

Original Research Article

Bioactive and Sensory Properties of Sweet Italian (*Capsicum annuum*) and Habanero (*Capsicum chinense*) Pepper commonly consumed in Benue State of Nigeria

ABSTRACT

This study investigated the bioactive and sensory properties of Sweet Italian (*Capsicum annuum*) and Habanero (*Capsicum chinense*) pepper varieties, which are the common varieties of pepper consumed in Benue State, Nigeria. The two varieties of pepper were harvested fresh and dried using local drying equipment and milled into powder using a laboratory blender. The bioactive, phytochemical and sensory properties of the two pepper varieties were evaluated using standard methods. Results showed that the bioactive components of Habanero (HNP) and Sweet Italian (SIP) pepper varieties were vitamin C (60.32 and 51.50), lycopene (142.00mg/g and 118.00mg/g) and carotene (52mg/g and 40.10mg/g) for HNP and SIP respectively. The phytochemical content include total phenolic content (136.0±0.98 and 173.0±1.4 mg GAE/g), total flavanoid content (78.6±1.1 and 62.7±0.137 mg RUTIN/g), Tannin (1.53±0.16 and 1.55±0.09 mg/g), Saponin (0.13±0.02 and 0.15±0.05 mg/g), Steroids (0.91±0.04 and 0.89±0.09 mg/g) and Alkaloids (6.75±0.53 and 7.00±0.35 mg/g). The values for sensory properties of the pepper samples ranged between (6.25 - 8.40) for appearance, (5.95 - 7.85) for aroma, (5.55 - 7.5) for taste, (5.20 - 7.90) for mouth feel and (5.75 - 7.90) for overall acceptability. The *Suya* from HNP peppers was most preferred for all the sensory attributes measured except for appearance the *Suya* prepared from SIP and HNP exhibited more acceptable sensorial attributes than the commercial *suya*, The results thus suggest that these peppers have bioactive properties that could be utilized in the prevention and management of degenerative chronic diseases such as oxidative stress and diabetes in our communities.

Key words: *Bioactive, phytochemical, sensory, sweet Italian (Capsicum annuum) and Habanero (Capsicum Chinense)*

1. INTRODUCTION

In Nigeria and other developing countries of the world today, a large population of the people across all demography have been faced with the problem of nutritional deficiency [1]. Pepper is one of the oldest cultivated crops which has been used in cooking and other applications. Pepper is a *Solanaceous* crop belonging to the *Capsicum annuum* L. species. As a spice, it is one of the most widely consumed vegetables in human diets. Pepper is the second most important *solanaceous* vegetable after tomato [2]. “Peppers varieties such as *capsicum annuum* and *Capsicum Chinese* are good source of bioactive compounds including flavonoids, phenolic acids, carotenoids, vitamins C, E, A, capsaicinoids as well as natural colours and aromas. These Phytochemicals have been linked to biochemical and pharmacological effects including anti oxidation and anti-inflammatory activities as well to promote energy consumption and to suppress fat accumulation, increase body temperature in humans can go a long way to modulate body activities” [3].

“Phytochemicals are bioactive, non-nutrient, naturally occurring plant compounds found in vegetables, fruits and spices” [3]. “Phenolic compounds are some of the most widespread molecules among plant

secondary metabolites are known to act as natural antioxidants” [4, 5]. “Among the phytochemical compounds, polyphenols are of particular interest due to their property of scavenging free radicals both *in vitro* and *in vivo*. Epidemiological studies have shown a possible association between the consumption of polyphenols and a lower risk of coronary disease and cancer” [6, 4, 7]. “These benefits are due to its high antioxidant activity as antioxidants have been hypothesized to play an important role in chronic disease prevention, because they might be able to prevent oxidative damage caused by reactive oxidant species to vital biomolecules such as DNA, lipids and proteins” [4].

Tannins are astringent-tasting polyphenols found in plants that can bind and precipitate proteins [8]. Studies have shown that saponin has several health benefits, including its ability to reduce cholesterol levels, inhibiting diseases causing bacteria; hence saponin is classified as a phytochemical [9]. Alkaloid is seen as by-products of plant metabolism and they also act as protein reservoirs [10]. Alkaloids play important role in defense systems against pathogens and animals.

“Vitamin C is a water-soluble vitamin and is not synthesized by the human organism. Vitamins are generally a class of complex compounds that can be found in the majority of edible foods in form of micronutrients and, although in small amounts, they are essential for the proper function of several physiological processes of the human body. Interest in carotenoids and lycopene, has grown rapidly owing to studies suggesting a role in human health and disease. Lycopene is not toxic and has antioxidant, anti-inflammatory and chemotherapeutic effects in cardiovascular or neurodegenerative diseases and in some cancers” [11]. The culinary properties and biological effects of bioactive compounds and their sensory attributes make *C. annuum* and *C. chinese* peppers extremely important mostly in pharmacological substrates. The combination of *C. annuum* and *C. chinese* (1:1) exhibited additive antioxidant properties [12].

In as much as pepper is highly rich in phytochemicals and other quality attributes required for healthy growth and development, little attention has been given to the phytochemicals and sensory attributes in sweet Italian (*C. annuum*) and Habanero (*C. Chinese*) unlike other fruits and vegetables which are widely recognized in human diets [13, 14]. The present study attempts to add more information on the phytochemicals and sensory properties of Sweet Italian (*C. annuum*) and Habanero (*C. chinese*) pepper varieties consumed in Benue State, Nigeria as well as prevent oxidative damage and management of degenerative chronic diseases.

2. MATERIALS AND METHODS

The two varieties of pepper, sweet Italian (*C. annum*) and Habanero (*C. annum*) were harvested fresh from local farms in their ripened stage and dried using local drying equipment and milled into powder using a laboratory blender.

2.2 Processing of Pepper Powder:

The modified method of [15] was used for pepper powder production as shown in Fig.1. The red pepper were selected to uniform sizes, shapes, and without any defect on visual inspection and thoroughly cleaned before manually sorting. The sorted red peppers were washed in cold water to remove soil and dust particles. The thoroughly cleaned samples were manually graded on the basis of their size. Washed red peppers were sliced with knives as approximate sizes of 15 mm x 15 mm of uniform slices with thickness of 2–4 mm. After slicing, the slices were blanched with hot water at 95 °C for 3-5 min. The method of blanching is similar to that of [16, 17]. “The blanched slices were dried using locally fabricated electric dryer, where the red pepper slices were spread on the shelves of the drying bin and the hot air was passing through these dryer upward from the electric air collectors at the temperatures between 65-70 °C.

Drying time and final moisture content for product was controlled. Also, the red pepper slices were shifted alternatively inside the electric bin in order to give the same chance for the red pepper slices to have the same drying conditions. The red pepper was ground and kept until used” [16, 17] .

2.3 Beef *Suya* Preparation

The modified method of [18] was used for Beef *Suya* preparation as shown in Fig. 2. Two kg of Beef Meat was cut into very thin fillet, the pepper samples mixed with *suya* ingredients were put in a flat bowl with groundnut oil, little quantity of salt was added and mixed. With the use of a cooking brush, the mixture was rubbed on the threaded fillets of beef. In a wide dish, the *Suya* spices was spread spices and dab the threaded fillets of beef in the spices so that the beef takes up as much of the spices as possible. All the beef was covered with spices. The spiced beef were placed on the flat plate, covered with a thin film and left to marinate for one hour. After one hour, it was preheated for 10minutes, before proper roasting which last for 15-20minutes. The beef was flipped to roast the underside again. The length of roasting time depends on the thick of the fillets. The total roasting time for both sides of beef is 30-40minutes. Rub the remaining spices and roast either sides for 5 minutes each.

2.4 Phytochemical Properties of Pepper Powders

2.4.1 Determination of total phenolic content (TPC) of pepper powders.

Determination of total phenolic content was carried out using Folin-Ciocalteu’s phenol reagent reaction as described by [19]. The calibration curve solutions will be prepared by pipetting 0.0, 0.2, 0.4, 0.6, 0.8, and 1.0 ml of gallic acid standard solution (1.0 mg/ml gallic acid) in triplicate into clean dried test tubes. Each test tube is made up to 1.0 ml with distilled water. To each of the test tube is added 1.5 ml of Folin-Ciocalteu’s reagent, incubated at room temperature for 5 min followed by the addition of 1.5 ml of 10% (w/v) NaHCO₃ solution to give a total volume of 4.0 ml. The reaction mixtures are further incubated for additional one and half hours and the absorbance is read at 725 nm against blank containing all reagents except the standard gallic acid which is replaced with distilled water. The standard curve is obtained by plotting absorbance against the concentration. The determination of total phenol in methanolic extract of *A. esculentus* is done by pipetting 0.5 ml each of 1 mg/ml methanolic extract into clean dry test tubes in triplicate. The volume is adjusted to 1.0 ml with distilled water. To each of the tubes is added 1.5 ml of Folin-Ciocalteu’s reagent. The reaction mixture was incubated at room temperature for 5 min. To the reaction mixture is added 1.5 ml of 10% (w/v) NaHCO₃ solution. The reaction mixture is incubated for one and half hour (1½ hr). The absorbance is read at 725nm against blank containing the extract and all reagents except Folin-Ciocalteu’s reagent which is replaced with distilled water. The concentrations of the phenolics in the extract is extrapolated from standard curve and expressed as milligram gallic acid equivalent per g of extract (mg GAE/g extract).

2.4.2 Determination of total flavonoid content (TFC) of pepper powders

The content of flavonoids in the extract was determined spectrophotometrically according to the procedure of [20]. The extract (0.01 g) will be dissolved in about 5 ml of extraction solvent and made up to 20 ml to give a final concentration of 0.5 mg/ml. To clean dry test tubes (in triplicate) will be pipetted 0.5 ml of working solution of the sample and diluted with 4.5 ml distilled water. To each test tube is then added 0.3 ml of 5% (w/v) NaNO₂, 0.3 ml of 10% AlCl₃ and 4 ml of 4% (w/v) NaOH. The reaction mixtures will be incubated at room temperature for 15 min. The absorbance will be read at 500nm against reagent blank containing all reagents except the extract or standard quercetin in the case of standard curve solutions. The standard calibration curve will be prepared by pipetting 0.0, 0.2, 0.4, 0.6, 0.8, 1.0 ml of 1 mg/ml quercetin (1 mg / ml of quercetin standard solution) into clean dry test. The volumes will be made

up to 5 ml with distilled water. The reaction mixture is incubated at room temperature for 15 min. Absorbance is taken at 500nm and was plotted against the concentration to give the standard calibration curve. The concentrations of the flavonoids in the extract will be extrapolated from standard calibration curve and expressed as milligram quercetin equivalent per g of extract (mg QE/g extract).

2.4.3 Determination of tannin content of pepper powders.

“0.2g of finely ground sample was weighed into a 50ml sample bottle. 10ml of 70% aqueous acetone was added and properly covered. The bottle were put in an ice bath shaker and shaken for 2h at 300C .Each solution was then centrifuge and the supernatant store in ice. 0.2ml of each solution was pipetted into the test tube and 0.8ml of distilled water was added. Standard tannin acid solutions were prepared from a 0.5mg/ml of the stock and the solution made up to 1ml with distilled water. 0.5ml of Folin-ciocalteu reagent was added to both sample and standard followed by 2.5ml of 20% Na₂CO₃ the solution were then vortexed and allow to incubate for 40minutes at room temperature, its absorbance was read at 725nm against a reagent blank concentration of the same solution from a standard tannic acid curve was prepared” [21].

2.4.4 Determination of saponin content of pepper powders.

The spectrophotometric method of [22] will used for saponin determination.

“2g of the finely grinded sample was weighed into a 250ml beaker and 100ml of Isobutyl alcohol was added. Shaker was used to shake the mixture for 5hours to ensure uniform mixing. The mixture was filtered with No 1 Whatman filter paper into 100ml beaker containing 20ml of 40% saturated solution of magnesium carbonate (MgCO₃).The mixture obtain again was filter though No 1Whatman filter paper to obtain a clean colourless solution. 1ml of the colourless solution was taken into 50ml volumetric flask using pipette, 2ml of 5% iron (iii)chloride (FeCl₃) solution was added and made up to the mark with distill water. It was allowed to stand for 30minutes for the colour to develop. The absorbance was read against the blank at 380nm” [22].

2.4.5 Determination of alkaloid content of pepper powders.

“5g of the sample was weighed into a 250ml beaker and 200ml of 10% acetic acid in ethanol was added and allowed to stand for 4min. This was filtered and extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide added drop wise to the extract until the precipitation was completed. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is then alkaloid which was dried and weighed” [23].

% Alkaloid = $\frac{\text{weight before} - \text{weight after}}{\text{weight before}} \times 100$

2.4.6 Quantitative estimation of steroids in the pepper powders.

“1ml of test extract of steroid solution was transferred into 10ml volumetric flasks. Sulphuric acid (4N, 2ml) and iron (III) chloride (0.5% w/v, 2 ml), were added, followed by potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5 ml). The mixture was heated in a water-bath maintained at 70±2°C for 30 minutes with occasional shaking and diluted to the mark with distilled water. The absorbance was measured at 780nm against the reagent blank” [24]

2.5. Bioactive Components of Pepper Powders

2.5.1 Determination of beta-carotene and lycopene contents of pepper powders using UV-Vis spectroscopy analysis.

A UV visible spectrophotometer was used for the analyses as described by [25]. The pepper samples (3 mg) were weighed into a round bottom flask equipped with a sensitive balance and 5 ml of n-hexane was then added into the flask to make a sample suspension. The suspension was then poured into a 50 mL flask and washed with 25 ml of fresh n-hexane and the process repeated for six times at 20 minutes interval until colorless suspension was obtained. The mixture was then filtered with whatman number 1 filter paper and kept in the dark for 40 min for phase separation into the upper and lower layers which were collected for carotene-hexane and lycopene-hexane analyses using spectrophotometer at wavelength 450 and 470 nm, respectively. The standard carotene and lycopene were prepared in a similar manner but at concentration of 0.1 to 1.0mg/ml for calibration curve. The quantity of carotene and lycopene was determined through extrapolation from the standard curve.

2.5.2 Estimation of ascorbic acid content of pepper powders.

Ascorbic acid content in the samples was estimated by titrimetric method described by [26]. Five millilitres of standard ascorbic acid (100 µg/mL) were measured into a conical flask containing 10 mL 4% oxalic acid. The mixture was titrated against the 0.0005M of 2, 6-dichlorophenol indophenols dye (DCPIP) (prepared by dissolving 145 mg DCPIP in 100 mL hot distilled water and a subsequent addition of 300 mL of 0.066 M phosphate buffer, pH = 6.98, previously prepared by mixing the respective volumes of sodium dihydrogen phosphate and sodium mono-hydrogen phosphate solutions (2/3 ratio) and distilled water was added to the final volume of 1000 mL). The appearance and persistence of pink colour for 30 seconds is taken as the end point. The amount of dye consumed (V1 mL) is equivalent to the amount of ascorbic acid. Five millilitres of sample (prepared by taking 5 mL of juice in 100 mL 4% oxalic acid) was measured inside a conical flask containing 10 mL 4% oxalic acid in a conical flask and titrated against the dye (V2 mL). The amount of ascorbic acid was calculated using the formula;

$$\text{Ascorbic acid (mg/mL)} = \frac{X_i \text{ mg}}{V_1} \times \frac{V_2}{15 \text{ ml}} \times \frac{100 \text{ mL}}{\text{mL of sample used for analysis}} \quad (3.31)$$

X_i (mg) = quantity of ascorbic acid dissolved in a known volume of oxalic acid V_1

= volume of dye consumed by the standard

V_2 = volume of dye consumed by the sample

15 mL = total volume of sample and oxalic titrated

100mL = volume of oxalic acid solution used in dissolving the sample

2.6 Statistical Analyses of the Samples

Data was subjected to Analysis of Variance (ANOVA) followed by T-Test and means were separated by Duncan multiple range test; significant levels were obtained at 95% ($P > 0.05$). SPSS version 17 software was used.

3. RESULTS AND DISCUSSION

3.1 Phytochemical properties of pepper powder.

Table 1 shows the results for the phytochemical properties of Sweet Italian and Habanero peppers. The HNP pepper sample was observed to show higher contents of total phenolic content (TPC) (173 mgGAE/g), tannins (1.55mg/g), saponins (0.15 mg/g) and alkaloids (7.00 mg/g) contrasting corresponding lower values of these phytochemicals (136 mgGAE/g, 1.53 mg/g, 0.13 mg/g and 6.75%, respectively) exhibited by SIP sample. On the other hand, SIP sample was higher in total flavanoid

content (TFC) which was 78.6 mg RUTIN/g compared to a significantly ($p<0.05$) lower value of 62.7 mg RUTIN/g found in the HNP sample. Similarly, SIP sample was significantly ($p<0.05$) higher in its steroid content (0.91 mg/g) when compared to the steroid content of HNP (0.89 mg/g). SIP and HNP samples have shown that they possess varying amounts of phytochemical which could prevent or mitigate different health challenges and help manage chronic diseases [6, 4, 7].

3.2 Bioactive components of Sweet Italian and Habanero pepper powders.

Table 2 presents three selected bioactive components reported to be contained in SIP and HNP. The contents of the three bioactive compounds, ascorbic acid (vitamin C), lycopene and beta-carotene were observed to be highest in the SIP than in the HNP samples. The distribution of ascorbic acid, lycopene and beta-carotene contents in the SIP and HNP samples were as follows: 60.3 and 51.5 mg/g; 142.0 and 118.0 mg/g and 52.0 and 40.1mg/g with the content of each bioactive component being significantly ($p<0.05$) higher in SIP than the HNP samples. These bioactive compounds will provide adequate nutrition, provide health promoting activities that will prevent, ameliorate and manage morbidities [4, 7].

3.3 Sensory properties of beef *Suya* made with Sweet Italian and Habanero peppers in comparison to commercially prepared beef *Suya*.

To carryout sensory evaluation on the SIP and HNP pepper powders, beef *suya* was used as a carrier and processed using the SIP and HNP pepper powders as ingredients and determine their sensory attributes compared to that of the commercially prepared beef *suya*. The results of the sensory properties of *suya* prepared using the SIP and HNP pepper powders are presented in Table 3. For appearance, SIP had the highest sensory score of 8.40, followed by the HNP (7.40) and CSS recorded the lowest score (6.25) for appearance among the *suya* samples evaluated and were all significantly ($p<0.05$) from each other. In other words, the panel of judges preferred the appearance of the SIPS most and that of the CSS least. In contrast, for the aroma, taste, mouthfeel and overall acceptability attributes, the HNPS Sample was most acceptable with scores of 7.85, 7.75, 7.35 and 7.90, respectively, compared to the CSS sample which was least preferred for these sensory characteristics as they recorded the least sensory scores of 5.95, 5.55, 5.20 and 5.75, respectively. The aroma, taste, mouthfeel and overall acceptability the SIPS, HNPS and CSS were significantly ($p<0.05$) different from each other. The sensory evaluation revealed that the SIPS and HNPS were preferred (better) for all the sensory attributes evaluated than the commercial *suya*. The results of the sensory properties indicated that the *suya* made from the two pepper samples exhibited better sensorial features than the commercial *suya*, suggesting that they have better potential for acceptability in food preparations [2].

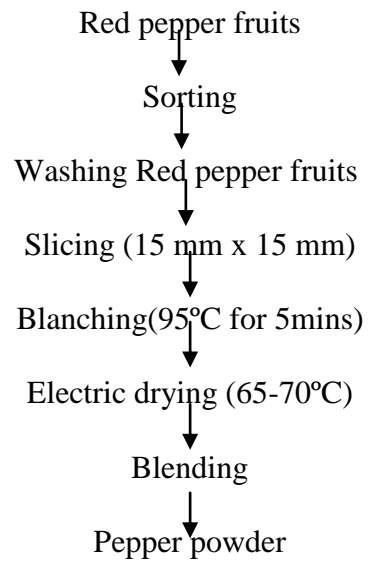


Figure 1: Flow chart for pepper powder preparation.

Source: Modified Raja and Sethuraman [15]

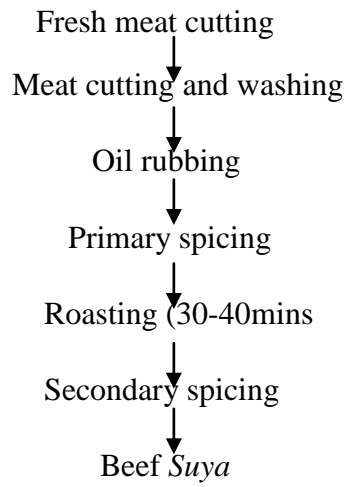


Fig. 2: Flow chart for the preparation of Beef Suya

Source: Olaoye et al. [18]

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Table 1: Phytochemical properties of Sweet Italian and Habanero pepper powders

Parameters	Sweet Italian Pepper	Habanero Pepper
Total phenolic content (mgGAE/g)	136.0 ^a ± 0.98	173.0 ^b ± 0.14
Total flavonoid content (mg RUTIN/g)	78.6 ^b ± 1.07	62.7 ^a ± 0.14
Tannins (mg/g)	1.53 ^a ± 0.16	1.55 ^a ± 0.09
Saponin (mg/g)	0.13 ^a ± 0.02	0.15 ^a ± 0.05
Steroids (mg/g)	0.91 ^a ± 0.04	0.89 ^a ± 0.09
Alkaloids (%)	6.75 ^a ± 0.58	7.00 ^a ± 0.35

Table 2: Bioactive components of Sweet Italian and Habanero pepper powders

Parameters	Sweet Italian pepper	Habanero pepper
Ascorbic acid (mg/g)	60.3 ± 0.98	51.5 ± 0.77
Lycopene (mg/g)	142.0 ± 0.21	118.0 ± 0.08
Beta-carotene (mg/g)	52.0 ± 0.32	40.1 ± 0.06

Table 3: Sensory properties of *Suya* prepared with Sweet Italian and Habanero powders compared to commercially prepared beef *Suya*.

	CPBS	SIPS	HNPS
Appearance	6.25±0.97 ^c	8.40±0.88 ^a	7.40±0.59 ^b
Aroma	5.95±0.68 ^c	7.25±0.67 ^b	7.85±0.78 ^a
Taste	5.55±1.07 ^c	7.00±1.26 ^b	7.75±1.23 ^a
Mouth-feel	5.20±0.87 ^c	6.90±1.29 ^b	7.35±1.23 ^a
Overall acceptability	5.75±1.28 ^c	6.95±0.64 ^b	7.90±1.23 ^a

Values are means ± standard deviation of duplicate determinations. Means with same superscript across the rows are not significantly ($p < 0.05$) different. Key: CS=Commercial *Suya*, SIPS= *Suya* made with Sweet Italian pepper, HNPS= *Suya* made with Habanero pepper.

4. CONCLUSION

The phytochemical content of SIP and HNP were as follows: TPC (136.0±0.98 and 173.0±1.4 mg GAE/g), TFC (78.6±1.1 and 62.7±0.137 mg RUTIN/g), Tannin (1.53±0.16 and 1.55±0.09 mg/g), Saponin (0.13±0.02 and 0.15±0.05 mg/g), Steroids (0.91±0.04 and 0.89±0.09 mg/g) and Alkaloids (6.75±0.53 and 7.0±0.35 mg/g), respectively. The results suggest that utilization of these peppers in foods will provide bioactive properties that could help prevent, manage degenerative chronic diseases and maintain healthy living. These bioactive compounds will provide adequate nutrition, health promoting activities that will prevent, ameliorate and manage morbidities. The results of the sensory properties indicated that the *suya* made from the two pepper samples exhibited better sensorial features than the commercial *suya*, suggesting that they have better potential for acceptability in food preparations.

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