Research Article

Effect of NPK Fertilizer on Chemical Composition of Pumpkin (Cucurbita pepo Linn.) Seeds

F. M. Oloyede,¹ I. O. Obisesan,¹ G. O. Agbaje,¹ and E. M. Obuotor²

¹ Department of Crop Production and Protection, Obafemi Awolowo University, Ile-Ife, Nigeria
² Department of Biochemistry, Obafemi Awolowo University, 220005 Ile-Ife, Nigeria

Correspondence should be addressed to F. M. Oloyede, funmilayooloyede@yahoo.co.uk

Received 31 October 2011; Accepted 12 December 2011

1. Introduction

Pumpkin seed vegetable is valued in many countries such as Japan, Czech Republic, Hungary, Germany, Austria, Romania, Italy, and West and South Ukraine. The seeds are a good raw material for the production of oil used in food preparation and in medicine [1, 2]. According to Jariene [3], the seeds contain about 50% fats, approximately 30% protein, sugar, B vitamins, ascorbic acid, Phytoestrogens, Phyto, lecithin, oxycerotine, tyrosine, salicylic acid, and resins. The seed oil is also rich in glicerides of linoleic, oleic, palmitin, and stearine acids. Omega-3 fatty acids was found to be present in pumpkin seeds; it helps to prevent artherosclerosis, high blood pressure, and heart diseases; it also stimulates metabolism of accumulated fats. Oil-cake fats from pumpkin seeds contain large amounts of (almost 60%) of omega-3 acids twice that of cod liver oil [3–6]. Pumpkin seed powder is used in China and the United States as an ingredient of salad dressings and in baked products. The seed oil is used as salad oil in Europe, and in India for cooking and lighting. The seed is used medicinally in the prevention of kidney stones. Seeds are eaten as an anthelmintic. In Mauritius an infusion of the seeds is used internally to treat hypertension and prostate complaints and externally to treat erysipelas [7].

Seeds form a major part of the diet of Nigerians; They are consumed as a meal as well as ingredients of local soups. In southwestern Nigeria, pumpkin seeds are used locally as an alternative to “egusi” melon (Citrullus vulgaris Schrad) seed. Melon seeds are milled and used to prepare the popular “egusi” soup where they act as food thickeners. Pumpkin seeds are used alone or in combination with leafy vegetable.

Information is scanty on the nutrient and antioxidant composition of pumpkin seeds in Nigeria. Due to reduction or loss of soil fertility in most Nigerian soils, chemical fertilizers are used to boost crop yield; this consequently has bearing on the chemical composition of the crops grown on such lands. This study thus aimed at evaluating the influence of NPK fertilizer on the proximate composition and antioxidant profile of pumpkin seeds.
2. Materials and Methods

2.1. Field Study. Pumpkin fruits were harvested after 15 weeks at the Teaching and Research Farm, Obafemi Awolowo University, Ile-Ife, Nigeria for 2 seasons in 2010. The experiment was a randomized complete block design consisted of 6 rates of NPK 15:15:15 fertilizer at 0, 50, 100, 150, 200, and 250 kg/ha. There were 6 replicates of 10 m × 12 m plot size. At maturity, 5 fruits each at random from all the plots were chosen and their seeds extracted, rinsed, and dried at 50°C. Six composite samples from the 6 replicates were milled and stored in the refrigerator.

2.2. Laboratory Analyses. For the antioxidant assays, about 5 g of the composite samples were extracted by cold extraction, that is, extraction not involving heat, for 24 hours using 80% methanol. The crude extract was obtained by evaporation of the methanol soluble extract to dryness. The hydrogen donating or radical scavenging of the extract was determined using the stable radical DPPH (2,2-diphenyl-1-picrylhydrazyl hydrate) according to the method described by Brand-Williams et al. [8]. DPPH reacts with an antioxidant compound which can donate hydrogen, and it is reduced. The change in colour from deep violet to light yellow was measured spectrophotometrically at 517 nm. Crude fibre, Ether extract (fat), and Moisture contents were determined using the routine chemical analytical methods of Association of Official Agricultural Chemists (AOAC) [13]. All data were subjected to combined analysis of variance SAS [14]. Means squares, significantly different, were separated using Duncan Multiple Range Test (DMRT) at 5% level of probability.

3. Results

The protein, fat, ash, crude fibre, and carbohydrate in pumpkin seeds were significantly influenced by season and fertilizer. Interaction between season and fertilizer was significant on protein, ash, and carbohydrate contents (Table 1). Seasonal influence showed higher nutrient values during the early season than in the season except for carbohydrate. Variation between the nutrient values ranges from 1.4 to 25%. Protein was 10% higher in early season while carbohydrate in late season was higher by 25% than in the early season (Table 2). Fertilizer influence showed that proximate values of protein, fat, ash, and crude fibre in pumpkin seeds were similar at 0, 50 and 100 kg NPK/ha. The nutrient values reduced significantly when compared to the control from the application of 150 kg/ha of NPK and this continued to the highest fertilizer rate (250 kg NPK/ha) with the exception of carbohydrate content whose values increased with increased fertilizer rates. Carbohydrate concentration in pumpkin seeds was highest with the application of 250 kg/ha (Table 3). The influence of season and fertilizer interaction on protein and ash contents showed that the nutrient concentration was higher across fertilizer rates in the early season than the late season. The values were significantly lower than in the control when fertilizer application was above 100 kg/ha both in the early and in the late seasons (Figures 1 and 2). On
Table 3: Proximate composition of Pumpkin seeds as affected by NPK fertilizer levels.

| NPK level (kg ha\(^{-1}\)) | Protein (g/100 g) | Fat (g/100 g) | Ash (g/100 g) | Crude fibre (g/100 g) | Carbohydrate (g/100 g) |
|-----------------------------|-------------------|--------------|--------------|-----------------------|------------------------|
| 0                           | 27.0\(^a\)        | 59.2\(^a\)   | 1.55\(^a\)   | 0.56\(^a\)            | 11.7\(^c\)             |
| 50                          | 27.0\(^a\)        | 59.2\(^a\)   | 1.55\(^a\)   | 0.56\(^a\)            | 11.7\(^c\)             |
| 100                         | 27.0\(^a\)        | 59.2\(^a\)   | 1.58\(^a\)   | 0.56\(^a\)            | 11.8\(^c\)             |
| 150                         | 25.9\(^b\)        | 58.1\(^b\)   | 1.39\(^b\)   | 0.51\(^a\)            | 14.2\(^b\)             |
| 200                         | 25.6\(^bc\)       | 58.0\(^b\)   | 1.37\(^b\)   | 0.49\(^c\)            | 14.6\(^b\)             |
| 250                         | 24.9\(^c\)        | 57.8\(^b\)   | 1.33\(^c\)   | 0.49\(^c\)            | 15.4\(^a\)             |

Means with the same letter in each column are not significantly different at 5% level of probability using Duncan’s multiple range test.

the contrary, the carbohydrate content of pumpkin seeds was higher in late season than that in the early season (Figure 3).

Antioxidant activities and its components in pumpkin seeds were significantly influenced by season and fertilizer. The interaction of season and fertilizer was significant on antioxidant activities, phenol, and proanthocyanidin (Table 4). Season had a greater influence on antioxidant activities of pumpkin seeds; however, flavonoid content was not significantly affected by season. Seasonal variation revealed higher values during the early season in all the determined profiles when compared to the late season. The values in flavonoid were similar in both seasons (Table 5). Fertilizer influenced showed consistent decrease in values of antioxidant activities and its components as fertilizer rates increased. The control had significantly higher values than the values from the application of 150 to 250 kg/ha. The addition of 50 and 100 kg gave similar antioxidant activities and component values with the zero fertilization at 0.05 probability level (Table 6). The significant influence of interaction (season \times fertilizer) on antioxidant activities, phenol, and proanthocyanidin concentration showed that early season had higher values than late seasons across fertilizer rates. The concentration at both seasons was similar between the control and 50 kg and 100 kg fertilizer. A significant reduction in values was found from 150 to 250 kg NPK fertilizer rate application (Figures 4, 5, and 6).

4. Discussion

In this study, application of fertilizer above 100 kg NPK/ha reduced the seed oil yield, fibre, and protein. However, Jariene et al. [3] reported a reduction in seed fat percentage but an increase in seed protein due to fertilizer application, and in the case of crude fibre in seed there was no significant response to fertilizer. Antioxidant properties of C. pepo seeds were higher at 0, 50, and 100 kg/ha levels of NPK than all other levels. This corroborated other findings on fruits and vegetables that the use of mineral fertilizer, particularly Nitrogen at high rates, negatively affects the antioxidant properties of fruits and vegetables [15]. The proximate contents and antioxidant properties of pumpkin seeds at control were similar to those of 50 and 100 kg/ha NPK fertilizer rates in this study. This means that at those...
Table 4: Combined analysis of variance showing means squares for antioxidant activities and its components in Pumpkin seed as influenced by season and NPK fertilizer.

| Source                        | DF | Antioxidant activities (%) | Phenol (mg/100 g) | Flavonoid (mg/100 g) | Anthocyanin (mg/100 g) | Proanthocyanidin (mg/100 g) |
|-------------------------------|----|----------------------------|-------------------|----------------------|------------------------|-----------------------------|
| Season                        | 1  | 2227.23*                  | 567473200*        | 21327079             | 8618.46*               | 254.80**                    |
| Rep within season             | 2  | 24.46                     | 47581426          | 8545837              | 522.83                 | 0.205                       |
| Fertilizer                    | 5  | 1071.78**                 | 1439581031**      | 81034624**           | 5491.33**              | 81.54**                     |
| Season × Fertilizer           | 5  | 117.85**                  | 78169287**        | 600093               | 295.96                 | 16.16**                     |
| Pooled error                  | 10 | 18.73                     | 14234829          | 1634308              | 296.16                 | 0.35                        |
| CV (%)                        |    | 5.6                       | 11.9              | 19.2                 | 26.3                   | 6.5                         |

*: significant at 0.05 level of probability.  
**: significant at 0.01 level of probability.

Table 5: Antioxidant activities and its components in Pumpkin seeds as affected by season.

| Season     | Antioxidant activities (%) | Phenol (mg/100 g) | Flavonoid (mg/100 g) | Anthocyanin (mg/100 g) | Proanthocyanidin (mg/100 g) |
|------------|---------------------------|-------------------|----------------------|------------------------|----------------------------|
| Early season| 86.5                      | 36.70             | 7.59                 | 0.085                  | 0.012                      |
| Late season | 67.2                      | 26.97             | 5.70                 | 0.047                  | 0.006                      |
| LSD (0.05) | 3.9                       | 6.94              | NS                   | 0.018                  | 0.0002                     |

NS: not significant at 5% level of probability.  
Values are means of duplicate analyses expressed on dry matter basis.

Figure 3: Carbohydrates content of pumpkin seeds as affected by season by NPK fertilizer interaction.

Figure 4: Antioxidant activities of pumpkin seeds as affected by season by NPK fertilizer interaction.

rates there is a complimentary balance between seed yield and nutrient concentration in the seed. However, there was reduction in antioxidant activities of C. pepo seeds under fertilizer rates of 150–250 kg/ha (53–77%) compared with that of 0–100 kg/ha (90%). The result is similar to the reduction in total phenolics and antioxidant activities observed in Mustard leaf due to increased N fertilization. Kader [16] noted that soil type affects antioxidant properties in crops. The crops grown in a sandy soil tend to retain fewer nutrients than those grown in clay soils and hence increase in their antioxidant properties. If the nutrient
Table 6: Antioxidant activities and its components in Pumpkin seeds as affected by NPK fertilizer.

| NPK level (kg ha\(^{-1}\)) | Antioxidant activities (%) | Phenol (mg/100 g) | Flavonoid (mg/100 g) | Anthocyanin (mg/100 g) | Proanthocyanidin (mg/100 g) |
|-----------------------------|----------------------------|-------------------|---------------------|------------------------|-----------------------------|
| 0                           | 90.4\(^a\)                | 47.45\(^a\)      | 10.95\(^a\)         | 0.095\(^a\)            | 0.013\(^a\)                 |
| 50                          | 89.9\(^a\)                | 47.12\(^a\)      | 10.63\(^a\)         | 0.097\(^a\)            | 0.013\(^a\)                 |
| 100                         | 89.9\(^a\)                | 46.38\(^a\)      | 10.46\(^a\)         | 0.098\(^a\)            | 0.013\(^a\)                 |
| 150                         | 77.0\(^b\)                | 33.36\(^b\)      | 4.5\(^b\)           | 0.062\(^b\)            | 0.006\(^b\)                 |
| 200                         | 61.3\(^c\)                | 10.02\(^c\)      | 2.47\(^b\)          | 0.024\(^c\)            | 0.005\(^c\)                 |
| 250                         | 52.9\(^d\)                | 6.6\(^c\)        | 1.32\(^c\)          | 0.018\(^c\)            | 0.004\(^d\)                 |

Means with the same letter in each column are not significantly different at 5% level of probability using Duncan’s multiple range test.

is low, antioxidant activities is expected to increase. At the University of Illinois comparison was made of flavonoid content in tomatoes under conventional and organic agriculture practices. It was observed that flavonoid levels in tomatoes increased under organic management. Plants with limited N were shown to accumulate more flavonoids than those that are well supplied with inorganic fertilizers. It was concluded that synthetic fertilizers in which N is easily accessible to the plant may reduce the health benefits of tomatoes [17]. This agreed with the observation in this study that overfertilization at 150–250 kg NPK reduces the health benefits of pumpkin seeds.

Climatic conditions have a strong influence on the concentration of bioactive compounds [18]. Climatic factors vary with growing sites, seasons, and between years. Temperature, both in terms of total or average temperature and the extremes during the growth period, may influence the chemical composition in plants [18, 19]. In lettuce, light effects increased the concentrations of vitamin C, carotenoids, and flavonoids in the outer leaves than the inner leaves, which receive less light [20–22]. In this study, the pumpkin seeds obtained during the early season had significantly higher proximate contents and antioxidant profile than those of late cropping season. This could be due to excessive rainfall during the late season. High rainfall has been reported to reduce nutrient content and antioxidant composition in vegetable crops [18].

In conclusion, the cultivation of pumpkin at lower NPK fertilizer rate and under moderate rainfall could enhance the health benefits from pumpkin seeds. World Health Organization had estimated that 2.7 million lives could be saved annually by consuming fruits and vegetables. Hence the consumption and utilization of pumpkin seeds can effectively fill this gap if its potentials are harnessed and improved.

References

[1] S. E. Lazos, “Nutritional, fatty acids and oil characteristics of pumpkin and melon seeds,” Journal of Food Science, vol. 15, pp. 1382–1383, 1986.
[2] I. Elmadfa and D. Fritzsche, “Tabele witamin I składnikow mineralnych,” Muza S.A. Warszawa, pp. 39–96, 1999.
[3] E. Jariene, H. Danilcenko, J. Kulaitiene, M. Gajewski, and E. Venuskutoniene, “Quality of oil bearing pumpkin cultivars depending on the fertilization method,” in Spontaneous and Induced Variation for the Genetic Improvement of Horticultural Crops, P. Nowaczyk, Ed., pp. 189–196, University of Technology and Life Sciences, Bydgoszcz, Germany, 2007.

[4] E. U. Akwaowo, B. A. Ndon, and E. U. Etuk, “Minerals and antinutrients in fluted pumpkin (Telfairia occidentalis Hook.),” Food Chemistry, vol. 70, no. 2, pp. 235–240, 2000.

[5] I. Kreft, V. Stibilj, and Z. Trkov, “Iodine and selenium contents in pumpkin (Cucurbita pepo L.) oil and oil-cake,” European Food Research and Technology, vol. 215, no. 4, pp. 279–281, 2002.

[6] A. Paulauskiene, H. Danilcenko, V. Rutkoviene, and J. Kulaitiene, “The influence of various fertilizer on electrochemical properties of pumpkin fruits,” in Lietuvos Sodininkystės ir Daržininkystės Inst., LŽUU, vol. 24, no. 3, pp. 78–86, 2005.

[7] C. M. Messiaen and J. A. Fagbayide, “Cucurbita pepo Linn,” in Plant Resources of Tropical Africa 2. Vegetables, G. J. H. Grubben and O. A. Denton, Eds., pp. 273–277, PROTA Foundation, Wageningen, The Netherlands; Backhuys Publishers, Leiden, The Netherlands; CTA, Wageningen, The Netherlands, 2004.

[8] W. Brand-Williams, M. E. Cuvelier, and C. Beret, “Use of a free radical method to evaluate antioxidant activity,” LWT—Food Science and Technology, vol. 28, no. 1, pp. 25–30, 1995.

[9] V. L. Singleton and J. A. Rossi, “Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagent,” American Journal of Enology and Viticulture, vol. 16, pp. 144–158, 1965.

[10] J. L. C. Lamaison and A. Carnet, “Teneurs en principaux flavonoïdes des fleurs de Crataegus monogyna Jacq et de Crataegus laevigata (Poiret D.C) en fonction de la vegetation,” Pharmaceutica Acta Helvetiae, vol. 65, pp. 314–320, 1990.

[11] L. J. Porter, L. N. Hrstich, and B. G. Chan, “The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin,” Phytochemistry, vol. 25, no. 1, pp. 223–230, 1985.

[12] T. Fuleki and F. J. Francis, “Quantitative determination of anthocyanins 2. Determination of total anthocyanin and degradation index for cranberry juice,” Journal of Food Science, vol. 33, pp. 78–83, 1968.

[13] AOAC, Official Methods of Analysis, Association of Official Analytical Chemists, Washington, DC, USA, 15th edition, 1995.

[14] “Version 9.1,” SAS Institute Inc., Cary, NC, USA, 2003.

[15] B. Skwarylo-Bednarz and A. Krzepilko, “Diversified fertilization with NPK in wide-row cultivation of Amaranthus cruentus L. and total antioxidant capability of leaves and soil under amaranthus,” Acta Agrophysica, vol. 2, no. 1, pp. 173–181, 2008.

[16] A. A. Kader, “Influence of preharvest and postharvest environment on nutritional composition of fruits and vegetables,” in Horticulture and Human Health: Contribution of Fruits and Vegetables, B. Quebedeaux and F. A. Bliss, Eds., pp. 234–453, Prentice-Hall, Englewood Cliffs, NJ, USA, 1988.

[17] A. E. Mitchell and A. W. Chassy, “Antioxidant and the nutritional quality of organic agriculture,” 9 pages, October 2007, http://mitchell.ucdavis.edu/Is%20Organic%20Better.pdf.

[18] L. A. Weston and M. M. Barth, “Preharvest factors affecting postharvest quality of vegetables,” HortScience, vol. 32, no. 5, pp. 812–816, 1997.

[19] M. G. Lefsrud, D. A. Kopsell, D. E. Kopsell, and J. Curran-Celentano, “Air temperature affects biomass and carotenoid pigment accumulation in kale and spinach grown in a controlled environment,” HortScience, vol. 40, no. 7, pp. 2026–2030, 2005.

[20] N. Poulsen, A. S. Johansen, and J. N. Sørensen, “Influence of growth conditions on the value of crisphead lettuce - 4. Quality changes during storage,” Plant Foods for Human Nutrition, vol. 47, no. 2, pp. 157–162, 1995.

[21] M. Drews, I. Schonhof, and A. Krumbein, “Content of minerals, vitamins, and sugars in iceberg lettuce (Lactuca sativa var. capitata L.) grown in the greenhouse dependent on cultivar and development stage,” Gartenbauwissenschaft, vol. 62, no. 2, pp. 65–72, 1997.

[22] U. Hohl, B. Neubert, H. Pförr, I. Schonhof, and H. Böhm, “Flavonoid concentrations in the inner leaves of head lettuce genotypes,” European Food Research and Technology, vol. 213, no. 3, pp. 205–211, 2001.