

Assessment of Color, Capsaicinoids, Carotenoids and Fatty Acids Composition of Paprika Produced from Moroccan Pepper Cultivars (*Capsicum Annuum* L.)

Naima Zaki, Aziz Hasib*, Abdelmalek Hakmaoui, Fatima Dehbi and Aaziz Ouattmane

Laboratory of Environment and Valorisation of Agro resources. University Sultan Moulay Slimane. Faculty of Science and Technology. Beni-Mellal, Morocco.

* E-mail of the corresponding author: azhasib@yahoo.fr

Abstract

Analysed quality parameters of paprika powder (*Capsicum annuum* L.) collected from three localities in Morocco (Tadla, Gharb and Elkalaa des Sraghna) were color, total capsaicinoids, total carotenoid, fat content and fatty acid composition. The oil contents of paprika powder collected from the three localities varied relatively from 7.55g to 8.67 g/100 g. The main fatty acids among the different paprika oils were linoleic acid (60.1–70.9%), oleic acid (12.1–16.1%) and palmitic acid (7.7–14.5%). Remarkable amounts of capsaicinoids were found in the different locations, with a maximum obtained for Elkalaa des Sraghna paprika (184.97mg/kg). Total carotenoid content values averaged from 2323.66 to 3025.05 mg/kg dw with the high content obtained for the Tadla paprika. The ASTA Values differ significantly between the three localities; the high value of ASTA 20 was obtained for Tadla paprika. The present study showed that the different paprika powders are a potential source of valuable oil and Color that could be used for edible and industrial applications.

Keywords: *Capsicum annuum* L., Paprika, Oil, Fatty acid, Capsaicinoids, Total carotenoid, ASTA, Color.

1. Introduction

Paprika derived from pepper cultivars (*Capsicum annuum* L.), is a ground, dried fruit used to improve food taste and colour. It has significant importance in the Moroccan horticultural production. The main production area is Tadla region with more than 80% of the national production (Hakmaoui et al. 2011). The plant material and the sun drying method used means that the paprika produced in Morocco resembles the paprika produced in Murcia (southeastern Spain) (Escarabajal & Fernández-Trujillo 2009; Fernández-Trujillo & Escarabajal 2006). It is both a popular spice in the world as a delicious spice with characteristic color, taste, and heat (Estrada et al. 2002; Perucka & Oleszek 2000). Capsicums are important food additives in many parts of the world, valued for their sensory attributes of color, pungency and aroma (Estrada et al. 2002). Pepper used as food colorant has traditionally been in the form of ground powder.

Capsicum cultivars have been identified as potential solanaceous crop with high antioxidant activity [Ou et al., 2002]. 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 [Dehbi et al 2013; Thompson 1994]. Paprika powder comprises numerous other chemicals including steam-volatile oil, fatty oils, capsaicinoids, carotenoids, vitamins, protein, fiber, and mineral elements [Bosland and Votava, 2000].

Fat content of paprika is a significant quality parameter as plays an important role in dissolving of color compounds of epicarp and gives red color of dried products and grinded powder and results more homogenous, attractive spice. Fatty acids are accumulated in both the pericarp and the seeds (Marion & Dempsey 1964; Nosti Vega et al. 1982). A prerequisite for the value of vegetable oils or fats is either a nutritional quality or the technical benefit. Both depend on the fatty acid composition as well as on the composition of the accompanying constituents like vitamin E-active compounds or phytosterols. For nutritional purposes, the oil should be low in saturated fatty acid, high in monounsaturated fatty acids and well-balanced in ω -6- and ω -3-fatty acids with remarkable amounts of vitamin E-active compounds and phytosterols.

Pungency of peppers is attributed to capsaicinoids, which are a group of 12 or more alkaloids with a structure of vanillylamide of branched fatty acids with 9–11 carbons. Capsaicinoids are distinctive components of this vegetable. They are synthesized exclusively in the epidermal cells of the placenta in capsicum fruit and are accumulated in blisters along the epidermis (Cisneros-Pineda et al. 2007). Capsaicin and dihydrocapsaicin, which are responsible for 80 to 90% of the total pungency, are the most abundant principles of hot pepper. Among the capsaicinoids, capsaicin comprises more than 70% pungency of red pepper (Titze et al. 2002). The concentration of capsaicinoids in paprika variety ranged from 0.001% to 0.01%, and in strong chilli varieties the concentration ranges from 0.1% to <1% (Govindarajan et al. 1987). The capsaicinoids are present in hot pepper varieties at different amounts (Govindarajan 1986).

Also, red peppers are a good source of carotenoids, well known for their antioxidative effects (Bartley & Scolnik 1995). Pepper carotenoids are mainly capsanthin and capsorubin. Capsanthin accounts for 30% to 60% of total carotenoids in fully ripe fruits (Matsufuji et al. 1998). However, capsaicinoids and color value are considered the most important quality factors in peppers (Kim et al. 2002).

Industrial utilization of paprika powder is enhanced by knowledge of its fatty acid composition, its color and its capsaicinoids content either it's bioactive constituent. In the Moroccan land the pepper culture play a significant role on the national economy, through generating considerable export earnings or import substitution. However the research conducted on their proximate chemical composition is limited. To our knowledge there is still no data on the composition and the levels of these important parameters of quality in the paprika produced in Morocco. This is the first study undertaken to generate baseline information on the chemical composition and the quality of the Moroccan paprika. The aim of this work was to examine the range of Moroccan paprika oil content and the fatty acid composition. Their coloration, total capsaicinoids and carotenoid content were investigated.

2. Material and methods

The pepper fruits were harvested during 2010 from three localities in Morocco during November 2010 (*Tadla, Elkalaa des Sraghna* and *Gharb*) (Figure 1).

Paprika powder samples derived from pepper cultivars (*Capsicum annuum* L.) were obtained by means of the process previously reported that essentially includes fruit sun drying and grinding (figure 2) (Hakmaoui et al. 2011), but no extra oil was added. Powders were taken immediately after milling. The average particle size of the powders was 400 μm . The samples were stocked in hermetic containers in darkness and under refrigeration (4°C) until analysis. Three replicates were used per region obtained from three independent millings without adding oil. The samples for milling were taken randomly form different levels of the piles of sacks in which the collaborating companies store the sun dried peppers.

2.1 The content of Fat:

The oil content was analyzed gravimetrically after Soxhlet extraction following the AOAC No. 960.39 (Hortwitz 2002).

2.2 Fatty acid composition

The fatty acid composition of oil was determined in a mixture of subsamples of the three replicates following the European Standard ISO 12966 (2011). Briefly 0.1g of the oil was dissolved in 2 mL isoctane and 0.1 ml de KOH (2N). The closed tube was agitated vigorously for 1 min at room temperature. After addition of 2 ml NaCl (40%), the tube was centrifuged at 45 00 rpm for 10 min. The fraction of isoctane is removed in a test tube, which 1 g of sodium bisulfate monohydrat was added. After centrifugation at 4 500 rpm for 10 min, the top isoctane phase was transferred to a vial and injected into a Varian 5890 gas chromatograph with a CP-Sil 88 capillary column (100 m long, 0.25 mm ID, film thickness 0.2 mm; Varian Deutschland, Darmstadt, Germany). The peak areas were computed by the integration software, and percentages of fatty acid methyl esters (FAME) were obtained as weight percent by direct internal normalization.

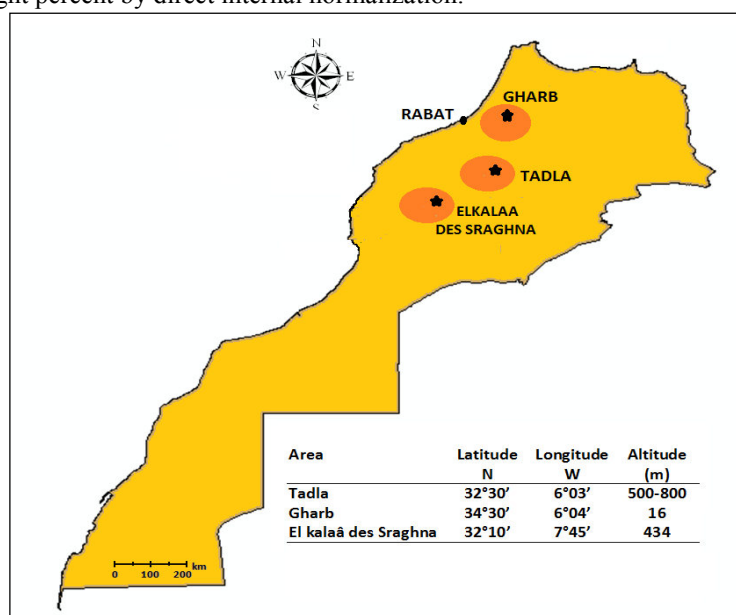


Figure 1: Repartition map of Moroccan paprika powder derived from pepper cultivars (*Capsicum annuum* L.) used in the study



Figure 2: A (to the left): the fruits of pepper cultivars (*Capsicum annuum* L.) are initially stacked in piles for the post-maturation without exposure to direct sunlight. B (to middle): the fruit is dispersed in the previously clean and compacted soil for a homogeneous drying in the Sun. C (to right): the paprika powder

2.3. Coloration

The ASTA color value of paprika powders was determined according to the official AOAC method 971.26 (Hortwitz 2002) with a slight modification. Samples (0.1 g) were 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:

$$\text{ASTA} = \text{Absorbance} * 16.4 * \text{devf} / \text{weight} \quad (1)$$

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

To measure skin color, a CR-300 Chromameter (Minolta, Osaka, Japan) was used for (C illuminant, 0° viewing) previously calibrating it with a white-plate standard. A glass Petri dish containing the samples was placed below the light source. The L, a(+), and b(+) values of each sample were determined in triplicate. Because chroma (C) and Hue angle (h°) have been shown to be more practical measures of color from a human sensorial point of view (McGuire 1992), both parameters were calculated:

$$C = (a^2 + b^2)^{1/2} \text{ and } h^\circ = \text{arc tan} (b/a).$$

2.4. Total carotenoids content

The total carotenoids content was determined according to method cited by Alasalvar et al. (2005). In brief, dried samples (0.5g) 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.

2.5. Total capsaicinoids content

The capsaicinoid content was determined as described by Collins et al. (1995). Samples of 1 g of paprika powder were mixed with 10 ml of acetonitrile and kept for 4 h at 80 °C with shaking in a capped Erlenmeyer flask. The supernatant was filtered into a 2 ml glass vial by using a 0.45 μm membrane filter (Millipore) and then used for HPLC inject. Capsaicinoids were quantified at 280 nm against a calibration curve with pure capsaicin and dihydrocapsaicin.

The results were converted to the Scoville Heat Value (SHV) by multiplying the individual capsaicinoid composition (in mg kg^{-1} dry weight of the paprika) by the coefficient of the heat value for each individual compound, 9.3 for nordihydrocapsaicin (NDHCAPS) and 16.1 for both capsaicin (CAPS) and dihydrocapsaicin (DHCAPS) (Todd et al. 1977).

$$\text{Total SHV} = [\text{CAPS} + \text{DHCAPS}] \times 16.1 + [\text{NDHCAPS} \times 9.3] \quad (2)$$

2.6. Statistical analysis

Analysis of variance of the data from each attribute was computed using the SPSS software. The Least Significant Difference test at 5% level of probability was used to test the differences among mean values.

3. Results and discussion

3.1. Fatty acid composition

Total lipids and fatty acid composition of paprika powder collected from three localities in Morocco were investigated (Table 1). Total lipid contents of spices ranged from 7.55 to 8.67% DW among the three localities paprika. These results are slightly higher than those obtained by Tchiegang et al. (1999) analyzing the chili in Cameroon. But they are inferior to those cited in the literature (12% to 22%) (Govindaraja 1985; Camara & Delamou 2003).

Eight different fatty acids were investigated (Table 1). As in most plants, the major fatty acids accumulated in

the paprika pepper are linoleic (18:2), palmitic (16:0), oleic (18:1), and linolenic (18:3) (Murphy et al. 1993; Pérez-Gálvez et al. 1999; Kwon et al. 2011). They represented 90% among the total fatty acids. The other fatty acids were detected in low percentage. The values obtained are (60.1 to 70.9 g/100 g) for linoleic acid, (12.1 to 16.1g/100 g) for oleic acid and (7.7 to 14.5 g/100g) for palmitic acid. Considerable differences in fatty acid composition between *Tadla*, *Gharb* and *ElKalaa des Sraghna* were observed probably due to climatic conditions. Paprikas investigated in our work were obtained by grinding all the dry fruit. Thus the fatty acid distribution in paprika is very similar to that found for the seed of *capsicum annum* L (Perez-Galvez et al. 1999)].

Unsaturated fatty acids of the different paprika ranged from 81.13 % to 88.62% of the total fat paprika powder. The lipid of paprika was rich based on especially linoleic acid (Omega-6), that human body is not capable of producing (Simopoulos 2008). Saturated fatty acids of paprika comprised between 11.38% and 18.87% of the total fat. Palmitic acid was the main saturated fatty acid in all paprika powders; the other saturated fatty acids were detected in small quantities. These results are similar to those obtained by Kim et al. (2002), for grain and slightly lower than those obtained for the paprika.

The SFA/UFA ratio of paprika powders ranged from 0.13 to 0.23 respectively for *Tadla* paprika and *ElKalaa des Sraghna* paprika, lower than those obtained for paprika powder (kim et al. 2002). The results are in agreement with those obtained for paprika powder investigated by Pérez Galvez et al. (1999) and in agreement with the seed ratio (kim et al. 2002). The S/U ratio in the seed is constant at around 0.16, similar to that of the oils of olive (0.17) and soybean (0.19), and better than that of palm oil (0.98) (Pérez Galvez et al. 1999). This difference in SFA/UFA is believed to be related to the stability of the powdered product.

Table 1: Oil content and fatty acids composition (%) of Moroccan paprika powder.

Localities	<i>Tadla</i>	<i>Gharb</i>	<i>Elkalaa des Sraghna</i>
Oil content g/100 g DW	7.55±0.39	8.67±0.74	7.97±0.44
C ₁₄ ⁰ : Myristic	1.0	1.1	0.9
C ₁₆ ⁰ : Palmitic	7.7	11.6	14.5
C ₁₆ ¹ : Palmitoleic	0.4	0.35	0.4
C ₁₈ ⁰ : Stéaric	2.2	4.35	3.2
C ₁₈ ¹ : Oléic	12.8	12.1	16.1
C ₁₈ ² : Linoléic	70.9	67.5	60.1
C ₁₈ ³ : Linoléic	3.6	2.2	4.1
C ₂₀ ⁰ : Arachidic	0.4	0.1	0.2
C ₂₀ ¹ : Gadoléic	0.3	0.13	0.15
Total Fatty acid	99.3	99.43	99.65
Palmitic + Oléic + Linoléic	91.4	91.2	90.7
Satured Fatty acid	11.38	17.25	18.87
Unsatured fatty acid	88.62	82.75	81.13
Ratio S/U	0.13	0.21	0.23

3.2. Coloration

The color of paprika powder can be measured either as extractable colour or surface colour. Extractable color is the official method used by the American Spice Trade Association (ASTA 1985). Tristimulus colorimetry has been used for color description as a rapid and simple method to specify visual perception of food products (Rocha et al. 1993). Surface color measurements will give some indication as to how the paprika powder will look to the eye. The lightness (L) value can give some indication of color differences, as powder of higher color intensity will have a lower L value. For chilli powder, a hue angle (h°) of 0° is red and 90° is yellow; therefore, the closer the value to 90°, the more orange a powder will appear (Jorge et al. 1997).

The ASTA extractable color results listed in Table 2 showed significant differences between the paprika samples. The *Tadla* paprika powder showed a high ASTA value 135, 47. Similar results were obtained by García et al. (2007) in Bola-type red peppers (91 to 150 units ASTA). The *Gharb* commercial ground paprika had the lowest ASTA value (104, 06 units), which is considered as still acceptable for home and industrial application (Tepić et al. 2008). Moreover, ASTA values are known to differ significantly depending on the cultivar and ripening stage, on the presence of seed (Garcia et al. 2007; Lozano et al. 2003). Either the ASTA unit varied strongly between years and between countries (García et al. 2007). Since paprika is used as food colorant both for industrial and culinary purposes, the red color intensity is considered as the most important attribute of paprika (kim et al. 2002).

Table 2: Color characteristics and Carotenoids content in Moroccan paprika powder

Localities	ASTA	Carotenoids*	L	a (+)	b (+)	Chroma (C)	Hue Angle (h°)
<i>Tadla</i>	135.47±0.73 _a	3025.05±35.7 _{9^a}	24.56±0.21 _c	29.14±0.65 _a	29.77±0.87 _c	41.66±0.78 _b	45.61±1.31 _b
<i>Gharb</i>	104.06±1.09 _b	2323.66±22.7 _{8^c}	27.01±1.12 _a	27.54±0.79 _b	33.34±1.22 _a	43.24±0.94 _a	48.32±0.65 _a
<i>El Kalaa des Sraghna</i>	115.54±1.44 _c	2579.78±43.4 _{6^b}	25.5±1.19 _b	24.89±0.69 _c	31.11±0.97 _b	39.84±0.77 _c	43.62±0.84 _c
ANOVA	***	***	***	***	***	***	***

Values are mean ± SD of 3 replicates. a-c Test values along the same row carrying different superscripts for each parameter are significantly different ($p < 0.05$).

* Total Carotenoid expressed as mg/kg dw

Table 3: Capsaicinoids content in Moroccan paprika powder

Localities	Capsaicin (CPS)		DHCPS*		NDHCPS**		Total Capsaicinoid	SHU***	CPS/DHCPS
	mg/kg DW	%	mg/kg DW	%	mg/kg DW	%	mg/kg DW		
<i>Tadla</i>	24.2±1.69 _c	36.85	23.98±2.22 _c	36.51	17.5±0.87 _c	26.64	65.67±6.19 _c	938.45±69 _c	1.01
<i>Gharb</i>	60.5±4.97 _b	51.01	33.25±1.88 _b	28.03	24.85±1.67 _b	20.95	118.6±7.45 _b	1740.40±54.97 _b	1.82
<i>El Kalaa des Sraghna</i>	99.09±6.78 _a	53.56	50.36±3.28 _a	27.22	35.53±2.33 _a	19.20	184.98±9.55 _a	2736.57±78.1 _a	1.97
ANOVA	***		***		***		***	***	

Values are mean ± SD of 3 replicates. a-c Test values along the same row carrying different superscripts for each parameter are significantly different ($p < 0.05$).

* DHCPS : Dihydrocapsaicin

** NDHCPS: Nordihydrocapsaicin

*** SHU : Pungency level

Reflected color of the paprika powders was presented in (Table 2). As three different localities colors were studied, the a(+) and b(+) values, Chroma (C) and hue angle (h°) were different between the sample's. In the *Tadla* paprika, the mean value of a(+) (contribution to red) was 29.14. While the *Gharb* and *ElKalaa des Sraghna* samples values were 27.54 and 24.89 respectively. The b(+) parameter (contribution to yellow) had a mean of 29.77 in the *Tadla* paprika, 31.11 in the *ElKalaa des Sraghna* paprika and 33.34 in the *Gharb* paprika. Due to the different values of a(+) and b(+), the h° significantly differed between the samples. The L values varied from 24.56 to 27.01. As it is difficult to interpret complex 'L' and 'h°' data, the standard technique used by the spice industry is to measure extractable color and to observe the powder visually for defects. Because surface color does not necessarily depend on the total amounts of pigments in chilies, color was additionally expressed in ASTA units, which are related to the total coloring capacity.

3.3. Total carotenoid content

Among Paprika samples, total carotenoid content values averaged from 2323.66 to 3025.05 mg/kg DW with the high content obtained for the *Tadla* paprika (Table 2). Significant difference was noted between all the samples. The total carotenoid concentration was within the range reported by Kims et al. (2002); Topuz & Ozdemir (2007); Tundis et al. (2012). On the contrary, these values were lower than those reported by Markus et al. (1999). Generally Greater variations both qualitative and quantitative carotenoid composition were observed in Capsicums (Hart & Scott 1995). Peppers are also a good source of oxygenated carotenoids or xanthophylls, which can vary in composition and concentration due to differences in genetics and degree of ripening (Markus et al. 1999).

3.4. Total capsaicinoids content

In the present study, the total capsaicinoids content and SHU of paprika were analyzed to determine the pungency of the paprika powder produced in the three localities in Morocco. Table 3 shows the composition quantities of capsaicinoids present in the paprika powder. Among the three paprika localities studied, *ElKalaa des Sraghna* paprika showed the high values of total capsaicinoids (184.97 mg/kg DW), followed by the *Gharb* paprika (118.6 mg/kg DW), and lastly by the *Tadla* paprika (65.67 mg/kg DW). The capsaicin and dihydrocapsaicin content analyzed from the paprika extract produced in *Tadla* area were respectively 24.2 and 23.98 mg/Kg DW. These data represent 73.37 % of the total capsaicinoids. Similar results had been previously reported by many authors for different *Capsicum annuum* varieties (Perucka & Oleszek 2000; Perucka & Materska 2001; Topuz & Ozdemir 2007). But these results are lower than those reported by Davis et al. (2007) for habanero pepper (80-90%). However for *Gharb* and *ElKalaa des Sraghna* paprika the content of these two capsaicinoids varied from 79.04 to 80.80 % respectively. This is in agreement with the results obtained by (Gahungu et al. 2011). The capsaicin contribution in pungency was 53.36 and 51.01 % respectively for *ElKalaa des Sraghna* and *Gharb* paprika. Norhydrocapsaicinoid was contained in low amount, ranging from 19.21 to 26.65% of the total capsaicinoids. In general, capsaicin and dihydrocapsaicin were relatively more abundant than nordihydrocapsaicin.

The capsaicin and dihydrocapsaicin ratio was 1.01, 1.82 and 1.97 respectively for *Tadla*, *Gharb* and *ElKalaa des Sraghna* paprika. For *Tadla* paprika similar result was reported by other authors who found values ratio around 1:1 in the *Capsicum annuum* (Govindarajan & Sathyanarayana 1991). However for the others paprika localities our results differ to the findings on *C. annuum* fruit but the same ratio (2:1) were reported for *Capsicum frutescens* (Govindarajan & Sathyanarayana 199; Collins et al. 1995; Estrada et al. 2002). The significant difference between the three paprika localities could be attributed to different factors (environmental, varietal, processing, time of harvesting). A number of previous authors found that the capsaicinoid levels and pungency values of *C. annuum* fruits have a broad range of variance (Perucka & Oleszek 2000). Govindarajan (1985) determined that there are great diversities in the contents and composition of capsaicinoids among fruits of *Capsicum* species, and even among cultivars. The environment, especially the climate, light, soil, moisture, fertilization and temperature during plant growth, is considered to have an impact on capsaicinoid levels, as does the age of the fruit (Estrada et al 2002; Titze et al. 2002). Processing after harvested *Capsicum* fruits also plays an important role on the level of capsaicinoids, in that both the drying conditions and the number of seeds included have an influence on pungency (Titze et al. 2002).

The capsaicin and dihydrocapsaicin contents and their corresponding pungency level measured in Total Scoville Heat Unity (SHU) are the main indicators for the hotness taste of the pepper (Topuz & Ozdemir 2007). The values found for SHU of each capsaicinoid were summed, and the total amount is reported in Table 4. From the sensorial point of view, the SHU values of the three paprika origins were ranged between 938.45 and 2736.57. Naturally, the paprika powder cultivars which have higher capsaicin and dihydrocapsaicin contents result in higher SHU values.

Therefore, from the SHU obtained in this study; the *Tadla* paprika can be qualified as sweet pepper according to classement of Bosland & Votava (2000) that ranks the sweet peppers in the range of 0 to 700 SHU. However the two other paprikas are qualified mildly pungent. The capsaicinoid concentration, and indirectly its pungency, is inevitably dependent upon species and varieties. However, it should be considered that the attributes are also highly influenced by a number of extrinsic factors, such as fruit age, water stress, temperature, soil and fertilizer (Estrada et al. 2002; Titze et al. 2002).

4. Conclusion

Results of the three paprika investigated showed a significant difference in terms of quality setting (extractable color, total capsaicinoids, total carotenoid, fat content and fatty acid composition). The predominant fatty acids in the three paprika localities are linoleic acid, oleic acid and palmitic acid. Either it has revealed that paprikas studied are mainly composed of capsaicin and dihydrocapsaicin as major capsaicinoids, thus responsible for its pungency taste. The significant difference between the compositions of the three paprika localities could be attributed to different factors as environmental, varietal, processing, time of harvesting and degree of ripening.

This study showed that the different paprika powders are a potential source of valuable oil with a high quality eatable vegetable oil. Therefore paprika can be recommended by nutritionists to be part of our diet. In the other hand, the paprikas studied are an excellent source of natural color that could be used for edible and industrial applications.

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