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Proximate, mineral and microbial assessment of fresh red pepper (*Capsicum annuum* L.) from markets in Benin City metropolis and environs

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Abstract

Red pepper (*Capsicum annuum* L) is the most economically important species in the *Capsicum* genus. They belong to the nightshade family Solanaceae. It varies in shape, size, colour, flavour, heat level and nutritional properties. This study aims to determine the proximate, mineral and microbial assessment of fresh *Capsicum annuum* purchased from five different markets in Benin City metropolis and environs. A total of 25 pepper samples were purchased; standard biochemical methods were used to analyze their proximate composition. The proximate composition included moisture (4.48±0.18 g/100g), ash (4.94±0.14 g/100g), carbohydrate (17.60±0.34 g/100g), protein (11.40±0.16 g/100g), fat (23.65±0.41 g/100g), crude protein (21.29±0.28 g/100g) and crude fibre (38.76±1.07 g/100g). Analysis of mineral element contents indicated that potassium was the most abundant (654.12±5.46 mg/100g), followed by magnesium (237.59 ±3.63 mg/100g), calcium (174.71±2.93 mg/100g), iron (17.49±0.25 mg/100g), sodium (12.38 ±0.12 mg/100g) and manganese (2.16±0.05 mg/100g). The microbial assessment of the pepper samples yielded eight microorganisms including *Bacillus* sp., *Candida* sp., *Staphylococcus aureus, Enterobacter* sp. and *Escherichia coli*. Samples procured from Uselu market (32.1%) were the most contaminated with microorganisms while those from Oliha market (12.5%) were the least infected. *Bacillus* sp. and *Staphylococcus aureus* were the most prevalent microorganisms with each of them having a prevalence of 23.2%. Bacterial contamination was more than fungi in the fresh red pepper investigated. It was also observed that the environment in which these red peppers were kept contributed greatly to their spoilage. Results from this study show that pepper can be an important dietary supplement for improving human health, but care must be taken to prevent microbial contamination during handling and storage.

Keywords: Proximate, Mineral, Pepper, Microbial, Spoilage

Introduction

Peppers (*Capsicum* spp.) are iconic and diverse plant species (Bosland and Votava, 2002). They vary in size, shape, colour, flavour, amount of heat, nutritional value, and application. There are as many varieties of peppers as there are growing regions and cultivators. *Capsicum* is a source of both spicy and non-spicy items that are employed globally. *Capsicums* are peculiar in that they are utilized as both a vegetable (or, strictly speaking, a fruit) and a spice (Greenleaf, 1986). They impart taste, color, and spiciness to dishes. In addition, they supply important vitamins, minerals, and nutrients. Extracts of pepper are utilized in medications, cosmetics, paintings, and pepper sprays. In addition to serving as a meal, condiment and medication, peppers are also employed for their aesthetic value. It is a member of the Solanaceae family and subfamily Solanoideae, is cultivated globally for its essential applications as foods, spices, decorations, medications, lachrymatories and vitamins (A and C) (Perry *et al*., 2007).

Micronutrient insufficiency in the human diet continues to be a massive global issue and is likely the root cause of several chronic health problems and illnesses. It is estimated that over two (2) billion people worldwide are deficient in important minerals and vitamins, particularly zinc, iodine, vitamin A, and iron, due mostly to poor food consumption. The elimination of micronutrient deficiencies in a sustained manner is only possible when the diets of vulnerable populations include appropriate levels of all essential elements. Among the several strategies for eradicating human nutritional deficit, consuming a wide variety of foods, particularly vegetables containing various micronutrients, is still seen as the most viable alternative. The widespread intake of chili peppers (*Capsicum annuum*), which are recognized for their rich nutritional content (including a wide variety of vitamins, minerals, phytochemicals, and dietary fiber), may contribute to the reduction of micronutrient shortages in humans. Incorporating nutrient-rich chili peppers into human meals might help prevent nutritional deficits by providing significant parts of the needed daily nutrients.

Fresh produce can have enormous microbial populations. Most research on produce-associated bacteria has concentrated on a small number of pathogens, so we know little about the variety and composition of bacterial communities on produce and how their structure differs between produce kinds (Leff and Fierer, 2013). Apples, grapes, lettuce, peaches, peppers, spinach, sprouts, and tomatoes host huge bacterial populations (King *et al*., 1991; Badosa *et al*., 2008; Ponce *et al*., 2008), but we're just beginning to investigate their variety. Since fresh food is commonly ingested uncooked, germs including *Listeria monocytogenes*, *Escherichia coli*, and *Salmonella* can cause illness outbreaks (Oliviera *et al*., 2010; Rastogi *et al*., 2012; Nguyen and Farlin, 1994). Produce microorganisms may have other less obvious effects on human health than producing disease. Exposure to non-pathogenic plant microorganisms may impact allergy development (Liao and Fett, 2001). Consuming raw food may introduce new commensal bacteria into the human gastrointestinal tract.

Only a few research have examined the makeup of produce-associated microbial communities using culture-independent techniques (Harris *et al*., 2003; Fatica and Schneider, 2011). From these previous works, a few key patterns emerge: Different produce types and cultivars can harbor different abundances of specific bacterial groups (Hanski *et al.,* 2012); Farming and storage conditions may influence the composition and abundances of microbial communities found in produce (Gram *et al.,* 2002; Flores *et al.,* 2012; Rudi *et al.,* 2002) and non-pathogenic microbes may interact with and inhibit microbial pathogens found on produce

surfaces (Lopez-Velasco *et al.,* 2011; Granado *et al.*, 2008; Otteson *et al.*, 2009; Shi *et al.,* 2009). Despite this effort, we have a limited understanding of produce-associated microbial communities, the variables that determine their composition, and the distributions of specific taxa among produce types (particularly those taxa that are difficult to culture).

This study aims to determine the proximate, mineral and microbial components of fresh pepper (*Capsicum annuum* L.) by using standard biochemical methods to analyze the proximate composition, the mineral element contents, and the microbial count as well as bacteria and fungi characteristics of the pepper samples collected from major markets in the Benin metropolis and environs. This aim will be achieved with the following objectives; access the availability and suitability for consumption of fresh pepper within Benin metropolis and environs, determine the proximate, mineral and microbial components of the red pepper samples by analyzing the microbial counts as well as fungi and bacteria characteristics and collect information on the public health risk of the microorganisms associated with fresh pepper collected from major markets in the Benin metropolis and environs.

Materials and Methods

Sample collection

An experimental study was carried out by randomly purchasing fresh pepper from different markets (Uselu, Oba, New Benin, Santana, and Oliha) in the Benin metropolis and environs. These markets were selected because they are major markets within the Benin metropolis and environs where pepper is sold at cheaper prices to consumers and has a lot of patronage.

A total of 25 fresh red pepper samples were collected from the markets at varying prices. These pepper samples were held in sterile "Polytene Ziploc" bags and sent to the laboratory for immediate laboratory investigation. Sterile scrape blades were used to cut the pepper open, before sterile swab sticks were used to collect samples from the pericarp and mesocarp, respectively. Before placing the pepper samples in the bag, the Ziploc bags were cleaned with distilled water and 70 % alcohol and left to stand for 5 minutes.

Laboratory analysis

In order to obtain a representative fraction, the samples were homogenized and mixed after collection. The homogenized fraction was subsequently evaluated for phytochemical and microbiological properties. Before incubation at 37°C for 18 - 24 hours, they were cultured on Chocolate agar, Nutrient agar, MacConkey agar, and Sabaraud agar. Sabaraud agar was incubated at room temperature for 3 - 7 days for fungus cultivation.

Identification of organisms

After incubation, the colonies of the organisms were examined for colonial morphology and morphological investigations, and Gram staining was performed to indicate the typical grouping of the cells. To identify bacteria isolates, biochemical tests (Indole, citrate, Urease, catalase, coagulate, and sugar fermentation tests) were conducted. Identification of fungi on Sabaraud agar was performed using Gram stain and a moist preparation.

Identification of Fungi species

Mounted with saline solution on a slide. Fungi possess features like hyphae, sporangiophore, and mycelium.

Candida spp.: At room temperature, their structure is identical to that of fungi. To understand the major Candida species, it was tested using a germ tube. After being injected, it was incubated for 2 to 3 hours and thereafter inspected under a microscope with X10 and X40 objectives.

Bacterial identification /classification

Gram staining was performed on all cultures. This was conducted after the swab sticks had been grown on various agars. When noticeable growth was observed, a test was conducted. This test distinguished between gram-positive and gram-negative organisms. Gram-positive bacteria stain purple, while gram-negative bacteria stain pink or red. *Bacillus* species are either long or short; *Streptococcus* or *Staphylococcus* is suspected if the bacterium is spherical.

Catalase test

Using H_2O_2 (hydrogen peroxide). Sift H_2O_2 onto the slide. *Staphylococcus* was confirmed by using an inoculation loop to drop the organism on the slide and spread it. The presence of bubbles indicated that the bacterium was catalase-positive, thereby confirming its identity.

Coagulate test

Slide coagulate test: On a grease-free microscope slide, a drop of normal saline was combined with a drop of plasma, and if clumping was detected after mixing and swirling, the resulting organism is *Staphylococcus aureus*.

Note: The organism releases coagulate, which transforms fibrinogen into fibrin.

Gram negative Bacilli

MacConkey agar was used to distinguish between lactose fermenting and non-lactose fermenting organisms. Biochemical assays, including an income test, citrate test and urease test were conducted to determine if lactose fermentation was occurring.

Indole test

This test is utilized for the identification of Enterobacteriaceae. The bacteria break down the amino acid tryptophan to generate indoles with a pinkish hue.

Method

1. The organism in question was injected into peptone water and cultured for 24 h.
2. This was followed by the addition of 3 drops of Kovac's reagent. Indole-positive organisms, such as *Escherichia* coli and *Klebsiella oxytoca*, were pink in hue, but the indole-negative organisms, *Klebsiella pneumonia* and *Enterobacter,* produced no pink hue.

Citrate test

This test was used to detect organisms belonging to the Enterobacteria group. Some bacteria use citrate to generate ammonia (NH_4) and carbon dioxide (CO_2).

Method

The probable organism was incubated with citrate reagent for twenty-four hours and its color was observed. If the solution is blue, it is citrate-positive. Organisms such as *Enterobacter, Citrobacter, Klebsiella sp., and Providencia sp.* come into this group.

Urease test

It is used to recognize the Enterobacteriaceae group of organisms.

Principle: Some gram-negative organisms generate urease by degrading urea to produce a pinkish coloration.

Method

The bacterium under suspicion was injected into urea reagent and was incubated for 24 hours, resulting in a color change. If a pinkish hue is noticed, organisms such as *Proteus vulgaris*, *Klebsiella oxytoca,* and *Proteus mirabilis* may be present; *Escherichia coli* was observed when the test was negative.

Oxidase test

This is a biochemical test used to distinguish *Pseudomonas* and *Alcalegenes* from other gram-negative organisms. Some organisms react with the oxidase reagent (tetramethyl para-amino benzaldehyde)

Method.

Observe a drop of Oxidase reagent on the Whatman number one filter paper for a purple colour shift within 5 to 10 seconds. It became purple, suggesting the presence of *Pseudomonas* species. *Pseudomonas vulgaris* was observed when negative; *Proteus mirabilis* was detected in that outcome.

Proximate analysis of pepper samples

The proximate composition of red pepper was evaluated using the methods of Aberoumand (2014); moisture was determined by weight loss after heating in a blast drying oven (DHG-9640A, Hecheng Instrument Co. Ltd., Shanghai, China) at 105 °C, ash in a muffle furnace (QSH-1700M, Quanshou Electric Furnace Co. Ltd., Shanghai, China) at 550 °C for 5 h. Crude fat was evaluated through extraction with petroleum ether using Soxhlet apparatus (Herch *et al.*, 2014). As proposed by Koyuncu *et al.* (2014), crude protein was estimated by multiplying the nitrogen value found by the Kjeldahl procedure by 6.25 to obtain the crude protein content. Total dietary fiber was determined by adding the soluble and insoluble fractions, according to the enzymatic gravimetric method of Prosky *et al.* (1988).

Determination of mineral elements contents of pepper

Mineral element content was estimated according to the method detailed by Tu *et al.* (2009) with slight modifications. In short, the sample (500 mg) was weighed into a beaker, and digested in 7 mL of HNO_3 -H_2O_2 (5:2, v/v) for 10 min, and the mixture was heated to near dryness. After cooling, the residue was treated with 0.1 M HNO_3 and brought to 50 mL with bi-distilled water. Mineral element was determined with an inductively coupled plasma-optical emission spectrometry (Teledyne Leeman Labs Ltd., Prodigy, Hudson, NH, USA). Calibration curves of standard elements (sodium, potassium, calcium, zinc, magnesium, iron and manganese) were prepared and their contents in samples were calculated by the regression equation.

Data analysis

Results from proximate and mineral analysis of the pepper samples were done in replicates and analyzed using SPSS version 21. The experimental results were expressed as means ± standard deviation (SD) of triplicates. Statistical analysis was performed using Fisher's F-test and p

Results

The proximate and mineral composition of the pepper samples collected from markets in Benin metropolis and environs are presented in Tables 1 and 2, respectively.

Table 1. Proximate composition of fresh pepper procured from markets in Benin City metropolis and environs

Chemical Constituent	Concentration (g/100 g)
Moisture	4.48 ± 0.18
Ash	4.94 ± 0.14
Carbohydrate	17.60 ± 0.34
Protein	11.40 ± 0.16
Fat	23.65 ± 0.41
Crude Protein	21.29 ± 0.28
Crude fibre	38.76 ±1.07

Mean value ± Standard Deviation (SD) of triplicates.

Table 2. Mineral composition of fresh pepper procured from markets in Benin City metropolis and environs

Mineral element	Concentration (mg/100 g)
Sodium	12.38±0.12
Potassium	654.12±5.46
Calcium	174.71±2.93
Zinc	7.97±0.13
Magnesium	237.59±3.63
Iron	17.49±0.25
Manganese	2.16±0.05

Mean value ± Standard Deviation in triplicates.

Contents of sodium, potassium, calcium, zinc, magnesium, iron, and manganese in pepper were 12.38, 654.12, 174.71, 7.97, 237.59, 17.49 and 2.16 mg/100 g, respectively (Table 2).

Out of 25 samples collected from fresh pepper (pericarp and mesocarp) and the environments in which they were stored in the five markets, eight (8) microorganisms were isolated using Chocolate, McConkey and Nutrient Agars (Plates 1 - 3). They included *Bacillus* sp., *Candida* sp., *Staphylococcus aureus*, *Klebsiella* sp., *Proteus vulgaris*, *Citrobacter* sp., *Enterobacter* sp. and *Escherichia coli*. Samples gotten from Uselu market (32.1%) were infected more with microorganism while those from Oliha market (12.5%) were the least contaminated with microorganisms.

The study showed that *Bacillus* spp. and *Staphylococcus aureus* were the most prevalent, with each of them having a prevalence of 23.2%, respectively. In this study it was observed that bacterial contamination was more than fungal contamination of fresh pepper. It was also observed that the environment in which these peppers were kept also contributed greatly to their spoilage (30.4%).

In this study *Candida* sp. was isolated from all the samples gotten from Edaiken market. *Citrobacter* sp. was the least prevalent in this study with a prevalence of 1.8%. It was only isolated in samples from Santana market. The results of microorganisms associated with pepper collected from the five major markets are present in Table 3 - 4.

Table 3: Microorganisms associated with fresh pepper procured from markets in Benin City metropolis and environs using Chocolate and McConkey Agars (see Plates 1 and 2)

Microorganisms	Uselu	Oba	New Benin	Santana	Oliha	Total
Bacillus sp.	4	3	3	2	1	13
Candida sp.	5	0	0	1	1	7
Staphyloccocus aureus	4	3	3	1	2	13
Enterobacter	1	0	2	2	1	6
Klebsiella sp.	4	2	2	2	1	11
Proteus vulgaris	0	0	1	0	1	2
Citrobacter sp.	0	0	0	1	0	1
Escherichia coli.	0	3	0	0	0	3
Total	18	11	11	9	7	56

Table 4: Microorganisms associated with epicarp, pericarp and mesocarp of fresh pepper in Benin City metropolis and environs

Microorganisms	Epicarp	Pericarp	Mesocarp	Total
Bacillus sp.	4	5	4	13
Candida sp.	3	1	3	7
Staphyloccocus aureus	4	5	4	13
Enterobacter	3	1	2	6
Klebsiella sp.	1	3	7	11
Proteus vulgaris	2	0	0	2
Citrobacter sp.	0	0	1	1
Escherichia coli.	0	0	3	3
Total	17	15	23	55

Discussion

Microbial assessment of pepper

In Nigeria, pepper is considered a basic cuisine and is eaten regularly across the country. As a result, there is a pressing requirement for research into the chemical and microbiological makeup of the fruity vegetable in its fresh state. The results of the present study have demonstrated the presence of a wide variety of microorganisms, ranging from bacterial to fungal contamination. The storage conditions, as well as the environmental conditions in which the fruit was exhibited for sale, made it easy for pollutants to contaminate the fruit. *Staphylococcus aureus*, *Proteus vulgaris*, *Enterobacter*, *Citrobacter* sp., *Bacillus* sp., *Klebsiella* sp., and *Escherichia coli* were shown to be the bacteria responsible for spoilage. Beuchat (1995) showed that *Bacillus* sp. was an agent of spoilage. It is possible that the sowing, gathering, transportation, and handling processes were the sources of contamination (Mailafia *et al.*, 2017).

Proximate and mineral composition of pepper

The moisture content of the pepper samples averaged 4.48 g/100 g. This figure is quite comparable to those reported by other authors (Al-Jasass and Al-Jasser, 2012), who reported moisture values of 4.68 g/100 g for black pepper (*Piper nigrum*) seeds and 4.36 g/100 g for mustard (*Sinapis alba*) seeds. The amounts of ash, crude fat, and crude protein which were 4.94, 23.65, and 21.29 g/100 g, respectively were higher in this study. Minerals have significant roles in the nutritional quality of a wide variety of plant foods and are involved in a wide variety of biological processes within the human body. In this study, the amounts of sodium, potassium, calcium, zinc, magnesium, iron, and manganese that were recorded per 100 g of pepper are shown in Table 4.

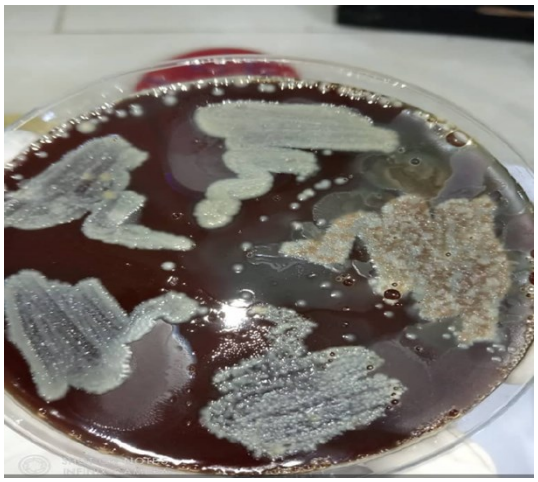

Plate 1: Microbial growth on Chocolate agar

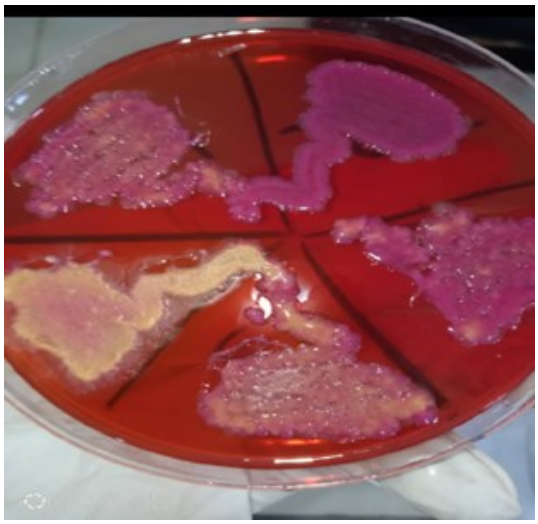

Plate 2: Microbial growth on MacConkey agar

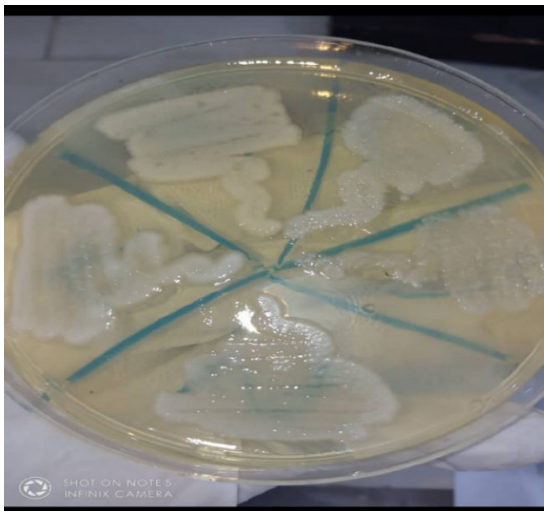

Plate 3: Microbial growth on Nutrient agar

In contrast to our finding, Park *et al.* (2006) reported calcium, iron, and zinc as the most plentiful minerals in pepper. All three of these minerals are very vital to the human body. The human skeleton needs calcium, making it one of its most essential components. Zinc is a crucial component for maintaining healthy immunological function, while iron is necessary for the production of red blood cells. As can be seen in Table 4, the levels of calcium, iron, and zinc found in our pepper samples measured 174.71, 17.49, and 7.97 mg/100 g, respectively, values which were roughly 1.07 - 1.20 times higher than the levels found in pepper investigated by El-Adawy and Taha (2001). Intake of these minerals (calcium, iron, and zinc), was frequently inadequate in the traditional Chinese diet, which was an essential fact to keep in mind (Jiang *et al.*, 2015). Consumption of food products containing pepper as an ingredient has the potential to raise the total amount of minerals in one's diet on a daily basis.

Capsicum annuum contains a range of bioactive compounds and essential nutrients that display various bioactivities such as antioxidant, antiviral, antimicrobial, anticancer, and anti-inflammatory activities. The results in this study suggest that peppers accommodate substantially different bacterial communities with hostile activities on their surfaces, regardless of agronomic practices employed and that the beneficial bacterial strains maybe more important for peppers established on open fields, which seem to be more susceptible to abiotic and biotic stresses. Analysis of mineral content indicated that the most abundant mineral in pepper was potassium, followed by magnesium, calcium, iron, zinc, sodium, and manganese showing that pepper can be an important dietary supplement for improving human health, but care must be taken to prevent microbial contamination and spoilage.

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