

Effect of γ -Irradiation Combined with Washing and Waxing Treatment on Physicochemical Properties, Vitamin C, and Organoleptic Quality of *Citrus clementina* Hort. Ex. Tanaka

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To enhance the shelf life of a late variety of Moroccan *Citrus clementina* (Nour), ionizing treatments were applied at 0.3 kGy, as well as washing (cold water) and waxing treatments. It has been found that, despite the irradiation treatment, the washing and waxing treatment do not improve the quality of *C. clementina*, but rather result in yellower peels, peel injury, and reductions of vitamin C content, acidity, and soluble solids. However, γ -irradiation alone enhanced significantly ($p \leq 0.05$) the level of vitamin C and the total phenolic content and maintained the color of the *C. clementina* during the entire storage period (49 days at 3 ± 1 °C and 84% relative humidity). Finally, sensory evaluation further confirmed the beneficial effect of γ -irradiation. Irradiated clementines were found to be sweeter. Also, the sensorial score of irradiated (I) and washed, waxed, and un-irradiated (LC) fruits was maintained over 7 days during 21 days as compared to 14 days for unwashed, unwaxed, and un-irradiated (C) and for washed, waxed, and irradiated (LCI) fruits.

KEYWORDS: *Citrus clementina* Hort. ex. Tanaka; γ -irradiation; cold water and waxing treatment

INTRODUCTION

The culture of citrus fruits plays an important role in the national economy of Morocco. However, citrus fruits are generally produced in regions that are quite far from the main cities and, thus, the main Moroccan markets. Moreover, fruits might ripen while the demand is very low or the supply overly abundant. In such situations, fruits may be stored over a very long period of time, in anticipation of more favorable market circumstances.

Once the fruits are harvested, they live at the expense of their own reserves and they mature progressively. During the storage, conditions favorable to microorganisms are generated (1–4), causing thus damages to the fruits. It is well-known and accepted that the main causes of damage of postharvest citrus fruits are due to the presence of stem rot, as well as green (*Penicillium digitatum* Sacc.) and blue (*Penicillium italicum* Wehmer) molds (5).

Many researchers have reported low-dose γ -irradiation as a potential tool for extending the postharvest life of many fresh fruits and vegetables (6–13). Several investigations were undertaken to improve storage conditions of citrus fruits, more precisely, oranges, lemons, and grapefruits (5, 14–16). These works have pointed out that citrus fruits are very sensitive to γ -irradiation. Indeed, to optimize the effectiveness of ionizing treatments of citrus fruits, the control of some parameters has been reported to be very important, namely, the dose, the dose rate, the storage temperature, preprocessing conditions, the degree of ripeness, and the original quality of the fruits.

Despite investigations reported so far, the behavior of small citrus fruits, such as clementines, mandarins, and tangerines, toward γ -irradiation is not well-known. Recent studies on tangerines (*Citrus reticulata*) (17) and clementines (*Citrus clementina*) (18) have shown promising results. Therefore, the purpose of this work is to report on the effect of γ -irradiation as well as washing (cold water) and waxing treatments on a late variety of Moroccan *C. clementina* (Nour). Several parameters were assessed during the storage of *C. clementina* (Nour), including physicochemical, nutritional, and organoleptic properties.

MATERIALS AND METHODS

Sampling. Moroccan clementines (*C. clementina* Hort. ex. Tanaka var. Nour) were used in this study. Fruits were collected from the

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Agricultural and Development Society (SODEA) of Rabat, Morocco. Selected fruits with the absence of bruises or other quality defects were divided in two groups. In the first group, unwaxed and unwashed fruits were packed in 30 paperboard boxes containing ~140 fruits. In the second group, fruits were treated with wax containing a fungicide (thiobendazole) at a concentration of 4000 ppm and packed in 30 paperboard boxes containing ~140 fruits. The day after, boxes were sent by Royal Air Maroc cargo shipment to Mirabel International Airport, Canada. Upon arrival at Mirabel, the fruits were on the same day trucked to the Canadian Irradiation Center (CIC), in the city of Laval. Upon arrival at CIC, the fruits were randomly distributed into four groups: unwashed, unwaxed, and un-irradiated controls (C); unwashed and unwaxed irradiated at 0.3 kGy (I); washed and waxed un-irradiated controls (LC); and washed and waxed irradiated at 0.3 kGy (LCI). Only fruits with the absence of bruises or other quality defects were used. During the experimentation, all spoiled fruits were destroyed by incineration.

Irradiation Treatment. Clementines were irradiated with a dose of 0.3 kGy in the ^{60}Co source carrier-type industrial irradiator (MDS Nordion International Inc., Kanata, ON, Canada) at the CIC (Laval, PQ, Canada). Optichromic dosimeters (Far West Technology) were used to validate the dose distribution. Upon completion of the irradiation at room temperature, all dosimeters were collected and dosimetry data recorded.

Storage. After completion of irradiation, the fruits (C, I, LC, and LCI) were stored at 3 ± 1 °C and at 84% relative humidity. Light was kept in the storage room both day and night. Analyses were begun on day 0 and repeated each week during 49 days.

Peel Injury. Damage consisting of rotted fruits was evaluated by noting the appearance of rot, molds, and pitting for each treatment and expressed as a percentage of the total fruits.

Color Measurements. The color of five clementines was determined with a standard Colormet colorimeter (Instrumar Ltd., St. John's, NF, Canada) fitted with a xenon flash lamp illuminating the sample area. The Colormet measures the spectrum of reflected light and converts it to a set of color coordinates such as L^* , a^* , and b^* , which are coordinates in a three-dimensional space containing all colors (19). The value of L^* represents lightness, which equals 0 for black and 100 for white. The a^* axis shows the amount of red (+) or green (-), whereas the b^* axis shows the amount of yellow (+) or blue (-). To interpret the color changes, calculation of the hue was carried out for the clementine peel [$\theta = \tan^{-1}(a^*/b^*)$]. A low hue value ($\theta = 0^\circ$) indicates a yellow peel color. A high hue value ($\theta = 90^\circ$) means a more reddish orange color of the peel.

Titrateable Acidity. The titratable acidity was determined according to AOAC methods 22.008 (1984) and 22.058 (1984). The acidity is expressed as grams of citric acid monohydrated per 100 g of the pulp.

Determination of Total Soluble Solids (°Brix). Total soluble solids content (°Brix) on 70 g of the homogenate pulp diluted in 200 mL of water was determined at 23 °C with a hand refractometer (Fisher 13.946.70C).

Determination of Vitamin C. Twenty grams of the clementine (5) homogenate pulp was used to determine the assay of vitamin C for each treatment investigated, according to AOAC method 96.721 (1990). The vitamin C content is expressed as milligrams per 100 g of the pulp.

Sensory Evaluation. Sensory evaluation of the clementine pulp was done for the overall appreciation of the taste, by a panel of usually 10 persons at the INRS-Institut Armand-Frappier. Panelists were asked to evaluate the samples of clementines from each of the four treatments on a nine-point hedonic scale (1 = dislike extremely; 9 = like extremely) during 4 weeks (20).

Statistical Analysis. Analysis of variance and Duncan's multiple-range tests with $p \leq 0.05$ were employed to analyze statistically all results. Student's t test was utilized at the time of the analysis of variance and paired-comparison with $p \leq 0.05$ (21). For each measurement, three replicates of three samples were tested.

RESULT AND DISCUSSION

Peel Injury. Figures 1 and 2 showed the effect of γ -irradiation in combination with and without the washing (cold water

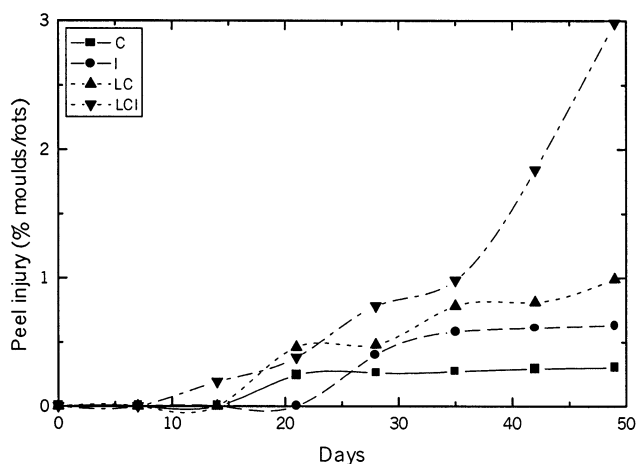


Figure 1. Effect of γ -irradiation with washing and waxing treatment on peel injury (molds and rots) of *C. clementina* Hort. Ex. Tanaka during storage (C, unwashed and unwaxed un-irradiated clementines; I, unwashed and unwaxed irradiated clementines; LC, washed and waxed un-irradiated clementines; LCI, washed and waxed irradiated clementines).

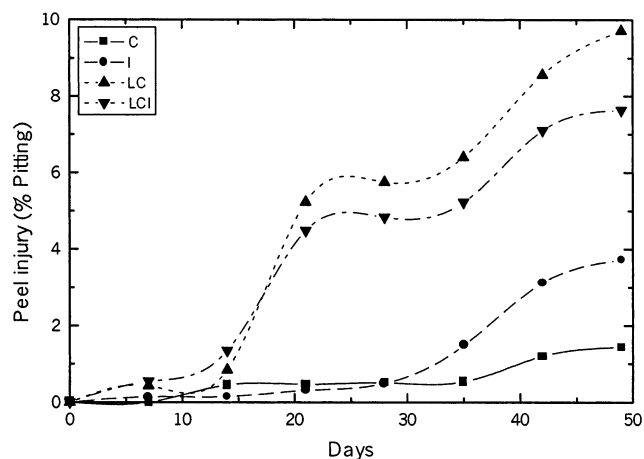


Figure 2. Effect of γ -irradiation with washing and waxing treatment on peel injury (pitting) of *C. clementina* Hort. Ex. Tanaka during storage (C, unwashed and unwaxed un-irradiated clementines; I, unwashed and unwaxed irradiated clementines; LC, washed and waxed un-irradiated clementines; LCI, washed and waxed irradiated clementines).

and waxing treatment on the quality of *C. clementina*, namely, unwashed, unwaxed, irradiated *C. clementina* (I) versus unwashed, unwaxed, non-irradiated ones (C) and washed, waxed, irradiated *C. clementina* (LCI) versus non-irradiated but washed and waxed *C. clementina* (LC). It is clear that the washing and waxing treatment generated *C. clementina* of lesser quality. The observed damages were related to the appearance of molds and rotting and to the pitting phenomenon (peel injury) (Figures 1 and 2, respectively). Less than 1% damage was noticed during the first two weeks of the storage period, in all four cases investigated (C, I, LC, and LCI). However, from day 21 to day 35, a significant difference ($p \leq 0.05$) could be observed between washed and waxed *C. clementina* LC and LCI and nonwashed and nonwaxed ones, C and I. No significant difference ($p > 0.05$) could be noticed between irradiated and the corresponding non-irradiated clementines, I versus C and LC versus LCI (Figures 1 and 2). At the end of the storage period, the percentage of molds was found to be 1–3% for, respectively, LC and LCI and almost negligible for C and I (0.25–0.50%, respectively) (Figure 1).

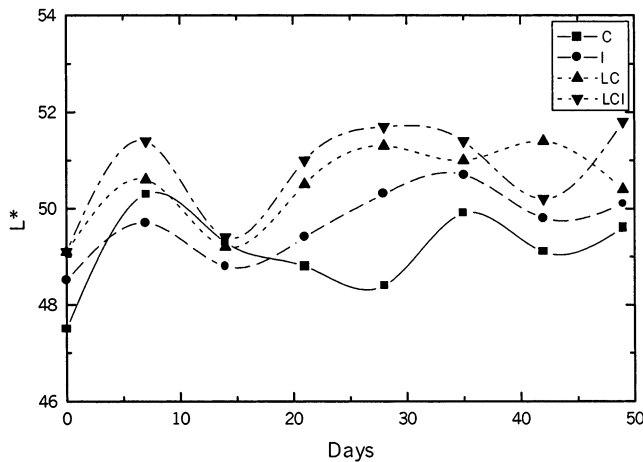


Figure 3. Effect of γ -irradiation with washing and waxing treatment on the L^* value of the peel of *C. clementina* Hort. Ex. Tanaka during storage (C, unwashed and unwaxed un-irradiated clementines; I, unwashed and unwaxed irradiated clementines; LC, washed and waxed un-irradiated clementines; LCI, washed and waxed irradiated clementines).

The pitting levels were found to be 10 and 7% for, respectively, LC and LCI fruits as compared to 3.8 and 1.5% for, respectively, I and C fruits. The pitting was significantly ($p \leq 0.05$) more important in washed and waxed *C. clementina*, LC and LCI (Figure 2), whereas only LCI fruits were more sensitive to molds and rotting (Figure 1). It has been reported that peel injury or pitting is related to the enzymatic activity changes that occur during the storage of fruits and vegetables, more particularly, to the activity of PAL, which is directly involved in the synthesis of phenolic compounds (22–25).

Color Measurements. During the entire storage, from day 0 to day 49, a significant increase ($p \leq 0.05$) of the L^* value was observed for all *C. clementina* investigated (Figure 3). This behavior indicates that *C. clementina* faded away. The L^* value was found to be strongly dependent on the nature of the treatment. Indeed, the washing and waxing treatment generated *C. clementina* with more important L^* values ($p \leq 0.05$), where a high L^* value expresses a lighter color. Ionizing treatment had less impact on the L^* value than the washing and waxing treatment. Nevertheless, γ -irradiation increased the L^* value. At the end of the storage, L^* values were, respectively, 45.0 ± 0.5 , 50.0 ± 0.8 , 50.5 ± 0.4 , and 52.0 ± 0.9 for C, I, LC, and LCI samples.

Color measurements revealed that the a^* value (associated with the red color) varied slightly during the storage period (Figure 4). However, as observed with the L^* value, the washing and waxing treatment had a more important impact ($p \leq 0.05$) than the ionizing treatment. Washed and waxed *C. clementina*, LC and LCI, exhibited a significantly lower a^* value ($p \leq 0.05$) than C and I fruits. At the beginning of the storage, day 0, C and I fruits showed a significantly higher a^* value than LC and LCI fruits, where the red color is being expressed by a high a^* value. This significant difference ($p \leq 0.05$) in a^* values was maintained throughout the entire storage period. γ -Irradiation also influenced the a^* value, although it was of lesser importance than the washing and waxing treatment. Results demonstrated that irradiated fruits, I, lost their red color ($p \leq 0.05$) with respect to control fruits, C. A similar trend was perceived between LC and LCI clementines. At the end of the storage, the a^* values were, respectively, 36.2 ± 0.4 , 36.5 ± 0.2 , 37.3 ± 0.5 , and 38.0 ± 0.6 for LC, LCI, I, and C samples. The loss of coloration generated by γ -irradiation and/or washing

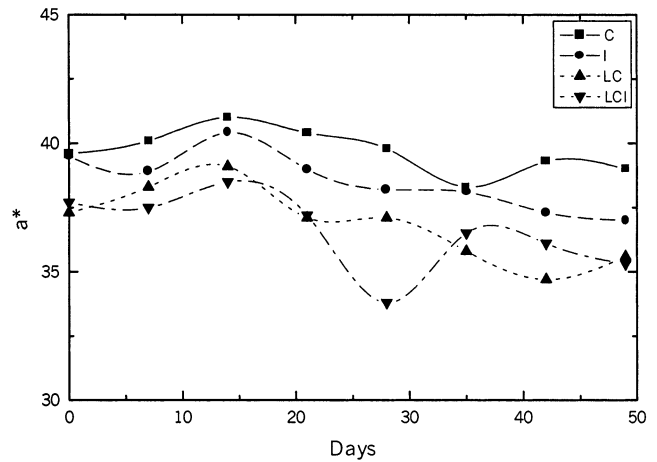


Figure 4. Effect of γ -irradiation combined with washing and waxing treatment on the a^* value of the peel of *C. clementina* Hort. Ex. Tanaka during storage (C, unwashed and unwaxed un-irradiated clementines; I, unwashed and unwaxed irradiated clementines; LC, washed and waxed un-irradiated clementines; LCI, washed and waxed irradiated clementines).

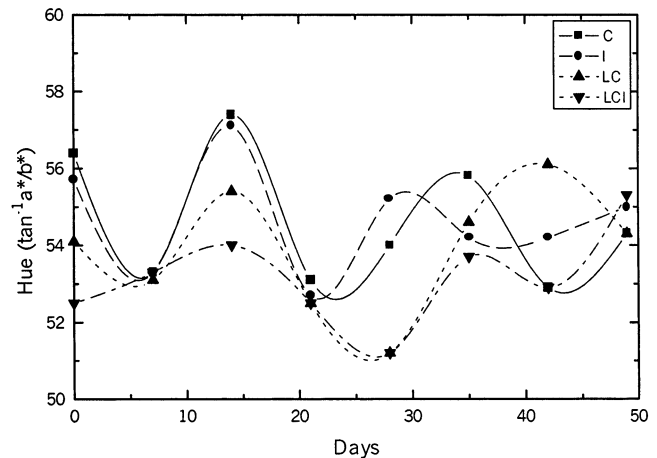


Figure 5. Effect of γ -irradiation combined with hot washing and waxing treatment on the hue of the peel of *C. clementina* Hort. Ex. Tanaka during storage (C, unwashed and unwaxed un-irradiated clementines; I, unwashed and unwaxed irradiated clementines; LC, washed and waxed un-irradiated clementines; LCI, washed and waxed irradiated clementines).

and waxing treatment was also observed in irradiated tangerines (*C. reticulata*) and citrus fruits (5, 17).

The variation of the hue value of the peel during the storage for the four groups of clementines studied is summarized in Figure 5. A significant effect ($p \leq 0.05$) of the treatment could be noticed on the hue value of the peel. First, the hue values of LC and LCI were significantly lower ($p \leq 0.05$) than those of C and I, at the beginning of the storage. These results indicate that the washing (cold water) and waxing treatment turned significantly yellower ($p \leq 0.05$) the color of the peel. Likewise, the peel behaved similarly toward the irradiation treatment. Indeed, the hue values of LCI and I were significantly lower ($p \leq 0.05$) than those of LC and C, at the beginning of the storage. The contrary occurred during the storage. The hue value of the peel increased significantly ($p \leq 0.05$) in order to be higher in irradiated clementines (I and LCI) than in non-irradiated ones (C and LC). Therefore, irradiation generated a more reddish orange color of the peel, during the 49 days of storage time at 3 °C and 84% relative humidity, whereas non-irradiated clementines turned yellower. At the end of the storage, hue values were, 54.3 ± 0.2 for L and LC samples and 55.0 ± 0.3

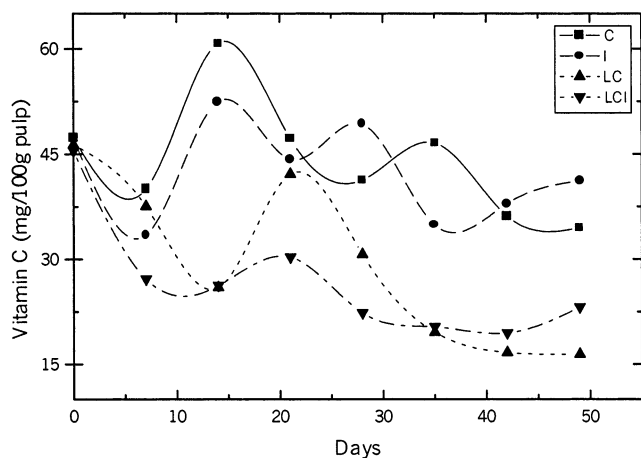


Figure 6. Effect of γ -irradiation with washing and waxing treatment on the total vitamin C of *C. clementina* Hort. Ex. Tanaka during storage (C, unwashed and unwaxed un-irradiated clementines; I, unwashed and unwaxed irradiated clementines; LC, washed and waxed un-irradiated clementines; LCI, washed and waxed irradiated clementines).

for I and LCI samples. Maxie et al. (26) observed a similar trend in irradiated Navel oranges, stored at 10 °C. However, O'Mahony et al. (27) reported the opposite in Navel oranges, whereas no difference was noticed between irradiated and control Kern Country oranges. A yellow color was attributed to the decrease of the concentration of some carotenoids during the storage (28).

Vitamin C. Figure 6 exhibits the evolution of vitamin C assay within *C. clementina* during the 49 days of storage at 3 ± 1 °C and 84% relative humidity. In all cases investigated, a significant decrease ($p \leq 0.05$) of vitamin C could be observed between the beginning (day 0) and the end of the storage period (day 49): from 47.0 ± 0.5 to 34.0 ± 0.2 mg/100 g in C, from 47.0 ± 0.5 to 41.0 ± 0.2 mg/100 g in I, from 46.0 ± 0.2 to 16.0 ± 0.1 mg/100 g in LC, and from 45.0 ± 0.3 to 23.0 ± 0.1 mg/100 g in LCI.

The washing (cold water) and waxing treatment resulted in a significant reduction ($p \leq 0.05$) of vitamin C and thus nutritional properties in *C. clementina* during the storage period: LC, LCI < C, I. According to Erdmand and Klein (29), vitamin C is sensitive to some treatments, such as bleaching and hot water treatment. This investigation clearly demonstrates that vitamin C is also sensitive to cold water and waxing treatment.

This investigation also demonstrated that irradiation did not have a significant impact ($p > 0.05$) on the vitamin C content of *C. clementina*: I ~ C and LCI ~ LC. This behavior is in agreement with previous works that have reported that irradiation of fruits, Valencia and Navel oranges, at doses ranging between 0.3 and 2 kGy does not affect ($p > 0.05$) the content of vitamin C throughout the entire storage period at various conditions: 7 weeks at 7 °C and 2 weeks at 21 °C (16, 30). A further investigation reported that an even higher irradiation dose did not produce a significant change ($p > 0.05$) in the vitamin C content of Navel oranges after 90 days of storage at 0 °C (26).

Titrateable Acidity. Results of titrateable acidity is showed in Figure 7. During the entire storage, the titrateable acidity was more greater ($p \leq 0.05$) in C and I clementines than in LC and LCI clementines: 0.8–1.1 versus 0.5–0.7 mg of ascorbic acid/100 g of pulp, respectively. Therefore, the washing (cold water) and waxing treatment caused a significant decrease ($p \leq 0.05$) of titrateable acidity.

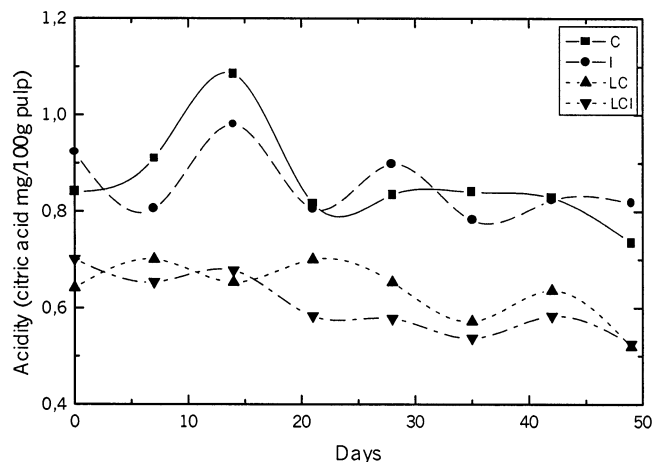


Figure 7. Effect of γ -irradiation with washing and waxing treatment on the titrateable acidity of *C. clementina* Hort. Ex. Tanaka during storage (C, unwashed and unwaxed un-irradiated clementines; I, unwashed and unwaxed irradiated clementines; LC, washed and waxed un-irradiated clementines; LCI, washed and waxed irradiated clementines).

The effect of γ -irradiation was significant at the early stage of the storage period. At day 0 irradiated clementines had higher titrateable acidity values ($p \leq 0.05$) with respect to non-irradiated ones: I > C and LCI > LC. Between days 0 and 14, the titrateable acidity increased significantly ($p \leq 0.05$), reaching maximum values in C and I clementines of, respectively, 0.96 ± 0.02 and 1.1 ± 0.02, whereas it remains almost stable in LC and LCI clementines with respective concentrations of 0.64 ± 0.02 and 0.68 ± 0.02. Control C and I clementines were significantly different ($p \leq 0.05$), but this was not the case ($p > 0.05$) for LC and LCI clementines. Between days 14 and 21, the titrateable acidity decreased ($p \leq 0.05$) suddenly in C and I clementines and remained almost stable until the end of the storage at day 49. Differences between C and I clementines were not significant ($p > 0.05$) from day 21. A smooth decrease ($p \leq 0.05$) of the titrateable acidity was observed in LC and LCI clementines between days 21 and 49. Differences between LC and LCI clementines were not significant ($p > 0.05$). Different investigations performed on oranges have shown that irradiation did not have a significant impact on the titrateable acidity, whatever the irradiation dose (16, 31–33).

Total Soluble Solids (°Brix). Results of total soluble solids are presented in Figure 8. Soluble solids varied between 13 and 14.5 °Brix in I, LC, and LCI clementines and between 14 and 14.5 °Brix in C clementines. At the beginning of the storage, control clementines (C) showed more soluble solids ($p \leq 0.05$) than the other clementines studied: C > I = LC = LCI. At day 7, a significant increase ($p \leq 0.05$) occurred in both C and I clementines, reaching a maximum value of 15.5 ± 0.08 °Brix. A decrease ($p \leq 0.05$) followed at day 14 in both control and irradiated clementines. From day 21 to day 35, soluble solids fell ($p \leq 0.05$) to reach a plateau in irradiated clementines (I), whereas a drop ($p \leq 0.05$) occurred in C clementines between days 42 and 49. At the end of the experiment, soluble solids contents in C and I samples were, respectively, 14.5 ± 0.07 and 14.0 ± 0.06 °Brix. The analysis of variance showed a significant difference between C and I clementines between days 21 and 49.

Soluble solids in washed (cold water) and waxed clementines (LC and LCI) remained stable until day 7. At day 14, a significant enhancement ($p \leq 0.05$) appeared, reaching maximum value of 14 ± 0.05 °Brix in LC clementines and 14.5 ± 0.05 °Brix in LCI clementines. From day 14 to day 28, soluble

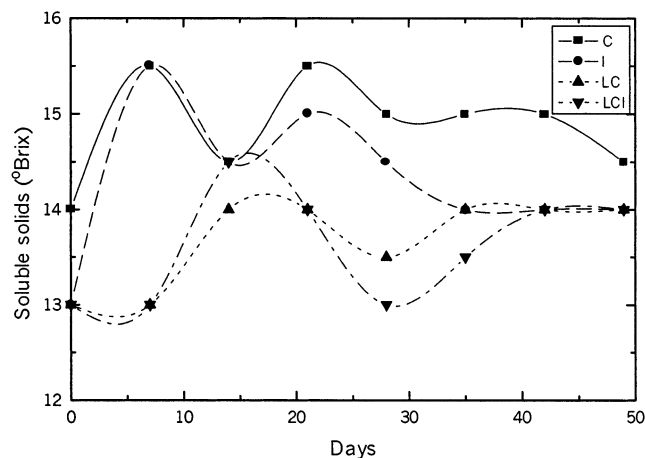


Figure 8. Effect of γ -irradiation with washing and waxing treatment on the °Brix of *C. clementina* Hort. Ex. Tanaka during storage (C, unwashed and unwaxed un-irradiated clementines; I, unwashed and unwaxed irradiated clementines; LC, washed and waxed un-irradiated clementines; LCI, washed and waxed irradiated clementines).

Table 1. Sensorial Evaluation (Hedonic Test) on the Overall Appreciation of Clementine Pulp during Storage^a

	day 1	day 14	day 21	day 28
C ^b	7.0 ± 1.0 a1	7.0 ± 1.3 a1	5.0 ± 1.5 a1	5.5 ± 1.5 a1
I	8.0 ± 1.2 b1	8.0 ± 1.2 b1	8.5 ± 1.5 c1	6.0 ± 1.5 a1
LC	6.5 ± 1.3 a1	9.3 ± 1.5 c2	8.0 ± 1.3 c1	6.0 ± 1.3 a1
LCI	7.0 ± 1.5 a1	7.0 ± 1.2 a1	6.0 ± 1.2 b1	5.5 ± 1.3 a1

^a Means followed by different letters in the same column are significantly ($p \leq 0.05$). Means followed by different numbers in the same row are significantly ($p \leq 0.05$). ^b C, unwashed and unwaxed un-irradiated clementines; I, unwashed and unwaxed irradiated clementines; LC, washed and waxed un-irradiated clementines; LCI, washed and waxed irradiated clementines.

solids were significantly reduced ($p \leq 0.05$) in both LC and LCI clementines. Washed, waxed, and irradiated (LCI and LC) clementines attained a plateau at day 42. At the end of the storage, both LC and LCI clementines had a mean amount of soluble solids of 14 ± 0.03 °Brix. The analysis of variance showed a significant difference between LC and LCI clementines between days 14 and 35.

Our observations differ from those reported by O'Mahony et al. (27), who did not notice significant differences in soluble solids between irradiated and control Navel oranges after 6 weeks of storage.

Sensory Evaluation. The results of the hedonic test for the overall appreciation of the taste of clementine pulp are shown in **Table 1**. Results showed that at day 1 of the storage, no significant difference ($p > 0.05$) was observed between treatments, except for I clementines for which a significantly higher score ($p \leq 0.05$) was observed. Values obtained were, respectively, 7.0 ± 1 , 8 ± 1.2 , 6.5 ± 1.3 , and 7.0 ± 1.5 for C, I, LC, and LCI fruits.

At day 14 of storage, a significant improvement of the score was observed for LC fruits with a score of 9.3 ± 1.5 . This value was significantly higher ($p \leq 0.05$) than values obtained for C, I, and LCI. Scores for these samples were between 7 and 8. At day 21, a significantly higher score ($p \leq 0.05$) was obtained for I and LC fruits with respective values of 8.0 ± 1.3 and 8.5 ± 1.5 as compared to 5.0 ± 1.5 and 6.0 ± 1.2 for C and LCI, respectively. At day 28, no significant difference ($p > 0.05$) was observed among all treatments. However, highest values were obtained for I and LC fruits with a mean value of $6.0 \pm$

1.4. These results showed that the sensorial quality decreased during storage for C and LCI fruits. Also, the quality of I and LC clementines was ≥ 7 during two weeks. The quality of LC fruits increased until day 1, and thereafter a decrease was observed until the end of storage.

Conclusion. This investigation clearly demonstrated that irradiation has a beneficial effect on the physicochemical, nutritional (vitamin C content), and organoleptic properties of *C. clementina* Hort. ex. Tanaka var. Nour (I) during the seven weeks storage at 3 °C and 84% relative humidity. The sensorial quality and nutritional properties of irradiated clementines were maintained during storage.

However, the washing (cold water) and waxing treatment combined or not with irradiation had rather a negative impact on the shelf life, the nutritional properties, and the appearance of clementines. Washing and waxing treatment yielded more vulnerable fruits as confirmed by the more significant peel injury (pitting) and the higher presence of molds and rots. The washing and waxing treatment also contributed to a decrease in the concentration of vitamin C of the clementine juice. This resulted in juice with impoverished nutritional properties. Finally, a loss of color was observed in washed and waxed clementines.

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