International Journal of Agriculture Innovations and Research
Volume 3, Issue 4, ISSN (Online) 2319-1473
Manuscript Processing Details (dd/mm/yyyy) :
Received : 02/12/2014 | Accepted on : 11/12/2014 | Published : 21/01/2015

Evolution of Capsaicinoids and Mineral Composition During Fruit Development in Some Hot Pepper Varieties (*Capsicum Annuum* L.)

Samia BenMansour-Gueddes
Regional Research Centre on Horticulture and organic Agriculture BP 47- 4042 Sousse, Tunisia.

Abstract: **Hot Pepper (*Capsicum annum* L) is a high value spice and vegetable crops used for commercial and industrial purpose. Pepper Fruit is an excellent source of nutritional compounds. It is the main source of capsaicinoids and macro-element, which varied strongly during fruit growth and maturation. The aim of the present study was to evaluate the effect of development stage of the fruit on capsaicinoids content and mineral composition in four Tunisian hot pepper varieties. The evolution of capsaicinoids showed significant variations among pepper varieties during eight stages of fruit development. Hence, capsaicin, dihydrocapsaicin, nordihydrocapsaicin and total capsaicinoids, increased continuously and reached a maximum at 20 days after fruit setting in Msarreh (1624,77 ± 13.40µg.g^{-1} DW) and Chaabani (902.52 ±23.3 µg.g-1 DW), at 30 days in Rouge Long (1449. 14 ± 25.62 µg.g^{-1} DW) and at 40 days in Baklouti Kairouan (339.3 ± 3.19µg.g^{-1} DW) and declined. Also, analysis of macro-element has revealed the influence of the ripening stage on the mineral content of the pepper fruit. Therefore, red pepper presents higher N, P, K, Ca and Mg contents than in green ones for different varieties. Under field conditions, the development stages of the fruits affect significantly capsaicinoids content and mineral composition.**

Keywords: ***Capsicum Annuum*, Fruits, Capsaicinoids, Macro-Element, Development Stage**

I. INTRODUCTION

Hot pepper is an important agricultural crop in the world, not only because of its economic importance, but also due to the nutritional and medicinal value of its fruits and spices used as food flavorings. Their fruits are considered a good source of antioxidant and biologically active compounds, such as carotenoids, flavonoids, vitamins, capsaicinoids and mineral elements (Lee et al., 2005). The major property of this genus is pungency, which is caused by the presence of alkaloid compounds named capsaicinoids. Capsaicin and dihydrocapsaicin are the main ingredient responsible for the hot pungent taste of peppers, are only found in the genus *Capsicum* (Huang et al., 2013). Many studies proved the bioactivities of capsaicin and its analogues which showed many beneficial effects in food, medical and pharmaceutical applications (Caterina et al., 1997; 2000; Spicer and Almirall, 2005; Gurung et al., 2011). These include their properties as chemopreventive and anticarcinogenic compounds (Surh et lee, 1995), their anti-inflamatory and analgesic properties (Spiller et al., 2008; Quian et al., 2010), the gastrointestinal protective effects (Huang et al., 2013) and antimicrobial properties (Gareaga et al., 2003).

Capsaicinoid content of peppers is one of the major parameters that determine its commercial and nutritional values (Al Othman et al., 2011). The two major capsaicinoids, responsible for up to 90% of pungency, are capsaicin and dihydrocapsaicin (Govindarajan et al., 1987), with at least nine more minor capsaicinoids occurring in pepper fruits (Suzuki et al., 1980; Kozukue et al., 2005). The type and amount of each capsaicinoid affect both the degree and the characteristics of pungency (Kozukue et al., 2005; Barbero et al., 2014). The degree of pungency depends on *Capsicum* species and cultivars. indeed, The amount of total capsaicinoids vary greatly but the proportions of capsaicin and dihydrocapsaicin range from 77 to 90% in fruits of the species *C. annuum* and from 89 to 98% in those from *C. frutescens* (Govindarajan et al., 1987).

The capsaicin contents may be affected by different factors such as the fruit age, size and developmental stage of fruits (Iwai et al., 1979, Salgado-Garciglia and Ochoa-Alejo, 1990; Estrada et al., 1997; 2000). Many studies showed that the capsaicinoids begin to accumulate in the early stages of fruit development, continuing their accumulation during ripening until reaching a maximum values when the fruit is at the full-ripened stage (Hall *et al*., 1987; Estrada et al., 1999; Barbero et al., 2014). At this moment, there is a rapid turnaround in the trend and later capsaicinoids levels decrease gradually. This diminution was attributed to degradation of capsaicin and dihydrocapsaicin by *capsicum* peroxidases (Bernal et al., 1993a, 1993b).

There are other factors than the development stage that affect the pungency of pepper fruit, such as the plant cultivars (Barceloux, 2009), growing conditions (Sung et al., 2005) and stress conditions (Estrada et al., 1999). Many studies reported that capsaicinoids content varied with the variety, and within the same variety according to the stage of maturity and different parts of the fruit. Capsaicinoids content was higher in the placenta than in other parts, but lowest in seeds as well as in green and red fruits (Nowkem et al., 2011; Ben Mansour Gueddes et al., 2012).

It has been reported that the level of capsaicinoid is influenced by genetics, environmental conditions, and nutrient accumulation in the placental tissue and is also regulated by a genotype and an environment interaction (Zewdie and Bosland, 2000; Garces-Claver et al., 2007; Rahman and Inden, 2012). The addition of mineral supplements to the pepper cultivation causes an increase in the capsaicinoid content (Estrada et al., 1998) and that nitrogen supply is essential for their synthesis. Hence, nitrogen and potassium availability may affect pepper pungency through its content in the fruit tissues (Monforte-Gonzalez et al., 2010).

Peppers have a high biological value, a rich content of minerals. Their fruits are an important source of minerals for humans. Their concentrations levels were greatly influenced by pepper varieties (Ekholm et al., 2007), ripening of fruits Buczkowska, 2007; Jadczak et al., 2010), plant nutrition (Materska and Perucka, 2005), and weather conditions during the growing (Buczkowska and Michałojć, 2012). Many studies showed that the ripening state of the samples exercises a strong influence on the mineral content of the pepper (Rubio et al., 2002). The phosphorus content in fruits harvested from the greenhouse was slightly higher than that from the field (Buczkowska and Michałojć, 2012).

Pepper Fruits was an extremely important crop in Tunisian State, but a few studies were interested on its capsaicinoids and minerals contents, and their interaction particularly how it may be influence fruit development and quality. In response, the present study aim was to analyze the effect of eight-development stage on capsaicinoid levels and minerals contents in pepper under field conditions.

II. Material And Methods

Pepper crops

The cultivation of four varieties of hot pepper was carried out in an open field (Figure 1). The seeds of these varieties were planted in the seedbeds in February and thirty plants samples/variety were transplanted in April, 2007 at a density of 3 plants.m^{-2}. During the culture, the plants were watered by a drip system; fertilization was carried out depending on the stage of plant development at each time according to Chaux and Foury (1994). The experiment was a completely randomized design with three replications of each treatment/plot. Only a single season was assessed.

Fig. 1. Pepper samples used in this study: (a) Msarreh; (b) Rouge Long; (c) Chaabani; (d) Baklouti Kairouan.

Plant material and sampling of fruits

The evolution of total and individual contents of three major capsaicinoids and five macro-element present in four hot peppers was studied. Peppers were marked at the begin of fruit set and so the age of each pepper at the time of collection was known. In June, the new flowers of plants of each pepper were marked with a temporal spacing of ten days. Simultaneously, fifteen mature fruits (collected for each variety) were harvested at eight stages of fruit development (10 to 80 days after fruit setting).

Capsaicinoid analysis

Extraction procedure

Samples for different ages, obtained from all varieties studied, were oven-dried at 60 °C until constant weight (2 to 3 days) and then ground by a mini- grinder for capsaicinoid analysis. Capsaicinoids were extracted and quantified according to the method of Collins et al. (1995). Two grams of dried pepper samples were extracted in 20 mL of acetonitrile and incubated in a water bath during 4 h at 80 °C. After cooling and centrifugation, the supernatant of each extract was filtered through a 0.45 µm nylon filter prior to High Performance Liquid Chromatography (HPLC) analysis.

Capsaicin and dihydrocapsaicin were analyzed using a HPLC analytical system (Waters, Milford, MA, USA). Elution was performed at 35°C with a flow rate of 1 mL min^{-1} with the following gradient of eluant A (10% methanol) and eluant B (100% methanol): from 0 to 10 min, 43% A and 57% B; from 10 to 20 min, 32% A and 68% B, and from 20 to 30 min, 43% A and 57% B, for a total of 30 min. The capsaicinoids were separated on a Nova-Pak C18, 4µm column (Waters, USA) and detected on a Scanning Fluorescence detector (Model 474 from Waters, USA). Excitation wavelength was set at 280 nm and emission wavelength at 338 nm. Retention times and quantities of capsaicin and dihydrocapsaicin were estimated by reference to standards (Sigma-Aldrich Co., St. Louis, MO).

Quantification of individual compounds

Standards of capsaicin and dihydrocapsaicin were obtained from Sigma Chemical Co. (St Louis, MO) and were used for verification of retention-time and instrument calibration. Quantifications were based on average peak areas of 10 µL injections obtained from external standard solutions of capsaicinoids prepared in methanol. The chromatogram shown in Figure 2 corresponds to a standard and extracted solution, respectively; they reveal that nordihydrocapsaicin (NDHC), capsaicin (C), dihydrocapsaicin (DHC) and are eluted at 16.32; 16.94 and 20.08 min, respectively.

Capsaicinoids analysis by High performance liquid chromatography (HPLC) currently allows a precise determination of the nature and quantity of these alkaloids compounds (González-Zamora et al., 2013). Extraction and quantization steps were carried-out in triplicate for each sample. The standard solutions used for the calibration curve were regularly injected at intervals between sample injections to confirm the retention times.

International Journal of Agriculture Innovations and Research
Volume 3, Issue 4, ISSN (Online) 2319-1473

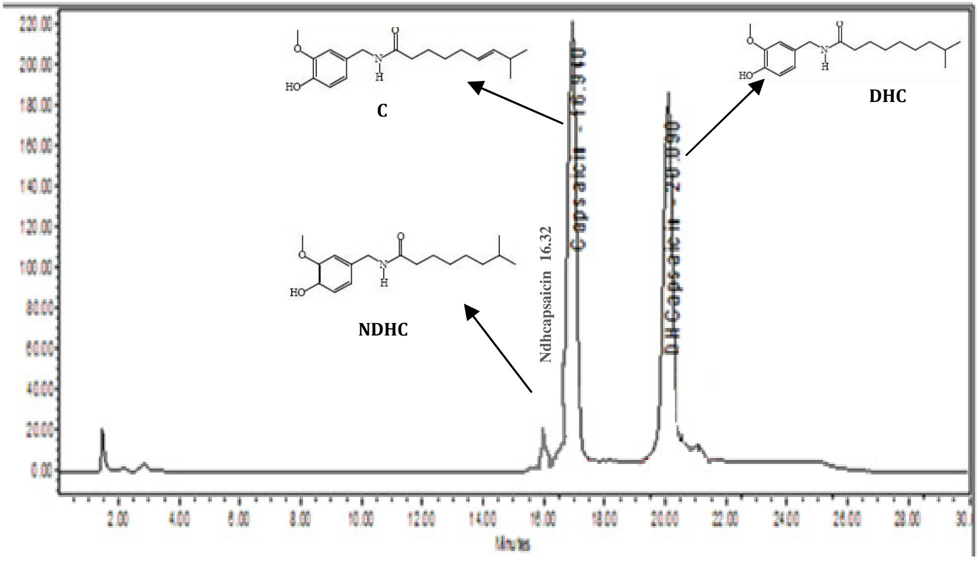


Fig. 2. Chromatogram of the standard solution corresponding and chemical structures of capsaicinoids nordihydrocapsaicin (NDHC), capsaicin (C) and dihydrocapsaicin (DHC). Fluorescence detection: excitation 280 nm, emission 310 nm.

The concentration of nordihydrocapsaicin was expressed as µg.g^{-1} dry weight (DW) equivalent capsaicin. The quantity of capsaicin and dihydrocapsaicin at each stage is expressed in µg.g^{-1} dry weight. These concentrations should allow used to calculate the Scoville Heat Units (SHU), to determine the pungency level of each type of pepper analyzed. All analyses were carried out in triplicate.

Scoville Heat Unit Conversions

According to the commonly accepted Scoville organoleptic test, the spicy strength of the investigated samples was designed by converting the capsaicin content expressed in grams of capsaicin per gram of dried pepper. For the general taste of hot pepper, the correlation between Scoville heat unit (SHU) and the two capsaicinoids obtained was calculated as shown in Table 2 by using the relationship between this content (µg.g^{-1} DW) and its SHU rating of approximately 15 SHU equivalent to 1 µg.g^{-1} of capsaicinoids (Mathur et al., 2000).

Mineral analysis

Samples of 100 mg of dried and ground pepper fruits were extracted and mineralized using a mixture of sulfuric and selenious acids, as described by Isaac and Johnson (1976). The N and P was quantified by spectrophotometric method with an automated continuous-flow injection analyzer using the method 13-107-06-2-D (Model QuikChem 8000, Lachat Instruments, Loveland,CO). The K, Ca and Mg was determinate by a Perkin-Elmer atomic absorption spectrophotometer, model 3100, equipped with hollow-cathode lamps was used.

For all the determinations, analytical quality controls were made by using reference certified reference materials (MDR or SRM). The mean Concentrations of macronutrients of hot pepper fruit are given in Table 1, which also includes recommendation norm of nutrient contents in tissue of pepper fruits.

Table 1. Certified concentration values of SRM1515a of NIST and concentration values obtained in this work

Elément	Certified value (mean ± SD)	Obtained value (mean ± SD)	Units
Azote	2.4±1.20	2.14±0.30	mg.g^{-1} D M
Potassium	1.61±0.02	2.37±0.31	mg.g^{-1}
Phosphorus	0.31±0.03	0.39±0.07	mg.g^{-1}
Calcium	1.52±0.015	0.20±0.08	mg.g^{-1}
Magnesium	0.27±0.008	0.15±0.02	mg.g^{-1}

a SRM (Standard Reference Material) No. 1515 (Apple Leaves) of the National Institute of Standards and Technology (NIST). SD: standard deviation.

Statistical analysis

The results were subjected to quantitative analysis by the SAS software (version 6) the values presented in the figures represent the mean ± standard error.

III. Results And Discussion

Evolution of the total capsaicinoid content in hot pepper

The plants of pepper of each variety began to producing fruits in the second week of June. The monitoring approved during maturation started ten days after the beginning of the first peppers and continued until states of above ripeness. From 70 days of fruits development, peppers showed beginning of dehydration and intense red coloration.

The present study showed a statistically significant effect of fruit development stage on capsaicinoids content (Figure 3). The total capsaicinoids are detected at the earliest stage of fruit development, increased progressively and reaches a maximum at twenty days after fruit set (DAFS) in Msarreh and Chaabani (1624.77 and 902.52

µg.g^{-1} dry weight respectively) at thirty days in Rouge Long (1449.14 µg.g^{-1} DW) and at forty days in Baklouti Kairouan (339.44 µg.g^{-1} DW) and declined progressively. At 50 DAFS there was a significant reduction of total capsaicinoids 83% decrease in Msarreh, 98% decrease in Baklouti Kairouan and Chaabani, and 55% decrease in Rouge Long relative to the optimum content. This decrease in the level of total capsaicinoid was reported to the correlation between the pattern of accumulation of capsaicin and the activity of the different peroxidase isoenzyme (Estrada et al., 2000) who is involved in the degradation of capsaicinoids in peppers.

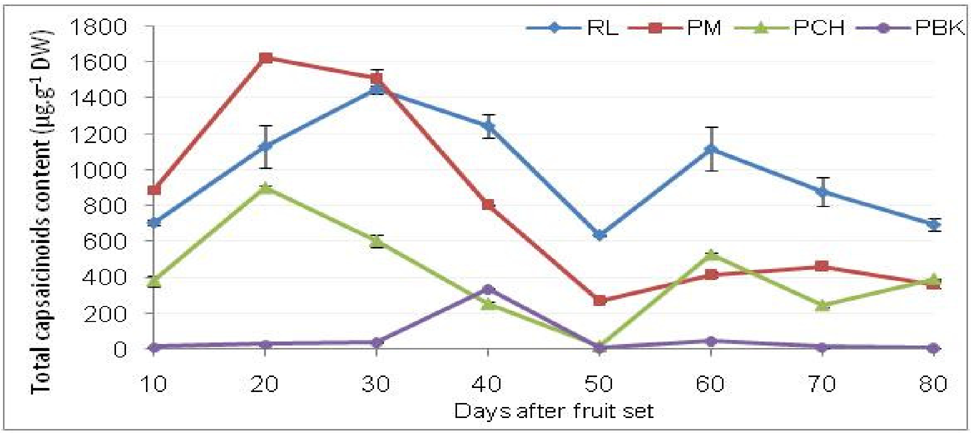


Fig. 3. Evolution of total capsaicinoids content during development and maturation of four hot pepper fruit. Vertical bars indicate standard deviation of the means. RL: Rouge Long, PM: Msarreh, PCH: Chaabani, PBK: Baklouti Kairouan.

After 50 day of fruit ripening, the results showed a little increase of total capsaicinoid. More capsaicinoids were determined in green fruits compared to fruits turning colour and red physiologically mature fruits. At 80 DAFS, capsaicinoids content was lower ~4.5 times in Msarreh, ~2.3 times in Chaabani than the maximum at 20 DAFS, thus, in Rouge Long total capsaicinoids was lower ~2 times than the maximum at 30 DAFS. In other hand, total capsaicinoids in Baklouti Kairouan was lower ~37 times than the maximum at 40 days after fruit setting. The level of total capsaicinoids in these cultivars Msarreh and Rouge Long was higher than that in other fruit varieties but accumulation patterns were similar (Estrada et al., 2000; Diaz et al., 2004). This result is in agreement with Robi and Sreelathaumary (2004) who reported that the red fruit stage have contained lower capsaicinoids content than the green fruits and higher than color changing stage.

Changes in capsaicinoids content at different maturation level

On considering the evolution of values of the three major capsaicinoids present in four hot pepper varieties, it can be seen that capsaicin, dihydrocapsaicin and nordihydrocapsaicin present a similar pattern of behavior.

Capsaicin and dihydrocapsaicin are the two major capsaicinoids present in four varieties other than nordihydrocapsaicin is the minor. Depending on the ripeness of the pepper fruits, these two capsaicinoids alternate as the major capsaicinoid (Fig. 4).

We have found differences in the levels of the predominant compounds of capsaicinoids. These differences were related with successive stages of fruit development. Thus in Msarreh, capsaicin is the major capsaicinoid in the first 10 days of development of the pepper. From 20 day of fruit ripening until day 50, the major capsaicinoids becomes dihydrocapsaicin. From this time on, capsaicin is once again the major capsaicinoid. In mature green or red peppers, for different varieties, capsaicinoid analysis showed that capsaicin was found to be higher than dihydrocapsaicin (Menichini et al., 2009). Although, in Chaabani at ten days after fruit set, dihydrocapsaicin is the major capsaicinoid. On day 20 of fruit ripening until day 50, capsaicin is the major compound. Starting 60 day the major capsaicinoid became dihydrocapsaicin.

The results of the present study confirm that the proportion of capsaicin, dihydrocapsaicin and nordihydrocapsaicin varied with variety and stage of maturity of the fruit.

During fruit ripening, capsaicin is present at levels between 44 % and 49 % in Msarreh, 26 % and 32 % in Rouge Long, between 44% and 48% in Chaabani, between 46 % and 63% in Baklouti Kairouan of total capsaicinoids (Fig. 5). Similarly, the amount of dihydrocapsaicin present between 40% and 47% in Msarreh, 49% and 54 % in Rouge Long, 36% and 47% in Chaabani, 29% and 44% in Baklouti Kairouan of total capsaicinoids. These two major capsaicinoids contribute between 88% and 92% in Msarreh and Chaabani, 78% and 85 % in Rouge Long, 80% and 95% in Baklouti Kairouan of total capsaicinoids during the development stage of pepper fruit. In addition, the level of nordihydrocapsaicin, depending on the fruit maturity stage, this is present at between 4% and 18% of total capsaicinoids for all varieties.

It can be observed (Fig. 5) that the decrease in the percentage of capsaicin that occurs between days 30 and 70 of ripening corresponds with an increase in the percentage of dihydrocapsaicin and nordihydrocapsaicin present in the Rouge Long. From day 70, the percentage of capsaicin begins to increase again. This leads to a decrease in the percentage of dihydrocapsaicin.

International Journal of Agriculture Innovations and Research
Volume 3, Issue 4, ISSN (Online) 2319-1473

However, between days 20 and 50 of ripening, the decrease in the percentage of capsaicin present in Msarreh corresponds with an increase in the percentage of the rest of the capsaicinoids. From day 50, the percentage of capsaicin begins to increase again. This is related with a decrease of dihydrocapsaicin and nordihydrocapsaicin. At over ripeness, 70 days of ripening, there is another decrease in the percentage of capsaicin, which is primarily attributable to an increase in the percentage of dihydrocapsaicin and nordihydrocapsaicin. The same results were found by Barbero et al. (2014) in cayenne pepper during ripening. In Chaabani, between days 20 and 40 of ripening, it can be seen a low increase in the percentage of capsaicin. This is associated with a low decrease of dihydrocapsaicin and nordihydrocapsaicin. At 50 days of ripening, a notable decrease of dihydrocapsaicin is attributable to an increase of nordihydrocapsaicin.

In Baklouti Kairouan, between 20 and 50 of ripening, it can be observed a decrease of capsaicin and dihydrocapsaicin. From day 50, the percentage of dihydrocapsaicin begins to increase again. Finally, in the state of over-ripening, starting at day 70 of ripening, there is another increase in the percentage of capsaicin and nordihydrocapsaicin, which is primarily attributable to a decrease in the percentage of dihydrocapsaicin.

The results of other studies showed different quantitative relations between particular capsaicinoids identified in hot pepper and they are evidence that the accumulation of capsaicinoids and their individual fractions is mainly determined by the genotype of a cultivar [Zewide and Bosland, 2000; Gibbs and O'Garro, 2004; Wang and Bosland, 2006; Wesołowska et al. 2011). Sung et al. (2005) proved that the rate of capsaicin, dihydrocapsaicin and nordihydrocapsaicin accumulation in a variety of pepper from different origins, differed among cultivars. The research of Contreras-Padilla and Yahia (1998). describes the evolution of capsaicinoids in the three varieties of hot Chilli peppers Habanero (*Capsicum chinense* Jacq.), De arbol (*C. annuum*) and Piquin (*C. annuum*) during the development, maturation and senescence of fruits. Capsaicin and dihydrocapsaicin were more abundant in the fruits of Habanero followed by De arbol and than by Piquin. Capsaicin was higher than dihydrocapsaicin in all three varieties for eight-development stage. Capsaicin, dihydrocapsaicin and capsaicinoids increased continuously and reached a peak at 45-50 days from fruit set in Habanero and De arbol and at 40 days in Piquin and than declined. The research showed that the peroxidase activity increased at the time when the concentration of capsaicinoids started to decrease. There was an inverse relationship between the evolution of capsaicin, dihydrocapsaicin and peroxidase activity that might indicate that this enzyme is involved in capsaicinoids degradation. Biles et al. (1997) and Bernal et al. (1993 a; b) reported that Pepper peroxidases have been implicated in the oxidation of capsaicin and dihydrocapsaicin, therefore influencing pungency. Estrada et al. (2000) suggested that capsaicin is a good substrate for this isoenzyme. Because the lignin level does not increase in pepper fruit throughout maturation this would suggest that, although the percentage of acidic peroxidases increases over the course of growth, the increment of these isoenzymes would not be related to lignifications processes as has been suggested by other researchers (Abeles and Biles, 1991).

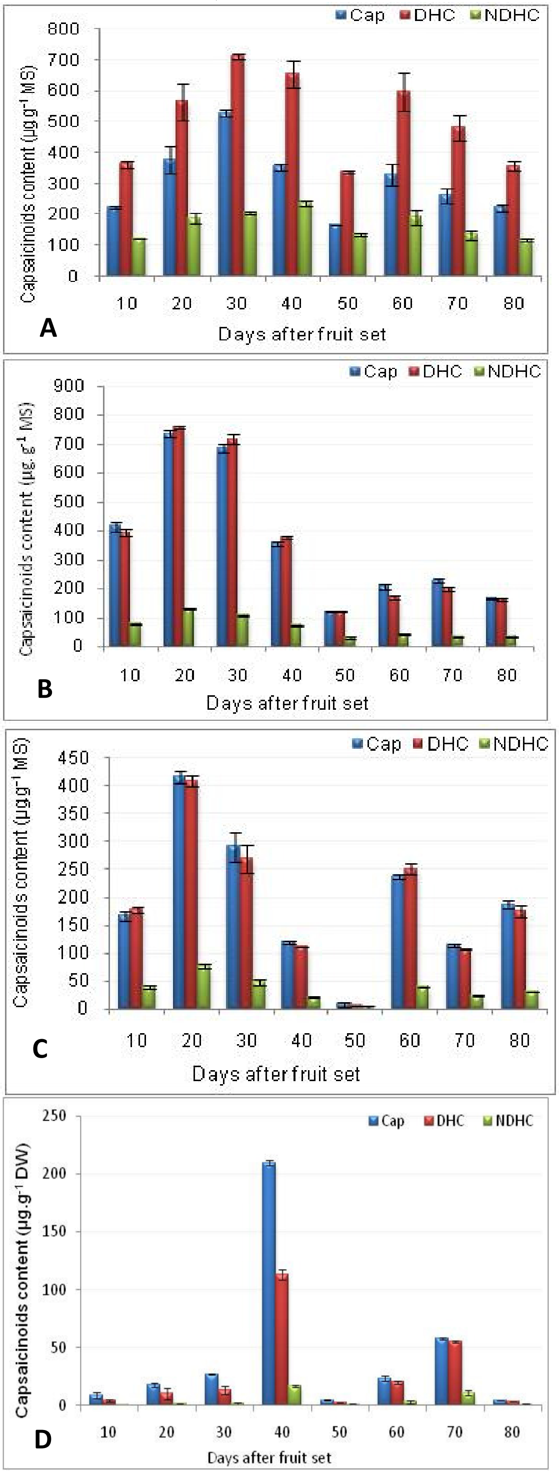


Fig. 4. Capsaicinoid concentration during development and maturation of four hot pepper varieties cultivated in an open field. A: Rouge Long; B: Msarreh; C: Chaabani; D: Baklouti Kairouan. Cap: capsaicin, DHC: dihydrocapsaicin, NDHC: nordihydrocapsaicin. Vertical bars indicate standard deviation of the means.

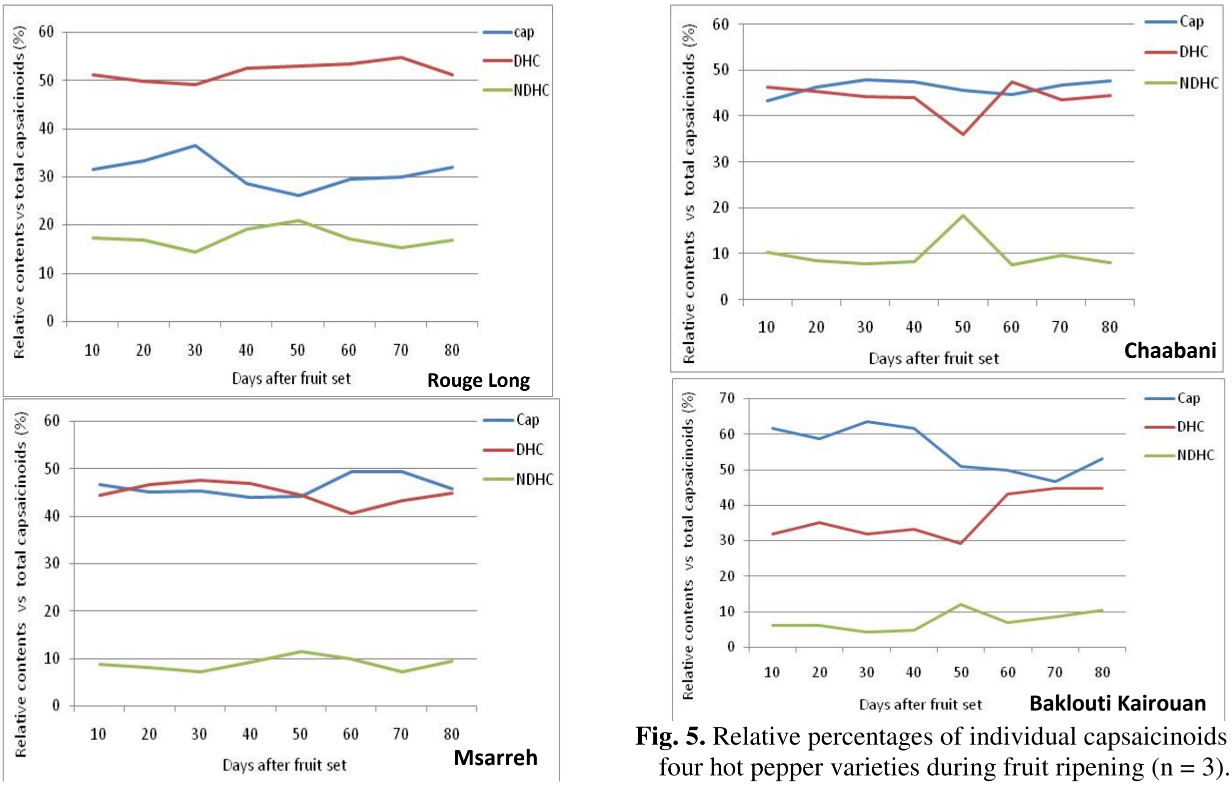


Fig. 5. Relative percentages of individual capsaicinoids in four hot pepper varieties during fruit ripening (n = 3).

Table 2. The maximum of capsaicinoids content, ratio of Cap: DHC and their Scoville Heat Unit (SHU) of four varieties of hot pepper.

Varieties	Rouge Long	Msarreh	Chaabani	Baklouti Kairouan
Capsaicin (µg.g^{-1} DW)	529.26±11.70	735.07±11.25	416.97±11.33	209.60±2.3
Dihydrocapsaicin (µg.g^{-1} DW)	712.79±8.97	758.88±5.24	408.95±9.19	112.94±4.4
Nordihydrocapsaicin (µg.g^{-1} DW)	207.10±5.47	130.82±2.81	76.61±3.95	16.78±0.8
Total capsaicinoids (µg.g^{-1} DW)	1449.14±25.62	1624.77±13.40	902.52±23.31	339.30±3.2
Total capsaicinoids (SHU)	21 800	24 400	13 550	5090
Stage of development (DAFS)	30	20	20	40
Ratio of Cap:DHC	0.67	0.97	1.02	1.85

In spite of large variations in capsaicinoids content within and between *Capsicum* spp. and due to cultivation conditions, the ratio of principal components, capsaicin and dihydrocapsaicin are about 1:1 for *C. annuum* and 2:1 for *C. frutescens* (Ravishankar et al., 2003).

The ratio of capsaicin to dihydrocapsaicin has been considered a characteristic of the species and is different among the cultivars (Ishikawa, 2003). The Table 2 indicates that the ratio of capsaicin to dihydrocapsaicin for four cultivars was 0.67 for Rouge Long, 0.97 for Msarreh, 1.02 for Chaabani and 1.85 for Baklouti Kairouan. These results are in agreement with those of Govindarajan et al. (1987) which state that, in the case of *Capsicum annuum* L., the ratio varies in a range from 0.64 to 1.94.

Macronutrient accumulation during fruit ripening

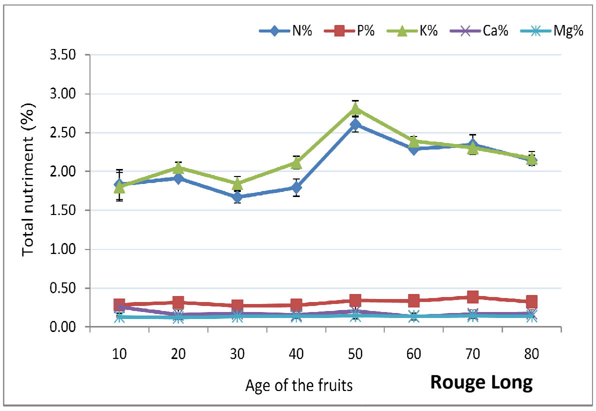
Analyses of variance showed a significant effect of the ripening stage on the level of five macroelements concentration. for all pepper varieties, the N and K were the most abundant of the analyzed elements in all samples, followed by P, Ca, Mg. The general order of the macroelements content is K > N> P >Ca>Mg (Fig. 6). The N, K, P and Ca present higher levels in red pepper than in green pepper. The biggest difference has been found for the N and K levels. During each development stage, Potassium was the mainly rich mineral in pepper fruit (Fig. 6).

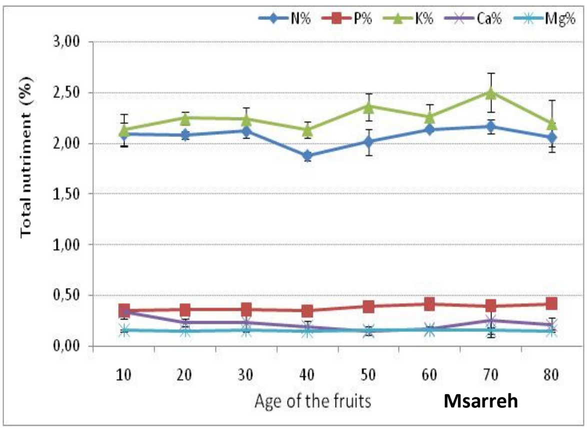
The levels of P, Ca and Mg found for the different ripening states can be considered as similar. Magnesium content in pepper fruits was similar to that of calcium. The first three most abundant mineral elements (K, N and P) were consistently highest in Baklouti Kairouan. The K values ranged from 180.20 (Rouge long) - 305.50 mg/100g (Chaabani) for all stages of fruits development. These results was in agreement with those of Rubio et al.

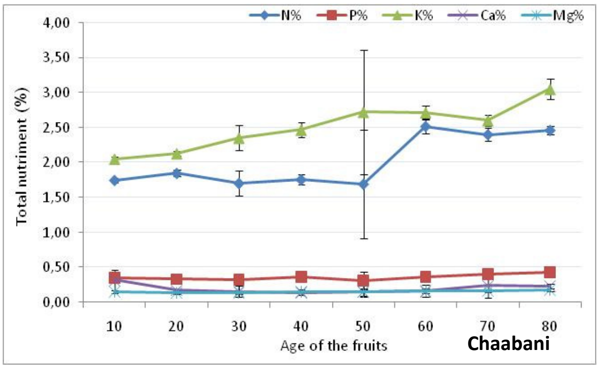
(2002) Who found the concentrations of macronutriment K, P, Ca and Mg present higher levels in red pepper than in green one. Although, Terbe et al. (2006) showed that the N, P and K levels detected in the individual organs were affected by soil nutrient levels to a considerable extent but differing from plant organ to plant organ. The smallest variation is detectable in the pepper fruit.

The daily adequate dietary intake of K is 4700 mg (Rohman et al., 2010). Therefore, 100 g of peppers in this study would supply about 3.8 - 6.5% of the K required by an average adult. Potassium is a vital mineral that constitutes about 70% of the positive ions in cells (Ogunlade et al., 2012). Potassium is essential in the regulation of acid-base and water balance of the cells (Rohman et al., 2010). The result showed that the pepper is a good source of potassium.

All the pepper varieties recorded higher content of P in red mature fruit than the green ones. Phosphorus is most abundant in Baklouti Kairouan than the other varieties. The dietary reference intake (DRI) for phosphorous is 700 mg (Biziuk and Kuczynska, 2007). Calcium and phosphorous are essential in the formation and development of bone. Food is considered 'good' if the Ca:P ratio is above 1 and 'poor' if the ratio is less than 0.5 (Aremu et al., 2011). Therefore, the Ca/P ratio in this study is considered poor for Baklouti Kairouan (0.45). While the Ca/P ratios for other pepper varieties can be considered fair as they recorded values less than 1 but greater than 0.5.







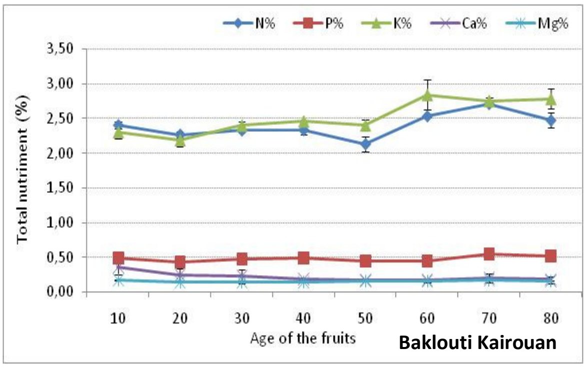


Fig. 6. Percentage of the mineral content of macroelements according to the ripening stage of pepper samples

IV. Conclusion

Based on the data in this study, significant differences were found in capsaicinoids and macro-element contents among hot pepper varieties and development stages. Results revealed that mature green stage (20 to 40 days after fruit setting) was ideal to acquire maximum pungency, while peppers at red ripe stage were best sources of macronutrient. Green pepper fruits contained higher levels of total capsaicinoids in all varieties tested. The pepper varieties can also be utilized as good sources of capsaicinoids and valuable minerals. At the same time, consumers should be educated about the benefits of including fresh and dried hot pepper fruit in their daily diet for the potential health advantages.

References

[1] Abeles, F.B. and C.L.Biles. 1991. Characterization of Peroxidases in Lignifying Peach Fruit Endocarp. Plant Physiology 95: 269-273.

[2] Al Othman, Z. A., Y. B. H. Ahmed, M. A. Habila, A. A. Ghafar. 2011. Determination of capsaicin and dihydro-capsaicin in Capsicum fruit samples using high performance liquid chromatography, Molecules, 16, 8919–8929.

[3] Aremu, M.O., B.G. Osinfade, S.K. Basu and B.E. Ablaku. 2011. Development and nutritional quality evaluation of Kersting's groundnut-Ogi for African Weaning diet. American Journal of Food Technology 6(12): 1021-1033.

[4] Barbero, G. F., A. G. Ruiz, A. L. Miguel Palm, J. C. Vera, C. G. Barroso. 2014. Evolution of total and individual capsaicinoids in peppers during ripening of the Cayenne pepper plant (Capsicum annuum L.). Food Chemistry 153: 200–206.

[5] Barceloux D.G., 2008. Pepper and Capsaicin (Capsicum and Piper Species). [In:] Medicaltoxicol-ogy of natural substances: foods, fungi, medicina l herbs, plants and ve nomous animals. Wiley (New Jersey), 71–73.
[6] [6] Ben Mansour-Gueddes S., N. Tarchoun, S. Ibn Maaouia-Houimli, S. Saguem, Capsaicinoids content in four Tunisian hot pepper varieties grown in an open field (Capsicum annuum L.). Cont. J. Agronomy 6(1): 8-13 (2012).
[7] Bernal, A.M., A.A. Calderon, M.A. Pedreno, R. Munoz, A.R. Barcelo, F.C. Merino. 1993a. Capsaicin oxidation by peroxidase from Capsicum annuum (var. annuum) fruits. J. Agric. Food Chem. 41: 1041-1044.
[8] Bernal, A.M., A.A. Calderon, M.A. Pedreno, R. Munoz, A.R. Barcelo, F.C. Merino. 1993b. Dihydrocapsaicin oxidation by Capsicum annuum (var. annuum) peroxidase. Journal Food Sciences 58: 611-613.
[9] Biles, C.L., G. D. Kuehn, M.M. Wall, B.D. Bruton and Wann E.V. 1997. Characterisation of chile pepper fruit peroxidases during ripening. Plant Physiol Biochem 35(4): 273-280.
[10] Biziuk, M. & J. Kuczynska. 2007. Mineral Components in Food — Analytical Implications, In: Mineral Components in Foods, Szefer P. And Nriagu J.O. (Eds), pp. 1-31, Taylor & Francis Group, ISBN 978-0-8493-2234-1, Boca Raton, FL.
[11] Buczkowska H., Dyduch J., A. Najda. 2013. Capsaicinoids in hot pepper depending on fruit maturity stage and harvest date. Acta Sci Pol Hortorum Cultus 12(6): 183-196.
[12] Buczkowska H., Michałojć Z. 2012. Comparison of qualitative traits, biological value, chemical compounds of sweet pepper fruit. J. Elem. 17(3): 367-377.
[13] Buczkowska H. 2007. Evaluation of yielding of Polish sweet pepper cultivars in the field cultivation in the aspect of breeding progress. Progress in Research on Capsicum & Eggplant, K. Niemirowicz-Szczytt (ed.) Warsaw University of Life Sciences Press, 257-265.
[14] Caterina, M. J., A. Leffler, A.B. Malmberg, W.J. Marti, J. Trafton, K.R. Petersen-Zeitz, M. Koltzenburg, A.I. Basbaum, D. Julius. 2000. Impaired nociception and pain sensation in mice lacking the capsaicin receptor. Science 13: 288- 306.
[15] Caterina, M.J., M.A. Schumacher, M. Tominaga, .A. Rosen, J.D. Levine, D.Julius. 1997. The capsaicin receptor: A heat activated ion channel in the pain pathway. Nature 389.
[16] Chaux, C.I. and C.I. Foury. 1994. Le piment, in Productions Légumières-Légumes Fruits, Lavoisier, Paris, pp. 271-295.
[17] Collins, M.D., L.M. Wasmund, P.W. Bosland. 1995. Improved method for quantifying capsaicinoids in capsicum using high-performance liquid chromatography. HortScience 30: 137-139.
[18] Contreras-Padilla M. and E.M. Yahia. 1998. Changes in capsaicinoids during development, maturation, and senescence of chili peppers and relation with peroxidase activity. J Agric Food Chem 46:2075-2079.
[19] Diaz, J., F. Pomar, A. Bernal, F. Merino. 2004. Peroxidases and the metabolism of capsaicin in Capsicum annuum L. Phytochemistry 3:141-157.
[20] Ekholm, P., H. Reinivuo, P. Mattila, H. Pakkala, J. Koponen, A. Happonen, J. Hellström, M.J. Ovaskainen. 2007. Changes in the mineral and trace element contents of cereals, fruits and vegetables in Finland. J Food Composit Analysis 20:487-495.
[21] Estrada, B., F. Pomar, F. Diaz, F. Merino, A. Bernal. 1997. Evolution of capsaicinoids in Capsicum annuum L. var. annuum cv. Padron at different growth stages after flowering. Capsicum Eggplant Newsletter 16: 60-64.
[22] Estrada, B., F. Pomar, J. Diaz , F. Merino, M.A. Bernal, 1998. The effect of the mineral supplementation regime on fruit development and the pungency level in PadroÂn peppers. J. Hortic. Sci. Biotechnol. 73(4): 493-497.
[23] Estrada B., J. Dıaz, F. Merino, M. A. Bernal, 1999. The effect of seasonal changes on the pungency level of Padron pepper fruits. Capsicum Eggplant Newsletter 18: 28-31.
[24] Estrada, B., M.A. Bernal, J. Diaz, F. Pomar, F. Merino. 2002.Capsaicinoids in vegetative organs of Capsicum annuum L. in relation to fruiting. J Agric Food Chem 50:1188-1191.
[25] Estrada, B., M.A. Bernal, J. Diaz, F. Pomar, F. Merino. 2000. Fruit development in Capsicum annuum: Changes in capsaicin, lignin, free phenolics, and peroxidase patterns. J Agric Food Chem 48: 6234-6239.
[26] Garcés Claver, A. 2007. Estudio de los componentes del carácter picante en pimiento (Capsicum spp.): técnicas de evaluación, análisis genético y molecular. Zaragoza University pp. 141.
[27] Gibbs, H.A.A., and L.W. O'Garro. 2004. Capsaicin content of west indies hot pepper cultivars using colorimetric and chromatographic techniques. HortScience 39(1): 132–135.
[28] González-Zamora, A., E. Sierra-Campos, J. G. Luna-Ortega, R. Pérez-Morales, J . Carlos Rodríguez Ortiz and J.L. García-Hernández. 2013. Characterization of Different Capsicum Varieties by Evaluation of Their Capsaicinoids Content by High Performance Liquid Chromatography, Determination of Pungency and Effect of High Temperature. Molecules 18: 13471-13486.
[29] Govindarajan, V.S., D. Rajalakshmi, N. Chand. 1987. Capsicum production, technology, chemistry, and quality. Food Sci Nutr 25:185-28.
[30] Gurung, T., S.Techawongstien, B. Suriharn and S.Techawongstien, 2011. Impact of Environments on the Accumulation of Capsaicinoids in Capsicum spp. Hortscience 46(12):1576–1581.
[31] [31] Hall, R. D., M. A. Holden, M. M. Yeoman. 1987. The accumulation of phenylpropanoid and capsaicinoid compounds in cell cultures and whole fruit of the chili pepper, Capsicum frutescens. Plant Cell, Tissue Organ Cult ., 8: 163-176.
[32] [32] Huang, X.F., J.Y. Xue , A.Q. Jiang , H.L. Zhu . 2013. Capsaicin and its analogues: structure-activity relationship study. Curr Med Chem. 20 (21):2661-2672.
[33] [33] Isaac, R.A., and W.C. Johnson. 1976. Determination of total nitrogen in plant tissue, using a block digestor. J. Assoc. Off. Anal. Chem. 59:98–100.
[34] Ishikawa, K. 2003. Biosynthesis of capsaicinoids, in Capsicum, Ed. by Amit Krishna pp. 87-95.
[35] Ishikawa K., 2003, Biosynthesis of capsaicinoids in capsicum. In: Capsicum. Amit Krishna (Eds) P: 87-95
[36] Iwai , K., T. Suzuki, H .Fujiwake, 1979. Formation and accumulation of pungent principle of hot pepper fruits, capsaicin, and its analogues, in Capsicum annuum var. annuum cv. Karayatsubusa at different stages of flowering. Agric Biol Chem 43: 2493-2498.
[37] Kozukue N., J.S. Han, E. Kozukue, S. J. Lee, J. A. Kim, K. R. Lee, C. E. Levin et M. Friedman, 2005. Analysis of eight Capsaicinoids in Peppers and Pepper-conteining Foods by High-Performance Liquid Chromatography-Mass Spectrométry. J. Agric. Food Chem. (53), 9172-9181.
[38] Jadczak, D., Grzeszczuk M., Kosecka D. 2010. Quality characteristics and content of mineral compounds in fruit of some cultivars of sweet pepper (Capsicum annuum L.). J. Elementol. 15(3): 509–515.
[39] Kozukue, N., J. Han, S. Lee, J. Kim, K .Lee, M. Park, C.E. Levin, M. Friedman. 2005. Analysis of seven capsaicinoids in peppers and pepper-containing foods by liquid chromatography-mass spectrometry. J Agric Food Chem 53: 9172-9181.
[40] Lee J. H., S.H. Kim, Y. H. Jung, J. A. Kim, M. O. Lee, P.G. Choi, W. Choi, K. N. Kim, and N.S. Jwa, 2005. Molecular cloning and functional analysis of rice (Oryza sativa L.) on defense signaling pathway. Plant Pathol. J., 21: 149–157.
[41] Materska, M. and I. Perucka. 2005. Antioxidant activity of the main phenolic compounds isolated from hot pepper fruit (Capsicum annuum L.). J Agric Food Chem 53(5): 1750-1756.
[42] Mathur, R., R.S.Dangi, S.C. Dass, R.C. Malhotra 2000. The hottest Chilli in India. Current Science 79(3): 287-288.
[43] Menichini F., R.Tundis, M. Bonesi, M. R. Loizzo, F. Conforti, G. Statti, B. De Cindio, P.J. Houghton, F. Menichini, 2009. The influence of fruit ripening on the phytochemical content and biological activity of Capsicum chinense Jacq. cv Habanero. Food Chemistry, 114:553–560.
[44] Monforte-Gonzalez, M., A. Guzman-Antonio, F. Uuh-Chim, , & F. Vazquez-Flota. 2010. Capsaicin accumulation is related to nitrate content in placentas of habanero peppers (Capsicum chinense Jacq.). Journal of the Science of Food and Agriculture, 90(5): 764–768.
[45] Nwokem CO, Nwokem NC, Usman Odjobo BO, Ocholi OJ, Yebpella GG, Batari ML. 2011. Determination of capsaicin

content in various parts of some peppers grown in Nigeria. Continental Journal of Agronomy, 5 (1): 6-10.

[46] Ogunlade I., A.A. Alebiosu and A. I. Osasona. 2012. Proximate, mineral composition, antioxidant activity, and total phenolic content of some pepper varieties (Capsicum species). Int. J. Biol. Chem. Sci. 6(5): 2221-2227.

[47] Quian, H.1., Z. Fu , W. Huang , H . Zhang , J. Zhou , L. Ge , R. Lin , H. Lin , X. Hu . 2010. Synthesis and preliminary biological evaluation of capsaicin derivatives as potential analgesic drugs. Med Chem. 6(4): 205-210.

[48] Rahman, M.J.and H. Inden. 2012. Effect of nutrient solution and temperature on capsaicin content and yield contributing characteristics in six sweet pepper (Capsicum annuum L.) cultivars. Journal of Food, Agriculture & Environment10 (1): 524-529.

[49] Ravishankar, G.A., B. Suresh, P .Gridhar, R. Ramashandra and J.T. Sudhakar. 2003. Biotechological Studies on Capsicum for metabolite production and plant improvement, in Capsicum, Ed. by Amit Krishna pp. 97-122.

[50] Robi, R. and I. Sreelathakumry. 2004. Influence of maturity at harvest on capsaicin and ascorbic acid content in Hot Chilli (Capsicum Chinense jacq.). Capsicum and Eggplant Newsletter 23:13-16.

[51] Rohman, A., S. Riyanto, N. Yuniarti, W.R .Saputra , R.Utami and W. Mulatsih,. 2010. Antioxidant activity, total phenolic, and total flavonoid of extracts and fractions of red fruit (Pandanus conoideus Lam). International Food Research Journal 17: 97-106.

[52] Rubio C., A. Hardisson, R. E. Martín, A. Báez, M. M. Martín,· R. Álvarez. 2002. Mineral composition of the red and green pepper (Capsicum annuum) from Tenerife Island. Eur Food Res Technol. 214:501–504.

[53] Salgado-Garciglia, R. and N. Ocho-Alejo. 1990. Increased Capsaicin Content in PFP-Resistant Cells of Chili Pepper (Capsicum annuum L.). Plant Cell Reports 8(10):617-620.

[54] Spiller, F., Alves, M.K., Vieira, S.M., Carvalho, T.A., Leite, C.E., Lunardelli, A., Poloni, J.A., Cunha, F.Q., de Oliveira, J.R., 2008. Anti-inflammatory effects of red pepper (Capsicum baccatum) on carrageenan- and antigen-induced inflammation. The Journal of Pharmacy and Pharmacology 60, 473–478.

[55] Sung, Y., Y.Y. Chang and N.L. Ting. 2005. Capsaicin biosynthesis in water-stressed hot pepper fruits. Botanical Bulletin of Academia Sinica 46: 35- 42.

[56] Surh, Y. J., S. S. Lee, 1995. Capsaicin, a Double-Edged-Sword - Toxicity, Metabolism, and Chemopreventive Potential. Life Sciences 56(22): 1845-1855.

[57] Suzuki, T., H. Fujiwake and K. Iwai. 1980. Intracellular localization of capsaicinoid, in Capsicum fruit. Microscopic investigation of the structure of the placenta of Capsicum annuum var. annuum cv. Karayatsubusa. Plant Cell Physiol 21(5):839-853.

[58] Terbe, I., Zs. Szabó, N. and Kappel,. 2006. Macronutrient accumulation in green pepper (Capsicum annuum L.) as affected by different production technologies. International Journal of Horticultural Science, 12 (1): 13–19.

[59] Wang, D. and P.W. Bosland, 2006. The genes of Capsicum. HortScience 41(5):1169–1187.

[60] Wesołowska, A., D. Jadczak and M. Grzeszczuk. 2011. Chemical composition of the pepper fruit extracts of hot cultivars Capsicum annuum L. Acta Sci. Pol. Hortorum Cultus 10(1): 171-184.

[61] Zewide, Y. and P.W. Bosland. 2000. Evaluation of genotype, environment, and genotype-by environment interaction for capsaicinoids in Capsicum annuum L. Euphytica 111: 185-190.