

Bioactive Compound Composition of Three Different Pepper Varieties: a Comparative Study

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ABSTRACT

Capsicum chinense, *Capsicum frutescens*, and *Capsicum annuum* var *longum* are three popular pepper varieties indigenous to Nigeria, each renowned for its distinctive flavor and culinary versatility. These peppers are not only culinary delights but also rich sources of bioactive compounds which have been associated with a plethora of health benefits. This study delves into the comparative bioactive compound composition of *Capsicum chinense*, *Capsicum frutescens* and *Capsicum annuum* var *longum*. The pepper samples (10g) each was extracted and used for the assay. The study unveiled distinct bioactive compound profiles among the three pepper varieties. *Capsicum chinense* had higher levels of phytonutrients; Phenol ($491.86 \pm 0.820 \text{mg/g}$) and Flavonoid ($20.42 \pm 2.015 \text{mg/g}$). All three pepper varieties displayed similar Phenol and Flavonoid contents suggests that all three peppers can contribute to this phytonutrients in diets along with their potential health benefits. *Capsicum chinense* demonstrated the highest antioxidant activity ($1178.44 \pm 2.376 \text{mg/g}$). This suggests that *Capsicum chinense* provides stronger antioxidant activity, while *Capsicum annuum* var *longum* had the lowest levels. The Lycopene and Beta-Carotene contents in the three varieties of *Capsicum* were significantly different from each other. Other bioactive compounds (phenol, flavonoids, and ascorbic acids) were detected in all but not significantly different among the three varieties. These differences may contribute to the unique flavors, heat levels, and potential health benefits associated with each pepper type.

Keywords: pepper, phenol, flavonoids, ascorbic acid, capsicum, bioactive compounds

INTRODUCTION

Pepper is a fruit of the *Capsicum* plant species. It is widely used as a spice and a vegetable in various cuisines around the world. Peppers are a diverse group of plants, with over 30 species and hundreds of varieties. Peppers come in a wide range of shapes, sizes, colors, and heat levels¹. Pepper is known for its pungent taste and aroma, which is due to the presence of capsaicinoids, a group of compounds that are responsible for the spiciness of pepper. Capsaicinoids are also known to have several health benefits, including anti-inflammatory, analgesic, and anti-cancer properties². Amidst the rich tapestry of pepper types, *Capsicum annuum* (Shombo), *Capsicum chinense* (Red Pepper), and *Capsicum annuum* var. *longum* (Yellow Pepper or Nsukka Pepper), the spotlight falls on each contributing a distinct culinary experience.

Cayenne pepper and Scotch Bonnet peppers are two varieties of *Capsicum annuum* L. that are known for their bioactive compounds and antioxidant properties. Cayenne pepper (shombo), also known as *Capsicum annuum* or African Bird's Eye Chili, is a plant species that belongs to the Solanaceae family. It is called 'shombo' in Yoruba, 'Ose' in Igbo, and 'tatashi' in Hausa. It is a small chili pepper variety native to West and Central Africa; widely cultivated and used in various culinary traditions across the region. Shombo is a perennial herbaceous plant that grows to a height of about 1 meter.

It typically has a branching structure with numerous small, green leaves that are elliptical to lanceolate in shape³.

Scotch bonnet (red pepper) *Capsicum chinense* is also referred to as Bonney peppers or Caribbean red peppers. It has a resemblance to a Scottish tam o' shanter bonnet⁴. It is called 'ata rodo' in Yoruba, 'Ose Oyibo' in Igbo and 'barkono' in Hausa. It look like "small bell peppers," with a round or slightly squashed shape. Their spiciness is intense. It's hotter than its closely related cousin in the *Capsicum* family, the habanero pepper. The red flavor is used for spicy sauces. Pepper is a rich source of bioactive compounds such as ascorbic acid, flavonoids, total phenolics, and capsaicinoids, which have antioxidant and other health-promoting properties⁵.

The importance of bioactive-rich compounds plants in the recent times across the globe cannot be over-emphasised because of their usefulness in human health and nutrition^{6,7}. *Capsicum* spp. are commonly cultivated in the tropical and subtropical regions of the world as spices and vegetables⁸. They are nutritionally rich and economically valuable. The reports of several researchers confirmed that the fruit of *Capsicum* spp contains many antioxidants and phytochemicals such flavonoids, phenolics, carotenoids⁹. Some varieties of pepper also play useful roles in the pharmaceutical industries and for various therapeutic purposes.

The main active ingredient in peppers that confers a high degree of medical and pharmacological properties is the capsaicinoids (pungent alkaloid)¹⁰.

Research shows that phytochemicals such as flavonoids, phenolic acid and antioxidants are abundant in fruits and vegetables; this explains their importance in human diet^{9, 11}. They protect the cells in human by disallowing the oxidation process of free radicals in the body. Sequence of reactions caused by free radicals destroys the membrane and disrupts the metabolic pathways thereby triggering a spontaneous change in the DNA (mutations) and alteration of platelet function among others^{12,13,14}. In the recent times, attention has been shifted to natural foods such as vegetables rich in phytochemicals because studies proved that consumption of these foods reduces risk of having some diseases such as stroke, cancer and cardiovascular diseases.¹⁰. However, pepper is categorized as a vegetable with a rich source of phytochemical and numerous other bioactive compounds with potential health-improving properties.

MATERIALS AND METHOD

Experimental Site

This study was carried out at the Department of Botany, Faculty of Biosciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria, and lies between Latitude 60 06'N and 60 16'N, Longitude 70'01'E and 70 10'E. The climatic condition of this area is tropically dominated by rainfall patterns ranging from 1828 – 2002mm.

Collection of *Capsicum annum*, *Capsicum chinense*, and *Capsicum annum*

Peppers were obtained from three different markets which includes; Eke Awka, Otuocha Anam, and Ifite Awka, all in Anambra state. They were put in a sterile polythene bag and brought to the Department of Botany laboratory, Nnamdi Azikiwe University, Awka for determination of their fresh weights.

Sample Extraction

The pepper samples (10g) each were extracted by homogenizing 10g of the sample with pestle and mortar in 100ml of 70% ethanol. This was filtered using Whatman Paper (NO. 4). The extract was used for the assay.

Assay of Phytonutrients

Total Phenol

The total phenol content of the extract was determined using the method of Barros *et al.*¹⁵. The stock solution of the sample extract was diluted to 1mg/ml. One mill of the diluted extract solution was mixed with Folin and Ciocalteu's phenol reagent (1 ml). The saturated sodium carbonate solution (1 ml) was added to the mixture and adjusted to 10 ml with distilled water after three minutes. The reaction was kept in the dark for 90 minutes, after which the absorbance was read at 725 nm using a spectrophotometer. The standard used was gallic acid while the results were expressed as mg of gallic acid equivalents (GAEs) per g of extract.

Total Flavonoids

The method of Barros *et al.*¹⁵ was adopted in determining the flavonoid content. A mixture of aliquot, 0.5 ml diluted sample solution (250µg/ml), with 2 ml of distilled water and subsequently with 0.15 ml of 5 % NaNO₂ solution was prepared. After 6 minutes, 0.15 ml of 10% AlCl₃ solution was added and

allowed to stand for 6 minutes. Two mill of 4% NaOH solution was added to the mixture. The final volume was brought to 5 ml, thereafter the mixture was thoroughly mixed and allowed to stand for another 15 minutes. The absorbance of the mixture was read at 510 nm. The analyses were performed in triplicate. The results were expressed as mg Catechin equivalents per 100g of sample (mg CE/100 g).

Beta Carotene and Lycopene Content

These were determined by the method of Barros *et al.*¹⁵. The concentrated extract (100 mg) was vigorously shaken with 6 ml acetone-hexane mixture in the ratio of (4:6) for one minute and filtered using Whatman No.4 filter paper. The absorbance of the filtrate was read at 453, 505 and 663 nm respectively. The contents of lycopene and β-carotene were calculated according to the following equations:

Lycopene (mg/100ml)

$$= -0.0458A_{663} + 0.372A_{505} + 0.0806A_{453}$$

$$\beta\text{-carotene (mg/100ml)} = 0.216A_{663} + 0.304A_{505} + 0.452A_{453}$$

Ascorbic Acid

The method of Klein and Perry¹⁶ was adopted in determining the ascorbic content. Ten mill of 1% metaphosphoric acid was used to extract the aliquot. This allowed to stand for 45 min at a temperature of 28°C (Laboratory temperature) then it was filtered through Whatman No.4 filter paper. An aliquote (1ml) of the filtrate was mixed with 9ml of 50µM 2,6-dichlorophenolindophenol sodium salt hydrate. The result was expressed as mg ascorbic acid equivalent per gram (mgAE/g) of the sample.

Statistical Analysis

Data collected from the research were subjected to Analysis of Variance (ANOVA) at 0.05% level of significance and the mean separated by Duncan Multiple Range Test (DMRT) results. The Duncan's multiple range test significance was used to separate the mean and to test the difference among treatments.

RESULTS

Comparative Phenol analysis of the three species of *Capsicum*

From Table 1, it was observed that the Phenol content of *Capsicum frutescens* had the highest value of (502.04±58.782mg/g). This was followed by *Capsicum chinense* with a value of (491.86 ± 0.820mg/g). The least Phenol content was observed in *Capsicum annum var longum* with (486.77±5.544mg/g). There was no significant difference in the Phenol content of the different pepper species (P<0.05).

Table 1: Phenol composition of the three species of *Capsicum* (Pepper)

Species	Phenol Contents mg/g
<i>Capsicum frutescens</i>	502.04±58.782 ^a
<i>Capsicum chinense</i>	491.86 ± 0.820 ^a
<i>Capsicum annum</i>	486.77 ± 5.544 ^a

Values with same superscripts are not significantly different (P<0.05).

Comparative Flavonoid content of the three species of *Capsicum*

The result of the concentration of the phytonutrients of three species of *Capsicum* species showed that *Capsicum chinense* had the highest concentration of flavonoid with (20.42±2.015mg/g) while *Capsicum annum var longum* had a value of (20.26±1.789mg/g).

Capsicum frutescens had the least concentration of flavonoid with a value of (15.66±58.782mg/g). However, there was no significant difference in the flavonoid contents of the three species of *Capsicum*.

Table 2: Flavonoid Phytonutrient Composition of the three Capsicum Species

Species Flavonoid Contents mg/g
<i>Capsicum frutescens</i> 15.66±3.352 ^a
<i>Capsicum chinense</i> 20.42±2.015 ^a
<i>Capsicum annum var longum</i> 20.26±1.789 ^a

Values with same superscripts are not significantly different ($P < 0.05$).

Ascorbic Acid Phytonutrient Concentration of Three Species of Capsicum

It was observed that the Ascorbic acid concentration of *Capsicum annum var longum* was highest with a value of (180.67±3.302mg/g). This was followed by the concentration value of Ascorbic acid in *Capsicum frutescens* with (168.65±3.323mg/g). *Capsicum chinense* had the lowest concentration of Ascorbic acid with the value of (165.10±4.950mg/g).

Table 3: Ascorbic Acid Phytonutrient Compositions of the three Species of Capsicum

Species Flavonoid Contents mg/g
<i>Capsicum frutescens</i> 168.65±3.323 ^a
<i>Capsicum chinense</i> 165.10±4.950 ^a
<i>Capsicum annum var longum</i> 180.67±3.302 ^a

Values with same superscripts are not significantly different ($P < 0.05$).

Comparative Lycopene Concentrations of three species of Capsicum

The result of Lycopene concentration in three species of *Capsicum* was found to be significantly ($P < 0.05$) highest in *Capsicum chinense* with the value of (905.76±15.867mg/g). This was followed by the Lycopene content of *Capsicum annum var longum* with the value of (729.82±10.691mg/g) while the least value of (260.41±1.061mg/g) was found in *Capsicum frutescens*; this was significantly lower than the value obtained in *Capsicum annum* and *Capsicum chinense*.

Table 4: Lycopene Contents of the three Species of Capsicum

Species Lycopene Contents mg/g
<i>Capsicum frutescens</i> 260.41±1.061 ^c
<i>Capsicum chinense</i> 905.76±15.867 ^a
<i>Capsicum annum var longum</i> 729.82±10.691 ^b

Values with different superscripts are significantly different ($P > 0.05$).

Beta-Carotene Contents of three Species of Capsicum

The results revealed that *Capsicum chinense* had the highest beta-carotene concentration of (1178.44±2.376mg/g) while *Capsicum frutescens* had a beta-carotene concentration of (795.10±8.118mg/g). The least concentration was found in *Capsicum annum var longum* with (237.58±0.141mg/g). The values among the three varieties were significantly ($P > 0.05$) different from each other.

Table 5: Beta-Carotene Content of Three Species of Capsicum

Species Beta-Carotene Content mg/g
<i>Capsicum frutescens</i> 795.10±8.118 ^b
<i>Capsicum chinense</i> 1178.44±2.376 ^a
<i>Capsicum annum var longum</i> 237.58±0.141 ^c

Values with different superscripts are significantly different ($P > 0.05$).

DISCUSSION

Phytochemicals especially phenolic compounds and antioxidants are present in large quantity in vegetables and fruits; these bioactive compounds have been proven to be important part of the human diet⁷. Bioactive compounds fight off free radicals in the body; this also helps in preventing the process of oxidation in the human body. There is evidence that membrane damage may be caused by reactions triggered by free radicals. This affects the metabolic pathway and alteration of platelet function⁷.

The result of this study revealed the concentration of phytonutrients of three species of *Capsicum* species as presented in Table 1. The result indicated that *Capsicum frutescens* had the highest total phenol (502.04mg/g) content followed by the *Capsicum chinense* (491.86mg/g) while *Capsicum annum var longum* had the least (486.77mg/g). Phenolic compounds have antioxidant and antimicrobial properties^{17,18}.

Capsicum chinense had the highest flavonoid content (20.42±2.015mg/g) followed by *Capsicum var annum longum* (20.26±1.789mg/g) while *Capsicum frutescens* had the least (15.66±3.352mg/g). The most common group of polyphenolic compounds are the flavonoids; they are common in plants and very essential in human diet. Evidence shows that animals and man ingest high amount of flavonoids in their food this is because they have many varieties and are relatively low toxic¹⁹. Foods rich in flavonoids are onions, citrus, banana, berries, green and black tea. Flavonoids have very high biological and pharmacological significance. They have anti-allergic, anti-inflammatory, antioxidant, antibacterial, antifungal, antiviral, anti-cancer, and anti-diarrheal potentials^{20,21}. According to Pietta²², Flavonoids have significant antioxidant properties that protect the human body from free radicals and reactive oxygen species. The result further showed that *Capsicum var annum longum* had the highest ascorbic acid content (180.67±3.302mg/g) followed by *Capsicum frutescens* (168.6±3.323mg/g) while *Capsicum chinense* had the least (165.10±4.950mg/g). Comparative analysis revealed that there was no significant ($p < 0.05$) difference in the total phytochemical composition among the three species.

Beta-carotene content (1178.44±2.376mg/g) was highest in *Capsicum chinense* followed by *Capsicum frutescens* (795.10±8.118mg/g) while the least is *Capsicum annum var longum* (237.58±0.141mg/g). *Capsicum chinense* had the highest Lycopene values (905.76±15.867mg/g), followed by *Capsicum frutescens* (729.82±10.691mg/g) while *Capsicum annum var longum* had the lowest (260.41±1.061mg/g). There was a significant ($p < 0.05$) difference in the beta-carotene and lycopene content among the three species. Based on this result, Takahashi et al.²³ reported high antioxidant properties of the

fruit extracts of *C. frutescens*. Thus, peppers as a rich source of essential phytochemical substances has a potential health improving potentials. However, generic control influences the presence of different kinds of bioactive compounds. Its been proven through empirical research that eating foods rich in phytochemicals and antioxidants has the potential to lessen risk of cancer and other deadly diseases, hence much attention is recently given to natural foods especially vegetables and fruits that have abundance of these compounds^{7,24}.

CONCLUSION

The study unveiled distinct bioactive compound profiles among *Capsicum frutescens*, *Capsicum chinense*, and *Capsicum annum var longum*. *Capsicum chinense* generally had stronger antioxidant activity, while *Capsicum frutescens* had intermediate levels of these compounds, and *Capsicum annum var longum* had the lowest levels.

The Lycopene and Beta-Carotene contents in the three varieties of *Capsicum* were significantly different from each other. Other bioactive compounds (phenol, flavonoids and ascorbic acids) were detected in all but not significantly different among the three varieties. These differences may contribute to the unique flavors, heat levels, and potential health benefits associated with each pepper variety.

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