

Bioactive Components and Antioxidant Activity of Moroccan Paprika (*Capsicum annuum* L.) at Different Period of Harvesting and Processing

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Abstract

In the present study the total phenols, flavonoids, flavonols, carotenoids content and ASTA of extracts of paprika powder produced at four periods of harvesting and processing were investigated for their antioxidant activity. A different composition between the four periods was evidenced. Paprika produced in November presented a major content of phenols, carotenoids, and ASTA (1360 mg/100g DW, 3727.54 mg/kg DW, 167.15 unit) respectively. Also it showed the highest radical scavenging activity in DPPH assay (IC₅₀ of 260µg/ml). Total flavonol and total flavonoid contents show a little variation depending on the time of harvesting and processing. The total phenolic and carotenoid contents were highly correlated with DPPH values (R²= 0.95 and R²= 0.96) respectively. Therefore, the total phenolic and carotenoid contents can serve as a useful indicator for the antioxidant activity of paprika. The obtained results suggest that Moroccan paprika could be used as valuable flavor with functional properties for foods.

Keywords: Niora, ground paprika, ASTA, carotenoids, phenolics, flavonoids, flavonols, antioxidant activity.

1. Introduction

Sweet pepper (*Capsicum annuum* L.) also called bell pepper or pimento is an important vegetable crop all over the world (Peet 2006) which ranks third in worlds vegetable cycle after tomato and onion (Akinfasoye et al. 2006). It is estimated that more than 7.5 million acres of capsicum are grown around the world (Peet 2003). In Morocco, sweet pepper called niora has been grown for many years by peasant farmers in the eastern part of the country (Slassi Moutabir 1987). The potential area of production is Tadla region with more than 80% of national production (Hakmaoui et al., 2011; Zaki et al. 2013). Traditionally, paprika is obtained by sun drying (Condori & Saravia 2001). It takes about 7-20 days (depending on the weather conditions) to reduce the moisture content to 10-15% (Oberoi et al. 2005). In Morocco, the period of the operations of harvesting and sun drying and transformation may often take about four months, from September to December (Hakmaoui et al. 2011).

Capsicum cultivars have been identified as potential solanaceous crop with high antioxidant activity (Ou et al. 2002). Sweet peppers are important both economically and nutritionally, because they are excellent sources of natural colors and antioxidant compounds including flavonoids, phenolic acids and carotenoids (Lee et al. 1995, Zaki et al 2013). Intake of these compounds in food is an important health protecting factor. They are also helpful in prevention of widespread diseases. There are growing evidences suggesting that antioxidants may maintain health and prevent many chronic diseases, such as certain cancers, cardiovascular diseases and other aging-related diseases (Thompson 1994).

Sweet pepper comprises numerous other chemicals including steam-volatile oil, fatty oils, capsaicinoids, carotenoids, vitamins, protein, fiber, and mineral elements (Bosland & Votava 2000). Many sweet pepper constituents have importance for nutritional value, flavour, aroma, texture, and colour. Many of these compounds are antioxidants that exert their biological effects through free-radical scavenging, protein binding and interaction with human signal transduction pathways (Padayatty et al. 2003).

Phenolics are secondary metabolites in plants composed of phenolic acids and polyphenols (includes flavonoids). A number of studies have demonstrated phenolics and flavonoids to possess numerous biological, antioxidants, pharmacological, and medicinal properties, including antimutagenic, anticarcinogenic, anti-inflammation and anti-allergy properties, as well as having the ability to modify gene expression (Lee et al. 1995).

On the other hand *Capsicum* spp. exhibit great genetic diversity in terms of color, size, shape, and chemical composition. Researchers have recently recognized that *Capsicum* fruit also vary greatly in their content of antioxidant vitamins and photochemical. Although the introduction of the cultivation and production of pimento in Morocco dates back to 1925, there is still no data on the composition and the levels of these important phytonutrients in the paprika produced in Morocco. Thus, the goal of this research was to obtain a more comprehensive picture of the biochemical composition of paprika produced in Morocco. We determined the content of total flavonoids, total carotenoids, total flavonols and total phenols. Also, this study aims to

characterize the antioxidant activity of paprika produced at different periods of harvesting and transformation of niora in the Tadla-Azilal area.

2. Materials and methods

Paprika powder derived from niora cultivated and harvested in 2011 in the Tadla Azilal area was used for this investigation. Geographic characteristics such as altitude slice, central latitude and longitude as well as the mean precipitation of this provenance are summarized in Table 1. Samples were taken immediately after milling at four periods of harvest (September, October, November and December). The average particle size of the powders was 400 μm . the samples were stocked at 4°C until use. For each parameter analysis, experiments were carried out three times. All analysis was done by using analytical grade chemicals and reagents. Triplicate determinations were performed on each sample; data shown later represent the means of three measurements.

Table 1. Geographic and meteorological conditions of provenance of paprika used in the study.

Provenance	Tadla Azilal
Geographic region	Middle Atlas mountain
Latitude N	32°30'
Longitude W	6°03'
Altitude (m)	500-800
Rainfall (mm)	550

2.1. Extractable color (ASTA)

The ASTA color value of paprika powders was determined according to the AOAC International [2002] method with a slight modification. The amount of about 0.1 g of samples was extracted with 20 ml acetone for 3 h by using a water bath (axially shaken at 140 rpm) maintained at 25°C. Then the extract was diluted 1/5 with acetone. The absorbance of the diluted extract was measured against acetone at 460 nm by spectrophotometer. The extractable color of the samples was expressed in ASTA units:

$$\text{ASTA} = \text{Abs} * 16,4 * \text{If}/\text{weight}$$

Where Abs is absorbance of the extract, and If is the deviation factor of the spectrophotometer, which was calculated by dividing the theoretical absorbance ($A_t = 0.600$) by the real absorbance (A_s) of standard color solution ($\text{K}_2\text{Cr}_2\text{O}_7$ (0.001M) and $(\text{NH}_4)_2\text{Co}(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ (0.09M) in H_2SO_4 (1.8M)) at 460 nm.

2.2. Total carotenoids

The total carotenoids content was determined according to method cited by Alasalvar et al. (2005). In brief, dried samples (0,500 g) were extracted with 5 ml of acetone–water (9:1, v/v) and centrifuged at 3000 rpm for 10 min at 4 °C. The clear supernatant was withdrawn and extraction was repeated for another five or six times with 3 ml of acetone–water until no color was extracted. Extracts obtained were pooled and measured against an acetone blank at 471 nm using a UV-2100 spectrophotometer.

2.3. Methanolic extracts

Paprika extracts were obtained by stirring 1g of dry paprika powder with 10 ml of methanol/ water (80:20) for 30 min. Extraction was carried out using maceration at room temperature for 24h followed by filtration through whatman N°4 filter paper. The filtrate was centrifuged at 4000rpm for 15 min. The supernatants were concentrated at the temperature of 35°C and pressure 70 mbar (Peruka & Materska 2007) and then the extract was solubilized with MeOH/H₂O (8:2) and adjusts to a concentration of 0.1 g/ml. The extracts were conserved at 4°C until analysis.

2.4. Total flavonoid content

The Total flavonoids content was determined spectrophotometrically using a method based on the formation of a flavonoid–aluminum complex (Zhishen 1999). One milliliter of extract was placed in a 10 mL volumetric flask. Five milliliter of bidistilled and 0.3 mL of sodium nitrite were added and mixed. About 0.6 mL of 10% $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ was added after 5 min. Two milliliters of NaOH (1M) was added 5 min later in the solution mixture, vigorously. Absorbance at 510 nm was read immediately. Total flavonoid contents were calculated using a standard calibration curve, prepared from quercetin.

2.5. Total flavonols

Total flavonols content of extracts was determined by the method of Kumaran & Joel (2007). 2ml of extracts solution, 2ml of 20 g/l AlCl_3 ethanolic solution and 3ml of 50g/l sodium acetate solution were prepared. The absorbance was read after 2.5 h at 20°C at 440nm. Total flavonols content was expressed as Rutine (mg Rutine/ 100g of dry weight).

2.6. Total phenolic content

The total phenolic contents of paprika samples were determined using a modified Folin-Ciocalteu method cited by Wolfe et al. (2003). A 50 μl aliquot of the extract was added to the test tube and combined with 500 μl of Folin–Ciocalteu reagent. The tubes were vortexed for 15 seconds. About 1.5 ml of 7% sodium carbonate

solution was then added to the test tubes, and the mixture was diluted to 5 ml with distilled and de-ionized water. The tubes were stored at dark and color was developed for 90 min and the absorbance was measured at 727 nm. The measurement was compared to a standard curve of prepared gallic acid solutions and expressed as gallic acid equivalent in mg/100g of paprika.

2.7. Assay of DPPH radical scavenging activity

The free radical-scavenger activity was determined by the DPPH assay, as described previously by Campos et al. (2003). The antiradical activity of extracts was evaluated using a dilution series, in order to obtain a large spectrum of sample concentrations. This involved the mixing of 250 μ l of DPPH solution (6 mg of DPPH in methanol) with an appropriate amount of extract or compound, followed by homogenization. After incubation in the dark at room temperature for 30min, quantification of the remaining DPPH radicals was recorded by using absorption set at 517 nm. Antiradical efficiency was established using regression analysis at a 95% significance level ($P < 0.05$). Results are presented in IC₅₀ values which represent the weight of sample required to scavenge 50% of the DPPH radicals available.

2.8. Statistical analysis

The analysis was carried out in three replicates for all determinations. The mean and standard error of means were calculated. The data were analyzed by one way analysis of variance ANOVA). Significance of the differences was defined as $P < 0.05$.

3. Results and discussion

3.1. ASTA Unit

The color of paprika powder can be measured either as extractable red color or surface color. Extractable color is the official method used by the American Spice Trade Association [ASTA, 1985]. It show the total pigment concentration synthesized in the fruit and the relation between the red and yellow carotenoids. The ASTA extractable color results presented in Table 2 showed significant differences between the paprika samples. The ASTA values for Moroccan paprika ranged from 117 to 167 units. The paprika harvested and transformed in November period showed a high ASTA value (167). Similar results were obtained by Garcia et al. (2007) in Bola-type red peppers. Because high quality paprika powders usually show ASTA values above 100 (Tevini 1997), the Tadla commercial ground paprika is considered as acceptable for home and industrial application. However, ASTA values are known to differ significantly depending on the cultivar, the ripening stage, the presence of seed, the localities (Garcia et al. 2007). Also the ASTA color varied strongly between years (Garcia et al. 2007).

Table 2: Carotenoid and ASTA Unit of Moroccan paprika.

Time of harvesting and processing	ASTA Units	Carotenoid (mg/Kg DW)
September	124.79 \pm 1.47 ^b	2782.76 \pm 32.77 ^b
October	117.56 \pm 1.81 ^b	2621.60 \pm 40.31 ^b
November	167.15 \pm 8.66 ^a	3727.54 \pm 193.22 ^a
December	121.20 \pm 0.48 ^b	2702.79 \pm 10.81 ^b

3.2. Total carotenoid content

Total carotenoid content ranged from 2500 to 3200 mg/kg DW, with the high content obtained for the paprika produced in November. Significant difference was noted between all the samples. The mean value of total carotenoid is 2788 mg/kg de paprika. The total carotenoid concentration was within the range reported by Tundis et al. (2012), Hervet-Hernandez et al. (2010). The levels of carotenoids values in Korean red pepper powders (*Capsicum Annuum* L.) ranged from 1070 to 3350mg/kg DW (Kim et al. 2011). Although, higher carotenoid contents have been reported by Hornero-Mendez et al. (2000) and Markus et al. (1999). Generally peppers is a good source of carotenoids, which can vary in composition and concentration owing to differences in genetics and maturation (Markus et al. 1999). Greater variations both qualitative and quantitative carotenoid composition were observed in Capsicums (Hart and Scott 1995).

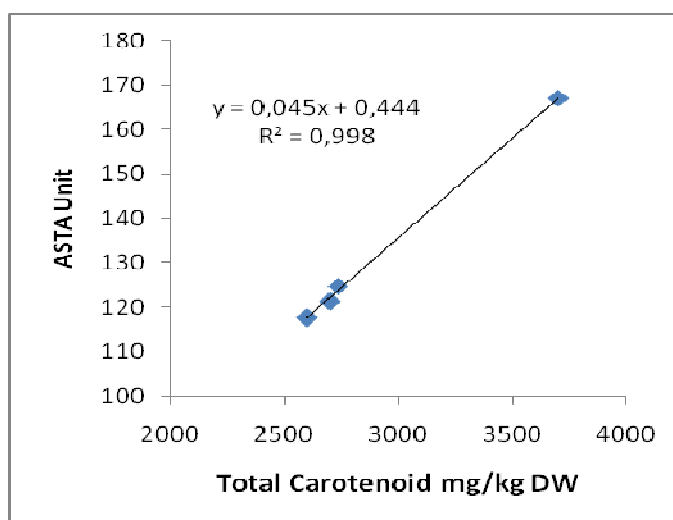


Fig 1: Correlations between ASTA and total Carotenoids of paprika powder

Correlation coefficients between ASTA units and total carotenoid content were positive and highly significant. This observation agrees with the work of Pérez-Gálvez et al. (2004) regarding ASTA units and total carotenoid content of paprika obtained through the traditional process, from dried fruits of *Capsicum Annuum* L.

3.3. Total phenolics content

Sweet peppers are an important source of total phenols, which are mainly localized in the peels (Marin et al. 2004). The results of total phenol contents of paprika samples are shown in table 3. That total phenolics content varied significantly among the period of production of paprika. Total phenol contents values ranged from 675 and 1360 mg/100g DW expressed as gallic acid equivalent. The highest total phenol content (1360 mg/100g DW) was observed in Paprika produced in November. Our results were found higher than those reported by Tripathi & Mishra (2009) for Indian paprika (500 mg/100g). However hot dried peppers had a high content of polyphenols (>2000 mg/100 g of dry matter), (Hervert-Hernandez et al. 2010). Our findings are in the same range as those obtained by Tundis et al. (2012) (843 and 748 mg/100g DW) for two different varieties of capsicum annum L, either by Deepa et al. (2007) for Flamingo red peppers (852 mg/100 g). Lee et al. (1995) and Menichini et al. (2009) reported that the total phenolic contents in raw pungent peppers were in the range (20–782 mg/100g) for Mexican type peppers.

Table 3: Total Phenols, Flavonoid, Flavonol and antioxidant activity of Moroccan paprika.

Time of harvesting and processing	Total phenols mg AG/100g DW	Flavonoid mg quercitin/100g DW	Flavonol mg rutine/100g DW	DPPH IC50 µg/ml
September	820 ± 72.63b	128.84±21.07	123.28±1.67	370±10.4
October	675 ± 63.83c	126.80±25.74	124.10±0.49	425±11.6
November	1360 ± 122.78a	130.20±3.89	123.45±0.86	260±7.4
December	871.66 ± 77.67b	121.36±10.65	142.45±1.58	395±6.4

The results show an important difference between period of harvest and transformation in total phenolics content. Nevertheless, the concentrations of total phenolics depend on cultivation, ripeness, storage and soil salinity, among other factors (Navarro et al. 2006).

3.4. Total Flavonoids and flavonols content

Phenolic compounds, especially flavonoids, possess different biological activities, but the most important are antioxidant activity, which is associated with a reduced risk of cancers and cardiovascular diseases (Czczot 2000; Kaur & Kapoor 2001). Sweet peppers contain a very rich polyphenol pattern, which includes hydroxycinnamates, flavonols and flavones (Marin et al. 2004). Flavonoids are a family of compounds with a C6–C3–C6 skeleton structure. Flavanols, flavonols and anthocyanins are included in this group. Among Paprika samples, the total flavonol and flavonoid contents values averaged from 123 to 142 and 121 to 130 mg /100 Dry weight respectively (Table 3). The flavonoid values were comparable to those reported for pepper flavonoids in the literature (Kim et al. 2011; Tundis et al. 2012). On the contrary, these values were higher than those reported by Bae et al. (2012) (62 mg/100gDW). In this research, the samples evaluated for total flavonol and total

flavonoid contents show a little variation depending on the period of harvesting and processing.

3.5. Antioxidant activity

Antioxidant activity is an important parameter to establish the health functionality of a food product. The antioxidant capacity of fruits and vegetable has been tested using a wide variety of methods. In the present study, free radical (DPPH) scavenging assay were used to evaluate the antioxidant activity of the paprika spice. This assay has frequently been used to assess antioxidant capacity (Deepa et al. 2007; Imeh & Khokhar, 2002). Data are reported in Table 3. All extracts were able to reduce the stable free radical DPPH to the yellow-colored DPPH. The radical scavenging activity (IC₅₀), exerted by Moroccan paprika, ranged from 260 to 425 µg/ml. The best free radical scavenging activity was exerted by paprika produced in November period with an IC₅₀ value of 260 µg/ml. This observation agrees with the work of Tundis et al. (2011), regarding the radical scavenging activity (IC₅₀) exerted by *C. annuum* var. *cerasiferum* (IC₅₀ values 463.0 µg/ml). Also, Conforti et al. (2007) reported a modest radical scavenging activity with an IC₅₀ value of 419.0 µg/ml for pepper at full maturity stage. Moreover, paprika produced in November was less active than red *C. annuum* var. *special* that exhibited an IC₅₀ value of 150.40 µg/ml (Kim et al. 2011). The same finding was reported by Tundis et al. (2012) for *C. annuum* var. *acuminatum* medium with an IC₅₀ value of 85.3 µg/ml.

3.6. Correlations

Linear regressions were performed with collected data in order to know which bioactive compounds are contributing to antioxidant activity (Fig. 2 and 3). Results revealed that the total phenolics and total carotenoid contents were highly correlated to antioxidant activity. The correlation coefficients were $R^2=0.95$ and $R^2=0.96$ respectively between antioxidant capacity and total phenolic contents and Total carotenoid content. In addition, red peppers are a good source of carotenoids, well known for their antioxidative effects (Bartley & Scolnik 1995). Earlier, Lee et al. (1995) reported that phenolic compounds correlated well ($r^2=0.86$) with antioxidant activity. However, total content of flavonoids and flavonols do not correlate with the antioxidant power observed for paprika powder in this study. On the contrary, Zimmer et al. (2012) reported that the contents of flavonoids and total phenolic compounds could be correlated with the antioxidant activities observed for *Capsicum annuum* L.

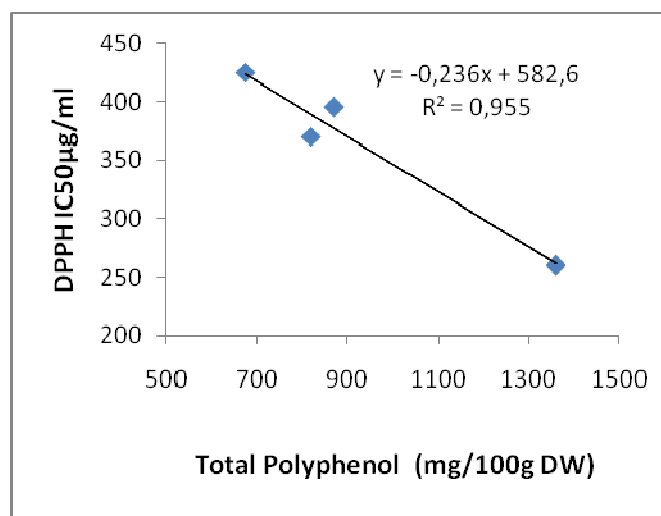


Fig 2: Correlation between antioxidant capacities (DPPH) and total phenolic of paprika powder

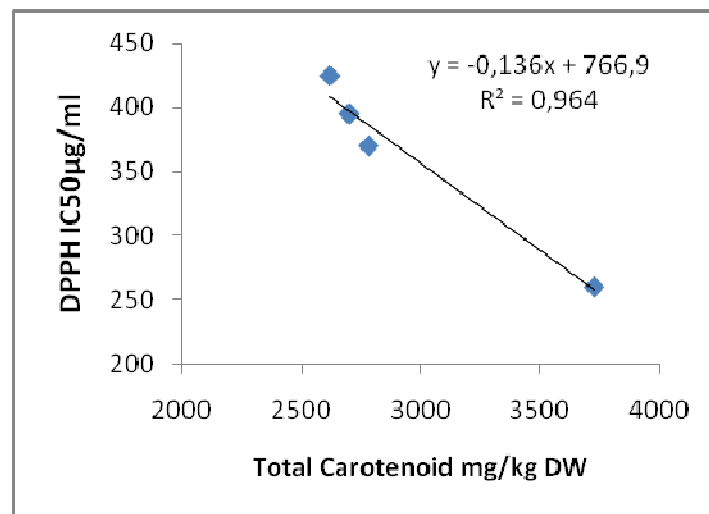


Fig 3: Correlation between antioxidant capacities (DPPH) and total carotenoids of paprika powder.

4. Conclusion

Significant variation in total phenolics, carotenoids content, antioxidant activity, between the Moroccan paprika powder periods was observed. Nutritionally, paprika produced in November period is a good source of mixture of antioxidants including, carotenoids and polyphenols which may offer potential health benefits.

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