

Original Research Article

Capsaicin and Ascorbic Acid Content in the High Yielding Chili Pepper (*Capsicum annuum* L.) Landraces of Northern Benin

A. Orobiyi^{1*}, H. Ahissou², F. Gbaguidi³, F. Sanoussi¹,
A. Hounghè³, A. Dansi¹ and A. Sanni⁴

¹Laboratory of Biotechnology, Genetic Resources and Animal and Plant Breeding (BIORAVE), Faculty of Sciences and Technology of Dassa, Polytechnic University of Abomey, BP14 Dassa-zoumé, Benin

²Laboratory of proteins Biochemistry and enzymology, Faculty of Sciences and Technology (FAST), University of Abomey-Calavi (UAC)

³Institute of Research and experimentation in Medicine and Traditional Pharmacopeia (IREMPT) BP 06, Oganla (Porto-Novo)

⁴Laboratory of Biochemistry and Molecular Biology, Faculty of Sciences and Technology (FAST), University of Abomey-Calavi (UAC), Cotonou, Republic of Benin

*Corresponding author

ABSTRACT

Keywords

Ascorbic acid,
Benin,
Capsaicin,
Chili pepper,
Landraces

Chili pepper constitutes one of more consumed spices in the world. It is very sought after in the pharmaceutical industries for its richness in capsaicin and in vitamin C. These two compounds are exploited for the production of insecticides (capsaicin) and several medicinal drugs use in the prevention of some illness. Capsaicin and vitamin C content belong to the parameters that determine chili pepper quality on the international market. For this reason, capsaicin and ascorbic acid content of 22 high yielding chili pepper landraces of northern Benin were determined by spectrophotometry. The results shown that landraceBO81 possesses the highest content in capsaicin (307.87 mg/100g of dry weight, corresponding to a value of 49.260 under the scale of Scoville) while landraceDO59D revealed the lowest content in capsaicin (76.5 mg/100g of dry weight, corresponding to a value of 12.260 under the scale of Scoville). Based on the scale of Scoville, all landraces analyzed are rich in capsaicin and vitamin C, and can be competitive on the market. Ascorbic acid (vitamin C) content varies from 84.64mg to 192.64 mg per 100g of fresh weight with an average of 125.70 mg. The higher value was recorded with landraceAL103B while the lowest was found with land race AT3. According to FAO (Food and Agricultural Organization) and WHO (World of Health Organization) recommendations, fifteen landraces (AL103B; AL109A; AT21A; DO58B; AL99; AT32A; BO94; DO52; BO48A; DO62; Do56; DO45A; DO59D; AT29C and AT28.) having more than 112.5 mg per 100g of fresh matter are rich in, and could be considered as potential sources of vitamin C.

Introduction

Food security, improvement of nutritional status and poverty alleviation became major preoccupations for the developing countries (Eromosele, 2008). In several countries of Africa, agricultural sector, through its intensification and diversification, help to achieve these objectives (Eromosele, 2008). Chili pepper belongs to the crops that are cultivated throughout the world for their nutraceutical (nutritional and medicinal) and economic value (Rahman et al., 2013). Member of the solanaceae family, chili pepper (*Capsicum annum* L.) is reported to be rich in proteins, lipids, fibers, mineral salts (Ca, P, Fe, K), vitamins (A, D3, E, C, K, B2 and B12) and in capsaicin (Chigoziri and Ekefan, 2013). Fresh green chili pepper contains more vitamin C than citrus fruits and fresh red chili pepper has more vitamin A than carrots (Chigoziri and Ekefan, 2013). Chili pepper is also suitable for the diets of the obese and is useful in the control of cancer of the stomach and colon (TayebRezvani et al., 2013; Dang et al., 2014). Chili pepper fruits are low in sodium and free cholesterol (Chigoziri and Ekefan, 2013) and are used in sauces, soups, stews and generally as a flavoring agent (Reyes-Escogido et al., 2011; Amruthraj et al., 2014).

In Benin, many landraces of chili pepper exist and cultivated only for food (Orobiyi et al., 2015) while in other countries the capsaicinoids that confer to chili pepper its hot taste and its medicinal properties (Liljana et al., 2013; Reddy and Sasikala, 2013) is internationally sought for by pharmaceutical industries hence offering to chili pepper an indubitable additional market value and competitiveness (Hachiya et al., 2007). Capsaicinoids, non-volatile alkaloids responsible for the pungent taste of chili pepper fruits (Diaz et al., 2004) contain:

capsaicin, dihydrocapsaicin, norcapsaicin, nordihydrocapsaicin, homocapsaicin and homodihydrocapsaicin (Nwokem et al., 2010; Al Othman et al., 2011). Among these, capsaicin and dihydrocapsaicin are the two most abundant and represent 90% of the total capsaicinoids content (Al Othman et al., 2011) out of which capsaicin, the active principal compound that confers to chili pepper its anti-carcinogenic, anti-mutagenic, anti-diabetic effects represents about 71% (Sanatombi and Sharma, 2008; Nwokem et al., 2010; Al Othman et al., 2011; Tayeb Rezvani et al., 2013). Capsaicin is also used like an analgesic against pain and inflammations (Al Othman et al., 2011; Dang et al., 2014) and also intervenes in the reduction of the rate of blood cholesterol (Chigoziri and Ekefan, 2013), and in the prevention of obesity and cardiovascular illnesses (Pamplona-Roger, 2007; Dang et al., 2014). Capsaicin-rich products have been primarily used to repel insects since ancient times (Sinha et al., 2011). Literature survey revealed that capsaicin has lethal effects on various invertebrates (Sinha et al., 2011).

Chili pepper is also known for its richness in ascorbic acid, a very essential antioxidant for human nutrition and proper functioning of the body (Igwe et al., 2013; Mohammed et al., 2013). Human body cannot synthesize vitamin C endogenously, so it is an essential dietary component (Li and Schellhorn, 2007). Vitamin C is instrumental in neutralizing free radicals, which are harmful to the body, assimilation of iron, healing of wounds, helps to build collagen which aids the skin, defense against bacterial and viral infection (Medina-Juarez et al., 2012). Deficiency of vitamin C in human can lead to a disease known as scurvy, whose symptoms include hemorrhaging especially in gums and skin, loosening of the teeth, joint pains and exhaustion (Phillips et al.,

2010; Igwemmar et al., 2013). It intervenes in the formation of the collagens and other proteins of the connective tissue, in the synthesis of the norepinephrine, adrenaline and the carnitine and in the activation of several hormones (Johnston et al., 2007; Phillips et al., 2010).

Among the great number of chili pepper landraces cultivated in Benin 22 were recently reported as high yielding (Orobiyi et al., 2015) and therefore can be valorized on the international market if having adequate capsaicin and ascorbic acid contents. The objective of this study was to determine capsaicin and vitamin C content of these high yielding landraces to better guide farmers, consumers and pharmaceutical industries in the choice of the Benin chili pepper landraces to focus on.

Material and Methods

Plant material

Twenty-two (22) landraces (Table 1, Figure 1) have been selected on the basis of their high yield for the assessment of capsaicin and vitamin C content. Fruits of these landraces cultivated for the purpose of the study on the experimental site of the Faculty of the Sciences and Technology of Dassa-Zoumé have been harvested in evening time and put in black bag. For determination of the content in capsaicin, one part of these fruits was dried in appropriate drying room during 12 days. The second part of the fresh fruits was immediately used for determination of ascorbic acid content.

Extraction and determination of capsaicin content

Capsaicin content in the samples was estimated by spectrophotometric measurement of the blue coloured component formed as a result of reduction

of phosphomolybdic acid to lower acids of molybdenum following Ademoyegun et al. (2011). One gram (1g) of each dry sample was extracted with 10 ml of dry acetone using pestle and mortar. The extract was centrifuged at 10,000 rpm for 10 min and 1ml of supernatant was pipetted into a test tube and evaporated to dryness in a hot water-bath (60°C). The residue was then dissolved in 0.4 ml of NaOH solution and 3 ml of 3% phosphomolybdic acid. The contents were shaken and allowed to stand for 1 h. The solution was filtered to remove any floating debris and centrifuged at 5,000 rpm for 15 min. Absorbance was measured for the clear blue solution, thus obtained, at 650 nm using reagent blank (5 ml of 0.4% NaOH⁺ 3ml of 3% phosphomolybdic acid). Capsaicin content calculated from the standard curve was expressed as mg/ 100g on dry basis. Capsaicin content of the chili peppers landraces analyzed was then converted in Scoville Unit by the multiplication of the gotten quantities (weight of capsaicin per dry chili pepper weight in grams) with 1.6×10^7 (Todd et al., 1977; Nwokem et al., 2010).

Extraction and determination of ascorbic acid content

Chilipepper fruits (0.5g) were washed with tap water and cut into small pieces and homogenized with the help of mortar and pestle by adding 5 ml of 4% oxalic acid. The homogenates were centrifuged at 5,000 rpm for 10 minutes then the supernatants were filtered with 540 Whatmann filter paper. The obtained residues were made up to 25 ml with 4% oxalic acid. The ascorbic acid content was estimated by using 2,4 dinitrophenylhydrazine reagent in conjunction with spectrophotometer at 540 nm (Sadasivam and Manickam, 1992; Kumar and Tata, 2009). Five samples from each lot were analyzed.

Results and Discussion

Capsaicin content

The standardization curve equation gotten is: $Y = 0.002X + 0.016$ (Figure 2). It was used to calculate the content of the capsaicin (expressed in mg/100g of dry weight) contained in each sample of chili pepper analyzed (Table 2). The content varied from a landraces to another. The highest content was 307.9 mg/100g of dry weight (accession BO81) while the lowest was 76.5 mg/100g of dry weight (DO59D) with an average of 195.8 mg/g (Table 2). Among the first five landraces having very high capsaicin content, one belongs to the class 2 (DO45A) and the other four belongs to the class 4 (BO81; DO63; AL99 and AT32). While considering the last five landraces having a low capsaicin content, two (DO62 and DO59) belong to the class 1 and three (AL109A; BO93B and BO84A) to the class 2. The average content (per class) of the capsaicin in the chili pepper fruits analyzed was: 248.2 mg/100g of dry weight (class 4), 190.4 mg/100g of dry weight (class 3), 189.7 mg/100g of dry weight (class 2) and 80.2 mg/100g of dry weight (class 1). These results show that in general the landraces of chili pepper belonging to the class 4 (chili pepper of frutescens group) are the richest in capsaicin while those of the class 1 have the lowest capsaicin content. According to Orobiyi et al. (2015), chili pepper varieties of class 1 would be hybrids between sweet pepper and hot chili pepper. These results also showed that the landraces of the class 1 (Tataché) are not sweet peppers. The fruits of the landraces of the class 3 (chili peppers of chinense group) and those of the class 2 (chili pepper of annuum group) have similar average capsaicin content and occupy the second and the third place respectively. Our results are similar to the works of Nwokem et al. (2010) that showed that in Nigeria, the

lowest landraces in capsaicin content are Tataché. This study confirms the Nigerian origin of Tataché chili pepper as reported by the producers surveyed in Benin. Ours results are also similar to those of Sanatombi and Sharma (2008) which revealed that the fruits of annuum group chili peppers are lower in capsaicin than those of chinense and frutescens groups. Other studies must be done in order to know the chili pepper group (annuum or chinense) to which Tataché belongs to. Landrace BO81 (richest in capsaicin) contains more capsaicin than Nsukka Yellow variety (81 mg/100g of dry weight) (Nwokem et al., 2010) and less capsaicin than CF1 (445 mg/100g of dry weight) (Tilahun et al., 2013), Tabasco (378.5 mg/100g of dry weight) and Orange Habanero variety (663.9 mg/100g of dry weight) (Garceas-Claver et al., 2006) which are popular varieties on International market because of their high capsaicin content (Garceas-Claver et al., 2006; Tilahun et al., 2013). However, the landraces of chili pepper analyzed can be used in the pharmaceutical industries for the value of the capsaicin. But other programs must be put in place in order to find within the whole existing diversity or to create some very productive and rich landraces of chili pepper in capsaicin and that will be very valuable and competitive in the international market.

Ascorbic acid (vitamin C) content

The equation of the standardization curve gotten is: $Y = 5 \times 10^{-5}X + 0.002$ (Figure 3). The ascorbic acid content of fresh fruits of chili pepper analyzed is shown in Table 3. The results obtained showed that the content of the ascorbic acid contained in the fresh fruits of chili pepper varies from 84.64 mg/100g to 192.64 mg/100g of fresh weight with an average of 125.70 mg/100g (Table 3).

Figure 1: Description of the 4 classes of chili pepper to which belong the samples analyzed



a) Class 1 (Tataché): Non-pubescent leaves, fruits very large and obtuse at the base as sweet pepper



b) Class 2 (Yèyèkouka): Leaves lightly pubescent on the dorsal surface and very elongated fruits



c) Class 3 (Bokinon): Leaves moderately pubescent, round fruits



d) Class 4 (Gninka): Leaves very pubescent at the dorsal side, small fruits

Figure.2 Standardization curve of the capsaicin (linear adjustment)

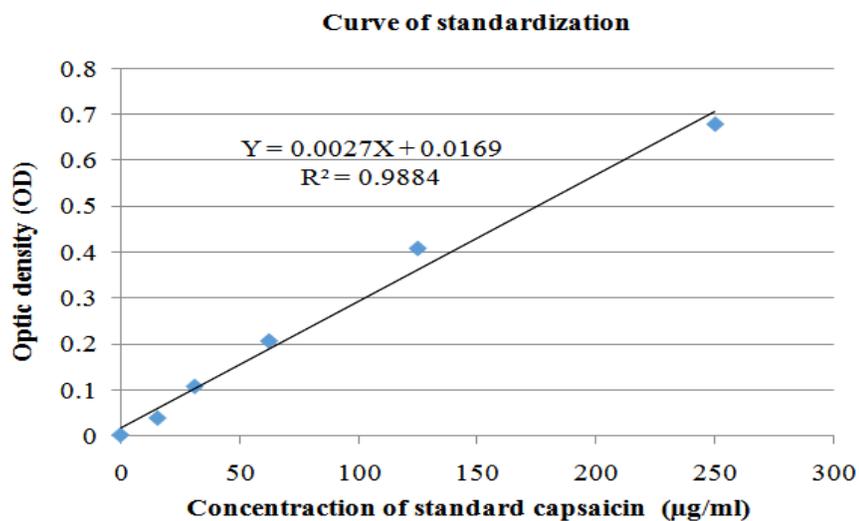


Figure.3 Standardization curve of ascorbic acid (linear adjustment)

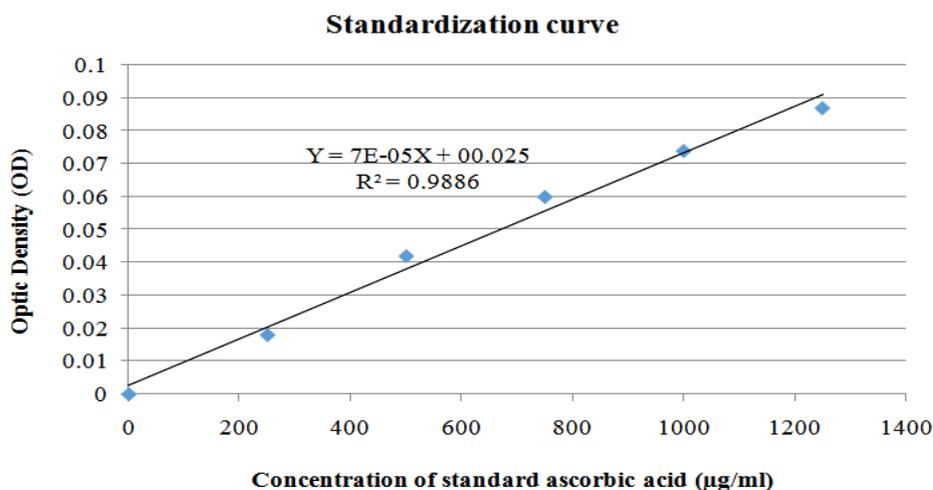


Table.1 List of chili pepper landraces analyzed

N°	Name of landraces	Codes	Morphologic Class
1	BargoudjèouPéto	AL99	Class 4
2	Berkpame	DO58B	Class 2
3	Bodanganda	AL103B	Class 2
4	Bokinon	AT3	Class 3
5	Djouè	BO93B	Class 2
6	Gbataki	DO52	Class 3
7	Gbataki	BO94	Class 3
8	Gninka	BO81	Class 4
9	Gnonnonzon	AL109A	Class 2
10	Kolamainma	AT32A	Class 4
11	Latogué	DO56	Class 4
12	Nkpankabouka	DO45A	Class 2
13	Tambowèwè	DO63	Class 4
14	Tataché	DO59D	Class 1
15	Tataché	DO62	Class 1
16	Tika	BO84A	Class 2
17	Yèbargou	AT38	Class 4
18	Yèyèkouka	AT29C	Class 2
19	Yèyèkouka	AT28	Class 2
20	Yèyèkouka	AT31B	Class 2
21	Yèyèkouokourè	AT21A	Class 2
22	Yèyèkouokourè	AT22	Class 2

NB: class 1: Tataché group or local sweet pepper; class 2: long chili pepper; class 3: round chili pepper; class 4: small chili pepper

Table.2 Capsaicin content of the dry fruits of chili pepper and their Scoville Unit

N°	Landraces	Capsaicin content (mg/100g of dry weight)	Scoville Unit
1	BO81	307.9	49,260
2	DO63	271.1	43,380
3	DO45A	270.4	43,260
4	AL99	247.1	39,540
5	AT32A	242.6	38,820
6	AT38	232.1	37,140
7	AT28	223.9	35,820
8	AT21A	222.7	35,640
9	AT3	215.2	34,440
10	AL103B	202.9	32,460
11	AT31B	202.1	32,340
12	DO58B	192.4	30,780
13	DO56	188.2	30,120
14	AT22	187.5	30,000
15	BO94	186.4	29,820
16	AT29C	186	29,760
17	DO52	169.5	27,120
18	AL109A	151.1	24,180
19	BO93B	138.4	22,140
20	BO84A	109.1	17,460
21	DO62	84	13,440
22	DO59D	76.5	12,240

Table.3 Ascorbic acid content of fresh fruits of chili pepper

N°	Landraces	Ascorbic acid content (mg/100g of fresh weight)
1	AL103B	192.64
2	AL109A	162.86
3	AT21A	154.71
4	DO58B	145.50
5	AL99	143.14
6	AT32A	142.71
7	BO94	140.57
8	DO52	140.14
9	BO84A	137.79
10	DO62	133.29
11	DO56	130.93
12	DO45A	127.57
13	DO59D	125.14
14	AT29C	115.07
15	AT28	114.00
16	BO81	105.00
17	DO63	102.43
18	BO93B	96.64
19	AT38	92.79
20	AT22	90.86
21	AT31B	87.00
22	AT3	84.64

The highest ascorbic acid content was found in landrace AL103B and the lowest with landrace AT3. When referring to the reports of Guil-Guerrero et al. (2006), the ascorbic acid content of AL103B landrace is more elevated than Bayadagi Kaddis (189.4 mg/100g of fresh weight) and Guindilla (168.5 mg/100g of fresh weight) landraces originated of while it is lower than Red Lamuyos (293 mg/100g of fresh weight) and Red California (348 mg/100g of cool weight) landraces (Rodriguez-Burruezo et al., 2009). The recommended ascorbic acid to be consumed by an adult according to FAO/WHO (2001) is about 45 mg/day for 40g of fresh fruits of chili pepper, then all landraces of chili pepper having ascorbic acid content greater than 112 mg/100g of fresh weight and can be considered as a potential source of the vitamin C. It is also very important to promote these chili pepper landraces so that consumers do not get just quantity but also quality products. Complementary studies should also be done in order to know the total number of landraces present in Benin republic and the ascorbic acid content of each of them. Because ascorbic acid is a thermo-labile compound chili pepper must be consumed fresh. For this, mass education and sensitization is needed. . Our study is beneficial to people who are allergic to the very high pungent chili peppers but need to consume chili peppers rich in vitamin C. Landrace AL109A is an example of chili pepper rich in ascorbic acid (162,86 mg/100g of fresh weight) but low in capsaicin (151,12 mg/100g of fresh weight).

This study showed that capsaicin and ascorbic acid contents vary from a chili pepper landrace to another and don't depend on any morphological similarity. The results showed that landraces of chili pepper rich in capsaicin and ascorbic acid exist in Benin. These landraces can be exploited by

pharmaceutical industries in the manufacturing of some remedies against cancer, diabetes, and cardiovascular illnesses. On the other hand, the consumption the fresh chili peppers whose fruits are rich in vitamin C is an ideal means of contributing to the problems of food insecurity and malnutrition in Benin. It is therefore necessary to follow up on the analyses within the whole existing diversity in order to find the landraces that are naturally rich in capsaicin.

Acknowledgments

This study has been financed by government of Benin through the Ministry in charge of Scientific Research. We would like to thank Dr Adéoti Kifouli for their advices. We also thank all the agricultural technicians and farmers met for fruitful discussion and knowledge sharing.

References

- Ademoyegun O.T., Fariyike TA. and Aminu-Taiwo RB. (2011). Effects of poultry dropping on the biologically active compounds in *capsicum annum* L (var. Nsukka yellow). *Agric. Biol. J. N. Am.*, 2(4): 665-672.
- Al Othman ZA., Ahmed YBH., MA. Habila and Ghafar AA. (2011). Determination of Capsaicin and Dihydrocapsaicin in Capsicum Fruit Samples using High Performance Liquid Chromatography. *Molecules*, 16: 8919-8929.
- Amruthraj NJ., Raj JPP. and Lebel LA. (2014). Effect of vegetable oil in the solubility of capsaicinoids extracted from *Capsicum Chinense* Bhut Jolokia. *Asian J. Pharm. Clin. Res.*, 7(1): 48-51.
- Chigoziri E. and Ekefan EJ. (2013). Seed borne fungi of Chilli Pepper (*Capsicum frutescens*) from pepper producing areas of Benue State, Nigeria. *Agric.*

- Biol. J. N. Am.*, 4(4): 370-374.
- Dang Y., Zhang H. and Xiu Z. (2014). Three-liquid-phase Extraction and Separation of Capsanthin and Capsaicin from *Capsicum annum* L. *Czech J. Food Sci.*, 32(1): 109-114.
- Diaz J., Pomar F., Bernal A., Merino F., (2004). Peroxidases and the metabolism of capsaicin in *Capsicum annum* L. *Phytochemistry Reviews*, 3: 141-157.
- Eromosele C.O., Arogundade L.A., Eromosele I.C. and Ademuyiwa O. (2008). Extractability of African yam bean (*Sphenostylis stenocarpa*) protein in acid, salt and alkaline aqueous media. *Food Hydrocolloids*, 22: 1622-1628.
- FAO/WHO. (2001). Human Vitamin and Mineral Requirements. Report of a Joint FAO/WHO Expert Consultation; Bangkok, Thailand: Food and Agriculture Organization/World Health Organization.
- Guil-Guerrero J.L., Martinez-Guirado C., Reboloso-Fuentes M.M. and Carrique-Perez A. (2006). Nutrient composition and antioxidant activity of 10 pepper (*Capsicum annum*) varieties. *Eur. Food Res. Technol.*, 224:1-9.
- Hachiya S., Kawabata F., Ohnuki K., Inoue N., Yoneda H., Yazawa S. and Fushiki T. (2007). Effects of CH-19 Sweet, a non-pungent cultivar of red pepper, on sympathetic nervous activity, body temperature, heart rate, and blood pressure in humans. *Biosci. Biotechnol. Biochem.*, 71: 671-676.
- Igwemmar NC., Kolawole SA. and Imran IA. (2013). Effect of Heating on Vitamin C Content of Some Selected Vegetables. *International Journal of Scientific and Technology Research*, 2(11): 209-212.
- Johnston C.S., Steinberg F.M. and Rucker R.B. (2007). Ascorbic acid. In: Zemleni J., Rucker R.B., McCormick D.B. and Suttie J.W. (Eds.), *Handbook of Vitamins*. 4th ed. CRC Press, Boca Raton, FL, USA, pp. 489-520.
- Kumar O.A., Tata S.S. (2009). Ascorbic Acid Contents in Chili Peppers (*Capsicum* L.). *Not. Sci. Biol.*, 1 (1): 50-52.
- Li Y. and Schellhorn H.E. (2007). New Development and Novel Therapeutic Perspectives for Vitamin C. *J. Nutr.*, 137: 2171-84.
- Liljana KG., Viktorija M., Marija SD. and Rubin G., Emilija IJ. (2013). Content of capsaicin extracted from hot pepper (*Capsicum annum* ssp. *microcarpum* L.) and its use as an ecopesticide. *Hem. ind.*, 67 (4): 671–675.
- Medina-Juarez LA., Molina-Quijada DMA., Toro-Sánchez CLD., González-Aguilar GA., Gamez-Meza N. (2012). Antioxidant activity of peppers (*Capsicum annum* L.) extracts and characterization of their phenolic constituents. *Interciencia.*, 37(8): 588-593.
- Mohammed GH. (2013). Effect of Seamino and Ascorbic Acid on Growth, Yield and Fruits Quality of Pepper (*Capsicum Annum* L). *Int. J. Pure Appl. Sci. Technol.*, 17(2): 9-16
- Nwokem C.O., Agbaji E.B., Kagbu J.A. and Ekanem E.J. (2010). Determination of Capsaicin Content and Pungency Level of Five Different Peppers Grown in Nigeria. *New York Science Journal*, 3(9): 17-21.
- Orobiyi A., Sanoussi F., Gbaguidi A., Dansi M., Korie N., Dansi A. (2015). Ethnobotanical study and varietal diversity of chili pepper (*Capsicum annum* L) in Central and Northern Benin (in press).
- Pamplona-Roger GD. (2007). Healthy Foods, *Editorial Safeliz*, Spain Pp375.

- Phillips K.M., Tarrago-Trani M.T., Gebhardt S.E., Exler J., Patterson K.Y., Haytowitz D.B., Pehrsson P.R and Holden J.M. (2010). Stability of vitamin C in frozen raw fruit and vegetable homogenates. *Journal of Food Composition and Analysis*, 23: 253-259.
- Rahman M.S., Al-Rizeiqi M.H., Guizani N., Al-Ruzaiqi M.S., Al-Aamri A.H., Zainab S. (2013). Stability of vitamin C in fresh and freeze-dried capsicum stored at different temperatures. *Journal of Food Science and Technology*, 52(3): 1691-1697.
- Reddy DMVB. and Sasikala P. (2013). Capsaicin and colour extraction from different varieties of green and red chilli peppers of Andhrapradesh. *International Journal of Advanced Scientific and Technical.*, 2(3): 554-572.
- Reyes-Escogido M.L., Gonzalez-Mondragon E.G. and Vazquez-Tzompantzi E. (2011). *Molecules*, 16:1253-1270.
- Rodriguez-Burruezo A., Prohens J., Raigon M.D. and Nuez F. (2009). Variation for bioactive compounds in Aji' (*Capsicum baccatum* L.) and rocoto (*C. pubescens* R. and P.) and implications for breeding. *Euphytica*, 170:169-181.
- Sadasivam S. and Manikkam A. (1992). Capsaicin. In *Biochemical methods for agricultural sciences*, Wiely Estern Ltd., Madras. pp. 193-194.
- Sanatombi K. and Sharma G.J. (2008). Capsaicin Content and Pungency of Different *Capsicum* spp. Cultivars. *Not. Bot. Hort. Agrobot. Cluj*, 36 (2): 89-90.
- Scoville W.L. (1912). Note on capsicum. *Journal of the American Pharmaceutical Association*, 1:453.
- Sinha R.K., Hahn G., Singh P.K. and Suhane R.K., Antho-nyreddy A. (2011). Organic Farming by Vermiculture: Producing Safe, Nutritive and Protective Foods by Earthworms. *Am. J. Exp. Agric.*, 1(4): 363-399.
- Tayeb Rezvani H., Moradi P. and Soltani F. (2013). The effect of nitrogen fixation and phosphorus solvent bacteria on growth physiology and vitamin C content of *Capsicum annum* L. *Iranian Journal of Plant Physiology*, 3 (2): 673-682.
- Tilahun S., Paramaguru P. and Rajamani K. (2013). Capsaicin And Ascorbic Acid Variability In Chilli And Paprika Cultivars As Revealed By HPLC Analysis. *J. Plant Breed. Genet.*, 01 (02) : 85-89.
- Todd P., Bensinger M. and Biftu T. (1977). Determination of Pungency due to Capsicum by Gas-Liquid Chromatography. *Journal of Food Science*, 42: 660-665.