



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 antioxidant capacity, varied significantly with temperature, moisture content (4 % / 28 ° C , 7 % / 28 ° C , 12 % / 28 ° C , 4 % / 45 ° C , 7 % / 45 ° C , 12 % / 45 ° C), and light exposure treatments. Drying in a hot-air oven induced a significant loss of epicatechin, cyanidin -3-O- galactoside, phloridzin and quercetin glycosides concentrations. Vacuum-drying red peppers at different temperatures, ranging from 20 to 45° C, had no significant effect on concentration of all phenolic compounds compared to the conventional drying method (28-45 ° C). The antioxidant activity was proportional to the samples' moisture and decreased initially from 45.25% to 35% and 28% at a humidity level of 4% and 12%, respectively. Flavonoid concentrations were sensitive to thermal processing. Flavonoid rates were reduced significantly ($p < 0.0024$) under all thermal conditions at both 4% and 12% humidity. Light exposure had significant effect on red pepper bioactive compounds. Light-exposed samples recorded lower total polyphenol concentrations, flavonoids, and antioxidant activity compared to those stored in darkness. Compared to other drying methods, hot-air oven drying resulted in a significant reduction in antioxidant capacity measured in terms of the absorption capacity of oxygen radicals. As expected, different storage conditions affected the concentration of few bioactive compounds. However, under appropriate storage conditions, the Moroccan red pepper cultivar showed promising future use in the agro-industry.

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

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 niorais grown in the eastern part of the country Slassi Moutabir, (1987). The main area of production is the Tadla region with more than 80% of national production Hakmaoui et al. (2011); Zaki et al. (2013). 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 Vega-Galvez et al. (2008). Red sweet and fresh peppers are an excellent source of

vitamin C and antioxidants including flavonoids, phenolic acids, ascorbic acid and carotenoids Castro et al. (2008). These compounds show potential action against certain cancers, stimulate the immune system, prevent cardiovascular diseases and protect against age-related macular degeneration. Some studies have addressed the influence of drying conditions on the quality characteristics of the dehydrated product Simal et al. (2005). Perez-Galvez et al. (2005) 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 color and 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 the red 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 polyphenol degradation depends very much on the processing time and the size of the vegetables (Ewald, Fjellkner-Modig, Johnson, Sjöholm, and Akesson (1999). 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 substituents 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 rich in bioactive compounds as reported by Ayaprakasha, G., Rao, L.J.M, (2011). 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 Ramesh et al, (2001). The effect of drying processes was evaluated by measuring total polyphenol content (Folin-Ciocalteu assay), flavonoids, 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 drying, 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.

Materials and Methods

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) north of Marrakech. The dried red sweet pepper, parsley and lovage were 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.

Total phenolic content:

For quantification of total polyphenol content, the Folin-Ciocalteu's method was used (Singleton et al. (1999). 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 2 hours and then the absorbance was measured at 765 nm with spectra physic Jasco 430 UV/vis spectrophotometer, France Instrument. 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 ($A_{765\text{ nm}} = 2,1169 [\text{GA}] - 0,0831$). The results of total polyphenol content were expressed as mg of gallic acid equivalents per ml of sample (mg GAE/ml), Zaki et al. (2013)

Flavonoids contents:

The total flavonoids content of paprika samples were estimated using the aluminum chloride colorimetric method Lee et al (1995) with slight modification. 1 ml of the ethanolic solution of AlCl_3 (2%) was added to 1 ml of powder paprika. After incubation at ambient temperature, the absorbance was read at 420 nm. The flavonoids content was expressed in terms of quercetin of mg EQ/per g of paprika extract.

DPPH Antioxidant activity

Antioxidant activity was evaluated by measuring the radical scavenging effect of dried peppers' methanolic extracts towards the 2,2-diphenyl-1-picrylhydrazyl (DPPH*) as reported previously by Sanchez et al, (1998) and Scherer (2009). 5 mL 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 430, France). Radical scavenging activity was expressed as inhibition percentage and was calculated using the following formula: Percent radical scavenging activity = $\frac{\text{control OD} - \text{sample OD}}{\text{control OD}} \times 100$.

Statistical analysis:

The different parameters were analyzed by ANOVA and Duncan's (1955) 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 ± 1 0. : The means Mean ^(abcd) 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%

Results

Phenolic compounds are the principal antioxidant constituents of natural products and are composed of phenolic acids and flavonoids, which are potent radical terminators Kahkonen et al. (1999) ;Shahidi, (1992). Our results showed that total phenolics content was dependent on storage conditions (Mahmood Ghasemnezhad et al. (2011).This may be attributed to the inactivation of the polyphenol oxidase enzyme during heating, leading to the inhibition of polyphenols degradation Yamaguchi et al. (2003).

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.1) 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. Velioglu et al. (1998) reported a strong relationship between total phenolic content and antioxidant activity in fresh fruits.

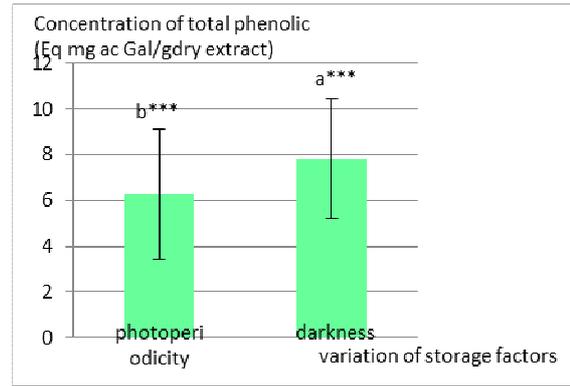


Figure. 1. Effect of darkness and photoperiodicity on total phenolic content of paprika with means comparison (ab), $P < 0,0001$ *** (Test de Duncan)

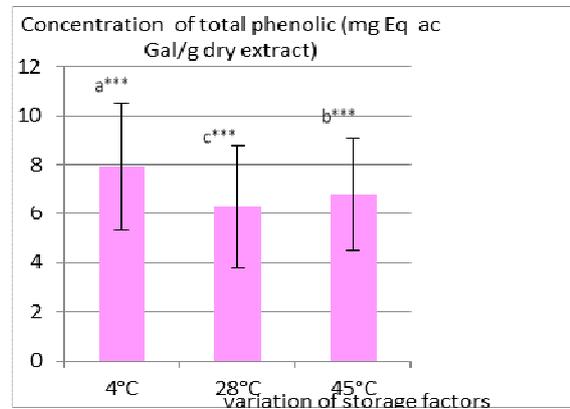


Figure 2: Effect of thermal condition on the total polyphenolic of the paprika with means comparison (abc), $P < 0,0001$ *** (Test of Duncan)

Figures 1 and 2 illustrate 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 phenolics and ascorbic acid) and changes during storage of minimally processed sweet peppers Riccardo et al. (2012).

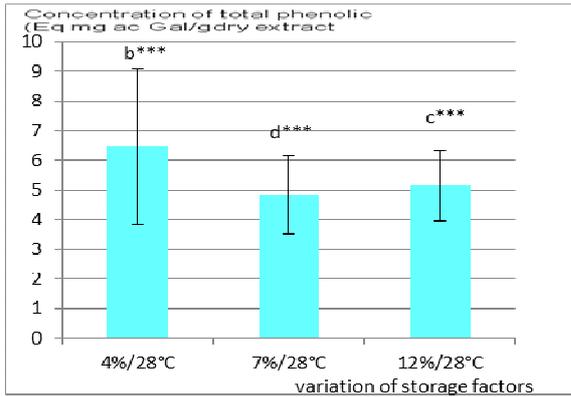


Figure.3. Effect of moisture content on the polyphenolic total of paprika at 28°C with means comparison (bdc), $P < 0,0001$ *** (Test de Duncan)

The concentration of total polyphenolic content in samples with different moisture content of 4, 7 and 12% presented a highly significant decrease ($P < 0,0001$) at 5% level (Figure 3 and 4) 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. Loncin (1975) reported that the moisture of food and the temperature of storage affect the physicochemical and microbiological quality of food, Labuza, (1975).

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 $0,07 \pm 0,0005$ 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.

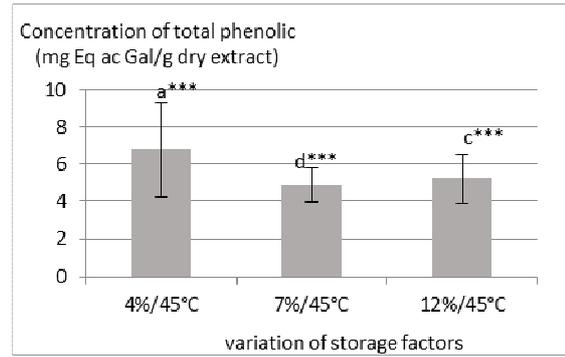


Figure.4. Effect of moisture content on the polyphenolic total of power paprika at 45°C with means comparison (adc), $P < 0,0001$ *** (Test of Duncan)

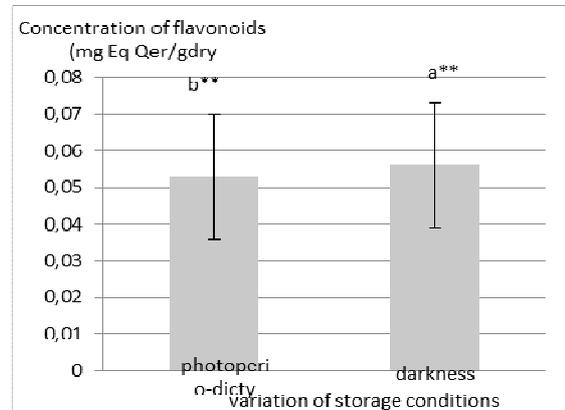


Figure.5. Effect of darkness and photoperiodicity conditions on flavonoids content of paprika with means comparison (ab), $P < 0,0011$ ** (Test of Duncan)

From the results (Figure.5), 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.

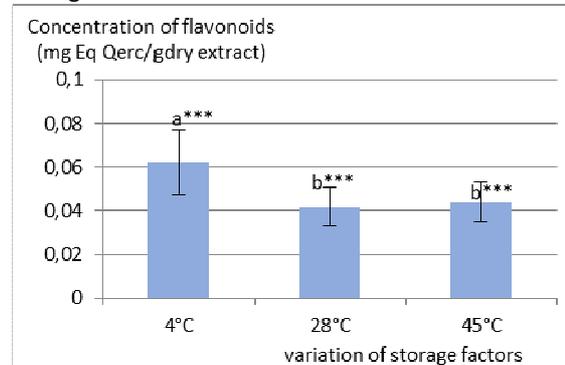


Figure.6. Thermal effect on flavonoids content of paprika with means comparison (abb), $P < 0,0001$ *** (Test of Duncan)

From the result (Figure 6), 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 higher 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. Shi et al. (1999) reported that color degradation of tomato was less severe when the drying temperature was lowered from 90 to 55°C.

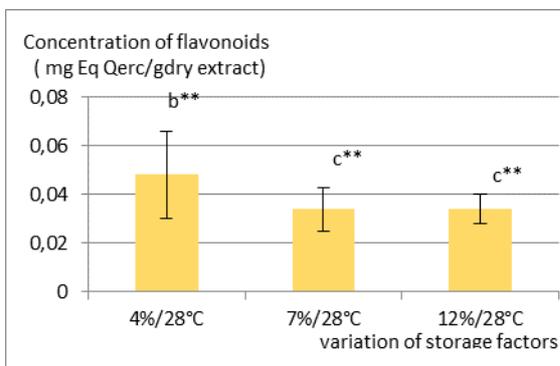


Figure .7.Effect of the moisture content on the flavonoids of paprika at 28°C with means comparison (bcc), $P < 0,0024^{**}$ (Test of Duncan)

The figure 7 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.

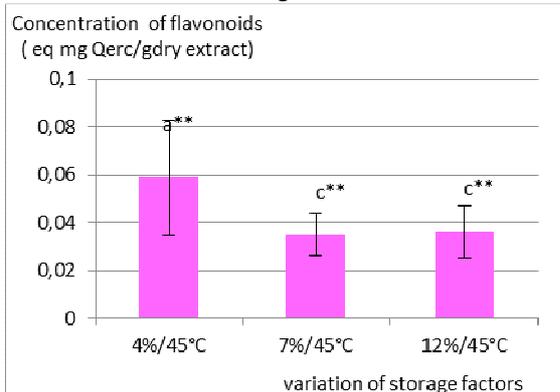


Figure.8. Effect of the moisture content at 45°C on flavonoids of paprika with means comparison (acc), $P < 0,0024^{**}$ (Test of Duncan)

The flavonoids concentration is decreased significantly ($p < 0.0024$) (Figure. 8) for all samples with moisture content of 4, 7 and 12%. The most noticeable decrease in flavonoids concentration is

obtained from samples with a water content of 7%. 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. The result of studies on the effect of heat treatment on the content of phenolic compounds are mixed, some works found an increase in the content of phenolic compounds while others have observed a decrease, Chipurura & Muchuweti (2010). 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, Arslan; Özcan, (2011).

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 Jun et al, (2004).

The antioxidant power of the paprika has been evaluated by the DPPH method. A methanol solution of DPPH• (2-2-diphényl-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 absorbance Perez et al, (2007).The color change of DPPH methanolic extract in the presence of each of the test products was measured at 517nm.

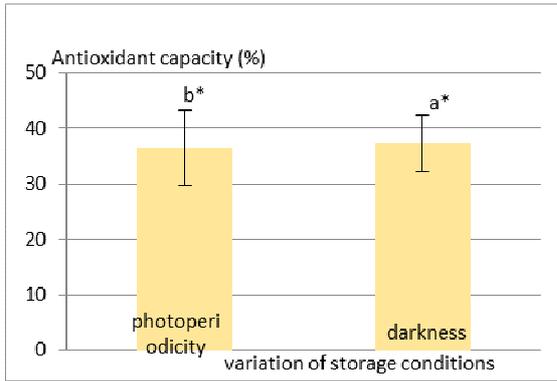


Figure.9.Effect of darkness and photoperiodicity on the antioxidant capacity of paprika with means comparison (ab), $P < 0,0104^*$ (Test of Duncan)

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.9). 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.

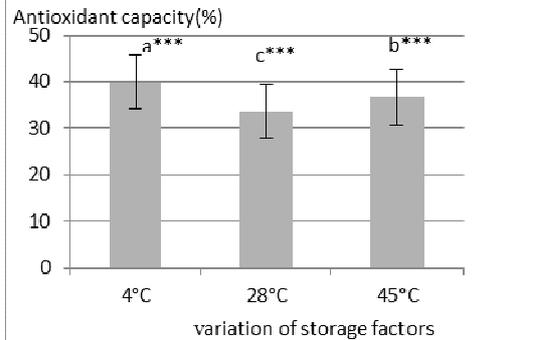


Figure. 10. Thermal effect on the antioxidant capacity of Paprika with means comparison (abc), $P < 0,0001^{***}$ (Test of Duncan)

The antioxidant capacity of samples reported a highly significant reduction ($p < 0.0001$) with a significant difference at the 5% level (Figure.9) and Figure.10. A positive and significant relationship between the decrease in antioxidant capacity and the effect of 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, Elzbieta Sikora et al, (2014)

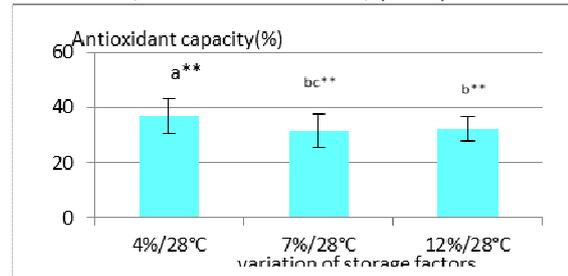


Figure.11. Effect of moisture at 28°C on the antioxidant capacity of Paprika with means comparison (abc), $P < 0,0019^{**}$ (Test of Duncan)

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.11)

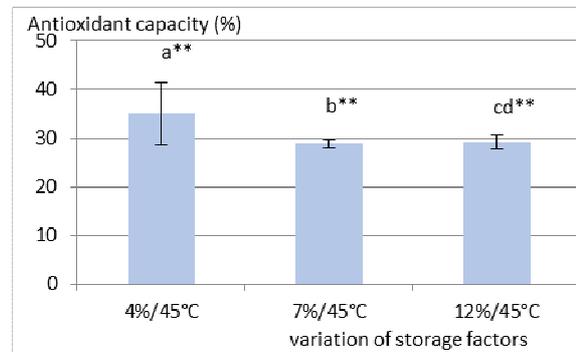


Figure.12. Effect of moisture at 45°C on the antioxidant capacity of Paprika with means comparison (abc), $P < 0,0019^{**}$ (Test of Duncan)

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 (Figures 11 and 12). Those with water content of 7% recorded the most 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 Madrau (2009) who reported a significant increase in antioxidant activity of apricots from Cafona variety after air drying at 75 °C, Inchuen et al. (2010) who reported an improved antioxidant activity of the red curry powder and Oboh and Akindahunsi (2004) who reported that sun-drying cause significant increases in the antioxidant properties of the green leafy vegetables Oboh et al, (2004). It was previously reported that long dehydration times together with high temperatures Perez-Galvez et al. (2005) lead to poor quality products due to

caramelisation, Maillard reactions, enzymatic reactions, pigment degradation and l-ascorbic acid oxidation, Horner, (1993)

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 Kim et al. (2006) 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.

Conclusion

The results of our study supplied detailed information regarding the variation of Bioactives properties 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 annum 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%. We conclude that 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.

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 fresh-cut 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.

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