

Comparative Study of Certain Hot Pepper Genotypes (*Capsicum Annum L.*) Cultivated in Oltenia, România



Chemistry

KEYWORDS : *Capsicum annum*, local genotypes, vitamin C, capsaicin, carotene

Maria Dinu

University of Craiova, Department of Horticulture and Food Science, 13 A.I.Cuza Street, nr.13, Craiova Dolj

Mihaela Gabriela Dumitru

University of Craiova, Department of Chemistry, 107i Calea București Street, Craiova, Dolj.

Ioan Pintilie

Associate Professor, University of Craiova, Department of Horticulture and Food Science, 13 A.I.Cuza Street, nr.13, Craiova Dolj.

ABSTRACT

The purpose of this study is to focus on the qualities that some of the local hot peppers varieties have as compared to a variety created, by analysing the content of the main bioactive compounds.

The study refers to 7 genotypes of hot peppers, 6 of which, originated from different areas of Oltenia (the Southern part of Romania), are well known and are cultivated by private producers with tradition in the area, and another one, the De Cayenne variety, which has been used as witness. The cultivars of such genotypes have not been treated against diseases, no pest control treatments have been applied due to their natural genetic resistance and they have not been chemically fertilised in vegetation.

In the present study the variation of the bioactive compounds has been evaluated in all seven pepper fruit varieties cultivated in Oltenia. The statistical analysis of the results obtained showed significant differences amongst local populations as well as between them and the variety created.

The content of ascorbic acid varied compared with the witness sample by 14.3% in case of sample V_3 , by 24.2% in case of sample V_6 and by 38.72% in sample V_7 . The content of carotene recorded variations of the concentration compared with the witness sample by 21.74% in sample V_3 , by 17.98% in case of sample V_6 and 23.29 for sample V_7 . The capsaicin contained by samples showed concentration changes compared with the witness concentration by 18.1% for V_3 , by 19.5% for V_7 , and by 21.6% for V_6 .

INTRODUCTION

Lately, the world has become more and more interested in organic fruits and vegetables rather than conventional ones. A high quality standards as well as a better taste are the reasons why these products have been on high demand (Heaton, 2001). It is worth mentioning that the way they are produced has a higher impact on our health in general (Williams, 2002), yet it is also common knowledge that we are not fully informed on the impact on health as well as on the need to learn as much as possible from the research studies made (Brandt și Leifert, 2005).

There is enough proof suggesting that an increase of the content of antioxidants inside the body can keep it healthy and can prevent several chronic diseases, such as some types of cancer, cardiovascular diseases, aging (Thompson, L.U. 1994). Antioxidants can suppress the forming of free radicals that slow down the oxidation process of proteins, lipids and DNA (Jacobs, D.R., and al., 1995).

Through its content of antioxidant vitamins, carotenoids, phenolic compounds and capsaicinoids hot peppers represent an important source of natural antioxidants.

Peppers are very important for the human nutrition with an increasing popularity lately and an ever increasing number of varieties and hybrids available on the market. Pepper fruits are important sources of vitamins C and E, provitamin A and carotenoids (Materska și Perucka, 2005).

Carotenoids have been known and used since ancient times when *Capsicum* fruits were used as natural food colorants. More than 30 different pigments have been identified in pepper fruits. They include green pigments (chlorophyll a and b), yellow-orange pigments (lutein, zeaxanthin, β -cryptoxanthin, violaxanthin, antheraxanthin and β -carotene) and red pigments (capsanthin, capsorubin and cryptocapsin) (Sun-Hwa Ha et al., 2007). In medicine, carotenoids are being used as detoxification agents for enzyme systems and they represent powerful antioxidants acting in the sense of preventing certain diseases connected to the aging process, such as cataract and cardiovascular diseases or certain forms of cancer, protecting the cells against aging, reducing the cardiovascular risk and strengthening the immunity system (Debjani Dutta et al., 2005).

The ascorbic acid is highly present in pepper fruits, content which varies depending upon the variety and the maturity stage of fruits reaching up to 139 – 160 mg/100g of fresh substance in the fruits at technological maturity and 211 – 300 mg/100g of fresh substance in the fruits at physiological maturity (Bernardo et al., 2008; Riefschneider, 2000), reaching high levels in certain *Capsicum* varieties, i.e. up to 400 mg/100g of fresh substance in *Capsicum frutescens*.

Peppers also contain phenols (Balasundram and al., 2006; Rodrigues, S. and al., 2008; Aniel, O. and al., 2010), different flavonoids and carotenoids (Materska și Perucka, 2005; Araceli M.V.G. et al., 2011). Carotenoids are important natural colorants in vegetables that mostly give the orange and red colour. The yellow-orange colour in peppers is given by α and β carotene, xanthine, lutein and β cryptoxanthin (Howard et al., 2000).

These compounds are antioxidants and can reduce the harmful reactions inside the human body, therefore the consumption of pepper can prevent several diseases which have been associated with the oxidation of free radicals, such as cardiovascular diseases (Pérez-Gálvez et al., 2003), cancer and neurological diseases (Harborne and Williams, 2000; Delgado-Vargas and Parede Lopez, 2003; Shetty, 2004).

Govindarajan and later Bosland (Govindarajan, 1985; Bosland, 1994), have established that there is a big diversity in the capsaicin content and composition of *Capsicum* fruits, even inside the same variety, also (Navarro et al., 2006; Conforti et al., 2007; Deepa et al., 2007) have learned that the composition of fruits changes depending upon the maturity stage of fruits and factors such as environment, light, humidity, soil and fertilisers present during the vegetation period are believed to have an impact on the capsaicin content. According to Titze et al., 2002, its content is even influenced by the age of the fruit.

Capsaicinoids are food additives largely known and highly appreciated for their sensory features like colour, heat and flavour in several regions of the world. The consumption of hot pepper is mainly owed to their hot taste which produces hot flavour and heat leading to its big sales. Capsaicin is one of the five chemical compounds forming the class of capsaicinoids (capsaicin, dihydrocapsaicin, nordihydrocapsaicin, homodihydrocapsaicin and

homocapsaicin) and the predominant compound in peppers (aprox.90%).

Capsaicin has been long studied during the last years and it was found that it can also have an antibacterial effect on a certain group of bacteria (Henderson & Slickman, 1999; Dorantes et al, 2000), as well as anti-inflammatory and antioxidant. It has been successfully used in treating cancer, heart attack, atherosclerosis, osteoporosis, psoriasis, etc.

Vitamin C, previously mentioned, also has a strong antioxidant character and can vary a lot depending upon the genotype and the period when fruits are harvested (technological maturity or physiological maturity).

As consequence, this study has been made in order to determine the content of vitamin C, capsaicin and carotene in certain local hot pepper genotypes whose cultivars have been fertilised with compost resulted from the decomposing of vegetal and household wastes.

MATERIALS AND METHODS

The study was made in Almăj, a town located in the South of Romania, in an area with transitional temperate continental climate. The experience was made during 2008-2009 and had seven variants. Each variant with three repetitions was represented by a local population (genotype), as follows:

V1(Mt) – De Cayenne; V2 - De Işalnița – Dolj; V3 - De Almăj – Dolj; V4 - De Corabia – Olt; V5 – Șapte frați highly spread throughout Oltenia; V6 - SCDL 1 – line resulted through selection from hybrid population; V7 - SCDL 2 - line resulted through selection from hybrid population.

De Cayenne has been used as witness as well as it is a homologated variety implemented for a long time in the cultivar and well known by field experts.

It should also be mentioned that since autumn, this experimental lot has been fertilised with 60 t/ha of compost resulted from the decomposition of vegetal and household wastes.

For statistical interpretation of the data, the ANOVA method has been used.

The determination of ascorbic acid

A sample of 5-10 g of pepper, previously ground with quartz sand has been put into a 100ml- balloon by using a solution of 2% hydrochloric acid. It has been stirred and after sedimenting it has been filtered into a dry glass. A 10-ml aliquot has been passed into a Berzelius glass, to which 30 ml of distilled water, 5 ml of 1% potassium iodate and 1 ml solution of amidon have been added. It has been then titrated with potassium iodate N/250 stirred until becoming bluish (Bița et al., 2009, Dumitru et al., 2010, Samuella et al., 2004, Roberts et al., 1987).

The calculation of ascorbic acid concentration is made by using the equation

$$\text{Vitamin C mg \%} = 352. \text{ n.f} / G$$

Where:

n - ml used for titration;

f – the factor of the potassium iodate N/250;

G – the sample weight in grams.

The data recorded were statistically processed by using the variant method and by establishing the limit differences (DL).

The determination of total carotenoids

The weighed samples, having been put separately in 95% in acetone (50 ml for each gram), were homogenized with Braun MR 404 Plus for one minute. The homogenate was filtered and was

centrifuged using the Hettich Universal 320/320R centrifuge at 2500 rpm for ten minutes. The supernatant was separated and the absorbances were read at 400-700nm on Cary 50 spectrophotometer. It was recorded that Chlorophyll a showed the maximum absorbance at 662 nm, chlorophyll b at 646 nm and total caroten at 470 nm and the amount of these pigments was calculated according to the formulas (Lichtenthaler et al., 1985, Dere et al., 1998, Wellburn et al., 1994, Grung et al., 1992), see Table 2.

$$\text{Ca} = 11.75 \text{ A662} - 2.350 \text{ A645}$$

$$\text{Cb} = 18.61 \text{ A645} - 3.960 \text{ A662}$$

$$\text{Cx+c} = 1000 \text{ A470} - 2.270 \text{ Ca} - 81.4 \text{ Cb}/227$$

Ca = Chlorophyll a, Cb = Chlorophyll b, Cx+c = Total carotene

The determination of capsaicin

Procedure

20g of finely ground chili peppers were placed in the extraction shell of a Soxhlet extractor and isopropanol was percolated through the sample until no more green color appeared in the percolated isopropanol. The extract was cooled and adjusted to a volume of 200 ml with isopropanol. Sg of charcoal was added to the colored extract. The slurry was heated to boiling for the duration of 3 min, filtered and the charcoal residue washed several times with isopropanol. The clear extract was then evaporated to dryness in the flash-evaporator.

The oil residue was dissolved in petroleum ether and transferred to a separatory funnel and washed with distilled water. After discard discarding the aqueous layer, the ether layer was evaporated to dryness in the flash-evaporator. The purified oily residue remaining was dissolved in isopropanol, collected in a volumetric flask, and total volume brought up to 25 ml with isopropanol. The absorbance was read at 281 nm against an isopropanol blank. Capsaicin standard solutions containing 10, 20, 30, 40 and 50µg/ml capsaicin were prepared in isopropanol and their absorbance values recorded at 281nm. A standard curve plotting absorbance against µg capsaicin/ml was prepared and the quantity of capsaicin in the samples was determined from this standard curve (Augustio Trejo-Gonzalez and Carlos Wildal Tamirano, 1973).

Statistical Calculation

The data recorded were statistically processed by using the analysis of the variant (ANOVA) and the calculation of the limit differences (DL).

Results and Discussions

The determinations were made on samples of fruits harvested at physiological maturity when their colour was red and they represent average values of the repetitions within the studied variants.

Green, yellow, red or orange peppers are remarkable for their capacity of strengthening the immunity system and their fight against free radicals, they represent one of the most important sources of vitamin C (red peppers contain three times more vitamin C than oranges), as well as of β - caroten (important compound in the fight against cancer), arguments which have determined several scientists to thoroughly study these miraculous species.

Regarding the content of vitamin C (Table 1) in the local populations under study one can notice that 6 out of the 7 cultivars had content higher or equal to that of the De Cayenne variety that was used as witness in our study. If we make a comparison with the data existent in the specialty literature (170 mg/100g⁻¹) we observe that our variants (V₆ and V₇) tend to a value double than the last ones.

The studies made showed that vitamin C varied within quite large limits depending on the cultivar and the fruit maturity stage at the moment when determinations were made (Howard

et al, 2000). In their study made in Turkey, Topuz and Özdemir (2007), showed that vitamin C recorded values between 15.2 and 64.9mg in 100 g⁻¹ of *C. annuum* fresh fruits. Same variety had vitamin C between 29.1 and 183.2 mg in 100 g⁻¹ depending on the cultivar as well as on the fruit harvesting stage (Araceli M.V.G. et al., 2011). Cruz-Pérez et al.,(2007) showed that vitamin C varied from 238.35 to 455.4 mg weight of 100g⁻¹ fresh substance of *C. pubescens*. Gnayfeed et al. (2001) observed that vitamin C recorded maximum values at the fruit physiological maturity (when turned red).

Segúnd Martinez et al. (2005) observed that vitamin C contained in the Fresno cultivar was 160 mg in 100 g-1 in fresh red fruits and 109 mg in 100 g-1 in green pepper fruits.

De Işalniţa cultivar had the same content as De Cayenne (269.28 mg in 100g-1), while Şapte fraţi, De Almăj, SCDL 1 and SCDL 2 had values exceeding the witness by far, the differences from the witness variety being positive and statistically covered.

One single cultivar, De Corabia, had a content of vitamin C (260.48 mg in 100 g -1) below De Cayenne, but as one can see in Table 1, this difference is highly significant and negative.

Based on over 650 scientific experiments made in the US, Italy as well as Sweden, it was proven that the substance called capsaicin contained by hot peppers giving the heat sensation inside the mouth when eaten, is directly responsible for easing several types of pain starting from common headaches to arthritis pains.

Table 1. The content of vitamin C in hot pepper fruits

Variant	mg/100g fresh substance (sp)	%	Difference vs. witness (mg/100g sp)	Significance
V1-Mt	269.28	100.0	-	-
V2	269.28	100.0	-	-
V3	283.58	105.3	+14.30	***
V4	260.48	96.7	-8.80	ooo
V5	272.80	101.3	+3.52	**
V6	293.48	108.9	+24.20	***
V7	308.00	114.3	+38.72	***

DL 5%=1.86; DL 1%=2.61; DL 0.1%=3.68

β-carotene and capsaicin were also determined from fresh fruits immediately after harvesting in the experimental field. The content of β - carotene (Table 2) recorded the highest values in De Işalniţa variety, SCDL 1, De Almăj variety and SCDL 2 (50.04 to 60.79 mg 100 g sp), which dropped in case of Şapte fraţi (42.20 mg 100 g sp) followed by the De Corabia cultivar with 39.15 mg 100 g sp and then followed by the lowest value in the De Cayenne, i.e. 37.50 mg 100 g sp.

All variants show differences compared with the witness variety and are statistically covered being very significant and positive.

According to Howard et al. (2000), the variations of the content of β-carotene in *C. annuum* and *C. frutescens* were determined by the maturity stage of pepper fruits.

Table 2. The content of β-carotene in hot pepper fruits

Variant	mg/100g fresh substance (sp)	%	Difference vs. the witness (mg/100g sp)	Significance
V1-Mt	37.50	100	-	-
V2	50.04	133.44	+ 12.54	***
V3	59.24	157.97	+ 21.74	***
V4	39.15	104.40	+ 1.65	***
V5	42.20	112.53	+ 4.70	***
V6	55.48	147.95	+ 17.98	***
V7	60.79	162.11	+ 23.29	***

DL5%= 0.48; DL1%=0.67; DL0.1%=0.95

The heat or piquancy of pepper fruits is mainly given by the concentration of capsaicin which determines the aroma in alimentary products when used as spice in different sorts of cooked foods. Such compounds are well known for their therapeutic effects on gastric ulcer and rheumatoid polyarthritis. (Sathyanarayana, 2006).

The capsaicin content in local populations under study recorded values between

129.1 to 151.4 mg/100 g in fresh substance.(Table 3). The lowest value was recorded in the De Corabia population (129.1), which was below the De Cayenne variety. The highest value of capsaicin was recorded in the "Şapte fraţi" variant (151.4), the difference vs. the witness variant being very significant and positive. The following local populations were found to have a high content of capsaicin: De Almăj (138.4), SCDL 1 (147.9) and SCDL 2 (149.3), with differences compared to the witness variant statistically covered, being very significant and positive.

Table 3. The content of capsaicin in hot pepper fruits

Variant	mg /100g fresh substance (sp)	%	Difference vs. the witness (mg/100g sp)	Significance
V1-Mt	129.8	100.0	-	
V2	130.0	100.15	+ 0.2	
V3	138.4	106.62	+ 8.6	***
V4	129.1	99.46	- 0.1	
V5	151.4	116.64	+ 21.6	***
V6	147.9	113.94	+ 18.1	***
V7	149.3	115.02	+ 19.5	***

DL5%=4.13; DL1%=5.79; DL0,1%=8.18

Conclusions

Analysing the hot pepper genotypes cultivated in Romania one could notice that except for the variant V₄, showing a drop of the concentration of ascorbic acid by 8.8% and of capsaicin by 0.1%, all the other variants showed an increase of the concentrations of ascorbic acid, carotene and capsaicin as compared to the witness sample.

The results obtained point out the top qualities that these hot pepper genotypes cultivated in Oltenia have by comparison with the De Cayenne variety with high recommendation for their usage on ever larger areas.

REFERENCE

- Aniel Kumar O., S. Appa Rao and S. Subba Tata (2010). Phenolics quantification in some genotypes of capsicum annuum L. *Journal of Phytology*, 2(6): 87-90. *Phytophysiology*. | Antoniali S, Leal PAM, Magalhães AM, Fuziki RT and Sanches J (2007). Physico-chemical characterization of 'zarco hs' yellow bell pepper for different ripeness stages. *Sci. Agric. (Piracicaba, Braz.)*, 64(1): 19-22. | Araceli M. V.G., J.L.C. Servia, J.C.C. Rodriguez and M.G. Lopez (2011). Phytochemical evaluation of wild and cultivated pepper (*Capsicum annuum* L. var *C.pubescens* Ruiz & Pav.) from Oaxaca, Mexico. *Chilean Journal of Agricultural Research* 71: 578-585. | Balasundram, N., K. Sundram and S. Samman (2006). Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. *Food Chem.* 99: 191-203. | Bernardo A., Sidonia Martinez, Maria Alvarez, Ana Fernandez and Mercedes Lopez (2008). The composition of two Spanish peppers varieties (Freston de la Vega and Benavente - Los Valles) in different ripening stages. *Journal of Food Quality*, Volume 31, Issue 6, pp.701-716. | Biță M.G., Valeria Ghivercea, Maria Dinu and Pelaghia Chilom (2009). Biochemical Changes During Red Peppers Preservation Process as a Function of Water Activity. *Revista de Chimie, București* Vol. 60, Nr. 11, pp.1181-1184. | Bosland, P.W. [1994]. History, cultivation and uses. In G.Charalambous (Ed.) *Spices, herbs and edible fungi (herbs)*.) pp . 347-366. Amsterdam: Elsevier | Brandt, K., and C.Leifert. (2005). Which aspect of health are likely to be affected by our choice of food quality, such as organic food and how can we investigate this question? <http://orgprints.org/8427/01/brandt240243.pdf> | Conforti, F., G.A.Statti and F.Menichini (2007). Chemical and biological variability of hot pepper fruit (*Capsicum annuum* var *varacuminatum*) in relation to maturity stage. *Food chemistry* 102: 1096-1104. | Cruz-Pérez, A.B., V.A. Gonzalez-Hernández, R.M. Soto- Hernández, M.A. Gutiérrez-Espinoza, A.A. Gardea-Béjar and M. Pérez-Grajales (2007). Capsaicinoids, vitamina C y heterosis durante el desarrollo del fruto de chile manzano. *Agrociencia* 41:627-635. | Delgado-Vargas, Fand Paredes-Lopez, O. (2000). Natural colorants for food and nutraceutical uses. Boca Raton, Fla.: CRC Press pp 257-305. | Dere S., Tohit Günes and Ridvan Sivaci (1998). Spectrophotometric Determination of Chlorophyll - A, B and Total Carotenoid Contents of Some Algae Species Using Different Solvents, *Tr. J. of Botany* 22, pp.13-17. | Deepa, N., C. Kaur, B.George, B.Singh, and H.C.Kapoor (2007). Antioxidant constituents in some sweet pepper (*Capsicum annuum* L) genotypes during maturity. *Food Science and Technology* 40:121-129. | Dorantes, L., Colmenero, R., Hernandezyáz, H., Mota, L., Jaaramillo, M.E., Fernandez, E. and Solano C. (2000). Inhibition of growth of some foodborne pathogenic bacteria by *Capsicum annuum* extracts. *International Journal of Food Microbiology* 57, 125-128. | Dumitru M.G. and Marinescu Gabriela (2010). Analize biochimice, Editura Universitaria Craiova, pp.195-196. | Debjani Dutta, Utpal Ray Chaudhuri and Runu Chakraborty Structure, health benefits, antioxidant property and processing and storage of carotenoids *African Journal of Biotechnology* Vol. 4 (13), pp. 1510-1520, December, 2005. | Govindarajan, V.S. (1985). *Capsicum* production, technology, chemistry and quality. 1. History, botany, cultivation and primary processing. *Critical Review in Food Science Nutrition*. 22 (2), 109-176. | Gnayfeed, M.H, H.G. Daood, P.A. Biacs, and C.F. Alcaraz (2001). Content of bioactive compounds in pungent spice red pepper (paprika) as affected by ripening and genotype. *J.Sci. Food Agric.* 81: 1580-1585. | Grung M.F, D'souza M.L. and M. Borowitzka (1992). Algal Carotenoids, Secondary Carotenoids, *J. Appl. Phycol.* 4, pp. 165-171. | Sun-Hwa Ha, Jung-Bong Kim, Jong-Sug Park, Shin-Woo Lee and Kang-Jin Cho (2007). A comparison of the carotenoid accumulation in *Capsicum* varieties that show different ripening colours: deletion of the capsanthin-capsorubin synthase gene is not a prerequisite for the formation of a yellow pepper. *Journal of Experimental Botany*, Page 1-10. | Harborne, J.B and C.A.Williams (2000). Advances in flavonoid research since 1992. *Phytochemistry* 55:481-504. | Heaton S., (2001). Organic farming, food quality and human health. A review of evidence. *The Soil Association, Bristol, U.K.*, 1-87. | Henderson, D.E., and A.M.Slickman (1999). Quantitative HPLC determination of the formation of lipid hydroperoxides of linoleic acid a comparative study against BHT and melatonin. *Journal of Agricultural and Food Chemistry* 47, 2563-2570. | Howard, L.R., S.T. Talcott, C.H. Brens, and B. Villalon (2000). Changes in phytochemical and antioxidant activity of selected pepper cultivar (*Capsicum* species) as influenced by maturity. *Journal of Agricultural Food Chemistry* 48: 1713-1720. | Jacobs, D.R., J. Slavin and L. Marquart (1995). Whole grain intake and cancer: a review of the literature. *Nutrition and Cancer*, 24: 221-229. | Lichtenthaler H.K. and A.R. Wellburn (1985). Determination of Total Carotenoids and Chlorophylls A and B of Leaf in Different Solvents. *Biol. Soc. Trans.*, 11, pp. 591-592. | 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: 1750-1760. | Martinez, S., M. Lopez, M. González-Raurich, and A. Bernardo (2005). The effects of ripening stage and processing systems on vitamin C content in sweet peppers (*Capsicum annuum* L.) *Int. J. Food Sci. Nutr.* 56:45-51. | Mougou A., Filali N., Verlodt H. and Y. Harbaoui (1988). *Capsicum* Newsletter, 7, pp. 44-47. | Navarro, J.M., P. Flores, C. Garrido, and V. Martinez (2006). Changes in the contents of antioxidant compounds in pepper fruits at different ripening stages, as affected by salinity. *Food Chemistry* 96:66-73. | Pérez-Gálvez, A., H.D. Martin, H. Siles, and W. Stahl (2003). Incorporation of carotenoids from paprika oleoresin into human chylomicrons. *British Journal of Nutrition* 89:787-793. | Pellitero M., Pardo A., Simon A., Suso Ma. L., and A. Cerrolaza (1993). Effect of irrigation regimes on yield and fruit composition of processing pepper (*Capsicum annuum* L.) - International Symposium on Irrigation of Horticultural Crops, ISHS Acta Horticulturae, pp. 335-342. | Pintilie I. and Maria Dinu (2008). The study and evaluation of some local populations of green pepper; *Buletin of University Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Horticulture*, Volume 65 (1), pp.95-99. | Reifschneider F.J.B. (2000). *Capsicum*. Pimentas e pimentões no Brasil. *Embrapa Comunicacao para Transferencia de Tecnologia, Brasilia*. | Roberts J. L. Jr.; Hollenberg J. L. and J.M. Postma (1987). *General Chemistry in the Laboratory*; W. H. Freeman: New York, pp. 427-432. | Rodrigues, S., G.A.S. Pinto and F.A.N. Fernandes (2008). Optimization of ultrasound extraction of phenolic compounds from coconut (*Cocos nucifera*) shell powder by response surface methodology. *Ultrasound Sonochemistry* 15: 95-100. | Rozin, P. "Getting to Like the Burn of Chili Pepper." In *Chemical Senses*, edited by B. G. Green, J. R. Mason, & M. R. Morley, pp. 231-269. New York: Marcel Dekker, (1990). | Samuella B. Sigmann and E. Dale (2004). Wheeler Quantitative Determination of Citric and Ascorbic Acid in Powdered Drink Mixes, *Journal of Chemical Education*, Vol. 81 No. 10. | Sathyanarayana, M.N. (2006). Capsaicin and gastric ulcers. *Critical Reviews of Foods Science and Nutrition* 46:275-328. | Shetty, K. (2004). Role of proline-linked pentose phosphate pathway in biosynthesis of plant phenolics for functional food and environmental applications: a review. *Process Biochem* 39: 789-803. | Shrivastava R. P. and Sanjeev Kumar (1994). *Fruit and Vegetable Preservation Principle and Practice*, International Book Distributing Company Lucknow, pp. 229- 232. | Somos A. (1984). The paprica Akademiai Kiado, Budapest. | Titze, P.K., Hiepler, C., Seitz, E.M and M. Petz (2002). Pungency in paprika (*Capsicum annuum*). 1. Decrease of capsaicinoid content following cellular disruption. *Journal of Agricultural and Food Chemistry*, 50, 1260-1263. | Thompson, L.U. 1994. Antioxidant and hormone-mediated health benefits of whole grains. *Critical Review in Food Science Nutrition*, 34: 473-497. | Augusto Trejo-Gonzalez and Carlos Wild-al Tamirano (1973). A new method for the determination of capsaicin in capsicum fruits, *Journal of Food science*-volume 38. | Williams, C.M. (2002). Nutritional quality of organic foods: shades of grey or shades of grey? *Proceedings of Nutrition Society* 61, 19-24. | Wellburn A.R. (1994). The Spectral Determination of Chlorophylls A and B, as well as Total Carotenoids, Using Various Solvents with Spectrophotometers of Different Resolution. *J. Plant Phys.* Vol. 144, pp. 307-313.