# Bioactive Components and Antioxidant Activity of Moroccan Paprika (Capsicum Annuum L.) under Different Storage Time and Conditions

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**Abstract:** The effects of the storage and drying process on active total polyphenols and flavonoid concentration, as well the antioxidant capacity of a Moroccan red pepper cultivar (Capsicum Annuum L.) were investigated. The concentrations of total phenolic compounds, flavonoids, and the extractable red color measured in the units of the American Spice Trade Association (ASTA) varied significantly with temperature, moisture content ( $4 \% / 28 \bullet C$ ,  $7 \% / 28 \bullet C$ ,  $12 \% / 28 \bullet C$ ,  $4 \% / 45 \bullet C$ ,  $7 \% / 45 \bullet C$ ,  $12 \% / 40 \pm 12 \%$ ,  $12 \% / 40 \pm 12 \%$ ,  $12 \% / 40 \pm 12 \%$ , 12

Keywords: bioactive compounds, Capsicum Anuum, antioxidant activity, drying process, flavonoids, total polyphenols content, antioxidant activity.

#### 1. Introduction

Pepper, specifically Capsicum Annuum L. is the general name for plants coming from Capsicum species of Solanaceae family, native to southern North America and northern South America. In Morocco, sweet pepper called called bell pepper or pimento « niorais" grown in the eastern part of the country [1], [2]. The main area of production is the Tadla region with more than 80% of national production [3] [4]. The red bell pepper (*Capsicum Annuum L*.) is in high commercial demand by the global food industry, based on its aromatic, coloring, and flavoring properties [5]. Red sweet and fresh peppers are an excellent source of vitamin C and antioxidants including flavonoids, phenolic acids, ascorbic acid and carotenoids [6]. These compounds show potential action against certain cancers, stimulate the immune system, prevent cardiovascular diseases and protect against agerelated macular degeneration. Some studies have addressed the influence of drying conditions on the quality characteristics of the dehydrated product [7],[8] determined the evolution of the carotenoid content during the dehydration process of red pepper fruits (Capsicum Annuum L.). Conventional drying of pepper causes a major loss of colorant texture quality of the final product. Undesirable changes in the color may lead to a decrease in its quality and marketing value. Loss of red color is caused by autoxidation of carotenoids. The stability of the main carotenoids of thered bell pepper during storage has been shown to depend on the drying conditions. Drying and storage conditions, particularly temperature and light, lead to vegetable modifications that can cause quality degradation. The degree of polyphenols degradation depends very much on the processing time and the size of the vegetables [9]. It is well known that polyphenol compounds are highly responsible for the health effects derived from consumption of plant origin food. They play a key role as antioxidants due to the presence of hydroxyl substituent and their aromatic structure which enables them to scavenge free radicals. Attention has been drawn to red sweet pepper and herbs due to numerous health-promoting properties of the products [10]. Peppers, such as habanero, cayenne, jalapeño and serrano, contain phenolics (flavonoids) [11] and carotenoids [12] and vitamin C, vitamin E,  $\beta$  carotene and carotenoid pigments content [13]. These compound play important roles in human health. In other studies, antioxidant activities in peppers were measured by radical-scavenging activity [14],[15], carotenoids Capsaicinoids and exhibit anticancer [16], [17], [19] [20]. Flavonoids have been shown to act as antioxidants, and they possess anti-inflammatory [21],[22]. It is reported that the stability of the quality of paprika during storage is dependent on drying conditions and the degradation rate of quality increases as the drying temperature increases. In view of the importance of this spice product, standardization of processing protocol is necessary [23]. Dehydration is an important preservation process which reduces water activity through the decrease of

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water content, avoiding potential deterioration and contamination during long storage periods [24]. However, the sudden removal of water decreases the nutritional and sensorial value of food and allows the presence of phenomena such as hardening and shrinkage [25].

Then in this subject, the aims of this study consiste to evaluate the effect of drying processes was evaluated by measuring total polyphenol content (Folin-Ciocalteu assay), flavonoids, ASTA colorant capacity and the reducing-power of the plant material towards DPPH stable free radical. Experimental design of drying processes established the optimal method and conditions of drving, thereby maximizing the antioxidant potential in function of the plant material and drying process characteristics. It is important to determine how the different environments and storage conditions could affect the quality of pepper destined for industry. The objective of this work was to analyze the effect of different sites and storage conditions on the quality of fresh pepper destined for industry at harvest and during storage. This study was aimed to evaluate the antioxidant activity and polyphenolic compounds from red pepper fruit during the storage conditions.

## 2. Materials and Methods

### 2.1 Sampling

The Moroccan red pepper fruits (*Capsicum Annuum L.*) were harvested at full maturity during September and October 2012 in the Tadla Azilal Region (Ouelad Ali, Fkih Ben Saleh). The red sweet pepper, are ground after the drying process and packed in food bags in order to protect them from light. Some aliquots were stored at -18 °C and others at 25°C until analysis.

#### **2.2 Total Phenolic Content**

For quantification of total polyphenol content, the Folin-Ciocalteu's method was used [26]. A volume of 0.5 ml of Folin-Ciocalteu's reagent was added to a dark flask, containing 0.5 ml of the diluted extract (1/10) methanolic solution (80%) of each sample. After 5 minutes, 8 ml of a 7.5% aqueous sodium carbonate solution was added to the mixture and the content was mixed thoroughly. The samples were kept in dark for 2hours and then the absorbance was measured at 765 nm with spectra physic Jasco UV/vis spectrophotometer. Three parallel samples were analyzed. Gallic acid was used for constructing the standard curve. Concentration range of gallic acid was of 0.05-0.5 mg/ml (A765 nm = 2, 1169 [GA]-0,0831). The results of total polyphenol content were expressed as mg of gallic acid equivalents per ml of sample (mg GAE/ml) [4].

#### 2.3 Flavonoids contents

The total flavonoids content of paprika samples were estimated using the aluminum chloride colorimetric method [27]. 1ml of the ethanolic solution of AlCl3 (2%) was added to 1ml of powder paprika. After incubation at ambient temperature, the absorbance was read at 420nm. The flavonoids content was expressed in terms of quercetin of mg EQ/per g of paprika extract.

#### 2.4 Determination of ASTA color values

The color value ASTA (American Spice Trade Association) of paprika powder is determined according to AOAC method [28],[29] with a slight modification. 0,1g sample was added to 20ml of acetone absolute and incubation in water bath for 3 hours at 25°C. The diluted extract (1/5 v with acetone) measured at 460nm using a spectrophotometer spectra physic JASCO. The ASTA color value was calculated using Eq.(1) below, where A: absorbance of the acetone extract; I: instrument correction factor calculated from a pattern solution of potassium dichromate. **Eq.(1)** : (Abs<sub>1</sub>-Abs<sub>2</sub>)/Abs<sub>1</sub>x100.

### 2.5 DPPH Antioxidant activity



**Figure 1:** chemical structure of DPPH scavening (2,2 diphenyl -1-Picryl-Hydrazyl)

Antioxidant activity was evaluated by measuring the radical scavenging effect of dried peppers' methanolic extracts towards the 2,2-diphenyl-1-picrylhydracyl (DPPH') as reported previously by [30], [31]. 5mL of a 0.1 ml methanol solution of DPPH (Aldrich) were added to 0.1 ml of several concentrations of methanol extracts of fresh and dried pepper samples. The tubes were allowed to stand at 27°C for 20 minutes. The absorbance at 517 nm was recorded in a spectrophotometer (Spectraphysic Jasco UV-vis spectrophotometer 1240). Radical scavenging activity was expressed as inhibition percentage and was calculated using the following formula: Percent radical scavenging activity = control OD – sample OD control OD  $\times$  100.

## 2.6 Statistical Analysis

The different parameters were analyzed by ANOVA and Duncan's [32] multiple-range test using SPSS (Version 13.0). Differences were considered statistically significant if the probability was greater than 99% (p value > 0,001) and p value> 0,05, the difference was not significant, (\*) 0,05 > p value > 0,01= is the least difference significant with 5% level (\*\*) p value > 0,001 = the difference is significant at 1% and (\*\*\*) p < 0,0001 the difference is highly significant difference at 1‰ level. All assays were performed by triplicate at temperature  $\pm 1.0$ . The means Mean <sup>(abcd)</sup> was affected with different letters, for the same factor of variation, are significantly different at 5% level

N : effective ; E.T. : Variance(\*\*), (\*\*\*) : the difference significantly respectively at 1% and 1‰

# 3. Results

The main value of the dry matter and the ash content are shown in table1. Moisture content and ash content are within the ranges (91,1%, 7,3%, respectively), results that are similar to those reported [33]. However, the amount of ash

content found in this work was higher than those reported by [34].

 Table 1: Dry matter content and ash content of paprika

(Capsicum A	nuum L	) powde	r studied
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Parameter	Quantity in %
Dry Matter	91,1
Ash content	7,3

These results were probably due to the soil and climatic factors and the diversity of pepper cultivars. The ash content was greater as ripening of the fruit progressed due to the greater degradation or biosynthesis of the polysaccharides and the accumulation of sugars.



**Figure 1:** Effect of the photo-periodicity and darkness conditions on the color value of paprika with means comparison (ab), P<0,0001\*\*\* (Test of Duncan).

The color value was significantly (P<0, 0001) affected by darkness and photo-periodicity (figure.1). Both samples have decreased significantly at 5% level (ab) for the dye color during the three months of storage. Those stored under the effect of photo-periodicity have recorded a greater reduction of color dye than those stored in dark.

During the storage period, highly significant decreases of color value occurred in paprika (P<0,0001).All samples stored at 4, 28 and 45°C have been decreased significantly with 5% level (abc) during three months of storage. These color value losses occurred while the temperature and storage time increased. This may be due to the formation of brown pigments by Mallard reaction. Statistical analysis was showed that interaction time–temperature factor had significant effect P<0,0001. Similar results were reported by [6],[35] when studying changes in the color values of strawberry jams stored at  $4^{\circ}$ C and  $20^{\circ}$ C.

At 28°C the samples with a moisture content of 4, 7 and 12% respectively (figure.3) had a highly significant decrease (P<0,0001)at the 5% level of the coloring power during storage. But the most important decrease of the coloring power is observed for the sample with moisture content of 7 and 12%. The loss in coloring power was related to non-enzymatic browning and carotenoid loss occurring during drying, which are major causes for the color degradation in

the red pepper [8]. In the previous studies, the color values were significantly influenced by the processing time, temperature, water activity and the color changes during processing and storage [36].



**Figure 2** : Effect of thermic on the paprika color value with means comparison (abc), P<0,0001\*\*\* (Test of Duncan)

The results allow us to observe highly significant decreases (P<0,0001) (figure.4). The samples with a moisture content of 4, 7 and 12% have all recorded a significant decrease at 5% of coloring power during the three months of storage, especially for those with 7% and 12% water content. The color value was greatly affected by storage moisture content 7% than 12% at 45°C.

The study shows that ASTRA value is significantly modified (P<0,05) depending of the cultivar, drying methods and storage period [37],[38],[39]. Effect of cooking on the antioxidant properties of colored peppers. The coloring power was significantly affected (P<0,0001) by the conditions of storage. The decrease in color for the samples stored in darkness at 4°C is less important than those humidified and stored at 28°C and at 45°C. A change in the rate of loss of paprika is observed from a temperature of 15°C. The lower degradation of carotenoids actually produces a water activity of 0,108 [40] such us heat treatment could obviously lead to a change in the original composition of the molecules of interest [41],[38], because the moisture content and storage temperature have been recognized as the most important parameters affecting stability of the powder during storage. A temperature below 25 °C, and a low water activity value are recommended for keeping a stable color of red chili powder [42]. In comparison with others compounds, other studies showed that the ascorbic acid reduces the quinones back to the phenolic acids, but during this reduction it is irreversibly oxidized to dehydroascorbic acid, thus allowing browning to occur upon its depletion. More stable forms of ascorbic acid derivatives, such as erythrobic acid 2- and 3-phosphate derivatives of ascorbic acid, phosphinate esters of ascorbic acid, and ascorbyl-6-fattyacid esters of ascorbic acid, have

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however been developed to overcome these problems [43].



Figure 3: Effect of the moisture content at 28°C on the color value of paprika with means comparison (acd), P<0,0001\*\*\* (Test of Duncan)

Phenolic compounds are the principal antioxidant constituents of natural products and are composed of phenolic acids and flavonoids, which are potent radical terminators [44], [45]. Our results showed that total phenolics content was dependent on storage conditions [46]. This may be attributed to the inactivation of the polyphenol oxidase enzyme during heating, leading to the inhibition of polyphenols degradation [47].

The autoxidation of polyphenolic compounds may be responsible for the pro-oxidant effects observed sometimes, especially during test involving antioxidant metal generators of oxidative stress. Statistical analysis shows that the storage under the effect of darkness and the photo-periodicity (figure.5) causes a highly significant (p < 0.0001) change in concentration during the three months of storage. The concentration of total polyphenolic of two samples has been reduced significantly at 5% level during the storage. Those stored under the effect of the photo-periodicity presented the most important regression. The role of phenols as antioxidants is supported by research and the recovery methods have a great importance for industrial use. It reported [48] that a strong relationship between total phenolic content and antioxidant activity in fresh fruits.



Figure 4 : Effect of the moisture content on the paprika color value at 45°C with means comparison (bde), P<0,0001\*\*\* (Test of Duncan)



Figure 5: Effect of darkness and photoperiodicity on total phenolic content of paprika with means comparison (ab), P <0,0001\*\*\* (Test de Duncan)





Figures 5 and 6 summarize the data for the effect of temperature on raw peppers and its changes after storage. The results present a significant correlation (p < 0.0001) between the losses in polyphenolic content and the effect of storage temperature.

According to these results, the loss of total phenolic compound may be due to the greater PPO activity in red peppers during the first days of storage, which is probably due to a greater enzyme affinity for the specific phenols (substrates) present in red fruits (Polyphenol oxidase, total phenolic and ascorbic acid) and changes during storage of minimally processed sweet peppers [49].



**Figure 7**: Effect of moisture content on the polyphenolic total of paprika at 28°C with means comparison (bdc), P<0,0001\*\*\*(Test de Duncan)

The concentration of total polyphenolic content in samples with moisture content of 4, 7 and 12% presented a highly significant decrease (P < 0,0001)at 5% level (figure 7, 8) during the three-month storage period. The samples recorded 7% decrease of total polyphenolic. The concentration of total polyphenolic is influenced by the conditions and storage period of paprika.

Comparatively, the initial concentration of  $10 \pm 0,013$  mg EAG/g of sample has undergone significant degradation (p< 0.0001) after three months of storage, because there is significant loss of total polyphenolic at 5% depending on storage conditions. The degradation is smaller in samples stored at 4°C followed by the sample stored in darkness and also the sample stored at 45°C. However, the hydrations of paprika cause a significant degradation of its phenolic compounds. Many works reported that the moisture of food and the temperature of storage affect the physicochemical and microbiological quality of food [50], [51].

The flavonoids are measured during a period of 12 weeks under different factors such as photoperiodicity, darkness, temperature and moisture content. The concentration of flavonoids at  $t_0$  is 0, 07 ±0, 0005 mg EQ/mg in comparison to the others samples at 0,05 for photoperiodicity conditions and 0,055mg Eq quercetin/g dry extract. The concentration of total polyphenolic content in samples with moisture

content of 4, 7 and 12% was highly significant (P < 0, 0001) at 5% level (figure.8) during the three months of storage. The samples recorded a 7% decrease of total polyphenolic. The concentration of total polyphenolic was influenced by the conditions and storage period of red pepper.



**Figure8** : Effect of moisture content on the polyphenolic total of power paprika at 45°C with means comparison (adc),  $P < 0,0001^{***}$  (*Test of Duncan*)



**Figure 9:** Effect of darkness and photoperiodicity conditions on flavonoids content of paprika with means comparison (ab), *P*<0,0011\*\* (*Test of Duncan*)

From the results (figure.9), the decrease of flavonoids concentration is highly significant (p < 0.0011) at 5% level, especially for two samples stored during the three months. However those stored under photoperiodicity conditions presented a very remarkable decrease of flavonoids concentration during three months of storage.





**Figure 10:** Thermal effect on flavonoïds content of paprika with means comparison (abb), *P* <0, 0001\*\*\* (*Test of Duncan*)

From the result (figure 10), the decrease of the flavonoids concentration is highly significant (p< 0.0001) at 5% level. The temperature factor has a significant influence on the flavonoids concentration losses. The samples stored at 28°C and at 45°C showed a bigger decrease during the three months of storage. These were observed in other studies which showed the effect of the longer drying time required during microwave drying at low output and convective heat transfer style. High temperatures involved in oven drying might lead to reductions in the redness of the samples. The other studies reported that color degradation of tomato was less severe when the drying temperature was lowered from 90 to 55°C [52].



Figure 11: Effect of the moisture content on the flavonoids of paprika at 28°C with means comparison (bcc), P < 0.0024 \*\* (Test of Duncan)

The figure 11 shows a significant decrease (p <0.0024) of flavonoids concentration for all samples with water content of 4%, 7% and 12% during the 12 weeks of storage. The sample with water content 7% presented the highest decrease for the 12 weeks of storage.



**Figure 12**: Effect of the moisture content at 45°C on flavonoids of paprika with means comparison (acc), P < 0.0024 \*\* (Test of Duncan)

The flavonoids concentration is decreased significantly (p < p0.0024) (figure 12) for all samples with moisture content of 4, 7 and 12%. The most noticable decrease in flavonoids concentration is obtained from samples with a water content of7%. The reactivity of flavonoids gives these molecules instability in different environmental conditions. The result showed that the flavonoids content in terms of the ethanol extracts of quercetin equivalent (EQ) is influenced by the conditions and duration of storage. Flavonoids were significantly reduced (p< 0.0011, p < 0.0001, p< 0.0024) over time with a significant difference at the 5% level, especially for hydrated samples showed a significant decrease during the three months of storage. At the same time we observed that flavonoids stored at 4°C in the dark are better than those stored under other conditions. Paprika is a food product that has considerable nutritional importance. In addition to the fat, fiber, carbohydrate, paprika contains vitamin C, carotene, unstable nutriment which must be preserved during storage to ensure product quality. Paprika stored in the dark at 4°C, is better preserved during a storage period of three months compared to the other conditions tested. The previously studies showed that the quality degradation in terms of color indices and antioxidant activity of bell-pepper slices undergoing sun, oven and microwave drying [53].

DPPH scavenging activity: DPPH forms a stable molecule on accepting an electron or a hydrogen atom and thus has applications in the determination of radical scavenging activity of natural products. In situ, free radicals like polyaromatic hydrocarbon cations have been linked with carcinogenesis. Thus, products that will scavenge DPPH in vitro may also scavenge polyaromatic hydrocarbon cations in vivo [54].

The antioxidant power of the paprika has been evaluated by the DPPH method. A methanol solution of DPPH•(2-2diphényl-1-picrylhydrazyl) has a dark violet color in the presence of an antioxidant, the reduced form of DPPH-H gives the solution a yellow color and therefore a decrease

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absorbance The color change of DPPH methanolic extract in the presence of each of the test products was measured at 517nm. The results presented in the figure 13 described the percentage of antioxidant capacity of paprika for free radical DPPH. The high potential of polyphenols to scavenge free radicals is due to their phenolic hydroxyl groups [55].



**Figure 13:** Effect of darkness and photoperiodicity on the antioxidant capacity of paprika with means comparison (ab), P < 0.0104\* (*Test of Duncan*)

DPPH was commonly used for evaluating the radical scavenging activity. The radical scavenging activity of the paprika extracts was analyzed by the DPPH assay, and the results were presented as %. The powder of red pepper has an antioxidant 45.25% power but during storage the initial value deteriorates (figure.13). The results showed that the antioxidant power reductions are less significant (p<0.0104) for both samples at the 5% level. The antioxidant power of samples due to photoperiodicity experienced a remarkable decrease.



**Figure 14**: Thermal effect on the antioxidant capacity of Paprika with means comparison (abc), *P* <0, 0001\*\*\* (*Test of Duncan*)

The antioxidant capacity of samples reported a highly significant reduction (p < 0.0001) with a significant difference at the 5% level (figure.14). We can state that there

is a positive and significant relationship between the decrease in antioxidant capacity and the storage temperature correlation. This correlation is more significant and positive for the samples stored at 28°C. In other published works it was demonstrated that technological procedures to which vegetables are subjected prior to their consumption may affect qualitative and quantitative changes in their chemical compositions. Similarly, the polyphenolic compounds and their antioxidant activity are strongly dependent on the technological treatments and it is expected that hydrothermal processes contribute to their losses. Knowledge of the factors and their impact on the content of phenolic compounds in vegetables may lead to the selection of technological processes which allow the most optimal retention of these components[56].



**Figure 15**: Effect of moisture at 28°C on the antioxidant capacity of Paprika with means comparison (abc), *P* <0, 0019\*\* (*Test of Duncan*)

DPPH radical scavenging capacities of the dried peppers at 28°C were in the range of 32–37%. The drying oven (28°C) with a moisture content of 4% peppers gave the highest DPPH radical scavenging activity.



**Figure 16**: Effect of moisture at 45°C on the antioxidant capacity of Paprika with means comparison (abc), *P* <0, 0019\*\* (*Test of Duncan*)

The antioxidant capacity of samples with water content of 4, 7 and 12% experienced a significant decrease (P < 0,0019) of the antioxidant capacity at the 5% level (figure 15 and 16). Those with water content of 7% recorded the most

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significant deterioration of antioxidant capacity. The high temperature, 45°C, did not negatively affect the antioxidant activity of pepper during oven drying. Similar results have been reported by various researchers such as [57] who reported a significant increase in antioxidant activity of apricots from Cafona variety after air drying at 75°C. In other works, it was reported an improved antioxidant activity of the red curry powder after thermal treatment [58], [59] and that sun-drying cause significant increases in the antioxidant properties of the green leafy vegetables [60]. However, it was mentioned that the cooking affect highly the color of pepper as reported by [39].

It was previously reported that long dehydration times together with high temperatures [8] lead to poor quality products due to caramelisation, Maillard reactions, enzymatic reactions, pigment degradation and l-ascorbic acid oxidation [60].

The sensory evaluation assessed by photosensitive molecular of minimally processed sweet pepper through the 12 weeks storage period, showed a good correspondence with changes of tested parameters. In addition to texture modifications, due to synergistic action of pectinase enzymes which also influenced the overall acceptance, the color changes on a weekly basis confirmed a higher potential suitability to storage of red pepper [61]. reported that modified drying, which is short time and low temperature drying of cut red pepper pods, was certainly more effective than conventional drying in reducing the destruction of the antioxidant activity, ascorbic acid and color.

## 4. Conclusion

The results of our study supplied detailed information regarding the physicochemical of red peppers (*Capsicum Anuum L*). It has been demonstrated the effect of thermal processing on the antioxidant capacity of flavonoids. The flavonoids are thermal sensitive. In addition, their antioxidant capacity varies but not proportional to the degradation of the flavonoids. Degradation products flavonoids play an important role in the biological properties of the solution after heat treatment. A storage at 28°C and 45°C induced changes on the physico-chemical parameters of pepper *Capsicum Anuum L*, but not as important as a storage at 4°C

The results showed a significant decrease (P<0.0019) of the antioxidant capacity of stored samples during storage with a significant difference at 5% level. The decrease in the antioxidant capacity of the samples with a moisture content of 7% is more important than those with a moisture content of 4% and 12%. Consequently, the antioxidant capacity of samples is influenced by the storage conditions compared to the initial value of the antioxidant capacity of paprika 45, 25 % had a significant decrease (p< 0.0104, p < 0.0001, p<0.0019) over time with a significant difference at the 5% level. In conclusion the decrease is less important for the samples stored at a temperature of 4°C in the darkness conditions against by decrease are important for the hydrated samples and stored at 28°C and 45°C under light exposition. Certainly, some additional agronomic, cultivating, technological, microbiological, physico-chemical and enzymatic factors (e.g. role of pectinases in product softening) could have a role in keeping the quality of freshcut sweet pepper. Knowledge of the factors of storage and their impact on the content of phenolic compounds, and antioxidant activity scavenging in red pepper may lead to the selection of technological processes drying and storage conditions, which allow the most optimal retention of these components. The information obtained in the present study finds its practical application because it showed the behavior of the jam during the period separating the production from the consumption. The results shown in this work may open new perspectives in the study of pigment stability. It would be interesting to investigate the possible influence of carotenoid content on capscisine and capsaicinoid stability on the one hand, and the other hand, our results would contribute to the controversial literature about the impact of technological process on healthy compounds in food and to promote the consumption of these two traditional bell pepper

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