

Microbiological Quality of unbranded curry and thyme sold in some markets in Port Harcourt, Rivers State Nigeria

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Abstracts: The total heterotrophic bacterial counts ranged between 5.3 to 9.6×10^6 cfu/g in C1 – C10, while the control curry gave 3.4×10^4 cfu/g, and 1.2 to 8.0×10^5 cfu/g in T1 – T10, while control thyme gave 5.2×10^3 cfu/g. Coliform count ranged between 2.0 to 3.8×10^3 cfu/100g in C1 –C10, while the control curry gave 1.5×10^2 cfu/100g and 1.0 to 4.0×10^3 cfu/100g in T1 – T10, while the control thyme gave 2.2×10^1 cfu/100g. The *E. coli* counts ranged between 1.2×10^1 to 3.3×10^2 cfu/100g in C1 – C10, while control curry gave 6.0 cfu/100g and 0.0 to 3.8×10^1 cfu/100g in T1 – T10, while the control thyme showed no growth. The fungal count ranged from 3.6 to 7.8×10^4 cfu/g in C1 –C5, while control curry gave 1.0×10^2 cfu/g and from 2.7 to 7.1×10^5 cfu/g in T1 –T10, while control thyme gave 7.0×10^2 cfu/g are shown in Tables 3 and 4. The pH and percentage moisture content of the thyme and curry are shown in Tables 1 and 2. Unbranded and branded spices (curry and thyme) retained in some Port Harcourt markets were investigated for their microbiological quality, to assess the total bacterial count, coliform count, fungal count, types of bacterial and fungal species present. Their physicochemical parameters, pH and moisture content were also assessed. Test results revealed pH and moisture content ranges of thyme from 5.1 to 5.8 and 4.1 to 4.9% respectively, the control thyme gave 5.0 and 3.8% for pH and moisture content respectively. While the pH and moisture content of the curry samples ranges from 5.6 to 6.4 and 8.3 to 10.6% respectively and the control curry gave 5.7 and 8.3 for pH and moisture content respectively. The total heterotrophic bacterial counts ranged between 5.8 to 9.8×10^6 cfu/g in C1 –C10, while the control curry gave 3.4×10^4 cfu/g, and 3.6 to 7.0×10^5 cfu/g in T1 – T10, while control thyme gave 5.2×10^3 cfu/g. Coliform count ranged between 2.0 to 3.8×10^3 cfu/100g in C1 –C10, while the control curry gave 1.5×10^2 cfu/100g and 1.0 to 4.0×10^3 cfu/100g in T1 – T10, while the control thyme gave 2.2×10^1 cfu/100g. The *E. coli* counts ranged between 1.2×10^1 to 3.3×10^2 cfu/100g in C1 – C10, while control curry gave 6.0 cfu/100g and 0.0 to 3.8×10^1 cfu/100g in T1 – T10, while the control thyme showed no growth. The fungal count ranged from 3.6 to 7.8×10^4 cfu/g in C1 –C5, while control curry gave 1.0×10^2 cfu/g and from 2.7 to 7.1×10^5 cfu/g in T1 –T10, while control thyme gave 7.0×10^2 cfu/g. Seven bacterial species namely *Bacillus cereus*, *Klebsiella species*, *Bacillus subtilis*, *Proteus species*, *Escherichia coli*, *Enterobacter species* and *Staphylococcus aureus*, and four fungal species; *Penicillium species*, *Aspergillus niger*, *Mucor species* and *Saccharomyces species* were isolated. Conclusively, the branded curry and thyme samples which served as the control showed that they are of better sanitary quality compared to unbranded curry and thyme samples. Poor sanitary nature of unbranded spices as observed in this study could be caused by air contamination, improper storage and insufficient drying before vending in the markets.

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Introduction

Spices are dried plant products used primarily for seasoning purpose. They have been used for thousand of centuries by many cultures to enhance the flavor and aroma of food. The term spices include various parts of dried aromatic plants and relates to natural dried components or mixtures thereof used in foods for flavoring, seasoning and imparting aroma. Early cultures also recognized the value of using species in preserving foods and for their medicinal value (Krishna and Bhaskor, 2003). Greeks, Romans, Chinas and Indians have used spices to combat snake bite, poor circulation, indigestion and hangovers (Krishma and Bhaskor, 2003). Spices have been used

throughout history to enhance flavors and fragrances of food, as well as for their medicinal purposes. Spices also have antioxidant properties that impede food rancidity (Lai, 2004). Volatile oils of these spices have been found to have antibacterial activity against many food-borne pathogens. (Dorman and Deans, 2000) In ancient times, spices, spice extracts, and herbs have been thought to cure diseases. In a study conducted in Turkey in 2005, it was shown that consumption of curcumin, found in the curry spice tumeric, reduced beta-amyloid and plaque burden in the brain, increasing cognitive function in elderly patients (Ng *et. al.*, 2006). *Bacillus* sp, *Citrobacter freundii*, *Escherichia coli*, *Klebsiella* sp *Serratia* sp,

Staphylococcus sp and *Streptococcus* sp have been isolated from black and white pepper as contaminants. Some locally produced spices have improved nutritional intake for human consumption, some still prevent risk of infection and poisoning (Gallo *et al.*, 1992). In general, spices are found to have antimicrobial properties but some fungi and bacteria can utilize spices for their growth (Sharma, 2004). Spices had great monetary value in the early ages as they were only available to the rich (Dhanya and Sasikumar, 2010). Spices have a vast range of functionalities which include flavoring of food, perfumery, cosmetics, medicinal use and preservative properties (McKee, 1995; Minakshi *et al.*, 1999; Bhattacharjee *et al.*, 2003; Sagoo *et al.*, 2008). Although spices added aroma and flavor characteristics to food, they were more valued as perfumery, cosmetic and medicinal agents in ancient times (McKee, 1995). Today large amounts of spices are utilized by the commercial sector of industrialized countries, primarily in food processing (Farag Zaied *et al.*, 1996). The use of spices pervades the food industry, for example spices are used in meat, fish, and bakery and vegetable products. However, spices as plants may be harmed even in the field and contaminated by bacteria and moulds before the beginning of drying and treatment. Later, due to the bad conditions of storage and ventilation and high percent of humidity, contamination of the stored amounts by pathogenic microorganisms frequently occurs (Omafuvbe and Kolawole, 2004). The greatest problem in that sense is growth of some species of moulds that may be producers of mycotoxins (Ochratoxin, aflatoxin, zearalenon), which can manifest its toxic and carcinogenic effect. Neither is negligible the presence of bacteria like *E. coli*, *Salmonella* species or sporogenous anaerobes (*Clostridium perferingens*), which are possible causes of infections and poisoning in humans. Spices such as *Thymus vulgaris*, *Murraya Koenigii* and *Piper nigerium* are subject to microbial contamination at various stages of preparation. The traditional method of harvesting and preparation of these product result in heavy contaminatin. Growth of bacteria and yeast may take place if the environment is damp; hence, the realization that there is a link between the environment and contamination of food. Equipment and food handlers have also been associated with contamination of food with various types of etiologic agent (Moro *et al.*, 2001). Other investigations have shown that spices and spice extracts can inhibit the growth of other bacteria as well. Turkish plant spices, such as thyme, cumin, mint, oregano, as well as other spices have been found to have inhibitory activity against several different Bacilli species. Eleven spices were tested at 1% and 2% against seven Bacilli

species. All of the spices, excluding cumin, were found to have some sort of antimicrobial activity against at least one of the seven Bacilli bacteria species tested (Sagdic *et. al.* 2006). In general, spices are found to have antimicrobial properties but some fungi and bacteria can utilize spices for their growth (Sharma, 2004). The aim of this study is to assess the microbial quality of some selected spices in Port Harcourt Market and to recommend the possibility of some health risk to consumers.

Materials and methods

Collection of samples

The selected spices were purchased from 10 markets namely **Choba Market, Rumuokoro Market, Rumuosi market, Mile 3 market, Mile 1 market, Ozuoba markets, Aluu market, Junction market, Oil mill market and Slaughter Market in Port Harcourt.** The selected spices were purchased from different locations in the markets and mixed together and a control sample (branded curry and thyme) was also purchased from listed markets and was transported aseptically to the laboratory for analysis.

Microbiological analysis of the samples

One milliliter of homogenously mixed sample was transferred using sterile one-milliliter pipette into sterile test tube containing 9ml physiological saline as diluent. One milliliter of the sample was transferred to other sterile 9ml diluent mixed properly. This ten-fold serial dilution continued until the required dilution was obtained. 0.1ml aliquot of the pre-enrichment broth of 10^{-1} and 10^{-4} dilutions was aseptically selected with a sterile pipette and spread plated in duplicates with flame sterilized glass spreader on dried agar plates which includes Nutrient Agar (Enumeration of Total Heterotrophic Bacteria Count), MacConkey Agar (Enumeration of Total coliforms, Eosin Methylene Blue (EMB) Agar (Enumeration of *Escherichia coli*), Potato Dextrose Agar (Enumeration of Total Fungi Count). The plates were incubated at appropriate temperatures for 24hr. The colonies were counted after 24hrs incubation and were expressed as **cfu/ml**. Identification of the isolates was based on their cultural morphology, microscopic examination, carbohydrate fermentation and other biochemical tests. References were made with the Bergey's Manual of determinative Bacteriology (1974)^{8th} Edition for the identification of bacteria. Morphological studies were carried out on different media plates for the isolation of the organisms. Pure colonies were isolated based on colony size, shape, pigmentation, elevation and texture of the individual organisms after 48 hours of the growth at 30°C.

The morphology was determined by examination of the plates directly under the microscope at low power (10x).

Physicochemical Analysis**pH Determination**

This was determined using a pH meter (Jenway 3015 model). The pH of the sample was measured by dissolving 1.0g of the sample in 10ml of distilled water, the electrode inserted in the solution and reading taken.

Moisture Content

The moisture content was determined using the method described by AOAC (1995).

Weigh moisture sample immediately and record as "wet weight of sample" Dry the wet sample to a constant weight, at a temperature not exceeding (115°C) using the suitable drying equipment. Allow the sample to cool. **Weigh the cooled sample again, and record as the "dry weight of sample."**

Results and discussion

The total heterotrophic bacterial counts ranged between 5.3 to 9.6×10^6 cfu/g in C1 – C10, while the control curry gave 3.4×10^4 cfu/g, and 1.2 to 8.0×10^5 cfu/g in T1 – T10, while control thyme gave 5.2×10^3 cfu/g. Coliform count ranged between 2.0 to 3.8×10^3 cfu/100g in C1 – C10, while the control curry gave 1.5×10^2 cfu/100g and 1.0 to 4.0×10^3 cfu/100g in T1 – T10, while the control thyme gave 2.2×10^1 cfu/100g. The *E. coli* counts ranged between 1.2×10^1 to 3.3×10^2 cfu/100g in C1 – C10, while control curry gave 6.0 cfu/100g and 0.0 to 3.8×10^1 cfu/100g in T1 – T10, while the control thyme showed no growth. The fungal count ranged from 3.6 to $7.8 \times$

10^4 cfu/g in C1 – C5, while control curry gave 1.0×10^2 cfu/g and from 2.7 to 7.1×10^5 cfu/g in T1 – T10, while control thyme gave 7.0×10^2 cfu/g are shown in Tables 3 and 4. The pH and percentage moisture content of the thyme and curry are shown in Tables 1 and 2. Test results revealed pH and moisture content ranges of thyme (T1 – T10) from 5.1 to 5.8 , and 4.1 to 4.8% respectively, the control thyme gave 5.0 and 3.8% for pH and moisture content respectively (**Table 2**). While the pH and moisture content of the curry samples (C1 – C5) ranges from 5.9 to 6.4 and 8.3 to 10.6% respectively and the control curry gave 5.7 and 8.3 for pH and moisture content respectively (**Table 3**).

Table 1: pH and Moisture content of Thyme

SN	PARAMETERS	T1	T2	T3	T4	T5
1	pH	5.8	5.3	5.4	5.1	5.5
2	Moisture content (%)	4.8	4.3	4.6	4.3	4.1

SN	PARAMETERS	T6	T7	T8	T9	T10	Control
1	pH	5.2	5.5	5.4	5.6	5.1	5.0
2	Moisture content (%)	4.6	4.9	4.5	4.4	4.7	3.8

Table 2: pH and Moisture content of Curry

SN	PARAMETERS	C1	C2	C3	C4	C5
1	pH	6.1	6.3	6.4	6.1	5.9
2	Moisture content (%)	8.8	9.6	10.6	8.3	9.4

SN	PARAMETERS	C6	C7	C8	C9	C10	Control
1	pH	5.7	6.2	6.3	5.9	5.6	5.7
2	Moisture content (%)	8.5	9.3	9.1	8.9	9.9	8.3

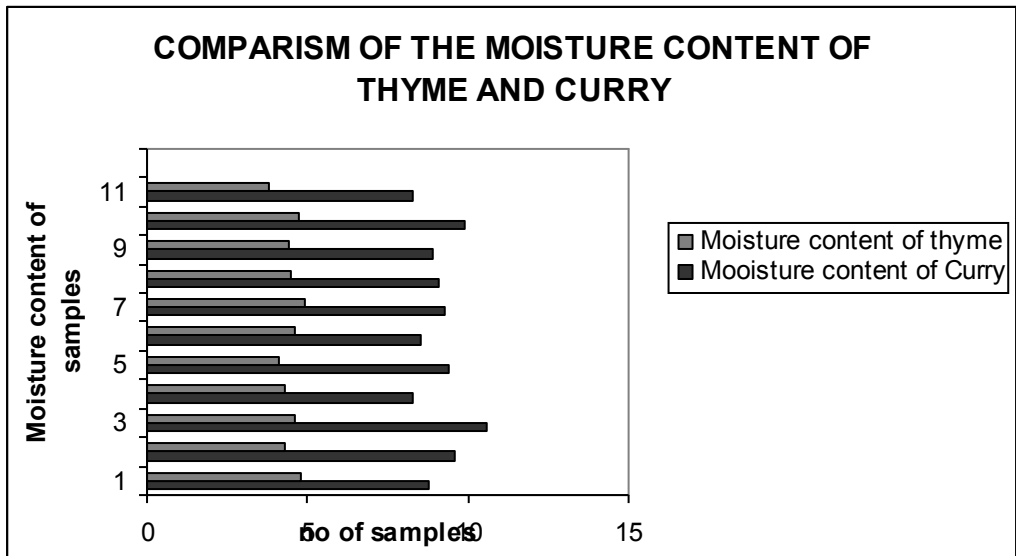
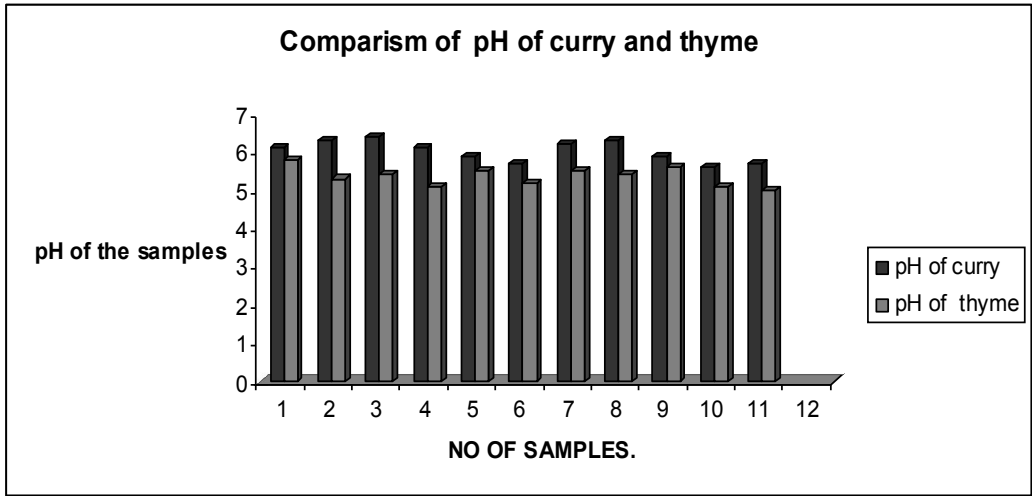
Table 3: Microbiological Quality of Thyme

SN	PARAMETERS	T1	T2	T3	T4	T5
1	Total Heterotrophic Bacteria (cfu/g)	3.6×10^5	6.8×10^5	1.2×10^5	7.0×10^5	5.7×10^5
2	Total coliforms (cfu/100g)	1.0×10^3	1.2×10^3	2.0×10^3	1.0×10^3	4.0×10^3
3	<i>Escherichia coli</i> (cfu/100g)	1.2×10^1	2.3×10^1	3.5×10^1	1.4×10^1	2.7×10^1
4	Total Heterotrophic Fungi (cfu/g)	5.1×10^4	2.7×10^4	6.0×10^4	6.8×10^4	7.1×10^4

SN	PARAMETERS	T6	T7	T8	T9	T10	Control
1	Total Heterotrophic Bacteria (cfu/g)	5.6×10^5	6.3×10^5	6.8×10^5	7.3×10^5	8.0×10^5	5.2×10^3
2	Total coliforms (cfu/100g)	1.8×10^3	1.5×10^3	1.7×10^3	1.5×10^3	1.9×10^3	2.2×10^1
3	<i>Escherichia coli</i> (cfu/100g)	1.3×10^1	2.4×10^1	2.5×10^1	3.5×10^1	3.8×10^1	0
4	Total Heterotrophic Fungi (cfu/g)	6.8×10^5	4.4×10^5	5.8×10^5	6.8×10^5	5.8×10^5	7.0×10^2

Table 4: Microbiological Quality of Curry

SN	PARAMETERS	C1	C2	C3	C4	C5	
1	Total Heterotrophic Bacteria (cfu/g)	5.6×10^6	7.8×10^6	3.2×10^6	8.0×10^6	5.3×10^6	
2	Total coliforms (cfu/100g)	2.0×10^3	2.2×10^3	2.0×10^3	2.0×10^3	4.1×10^3	
3	<i>Escherichia coli</i> (cfu/100g)	1.2×10^1	4.2×10^1	3.9×10^1	0	3.7×10^1	
4	Total Heterotrophic Fungi (cfu/g)	4.1×10^4	3.6×10^4	4.0×10^4	6.3×10^4	6.3×10^4	
SN	PARAMETERS	C6	C7	C8	C9	C10	Control
1	Total Heterotrophic Bacteria (cfu/g)	9.6×10^6	7.3×10^6	5.8×10^6	7.9×10^6	8.0×10^6	3.4×10^4
2	Total coliforms (cfu/100g)	3.8×10^3	1.8×10^3	1.2×10^3	1.2×10^3	1.5×10^3	1.5×10^2
3	<i>Escherichia coli</i> (cfu/100g)	1.2×10^2	2.0×10^2	2.0×10^2	3.0×10^2	3.3×10^2	6.0
4	Total Heterotrophic Fungi (cfu/g)	7.8×10^5	4.0×10^5	5.3×10^5	6.0×10^5	5.7×10^5	1.0×10^2



The pH values obtained shows that the spices are slightly acidic to neutral thereby indicating that spices could permit and tolerate the growth of bacteria and fungi (Frazier *et al.*, 1967). pH alone is not sufficient parameter to predict the chances of survival and proliferation of bacteria and fungi in spices (Marcus, 1997). The moisture content obtained is generally low. High moisture content has been reported to accelerate food spoilage (Prescott, 2008); if low moisture content is held under humid condition it is able to support growth of moulds. This is in conformity with this study.

The high bacteria and fungi counts obtained may be due to poor storage and or handling (Asta, 1999). Some of the organisms isolated have been implicated as causative agents of gastroenteritis (Nester *et al.*, 2001). The International Microbiological Standard recommended limit for

bacteria contaminants in spices are in the range of 10^1 to 10^3 cfu/g for coliform, 10^1 to 10^5 cfu/g total microbial plate count, 10^1 to 10^3 cfu/g for mould and yeast, 0/20g for *S. aureus*, 0/20g for *Bacillus cereus* and 0/20g for *E. coli* (FCD, 1972). It was observed that both the total bacterial count of the spices falls within the limits recommended while coliform and fungi counts values are above the recommended limits.

Gallo *et al.*, (1992) reported that faulty food handling techniques especially storage of food at improper temperature for long periods of time has been identified as one of the microbial proliferation in contaminated food. The incidence of *B. cereus* in spices is indicative of environmental contamination, which could have resulted from exposure of the spice to air or contact of utensils used with soil (Graven *et al.*, 1975).

B. cereus in food causes intoxication and is capable of causing non gastrointestinal infection. *S. aureus* in the sample is indicative of human contamination after processing. The organism is associated with enterotoxin characterized by short incubation period, violent nausea, vomiting and diarrhea. The most frequent cause of microbiological contamination of spices was the finding of total number of microorganisms. Such finding was expected considering that most of the species were plants that may be contaminated from the ground by different microorganisms during its growth in the field, or by animal feces, and later by inadequate storage. Finding of moulds in spices as the second cause of contamination is the outcome of inadequate drying and storage in storehouses, where due to the increased humidity, moulds developed. The presence of *Klebsiella* sp suggests faecal contamination (Umoh, 1995). The presence of *A. species* in the spices might be due to air contamination (Marcus *et al.*, 1997). From this, it could be predicted that the inability of the control curry and thyme samples to show the presence of *Aspergillus* species could be attributed to packaging of the control samples, which limit air contamination.

Generally, the results obtained in this study showed that the branded curry and thyme samples which served as control samples are of better sanitary quality compared to unbranded curry and thyme samples. Poor sanitary nature of unbranded spices as observed in this study could be caused by air contamination, improper storage and insufficient drying before vending in the markets. The conclusion drawn from this study showed that both the unbranded thyme and curry samples had values capable of causing deleterious effects to human health due to high microbial loads and relatively low pH to neutral which could harbor microbial load. While the branded curry and thyme samples showed that they are of better sanitary quality. This could be attributed to proper storage and drying conditions as well as proper packaging.

Recommendations

1. Production of these spices should be done under hygienic condition.
2. These spices should be properly dried, while contaminated raw materials should be removed from the final products before production and storage.
3. It is necessary to reassess production processes to ensure that techniques capable of reducing microbial contaminations are employed.
4. Care must be taken to select good leaves while infected leaves should be discarded, seeds must be properly dried prior to production.

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