis de varianza de los parámetros de calidad de cebolla a diferentes niveles de remoción de capas y 30 días de exposición a condiciones ambientales (temperatura de 25±5 °C y humedad relativa de 70±10%).

Source Variance	DF	Square means and significance			
		DM	F	C	L
Scale removal (C)	2	5.003 ^{ns}	52.186 ^{ns}	54.682 ^{ns}	18.579 ^{ns}
Days of exposure(D)	3	8.641 ^{ns}	146.249 ^{**}	92.007 ^{**}	77.199 [*]
CxD	6	4.638 ^{ns}	57.482 ^{ns}	38.065 ^{ns}	138.253 ^{***}
Error	171	4.204	33.142	22.366	22.887
Total	182				
CV(%)		20.024	15.895	44.319	5.673

Source Variance	DF	Square means and significance			
		h°	TSS	TA	pH
Scale removal (C)	2	2216.610 ^{***}	7.385 [*]	0.000 ^{ns}	0.181 ^{***}
Days (D)	3	59.528 ^{ns}	2.769 ^{ns}	0.104 ^{***}	0.731 ^{***}
CxD	6	118.422 [*]	5.446 [*]	0.003 ^{**}	0.112 ^{***}
Error	171	41.731	2.098	0.000	0.014
Total	182				
CV(%)		6.761	16.448	27.103	2.051

DF: degrees of freedom; DM: dry matter; F: Firmness (N); C*: chromaticity, L*: luminosity; h°: angle hue; TSS: Total soluble solids; TA: titratable acidity; pH: hydrogenation potential. ns: non-significant values; Significant differences: *p-value<0.05, **p-value<0.01 and ***p-value<0.001.

and RH 60–80%), with a subsequent cold storage (0±1 °C and HR 70–75%) period of 8 months.

Onion firmness was a characteristic not affected by C0, C1 and C2 (p>0.05), as was the interaction of scale removal level and exposure periods (p>0.05) (Table 1). However, when the exposure periods were independently compared, a 5.77% reduction in firmness was observed after 10 days of the onions exposure (Figure 2).

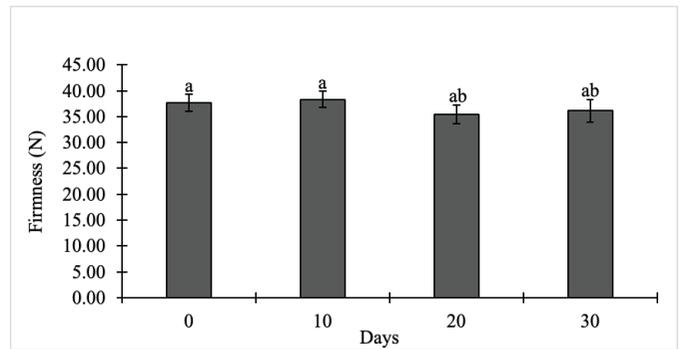


Figure 2. Onion firmness at 0, 10, 20 and 30 days of exposure to environmental conditions (temperature of 25±5 °C and relative humidity of 70±10%).

Figura 2. Firmeza de cebolla durante 0, 10, 20 y 30 días de exposición a condiciones ambientales (temperatura de 25±5 °C y humedad relativa de 70±10%).

Scale removal and exposure period were expected to have an effect on the onion dehydration firmness; but, the 30 days of exposure were not enough to significantly alter this attribute. Coolong *et al.* (2008) observed that in some onion cultivars the progressive loss of firmness occurs after four weeks of storage at 6.6 ±1.4 °C and HR 82±4.2%, while in other varieties is after eight weeks. Melo *et al.* (2012) point out that Optima variety increases in bulb firmness as the loss of weight increases during the 50 days of exposure to a temperature of 5 °C and relative humidity of 60%. However, this effect may vary depending on the variety. Islam *et al.* (2019) report a loss of firmness at 60 days after storage in different varieties. Variations in onion firmness are considered to be the result of genotype, storage time and exposed temperature interaction (Petropoulos *et al.*, 2016).

Chromaticity in the bulb's coloration is an attribute that does not vary due to scale removal level, nor to interaction effect of said factor with exposure time (Table 1). However, the Chroma (C*) value was altered by exposure time (p<0.01) at ambient conditions (25±5 °C and RH 70±10%). The chroma value remained stable between 9.3 and 10.7 in the first 20 days of exposure, while, at 30 days it increased significantly by 48.98% with respect to the value registered at 20 days (Figure 3). This increase indicates that the onion color changed

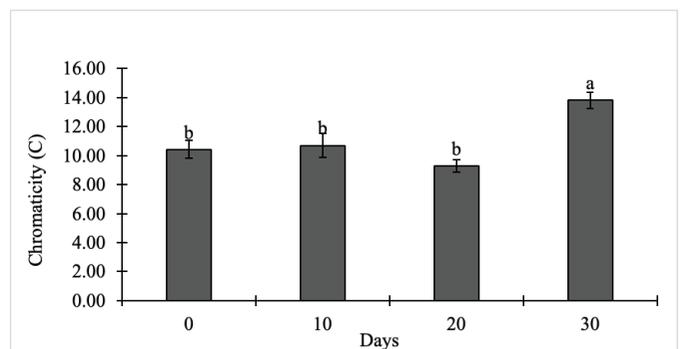


Figure 3. Onion chromaticity at 0, 10, 20 and 30 days of exposure to environmental conditions (temperature of 25±5 °C and relative humidity of 70±10%).

Figura 3. Cromaticidad durante 0, 10, 20 y 30 días de exposición a condiciones ambientales (temperatura de 25±5 °C y humedad relativa de 70±10%).

from opaque to a more saturated. Berno *et al.* (2014) reported an increase in chroma value 15 days after storing minimally processed purple onions at a temperature of 0 °C. However, saturation value (C*) depends on the variety (Petropoulos *et al.*, 2015) and position of the scales (Galsurker *et al.*, 2017).

The luminosity expressed in values from 1 to 100, where 1 is dark and 100 light, it was found that the bulbs presented values from 82.41 to 88.15 in a period of 30 days (Figure 4). The bulbs response with and without scales did not show differences either at the beginning (0 days) or the end (30 days) of the sampling. The average value registered in treatments at the beginning of experiment was 83.29 and at the end it increased 3.30% ($L = 86.04$). These differences between the values indicate that the onions are less shiny at the beginning and after a period of 30 days, they become shinier. In the intermediate sampling periods (10 and 20 days) of exposure, it was found that removal of two scales (C2) significantly affected brightness compared to the other treatments. This response was because C2 went from a hydrated condition at the beginning, to a dehydrated one in the next 10 days, giving appearance of a dark white color. Subsequently after 20 days, brilliance in C2 was significantly increased with respect to the other treatments. The onion condition with scales (C0) and without scales (C1), although they registered lower brightness at 20 days, the values were similar in the evaluated periods, which indicate stability in color clarity compared to C2.

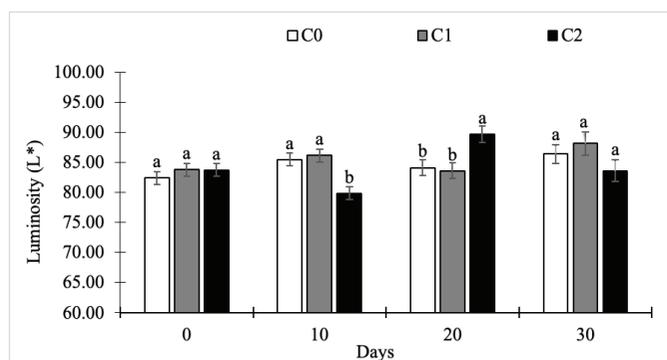


Figure 4. Onion lightness without removal of scales (C0), without a scale (C1) and without two scales (C2) at 0, 10, 20 and 30 days of exposure to environmental conditions (temperature of 25 ± 5 °C and relative humidity of $70 \pm 10\%$).

Figura 4. Luminosidad de la coloración de cebolla sin remoción de capas (C0), sin una capa (C1) y sin dos capas (C2) durante 0, 10, 20 y 30 días de exposición a condiciones ambientales (temperatura de 25 ± 5 °C y humedad relativa de $70 \pm 10\%$).

The onion brilliance is related to variety and storage conditions. Ríos-González *et al.* (2018), pointed out that Sierra Blanca variety registers values from 93.92 to 98.91, while Bola Precoce variety presents values of 67.26 (Cardoso *et al.*, 2016). In the present investigation, results obtained were intermediate and ranged from 82.15 to 88.15 between treatments. Onion stability brightness during storage is a trend that has been found when using controlled atmospheres for periods of up to 7 months (Ríos-González *et al.*, 2018). In another stu-

dy, it was found that luminosity increases in Beta Cristal and Optima varieties, when stored at a temperature of 5 °C and humidity of 85% (Melo *et al.*, 2012). It has also been reported that in the Bola Precoce variety, the external luminosity remained without significant difference with values between 59.86 and 61.88, when stored in silo at 30 °C for 14 days (Rego *et al.*, 2019).

The onion coloration based on values obtained from the hue angle was yellow (84.31 to 103.39 h°) in the different treatments during the 30 days of exposure (Figure 5). The values recorded without removal of scales (C0) were statistically the lowest (84.31 to 93.65), which is interpreted as a yellow hue with a slight orange hue. Bulbs without one (C1) and two scales (C2), presented hue values statistically higher than C0. Yellow coloration of bulbs C1 was higher (96.35 to 98.02), in comparison with C2 (99.19 to 101.91), where the yellow hue is reduced towards a green hue. The pattern of variation between the treatments within each evaluated period was consistent, showing an increase in the hue angle with respect to removal scales level.

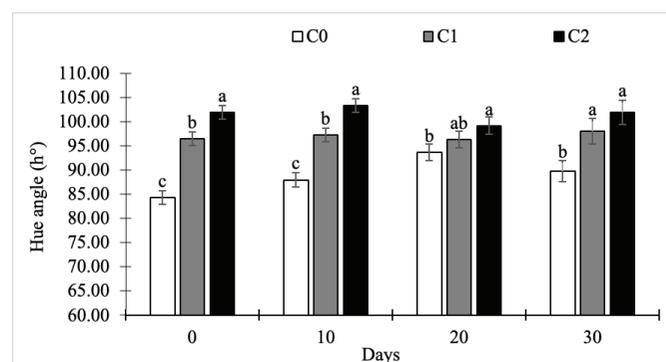


Figure 5. Onion hue angle without removal of scales (C0), without a scale (C1) and without two scales (C2) at 0, 10, 20 and 30 days of exposure to environmental conditions (temperature of 25 ± 5 °C and relative humidity of $70 \pm 10\%$).

Figura 5. Tonalidad de la coloración de cebolla sin remoción de capas (C0), sin una capa (C1) y sin dos capas (C2) durante 0, 10, 20 y 30 días de exposición a condiciones ambientales (temperatura de 25 ± 5 °C y humedad relativa de $70 \pm 10\%$).

According to Sharma and Lee (2016), the presence of yellowish-orange tones in onions that preserve scales, is caused by transformation of quercetin glucosides during the formation of dry skin. Grzelak *et al.* (2009) mention that the quercetin glucosides content in onion is high, and remains constant during storage. However, since they are mainly found in the outer scales (Lee and Mitchell, 2011), their total removal results in a loss of approximately 50% of polyphenols (Grzelak *et al.*, 2009) when trying to improve the bulb appearance. The exposure of bulbs without external scales to light during marketing, generates greening because they lack the protective layer generated in curing (Grzelak *et al.*, 2009).

Content of total soluble solids (TSS), titratable acidity (TA) and pH, were significantly modified ($P \leq 0.05$) when combining the scales removal level and exposure time (Table

1). Concentration of soluble solids varied from 7.69 to 9.51 °Brix (Figure 6). The onion that kept all the scales (C0) showed the highest concentration of TSS in each exposure period. Treatment without one scale (C1) presented a more stable behavior (8.33 to 8.98 °Brix) in comparison to C0 onions (8.84 to 9.74 °Brix), with similar values between both in the period of 10 and 20 d. At the end of the experiment (30 d), TSS concentration in C1 was reduced by 2.29% in relation to bulbs at 0 days. Onions without two scales (C2) registered a lower percentage of solids during the first two evaluation periods, with values of 8.15 (0 d) and 7.69 (10 d), but later, values were similar to bulbs C0 at days 20 and 30.

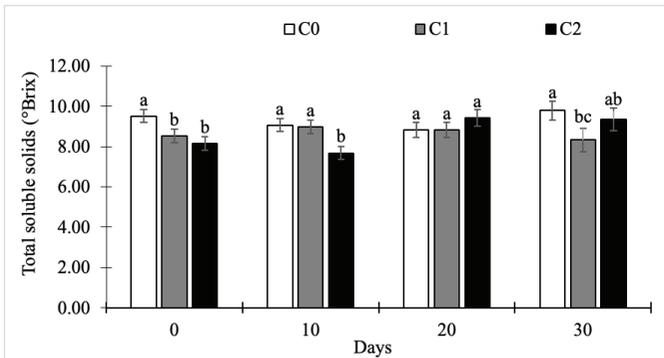


Figure 6. Onion total soluble solids without removal of scales (C0), without a scale (C1) and without two scales (C2) at 0, 10, 20 and 30 days of exposure to environmental conditions (temperature of 25 ± 5 °C and relative humidity of $70\pm 10\%$).

Figura 6. Contenido de sólidos solubles en cebolla sin remoción de capas (C0), sin una capa (C1) y sin dos capas (C2) durante 0, 10, 20 y 30 días de exposición a condiciones ambientales (temperatura de 25 ± 5 °C y humedad relativa de $70\pm 10\%$).

Titrateable acidity of onions during the evaluated period was variable between the different scale removal levels (Figure 7). Acidity values remained low (0.079) at 0 and 10 days, at 20 days they increased (0.178) and finally decreased again at 30 days (0.084). In comparison within the treatments, the greatest contrast was presented at 10 and 30 days after

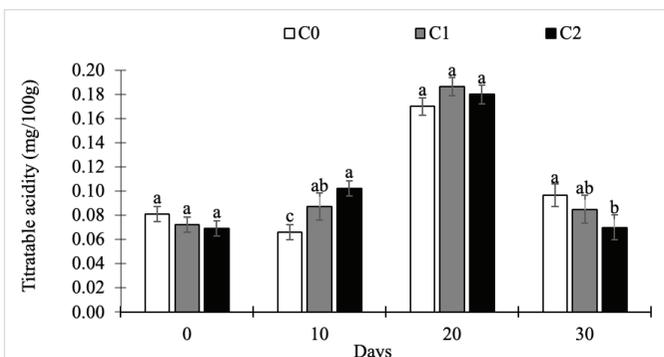


Figure 7. Onion titrateable acidity without removal of scales (C0), without one scale (C1) and without two scales (C2) at 0, 10, 20 and 30 days of exposure to environmental conditions (temperature of 25 ± 5 °C and relative humidity of $70\pm 10\%$).

Figura 7. Acidez titulable de cebolla sin remoción de capas (C0), sin una capa (C1) y sin dos capas (C2) durante 0, 10, 20 y 30 días de exposición a condiciones ambientales (temperatura de 25 ± 5 °C y humedad relativa de $70\pm 10\%$).

the onions were placed. At 10 days, titrateable acidity increased with scales removal level, while at 30 d the relationship was inverse, registering the lowest acidity in condition of onions without two scales.

The quantification of pH between treatments, registered variations from 5.25 to 5.82 during the 30 days of exposure (Figure 8). The lowest pH was recorded in onions without two scales (C2) at 30 days. Treatments that conserved all the scales (C0) and without one scale (C1), presented a similar pattern of pH variation during the days of exposure, with the exception of values recorded after 10 days, where value was lower in condition C0. The pH in bulbs C2 remained unchanged at 10 days, subsequently it increased by 1.7% at 20 days and at the end of the period (30 d), the lowest value was recorded of 8.11%.

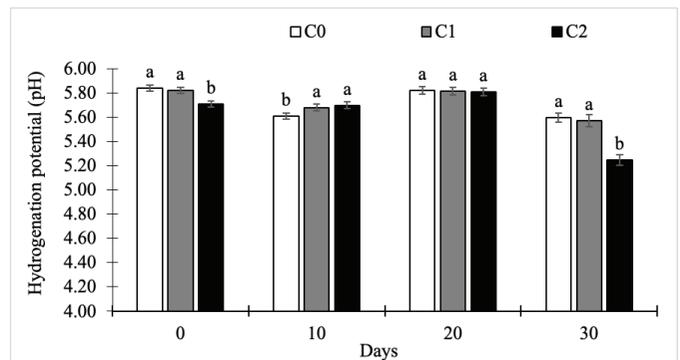


Figure 8. Onion hydrogenation potential without removal of scales (C0), without one scale (C1) and without two scales (C2) at 0, 10, 20 and 30 days of exposure to environmental conditions (temperature of 25 ± 5 °C and relative humidity of $70\pm 10\%$).

Figura 8. pH de cebolla sin remoción de capas (C0), sin una capa (C1) y sin dos capas (C2) durante 0, 10, 20 y 30 días de exposición a condiciones ambientales (temperatura de 25 ± 5 °C y humedad relativa de $70\pm 10\%$).

General behavior of soluble solids content, titrateable acidity and pH in onion with different degrees of scales removal and storage periods, includes high and low values but presenting statistical differences only in some periods. Studies carried out by Petropoulos *et al.* (2016) indicate that the soluble solids in the bulb of different varieties do not show a specific trend after storing onion at temperatures of 5 and 25 °C for 147 days. Melo *et al.* (2012) found that in onions Beta Cristal and Optima varieties, the content of titrateable acidity presents ups and downs when stored at a temperature of 5 °C. This behavior was also found in minimally processed onions stored at temperatures of 0 and 5 °C (Berno *et al.*, 2014).

Statistical variations of the variables TSS, TA and pH in treatments within some periods of exposure are considered to be the result of scales exposed during the experiment. Galsurker *et al.* (2018) pointed out different biological responses of onion according to scales exposed during thermal stress. Additionally, in aging process, the exposed scales present morphological and transcriptional changes (Galsurker *et al.*, 2017), which also contributes to changes observed in applied treatments.

Variations of lower TSS concentration in onions without one (C1) and two (C2) scales with respect to those without removal (C0), is attributed to the increase in respiration due to mechanical damage caused by removing scales. Islam *et al.* (2019) found that Bartio, Hylander and Summit varieties increase respiration rate during storage, while reports on Orlando variety, show that respiration rate increases significantly in bulbs without four scales compared to onions with outer layer, when exposed to temperatures of 33 °C for a period of 8 days (Galsurker *et al.*, 2018). Elimination of impermeable layer developed in the curing of onions, generates sensitivity to environmental factors and consequently produces greater activity of catabolism of substrates, mainly carbohydrates and other phytochemicals (Rutherford and White, 1982; Mogren *et al.*, 2007a, 2007b).

In a study carried out by Rodríguez *et al.* (2008) on the content of organic acids in six onion cultivars, pH values of 5.47 to 5.67 were found, similar to those obtained in this work, except for C2 at 30 days. Marinozzi *et al.* (2014) and Petropoulos *et al.* (2015) reported values between 5.40 and 5.90 in different onion cultivars. However, pH value also depends on genotype, storage time and temperature interaction (Petropoulos *et al.*, 2016).

CONCLUSION

Removal of one or two outer scales does not modify the dry matter content and bulb firmness during an exposure period of 30 days under ambient conditions (25±5 °C and RH 60% - 80%). This favors outer scales elimination to improve onion appearance during market exhibition. However, scales removal induces changes in appearance (shade and brightness) and biochemical quality of the onion. The similarity of quality in soluble solids content, titratable acidity and pH between bulbs with all the scales and without one scale, determines that elimination of one scale is an alternative to improve appearance, when it is required to eliminate traces of scale damaged by handling and storage periods. However, in subsequent investigations it is necessary to evaluate onion quality due to scale removal effect, considering environmental conditions and exposure period of 30 days, as well as onion previous storage conditions to be used in the study.

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