

The effect of physiological maturity on the content and stability of active compounds in chili pepper fruits (*Capsicum frutescens* L.) during storage

Muna Salih Muttar*, Taha Shihab Ahmed, Mohamed A Ahmed

Department of Horticulture and Landscape, College of Agriculture, Tikrit University, Iraq.

* Corresponding author: E-mail: muna.sa.mot338@st.tu.edu.iq

ABSTRACT

This study aimed to evaluate the impact of fruit maturity stage, storage container type, and storage duration on the content of active compounds and vitamins in hot pepper fruits (*Capsicum frutescens* L.), as these are important factors in maintaining the postharvest chemical quality and utility of food. The experiment was carried out in spring season of 2024 at the Horticultural Research Station, College of Agriculture, University Kirkuk, Its geographical coordinates are approximately (44.3) degrees east longitude and (35.3) degrees north latitude. according to Completely Randomized Design (CRD) and with three replication under three factors. The factors consisted of two maturity stages (green fruits A1 and red fruits A2), two storage container types (metallic B1 and clay B2) and three storage periods (45 days C1, 90 days C2, 135 days C3). Results were significant for all factors analysed. The maturity stage A 2 outperformed in phenols, alkaloids and flavonoids while capsaicin and the vitamins studied were most abundant at stage A2. Successive extractions Efficiency of retaining active compounds was greater in clay (B2) than metal containers. In relation to storage period, 45 days was best for most of the traits and the highest capsaicin content a peel was observed at 135 days. The interactions demonstrated the dominance of A2B2C1 treatment phenols, alkaloids, vitamin A, B6 and C (280.1 mg 100g⁻¹, 1.1033 %, 123.67 ppm, 40.67 ppm, 199.77 ppm) respectively.

The findings indicate that the late maturity and fermentation in clay pots, followed by short-term storage, help to decrease loss of oxidation-sensitive compounds because of the stabilization of storage conditions and minimization of metabolically mediated degradations. This validates the possibility of improving the chemical value of hot pepper fruits through proper postharvest handling.

KEYWORDS: Organic fertilizers, active ingredients, pinching, pepper plant.

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تأثير النضج الفسيولوجي على محتوى وثبات المركبات الفعالة في ثمار الفلفل الحار (*Capsicum frutescens* L.) أثناء التخزين

منى صالح مطر*، طه شهاب احمد، محمد عبد الله احمد

قسم البستنة وهندسة الحدائق، كلية الزراعة، جامعة تكريت، العراق

المخلص

هدفت هذه الدراسة إلى تقييم تأثير مرحلة نضج الثمار، ونوع أواني التخزين، ومدة الخزن في محتوى المركبات الفعالة والفيتامينات في ثمار الفلفل الحار (*Capsicum frutescens* L.)، لما لهذه العوامل من دور محوري في الحفاظ على الجودة الكيميائية والقيمة الوظيفية بعد الحصاد. نُفذت التجربة خلال الموسم الربيعي لعام 2024 في محطة البحوث البستنية/كلية الزراعة/جامعة كركوك، و إحداثياتها الجغرافية هي 44.3 درجة شرقاً و 35.5 درجة شمالاً. وفق التصميم العشوائي الكامل (CRD) وبثلاث مكررات، وباستخدام ثلاثة عوامل شملت مرحلتين نضج (ثمار خضراء A1 و ثمار حمراء A2)، ونوعين من أواني التخزين (معدنية B1 وفخارية B2)، وثلاث مدد خزن (45 يوم C1، 90 يوم C2 و 135 يوم)، ظهرت النتائج وجود تأثيرات معنوية لجميع العوامل المدروسة، إذ تفوقت مرحلة النضج المتأخر (A2) في محتوى الفينولات والقلويدات والفلافونيدات والكابسييسين والفيتامينات المدروسة، كما أظهرت الأواني الفخارية (B2) كفاءة أعلى في المحافظة على المركبات الفعالة مقارنة بالأواني المعدنية. وبخصوص مدة الخزن، تفوقت مدة 45 يوماً في معظم الصفات، في حين سجل أعلى محتوى للكابسييسين عند مدة 135 يوماً. وسجلت التداخلات تفوق معاملة A2B2C1 في الفينولات، القلويدات، و فيتامينات A، B6 و C (280.1 ملغم 100 غم⁻¹، 1.1033 %، 123.67 ppm، 40.67 ppm، 199.77 ppm) على التوالي.

تعكس النتائج أهمية النضج المتأخر والأواني الفخارية والخزن القصير في الحد من فقد المركبات الحساسة للأكسدة، نتيجة استقرار الظروف الخزنية وتقليل التحلل الأبيض، مما يؤكد إمكانية تحسين القيمة الكيميائية لثمار الفلفل الحار من خلال الإدارة المثلى بعد الحصاد.

الكلمات المفتاحية: الأسمدة العضوية، المكونات النشطة، التقليم، نبات الفلفل.

INTRODUCTION

The hot pepper plant (*Capsicum frutescens* L.), in the family Solanaceae, is an important horticultural crop both economically and nutritionally. This is attributed to its richness in bioactive products, capsaicinoids, phenolic compounds, alkaloids carotenoids and vitamins that make the plant valuable from healthiness and medicinal points of view (Guijarro-Real et al., 2023; Lahbib et al., 2023). The levels of these compounds are substantially affected by the consequence of ripening being associated with both physiological (tissue modifications), and chemical changes (changes in enzymatic activity, carbohydrate and secondary metabolites translocation within the fruits).

Numerous investigations have reported that the levels capsaicinoid compounds do not increase with maturity in a uniform manner and may remain stagnant or it can peak at an intermediate ripening stage depending on the cultivar, environmental conditions, and harvest time (Manikharda et al., 2018; Mercedes Vázquez-Espinosa et al., 2020). Moreover, at late ripening linked to the change of colour from green into red, the accumulation of carotenoids that induce red pigmentation through transformation of chloroplasts to chromoplasts is significantly increased that will also be mirrored in vitamin A traits and marketability (Zsófia Kovács et al., 2022).

In addition to the maturity stage, postharvest treatment, specifically storage container and length of storage; are a critical factor in controlling these compounds stability. Studies have demonstrated that the permeability of various storage materials to O₂, moisture and light is among key factors determining the increase/decrease rate of oxidation and degradation processes which results in a significant difference in the loss of active compounds over a wide range of storage duration (Versino et al., 2023; Hong et al., 2023). Some recent works indicated that materials having insulating properties or controlled porosity, like clay pots or metal tanks, have dramatic effect in maintaining the value of chemical content compared to materials with minimal barrier (Ibrahim et al., 2024; Fikiru et al., 2025).

The storage period is a composite temporal factor, long-term storage usually contributing to apparent losses in the concentration of phenols, vitamins and capsaicinoids as a result of continuous oxidative processes and loss of antioxidant capacity mainly if temperature or packaging were not controlled (Iqbal et al., 2015; Villa-Rivera et al., 2023).

Therefore, the need of investigating the maturational stage (SP), storage container type and storage period as an integrated system that describes how SP affects the fate of active compounds in dried HP fruit should be emphasized. We wanted to look at this within the environmental conditions of Kirkuk Governorate.

Although there are studies that have evaluated the effect of stage of maturity or storage period by separate, scientific information continues to be scarce with regard to the combined interactive effect between stage of maturity, type of container used in storing and length of storage period –

particularly when stored in clay pots as opposed to metallic containers – on active compounds content and vitamin stability in dried hot pepper fruits under local conditions.

Considering the above, and considering the importance of this plant, this research was carried out with the following objective: to evaluate fruit maturity stage, time of storage and type of storage container on active substance and vitamin content in hot pepper fruits and analyze their interaction. The objective is to find the most suitable preservation method which ensures maximum nutritional and chemical stability of food through storage.

MATERIALS AND METHODS

Field trial The field study was conducted on April 1st, 2024 using chili plants at the research station of the Department of Horticulture and Landscape Gardening, College of Agriculture, University of Kirkuk. The objective was to develop fruits and then add them to a storage study that follows drying. The experiment was conducted in a Complete Randomized Design (CRD) with three replications factors. The first factor was fruit maturity stage, early ripening (A1) and late ripening (A2). The second variable was the material of storage, which included clay containers (B1) and metal containers (B2). The storage time (45 days C1, 90 days C2 and 135 days C3) was the third studied factor.

The storage experiment for the dry and sorted plant fruits started on September 1, 2024. The storage was performed at standard laboratory conditions, with no regulation of temperature and relative humidity, as well as without exposing to direct sunlight as shown in Table (1). The following characteristics were then measured according to the study protocol:

- Total phenols in plant fruits ($\text{mg } 100\text{g}^{-1}$): Measured based on Kingne et al. (2018).
- Total alkaloids in plant fruits (%): Measured according to the method followed by Ajanal et al. (2012).
- Capsaicin compound (mg g^{-1}): The capsaicin content in the fruits was estimated based on Saksit Chanthai et al. (2012).
- Vitamin C (ppm): Vitamin C in hot pepper fruits was estimated according to the method adopted by Sharaa et al. (2019).
- Vitamins (ppm): Vitamins were measured based on Kozhanova et al. (2002). Vitamin B₆ was measured at a wavelength of 290 nm, Vitamin E at 292 nm, and Vitamin A at 450 nm.

Data were statistically analyzed using Genstat 12th ed (Payne et al., 2009 and Al-Qaisi, 2020). The Least Significant Difference (LSD) test was used to determine differences between means at a 0.05 probability level.

Table 1. Temperature of the storage room

Minimum Temperature(°C)	Maximum Temperature (°C)	Period
24.1	40.9	2024/9/1
24.7	42.9	2024/9/9
22.2	38.6	2024/9/16
21.2	37.3	2024/9/25
19.7	31.7	2024/10/4
19	31	2024/10/13
18.3	30.3	2024/10/18
17.6	29.6	2024/10/26
12.5	23.4	2024/11/6
11.6	21.9	2024/11/14
10.6	20.5	2024/11/22
9.7	19.3	2024/11/29
8.3	18.4	2024/12/5
8.2	19.2	2024/12/12
7.6	18.1	2024/12/22
6.5	16.4	2025/1/1
6.9	17.8	2025/1/7
5.2	15.7	2025/1/15

RESULTS AND DISCUSSION:

The results showed significant differences. As seen in the data of Figure No. 1, there were significant effects ($P \leq 0.05$) of fruit maturity stage, storage container type, and storage duration on the total phenol content in hot pepper fruits, in addition to significant two-way and three-way interactions between these factors.

Late maturity (A2) led to an increase in total phenol content by 51.8% compared to the early maturity stage (A1), reflecting an increase in the accumulation of phenolic compounds with the advancement of physiological fruit ripening.

The retention in phenol content was higher in clay (B2) storage containers (10.3 % increase) than in aluminium (B1). This proves the effect of physical properties of clay containers in minimizing the loss of active compounds during storage.

In terms of storage period, the findings indicated that phenol contents decreased progressively and significantly with increasing duration of storage. Total phenol content decreased by 34.8% on increasing the storage period from 45 to 135 days.

For the two-way interactions, phenols of early maturity (A1) significantly decreased in content with storage time from 45 to 135 days, decreasing by as much as 44.6% of minimum level at day-135 compared to day-45. By contrast, it was lower in late maturity (A2) and reached 27.9% for the above-mentioned period. This shows the capacity of late-maturity fruits to retain phenolic compounds even for long storage times.

In the three-way interaction between maturity stage, type of storage container and duration of storage, the control in the medium maturity stored for 45 days (A2B2C1) presented the highest phenol content. This was about 2.8 times the least observed treatment, Early maturity stored in aluminum containers for 135 days (A1B1C3). The integrative effect of these factors on the stability of phenolic compounds during storage was thus confirmed.

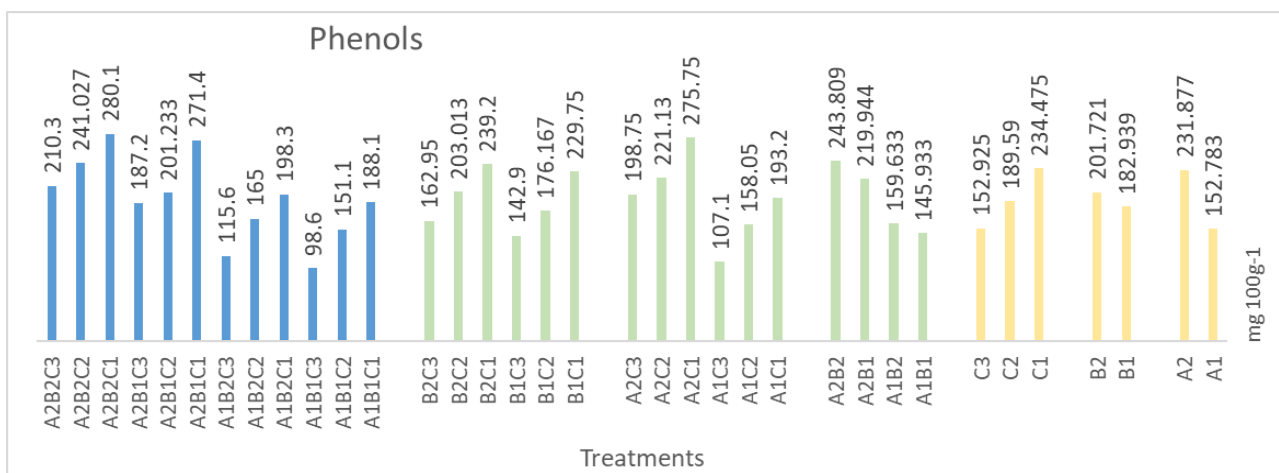


Figure 1. The role of fruit maturity degree, container type, and storage duration on the phenol content of hot pepper plants (mg 100g⁻¹). L.S.D A: 0.1237, B: 0.1237, C: 0.1515, A×B: 0.1749, A×C: 0.2142, B×C: 0.2142, A×B×C: 0.3030

According to the results of Figure No 2, we concluded that there was significance at a level of $P \leq 0.05$ between alkaloids too. Late stemmed (A2) caused a significant alkaloid content increase of ~217% (3.2 fold) as compared to early stemming (A1). This suggests a close relationship between the development of physiological maturity and increase in alkaloid content of the fruits.

The findings also demonstrated the significant ($p < 0.05$) alkaloid retentions of clay pot material (B2) compared to aluminum can material (B1) followed by an increase at 16.8% respectively. This is indicative of the influence of clay vessel properties in minimizing the dissipation of these volatile constituents during storage.

As for storage time, Alkaloid content lowered slowly and significantly with the prolongation of storage. Prolongation of the storage period from 45 to 135 days resulted in a reduction of the total alkaloid amount by 40.5%.

Regarding the two-way interaction, decrease in alkaloid content with increasing storage time was more pronounced in aluminum containers and decreased by 47.3% than that of clay's decrease

(by 33.9%). In addition, the reduction of average ACC with increases storage time in early maturity fruits (A1) had a more pronounced decreasing ratio was 49.1% while late maturity fruits (A2) were reduced by only 37.6%. This suggests the potential of less mature fruits to preserve alkaloid content over longer storage.

As for the interaction between maturity stage, storage containers type and storage period, late mature plants stored in clay pots for 45 days treatment (A2B2C1) had higher alkaloid than the lowest one which was early mature plants stored in aluminium containers at 135 days Burkil et al., 1975) of storage. This supported the integrative and dominant role of these factors affecting alkaloid content in hot pepper fruit after storage.

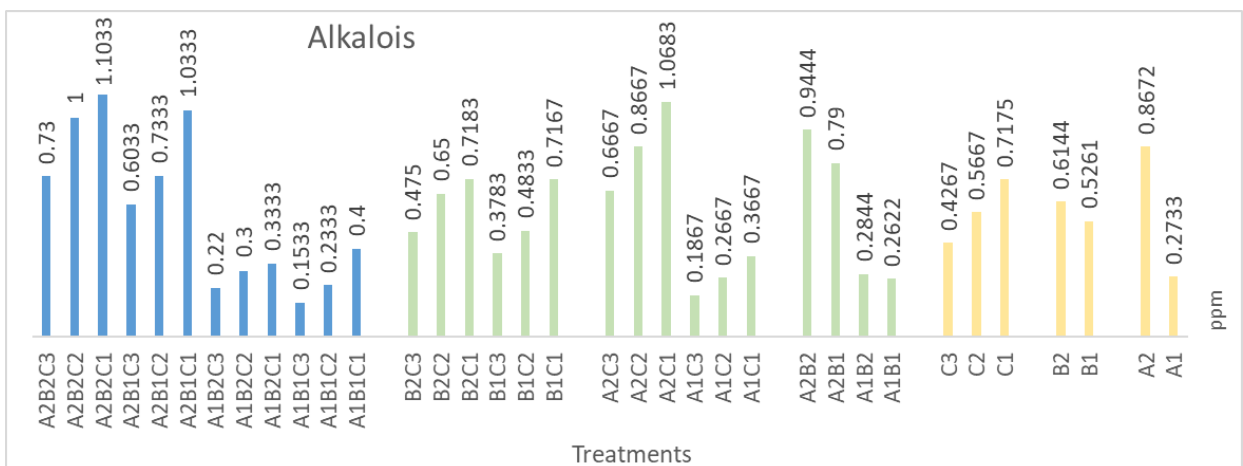


Figure 2. The role of fruit maturity degree, container type, and storage duration on the alkaloid content of hot pepper plants (%). L.S.D A: 0.03167, B: 0.03167, C: 0.03879, A×B: 0.04479, A×C: 0.05486, B×C: 0.05486, A×B×C: 0.07758

For capsaicin, we observe the central effects that Figure No. 3 demonstrates. At late maturity (A2), capsaicin accumulation increased up to 45.8% relative to that of early maturity (A1) indicating the relationship between high capsaicin accumulation and progressive develop in physiological ripening stage of fruits. The clay storage containers (B2) were superior to the metallic bins (B1), and capsaicin recovered was 23.8 g %. This indicates that they are able to better maintain the irritants inside them for storage.

In terms of storage period, the findings revealed a clear and positive effect of long retention on capsaicin. The content, between 45 and 135 days of storage, increased by 63.3%.

The two-way interactions indicated that capsaicin content increased more in late-maturity fruit stored in clay containers and for long storage periods. In this case, the three-way interaction presented for the treatment of late maturity in clay containers with 135 days (A2B2C3) recorded a maximum value of capsaicin content and increased by almost ≈196% compared to that of the lowest treated early maturity in aluminum container during 45 days (A1B1C1). This was an indication of the integrated effect of these factors on increasing the capsaicin content during storage and its

stability.

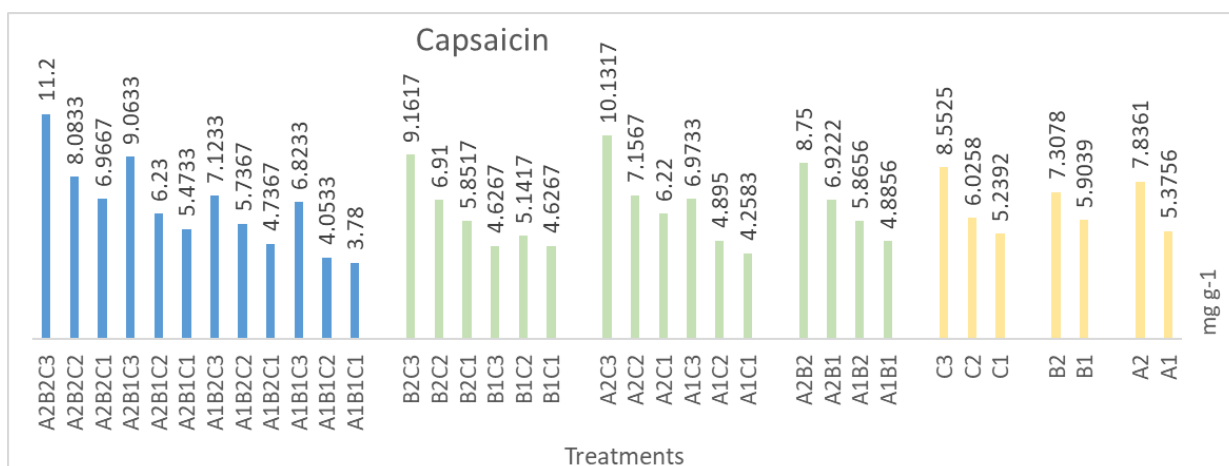


Figure 3. The role of fruit maturity degree, container type, and storage duration on the capsaicin content of hot pepper plants (mg g⁻¹). L.S.D A: 0.02064, B: 0.02064, C: 0.02528, A×B: 0.02919, A×C: 0.03575, B×C: 0.03575, A×B×C: 0.05055

The maturity stage of the fruit, type of storage container and duration of storage had significant effects ($P \leq 0.05$) on the vitamin A content in hot pepper fruits. The content of vitamin A in late maturity (A2) fruit was higher by 65.9% than that at the early stage of maturity (A1). Moreover, the clay containers (B2) showed higher efficiency in vitamin A retention, with an increase of 10.8% as compared to aluminum containers (B1).

As for storage time, the amount of vitamin A was found to have a marked diminution of (12.3%) when the duration of storage increased from 45 to 135 days. The type of storage container and the length of storage duration also showed a marked variation in loss. The values of the aluminum containers (B1) decreased 24.0% after 135 days of storage under conditions similar to those used in the first plateau that was formed with the 45 days, while those made with clay (B2) showed limited variation and did not exceed more than 0.7%.

The responses on two-way interactions indicated significant ($p < 0.05$) benefits of clay containers in both sorghum maturity stages, showing enhancement factors of 14.2% at A1 and 8.8% at A2 relative to aluminum ones. Regarding the triple-interaction among factors, considering the three levels of each factor tested, the greatest value found for vitamin A content was late maturity and stored in clay container and up to 45'days (A2B2C1), which showed an increase of ~135.9% compared with lower values obtained from early maturity and stored into aluminum containers for up to 135 days.(A1B1C3).

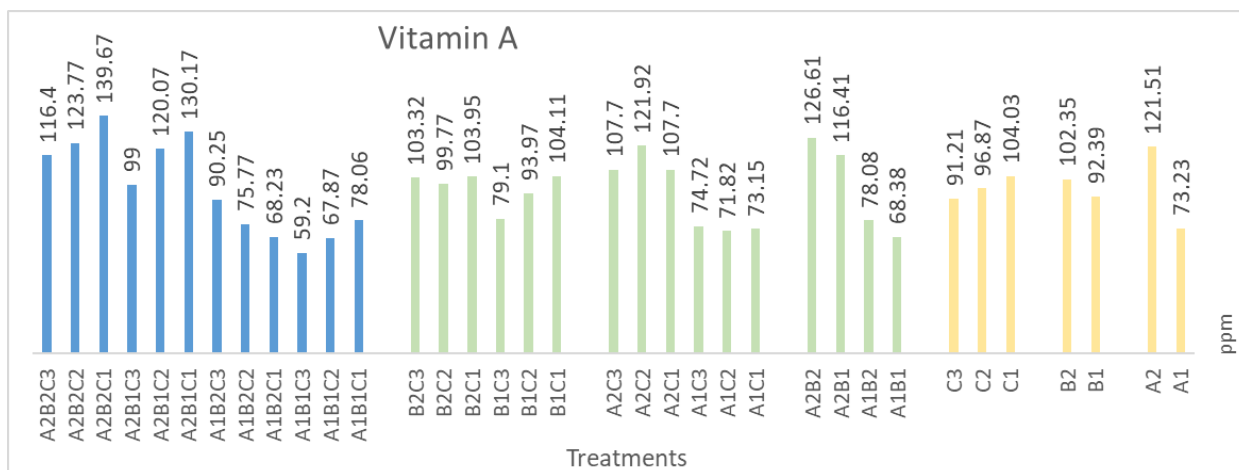


Figure 4. The role of fruit maturity degree, container type, and storage duration on the vitamin A content of hot pepper plants (ppm). L.S.D A: 0.707, B: 0.707, C: 0.866, A×B: 1.000, A×C: 1.225, B×C: 1.225, A×B×C: 1.732

The late maturity stage (A2) increased the vitamin B6 content by 224.4% compared to the early maturity stage (A1). Furthermore, clay containers (B2) outperformed aluminum ones (B1) by 12.4%. (Figure 5)

Regarding storage duration, the results in the figure show a decrease of 38.7% when extending the storage period from 45 to 135 days. The interaction between container type and storage duration revealed that aluminum containers (B1) recorded a decrease of 36.3%, while the decrease in clay containers (B2) was 40.7% for the same period. As for the interaction between maturity stage and container type, clay containers showed an increase of 34.9% in the early maturity stage and 6.1% in the late maturity stage compared to aluminum containers.

In the three-way interaction between factors, the highest value for vitamin B6 content was achieved with the treatment of late maturity stored in clay containers for 45 days (A2B2C1), showing an increase of 587.3% compared to the lowest value recorded for early maturity stored in aluminum containers for 135 days (A1B1C3).

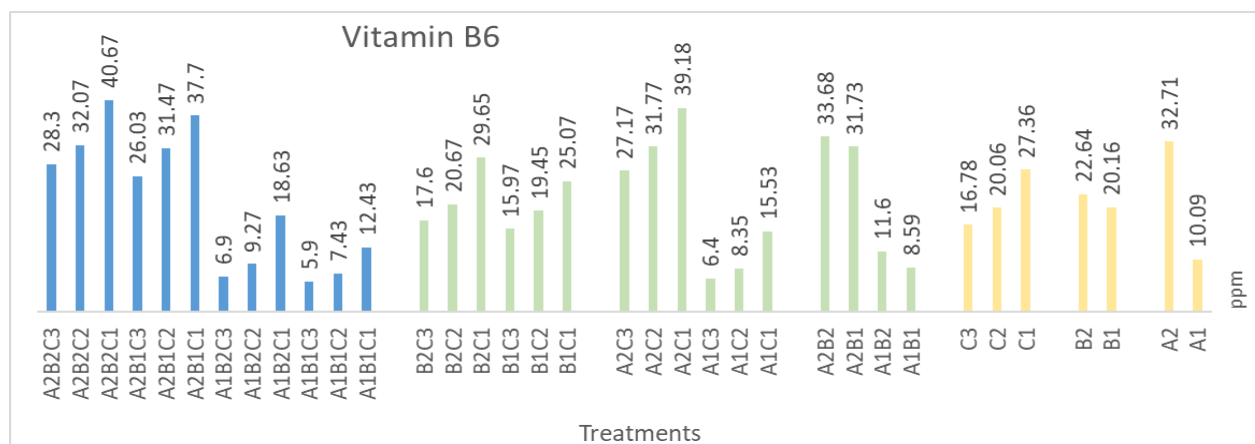


Figure 5. The role of fruit maturity degree, container type, and storage duration on the vitamin B6 content of hot pepper plants (ppm). L.S.D A: 0.471, B: 0.471, C: 0.576, A×B: 0.666, A×C: 0.815, B×C: 0.815, A×B×C: 1.153

The vitamin C content was significantly affected at a probability level of $P \leq 0.05$ by the studied factors. Late maturity (A2) increased the content by 61.6% compared to early maturity (A1), while clay containers (B2) recorded a 7.6% increase compared to aluminum containers (B1).

Regarding storage duration, a decrease of 23.1% was observed when extending the storage period from 45 to 135 days. The interaction between container type and storage duration showed that vitamin C content decreased by 24.5% in aluminum containers (B1) and by 16.2% in clay containers (B2) for the same period. As for the interaction between maturity stage and container type, clay containers showed an increase of 9.1% in the early maturity stage and 6.7% in the late maturity stage compared to aluminum containers.

In the three-way interaction between factors, the highest value was achieved with the treatment of late maturity stored in clay containers for 45 days (A2B2C1), showing an increase of 134.1% compared to the lowest value recorded for early maturity stored in aluminum containers for 135 days (A1B1C3).

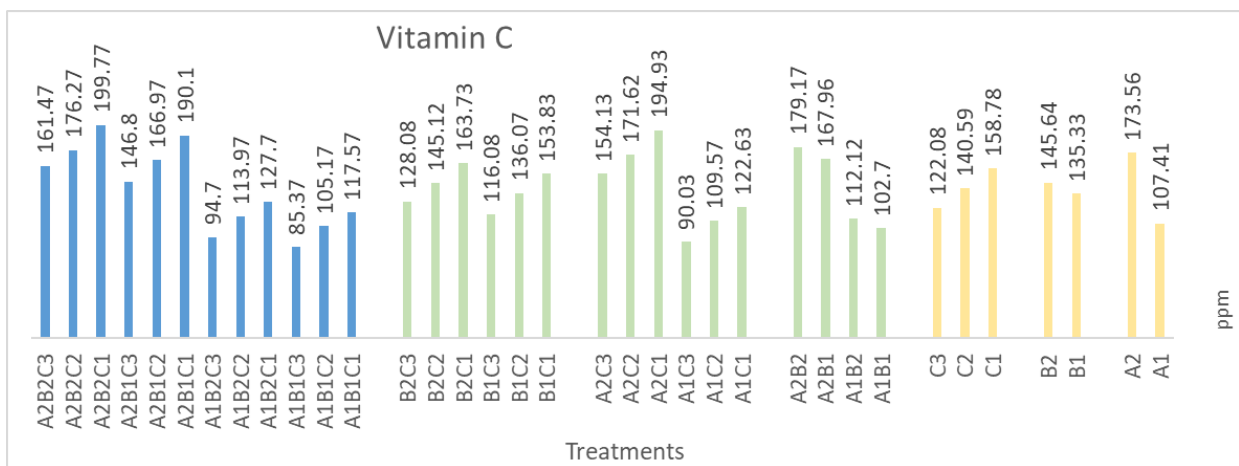


Figure 6. The role of fruit maturity degree, container type, and storage duration on the vitamin C content of hot pepper plants (ppm). L.S.D A: 0.666, B: 0.666, C: 0.816, A×B: 0.942, A×C: 1.154, B×C: 1.154, A×B×C: 1.631

On the other hand, late maturity (A2) recorded a 120.5% higher vitamin E level (Figure 7) compared to early maturity (A1). Clay (B2) containers showed significantly higher increase, attaining 12.6%, over the aluminum (B1) containers.

In terms of storage time, at 45–135 days longer duration, the content of vitamin E decreased by 36.0%. Regarding the interaction between container type and storage time, it was observed a loss of content for aluminum containers (B1) of 39.3%, with values changing from 48.45 to 29.37 ppm, whereas in clay pots (B2) the drop was reduced by only 32.9%, reducing from 52.60 to 35.33 ppm.

Regarding maturity stage x container type, at the early maturity stage clay containers had an increase of 18.7% (27.63 vs 23.28 ppm) and at late maturity stage a 120.9% (58.76 vs 53.49 ppm) compared to aluminum containers. The effect of maturity stage and storage duration was related to

the fact that early mature fruit lost 41.6% vitamin E from 32.87 to 19.22 ppm, whereas late mature fruit lost 33.3%, falling from 68.18 to 45.48 ppm (Table iii).

Between the maturity stages, container types and storage periods was observed a higher value of 69.80 ppm for the treatment (A2B2C1), which was 315.5% compared to the lowest concentration found in the treatment (A1B1C3) with 16.80 ppm

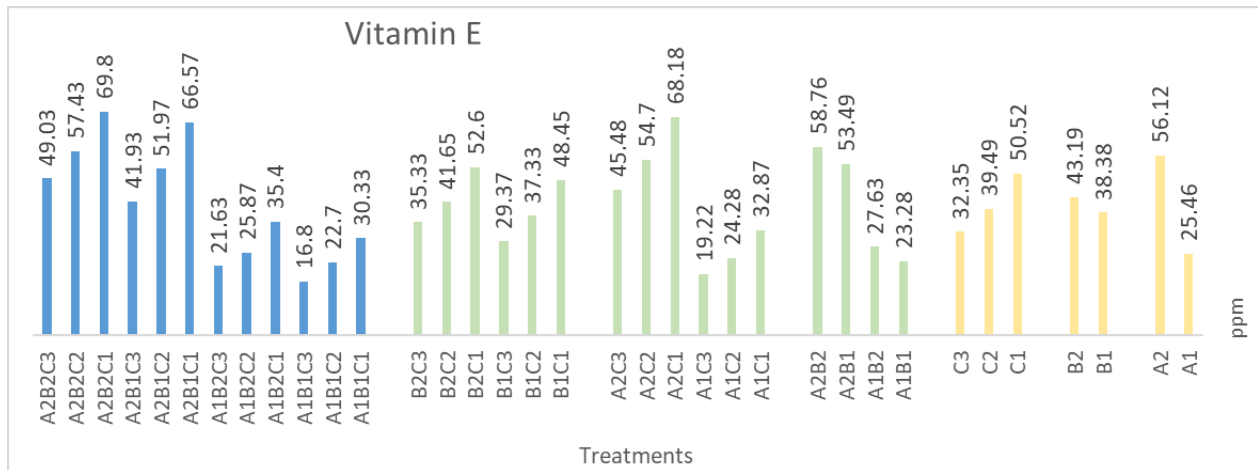


Figure 7. The role of fruit maturity degree, container type, and storage duration on the vitamin E content of hot pepper plants (ppm). L.S.D A: 0.619, B: 0.619, C: 0.759, A×B: 0.876, A×C: 1.073, B×C: 1.073, A×B×C: 1.517

DISCUSSION

The results of this study elucidated that the stage of fruit maturity had highly significant and clear effects on total phenol content in hot pepper fruits; mature red fruits (A2) showed superiority over green fruits (A1) in all treatments. This superiority is due to an enhanced activity of secondary metabolic paths which occur at the late ripening stages and especially of the phenylpropanoid path which gives rise to phenolic compounds, accumulating at higher concentrations in physiologically mature fruit. Recent studies also demonstrated that ripening allows an increase in the antioxidant content by increasing of phenolic compounds and other active molecules (Guijarro-Real et al., 2023; Lahbib et al., 2023). Significant was the storage container also type, being B2 superior to B1. This can be explained by the fact that although oxidation could cause loss of phenols during storage (, 2022), a reduction of exposure to oxygen and light may take place in fruit in clay containers as well(materials section) conceal damaged side.). On the other hand, the longer storage time reduced phenol content over time, due to indirect contact between these compounds and enzymatic and non-enzymatic degradation or oxidation during long periods (Santos et al., 2021). The two-way and three-way interactions were significantly different with interaction (A2×B2×C1) having the highest phenol content. This can be interpreted as to show that the combination of an high degree of maturity and brief storage at least in coins with some kind of containers is seem to be the best preservation condition for these compounds.

For alkaloid levels, maturity phase exhibited the most significant effect, where percent of alkaloids were greater in red fruits than green ones. This is due to the defensive nature of the alkaloids, which accumulate further, once fruits reach their full physiological growth (Kim et al., 2022). The container type helped in maintaining the compounds, although treatment B2 exhibited superior loss reduction during storage. On the other hand, long storage time resulted in a decrease in alkaloids percentage mainly attributed to their chemical instability which may cause the degradation or conversion of these compounds into less effective substances during prolonged storage (Ribes-Moya et al., 2020). A2×B2×C1 exhibited the maximum values (three-way interaction) which indicated that the three factors had combined effect.

Capsaicin was the compound most affected by all factors, according to the content results obtained. Red fruits surpassed green ones, which is explained by the persistence of synthesis and accumulation of capsaicinoids at late stages mainly on placenta where is the main site for these molecules' synthesis (Sora et al., 2021). Capsaicin content retention less in container type B1 but higher value observed with storage interval, while higher capsaicin content was found in container type B2 and it has also been increased slightly with increase of storage time. This is probably owing to the decrease in moisture content of fruit during storage that resulted in the concentration of effective compounds (Estrada et al., 2020). Mix (A2×B2×C3) produced the highest capsaicin content and suggest that its accumulation was increased by aging of fruits under optimal storage conditions after fully mature level.

Results of the present study revealed that levels of vitamins A, B 6, C and E of hot pepper fruits were significantly influenced by fruit maturity stage, storage container type and duration of storage. In all vitamins studied, ripe red (A2) were significantly higher than green fruits (A1). The main reason is that the metabolic activity of the tomato and also deposition of carotenoids, tocopherols, and water-soluble vitamins would increase in fruits at a more advanced ripening stage (Rodriguez-Amaya et al., 2022; Mozafar, 2019; Nisar et al., 2021). The kind of storage can also contribute that the effect is remarkable, as the B2 treatment allowed maintaining higher level of vitamins than B1. It is related to lower contact with oxygen and light leading lower oxidation and degradation reactions (Saini et al., 2021). A gradual reduction in the vitamin content (especially Vitamin C, highly susceptible to oxidation, and instability under extended storage) occurred with increasing storage time (Davey et al., 2021). Interactions between the two- and three-way were found to be highly significant, with interaction (A2×B2×C1) showing highest values for majority of the vitamins. This validates that mature fruits kept in a short time for suitable containers have the best conditions to preserve hot pepper fruits nutritional value.

CONCLUSIONS:

The present study showed that fruit maturity stage, storage container type, and storage duration significantly affect the content and stability of active compounds and vitamins in hot pepper fruits (*Capsicum frutescens* L.). Mature red fruits recorded higher levels of phenols, alkaloids, capsaicin, and vitamins compared to green fruits. Clay pots were more efficient than aluminum in preserving these compounds. Short-term storage (45 days) maintained the highest levels of most active compounds, and mature fruits stored in clay pots for 45 days achieved the best retention levels. Harvesting at full maturity and storage in suitable containers for short periods can enhance the nutritional and functional value, preserve active compounds, and maintain overall fruit quality.

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