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Assessment of microbial quality of some industrially packed food seasoning obtained from different retail outlets in Ota, Ogun State, Nigeria

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ABSTRACT

Qualities of curry, thyme and pepper commonly marketed in Nigeria were investigated. The total bacterial and fungal counts, types of microorganisms present and the physico-chemical qualities were also determined. These spices had a pH range from 5.92 to 6.44, with a moisture content of 6.08% to 36.32%. The bacterial load was within the range of 13.0×10^3 to 43.8×10^3 cfu/g with a lower fungal count of 6.5×10^3 to 35.2×10^3 cfu/g. Five bacterial and six fungal species namely *Aeromonas, Staphylococcus, Pseudomonas, Streptococcus* and *Bacillus;as well as Mucor, Aspergillus flavus, Penicillum, Candida, Aspergillus and Rhodotorula,* respectively, were isolated. The implication of these findings on the health of the populace was discussed from a microbiological viewpoint. The presence of these microbes in the spices may pose a serious threat which is of significance in public health.

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1. Introduction

Spices constitute an important group of agricultural commodities, which are virtually indispensable in the culinary art. They can be primarily defined as farm products used in various forms, namely fresh, ripe, dried, broken, powdered, etc. which contribute aroma, taste, flavour, color and pungency to food.

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Spices may be either bark, buds, flowers, fruits, leaves, rhizomes, roots, seeds, stigmas and styles, or the entire plant tops (1). Spices may be contaminated because of the conditions under which they were cultivated and harvested. Factors responsible for the contamination of products include moisture content, pH, temperature etc. Contaminated spices have been reported to have been the cause of certain food-borne illnesses and spoilage of foods (2). Indigenous microflora of plants, presence of microorganisms in the processing plant, air, dust, using contaminated water and animal/human excreta, and pre-and post-harvest procedure including

processing, storage and distribution may be the sources of microbial contamination of spices, therefore, some spices pose health problems because some are often added to foods without further processing or are eaten raw (3).

During the cleaning and processing procedure of spices, there is a progressive reduction in the number and types of microorganisms; those remaining are usually aerobic spore-forming bacteria and common molds. In addition to the contamination of raw food supplies that occurs during growing, shipping and processing, there is the problem of food contamination caused by people who are carriers of pathogens such as *Escherichia coli* and *Staphylococcus aureus* (2). Coliform bacteria occur sporadically and usually in small populations in species and are associated with fecal contamination. Yeast and mould densities vary considerably with the individual spices but are usually quite low (4). Spices are mainly used as ingredients to flavor food and drinks. Many spices have additional commercial uses, e.g., as ingredients in medicine, perfumes, incense and soaps. It is also used as a condiment. Spices are not only for our taste buds but also for our health (5).

Spices are cultivated and harvested in warm, humid areas of the world where the environment is conducive to the growth of a wide variety of microorganisms. The microbiological quality, the load of total heterotrophs or Enterobacteriaceae in particular, often acts an as indicator of the hygienic situation of a region where the spices are produced and processed (6). Contaminated spices may cause a microbiological problem, depending on the end use. Spices are the principal source of spore-forming bacteria in large volumes of foods, such as soups, casseroles, stews and gravies produced by catering establishments; under favorable conditions, they germinate and multiply to infective and toxic levels (7, 8).

Studies on the microbiology of spices have demonstrated profiles of microorganisms, including total heterotrophs, *Bacillus cereus, Clostridium perfringens, Escherichia coli, Salmonella* and toxigenic moulds. Spices also have antioxidant properties that impede food rancidity (9). It was reported that consumption of curcumin, found in the curry spice reduced bate-amyloid and plaque burden in the brain, increasing cognitive function in elderly patients (10). *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, and some still prevent the risk of infection and poisoning (11). As with many other agricultural products, spices and herbs became contaminated at any point from production to consumption (12). In addition, because of the overuse of antibiotics in plant agriculture and the use of contaminated fertilizer or irrigated water to croplands, antibiotic-resistant bacteria are of special concern, since these products are likely added raw or even minimally processed to foods and do represent some risk to public health (13).

The objectives of this study therefore include:

To determine the pH of five brands of different kinds of spices; curry, thyme and pepper seasonings in hot and cold water; to determine the moisture content of the different samples of curry, thyme and pepper; to assess the bacteria and fungi present in the five brands of food seasonings at room temperature (28^{o}C) and after boiling at 100^{o}C.

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2. Materials and Methods

2.1. Collection of samples

Samples of different curry, thyme and pepper were randomly purchased from different retail outlets in Ota, Ogun State, Nigeria. These samples were carefully examined to make sure they were intact (no tear or damage) and not expired. They were then analyzed at Bells University of Technology, Ota, Ogun State, Nigeria.

2.2. Identification of samples

These collected samples of five different brands of curry and thyme and pepper were designated as (D, M, G, T and E) and were also labeled as (CDC, CMC, CGC, CTC, and CEC) representing the five curry samples in cold water, while (HDC, HMC, HGC, HTC and HEC) represent the hot samples. For the thyme, CDT, CMT, CGT, CTT, and CET) represent the brands in cold water while HDT, HMT, HGT, HTT and HET represent the hot water samples respectively. And (CMP and HMP) represent the brand M pepper in cold and hot water. All these were designated for the determination of pH, isolation and characterization of bacteria and fungi in cold and hot samples respectively.

2.3. Determination of the moisture content and pH of the spices

The pH was determined with a pH meter (Hanna Instrument, pH 211, Microprocessor pH meter), while the moisture content was determined using the method described by AOAC (14).

2.4. Cultivation and enumeration of bacteria in the curry, thyme and pepper seasonings

Ten ten-fold serial dilution technique was carried out to reduce the microbial load of the samples. This was done in triplicate and incubated at 37^{o}C for 24 h and colony forming units were enumerated. The mean counts for triplicate cultures were recorded as the bacterial counts in each sample (15).

2.5. Cultivation and enumeration of fungi in the samples [curry, thyme and pepper]

Agar plates of potato dextrose agar [PDA] using both pour and spread plate techniques (pour plate for cold samples and spread plate for hot samples) served as media for this analysis. These were in triplicates and incubated at 28^{o}C for 72 h and colonies forming units were enumerated (16).

2.6. Isolation and identification of bacteria

Pure cultures of bacteria were obtained aseptically by streaking representative colonies of different morphological types which appeared on the cultural plates onto freshly prepared nutrient agar plates which were then incubated at 37^{o}C for 24 h. They were then identified based on Gram staining, catalase, production of hydrogen sulphide, indole, motility, citrate utilization, methyl red (MR) and coagulase tests (17).

2.7. Isolation and identification of fungi

This was done by morphological characteristics from pure cultures on potato dextrose agar, the arrangement and type of spores formed and details on colour of cultures. Ascospores and ballistospore tests were conducted for further identification (18).

2.8. Ascospore test

Gorodkovac's agar was prepared to contain 0.25% of glucose, 0.5% of sodium chloride, 1% lab lemco powder and 2% agar powder. The molten agar was dispensed and allowed to solidify in the Petri dishes. The media was inoculated with the pure fungal isolates and incubated at 28^{o}C for 8 days. After this period, small portions of the cultures were placed on clean

microscope slides with a drop of sterile distilled water added to prepare a smear of each culture using a sterile wire loop. The smears were air-dried and then heat-fixed. Malachite green dye was added in each case, allowed to steam over the Bunsen flame for 2 min and the slides rinsed under tap water for 2 min and then counterstained with safranin for about 30 s. They were then observed under the microscope (18).

2.9. Ballistospore test

Corn meal agar was prepared under aseptic conditions and allowed to cool. The molten agar was dispensed in equal amounts into two sets of sterile plates and allowed to solidify. The first set of Petri dishes was inoculated by streaking the fungal isolates over the agar surface in an S-shaped fashion. The second set of plates containing the solidified media were superimposed on the inoculated plates and sealed aseptically with masking tape. They were then incubated at 28^{o}C and 37^{o}C for 7 days, respectively, after which the superimposed plates were observed for growth. Smears from small portions from the positive plates were prepared on clean microscope slides adding a drop each of Carbol fuchsin and potassium hydroxide. The slides were then observed under the microscope (18).

2.10. Statistical analyses

All data collected was analyzed for statistical significance using analyses of variance (19).

3. Results

The pH values and percentage moisture contents of five brands of curry, thyme and pepper seasonings are shown in Tables 1-5. The pH values of these samples varied with brands, all of the brands were acidic. The pH range was between 5.92 and 6.44; with the moisture content between 6.08% and 36.32%. The cold water sample of G thyme (CGT) had the lowest pH value of 5.92 (Table 3), while the highest pH value of 6.44 was found in cold water pepper sample M(CMP) (Table 2). The thyme of D (CDT) had the least moisture content of 6.08% (Table 1), while the highest moisture content of 36.32% was found in the pepper of sample M (CMP) (Table 2). The pH values were within the range of 6.15 and 6.41 for sample D (Table 1), 5.97 and 6.44 for sample M (Table 2), 5.92 and 6.32 for sample G (Table 3), 5.97 and 6.18 for sample T (Table 4) and 6.04 and 6.19 for brand E (Table 5). The order of increase in pH values for Sample D (Table-1) is CDT>CDC>HDC>HDT, while for Sample M (Table 2) is HMT>CMT>HMC>HMP>CMC>CMP. Table 3 is CGC>HGC>CGT>HGT for Sample G; while Sample (Table 4) is CTC>HTC>HTT>CTT. The order of increase in pH values for Sample E (Table 5) is HEC>CET>HET>CE

A significantly higher microbial population was obtained in cold samples than in hot samples. The bacterial counts shown in Tables 1-5 were within the range of 13.0±0.75×10^{3} to 43.8±0.67×10^{3} cfu/g. This was between 15.0±0.0.50×10^{3} to 40.2±0.59×10^{3} cfu/g in curry, 13.0±0.75×10^{3} to 43.8±0.67×10^{3} cfu/g in thyme and 30.0±0.56×10^{3} to 42.3±0.58×10^{3} cfu/g in pepper. Table 1 shows the pH values, moisture content and the total bacterial count in sample D, cold D curry (CDC) had the highest count of 29.0±0.83×10^{3} cfu/g. In contrast, hot D curry (HDC) had the lowest count of 18.0±0.62×10^{3} cfu/g, the order of increase in bacterial counts of these samples is CDC>CDT>HDT>HDC. Table 2 shows the pH values, moisture content and total bacterial count in sample M, Hot M curry (HMC) had the least bacterial count of 19.4±0.69×10^{3} cfu/g

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while cold M thyme (CMT) had the highest bacterial count of 43.8±0.67×10^3 cfu/g. The order of increase in bacterial counts of these samples is CMT>CMP>CMC>HMP>HMT>HMC. Table 3 shows the pH values, moisture content and total bacterial count in sample G, Hot G thyme (HGT) had the least bacterial count 13.0±0.75×10^3 cfu/g while cold G curry (CGC) had the highest bacterial count of 33.7±0.74×10^3 cfu/g, The order of increase in the bacterial count of these samples is CGC>HGC>CGT>HGT. Table 4 shows the pH values, moisture content and total bacterial count in sample T. Hot T thyme (HTT) had the least bacterial count of 15.9±0.56×10^3 cfu/g. In contrast, cold T curry (CTC) had the highest bacterial count of 33.5±0.68×10^3 cfu/g, The order of increase in bacterial counts of these samples is CTC>HTC>CTT>HTT. Table 5 shows the pH values, moisture content and total bacterial count in sample E, Hot E curry (HEC) had the least bacterial count of 15.0±0.50×10^3 cfu/g. In contrast, cold E thyme (CET) had the highest bacterial count of 29.6±0.79×10^3 cfu/ml, The order of increase in bacterial counts of these samples is CET>HET>CEC>HEC. In all the brands, CMT which has a moisture content of 6.57%, with a maximum mean bacterial population of 43.8+ 0.67×10^3 cfu/g was not significantly different from CMP(42.3+0.59×10^3 cfu/g with a moisture content of 36.32% (Table 2). The highest mean fungal count was observed CET with a considerable decrease in the fungal load on heating (HET) (Table 5).

The total fungal counts shown in Tables 1-5 were within the range of 6.5±0.80×10^3 to 35.2±0.77×10^3. Table 1 showed that Hot D thyme (HDT) had the least fungal count of 7.0±0.53×10^3 while cold D thyme (CDT) had the highest value of 15.0±0.5.0×10^3.

The order of increase in fungal count of these samples is CDT>CDC>HDC>HDT. Table 2 showed that samples M (Mr. Chef) and hot M pepper (HMP) had the least fungal count of 11.6±0.56×10^3 cfu/g while cold M curry (CMC) had the highest value of 29.0±0.83×10^3 cfu/g. The order of increase in fungal count of these samples is CMC>CMP>CMT>HMC>HMT>HMP. Table 3 shows the total fungal count of samples G (Gino), Hot G thyme (HGT) had the least fungal count of 6.5±0.0.80×10^3 cfu/g while cold G thyme (CGT) had the highest fungal count of 16.4±0.68×10^3 cfu/g. The order of increase in these samples is CGT>CGC>HGC>HGT. Table 4 shows the total fungal count of samples T (Tiger), Hot T curry (HTC) had the least fungal count of 13.3±0.77×10^3 cfu/g while cold T thyme (CTT) had the highest fungal count of 20.5±0.64×10^3 cfu/g. The order of increase in fungal count of these samples is CTT>CTC>HTT>HTC. Table 5 shows the total fungal count of samples E (Euroma), Hot E thyme (HET) had the least fungal count of 17.5±0.57×10^3 cfu/g while cold E thyme (CET) had the highest fungal count of 35.2±0.86×10^3 cfu/g. The order of increase in fungal count of these samples is CET>CEC>HEC>HET.

Table 1. The pH values, percentage moisture content and total bacterial and fungal counts for sample D

Samples	pH values	Moisture content	Mean population bacterial (cfu/g)	Mean population fungi (cfu/g)
CDC	6.25	6.25%	$29.0\pm0.83X10^3$	$13.0\pm0.75X10^3$
HDC	6.16		$18.0\pm0.62X10^3$	$8.0\pm0.66X10^3$
CDT	6.41	6.08%	$28.0\pm0.79X10^3$	$15.0\pm0.50X10^3$
HDT	6.15		$23.4\pm0.57X10^3$	$7.0\pm0.53X10^3$

KEY: CDC; cold D curry HDC; hot D curry
CDT; cold D thyme HDT; hot D thyme

Table 2. The pH values, percentage moisture content and total bacterial and fungal counts for sample M

Samples	pH values	Moisture content	Mean population bacterial (cfu/g)	Mean population fungi (cfu/g)
CMC	6.35	10.17%	$40.2\pm0.59X10^3$	$29.0\pm0.83X10^3$
HMC	6.12		$19.0\pm0.69X10^3$	$15.2\pm0.56X10^3$
CMT	6.10	6.57%	$43.8\pm0.67X10^3$	$15.6\pm0.66X10^3$
HMT	5.97		$28.6\pm0.74X10^3$	$12.9\pm0.59X10^3$
CMP	6.44	36.32%	$42.3\pm0.59X10^3$	$16.0\pm0.55X10^3$
HMP	6.14		$30.0\pm0.56X10^3$	$11.6\pm0.56X10^3$

KEY: CMC; cold M curry. HMC; hot M curry. CTM; cold M thyme
HMT; hot M thyme. CMP; cold M pepper. HMP; hot M pepper

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Table 3. The pH values, percentage moisture content and total bacterial and fungal counts for sample G

Samples	pH values	Moisture content	Mean population bacterial (cfu/g)	Mean population fungi (cfu/g)
CGC	6.32	6.43%	$33.7\pm0.74X10^3$	$12.0\pm0.76X10^5$
HGC	6.11		$29.0\pm0.83X10^3$	$7.8\pm0.54X10^5$
CGT	5.92	6.76%	$22.0\pm0.53X10^3$	$16.4\pm0.68X10^3$
HGT	6.09		$13.0\pm0.75X10^3$	$6.5\pm0.80X10^3$

KEY: CGC; cold curry HGC; hot G curry
CGT; cold G thyme HGT; hot G thyme

Table 4. The pH values, percentage moisture content and total bacterial and fungal counts for sample T

Samples	pH values	Moisture content	Mean population bacterial (cfu/g)	Mean population fungi (cfu/g)
CTC	6.18	9.71%	$33.5\pm0.68X10^3$	$17.5\pm0.57X10^3$
HTC	6.10		$27.9\pm0.66X10^3$	$13.3\pm0.77X10^3$
CTT	5.97	7.22%	$24.4\pm0.56X10^3$	$20.5\pm0.56X10^3$
HTT	6.05		$15.9\pm0.56X10^3$	$13.9\pm0.66X10^3$

KEY: CTC; cold T curry HTC; hot T curry
CTT; cold T thyme HTT; hot T thyme

Table 5. The pH values, percentage moisture content and total bacterial and fungal counts for sample E

Samples	pH values	Moisture content	Mean population bacterial (cfu/g)	Mean population fungi (cfu/g)
CEC	6.19	26.87%	$22.0\pm0.54X10^3$	$25.0\pm0.86X10^3$
HEC	6.04		$15.0\pm0.50X10^3$	$17.6\pm0.72X10^3$
CET	6.08	7.26%	$29.6\pm0.79X10^3$	$35.2\pm0.86X10^3$
HET	6.15		$26.0\pm0.72X10^3$	$17.5\pm0.57X10^3$

KEY: CEC; cold E curry HEC; hot E curry
CET; cold E thyme HET; hot E thyme

Table 6. Microorganisms isolated from curry, thyme and pepper brands of packaged food seasonings

Samples	Bacterial isolates	Fungal isolates Brands
D	*Aeromonas and Staphylococcus spp.*	*Mucor sp, Aspergillus flavus and Penicillum sp*
M	*Pseudomonas and Streptococcus spp.*	*Candida and Aspergillus spp.*
G	*Bacillus and Staphycoccus spp.*	*Aspergillus and Penicillum spp*
T	*Bacilllus and Staphylococcus spp.*	*Mucor sp, Aspergillus flavus and Penicillum spp*
E	*Bacillus, Staphycoccus and Aeromonas spp.*	*Mucor, Aspergillus niger, Penicillum and Rhodotorula spp*

4. Discussion

The pH values obtained in all the food seasonings (D, M, G, T, and E) showed that the spices were slightly acidic (5.92-6.44), indicating that spices could permit the proliferation of acidophilic microbes (Tables 1-5). It must be noted that pH alone is not a significant parameter to predict the chances of survival and proliferation of bacteria and fungi in spices (20).

Smith and Fratamico (21) reported that the interplay of factors affecting microbial growth in foods such as water activity, pH and temperature ultimately determine whether a microorganism will grow in a given food. Often the results of such interplay are unpredictable; as poorly understood synergism or antagonism may occur. The moisture content was almost generally low (6.08% and 36.32%; Tables 1-5). High moisture content has been reported to accelerate food spoilage, however, if the low moisture content is held under humid conditions it can support the growth of moulds (16).

A significant increase in the microbial load of all cold samples; curry, thyme and pepper of all the five brands on nutrient and potato dextrose agar when compared to the hot samples (Tables 1-5).

The fact that there was a decrease in microbial load of all hot samples infers that the use of heat has an effect on the microbial quality of all brands as compared to that of the cold samples.

The high microbial contamination in curry, thyme and pepper seasonings in this study may result during production or packaging as individuals concerned during these processes may not take necessary precaution and as such microbial contamination is prominent or may be attributable to storage condition, contamination from the seals of sachets or from handlers before they were obtained for sampling.

It is important to note that these samples did not show any visible signs of spoilage. Thus, physical outlook may not be a good criterion for assessing the quality of spices.

The presence of bacteria such as *Staphylococcus, Pseudomonas, Streptococcus and Bacillus spp.* is of major concern as they are pathogenic and are of health risk to man (Table 6). This further highlights the need to

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safeguard the health of the consumers by proper washing and decontamination of these products which are sometimes consumed without heat treatment.

The food bacteria of greatest importance to human pathology are the most common cause of human infection and are extensively widespread in the environment of fast foods. These results are in agreement with the above studies and are supported by many researchers (8, 22). The findings are consistent with our results that revealed some pathogenic bacteria, fungi and yeasts were found in food especially traditional fast foods. Most investigators indicated that bacteria, fungi and yeasts may exert their pathogenic action either through infection of the body or as toxic substances demonstrated as contaminated foods. The most common infections causing food poisoning and other diseases are those associated with contamination due to fast foods and traditional fast foods to which spices belong (22).

The presence of fungi such as *Aspergillus flavus. Aspergillus niger, Mucor, Rhodotorula* and *Candida spp.*is of major health concern as Barnett *et al.* (23) reported that these may also produce mycotoxins in various foods (Table 6). *Aspergillus* and *Penicillium spp.* have been reported to produce aflatoxins, and the aflatoxins have been isolated from legumes, grains fruits, meats, spices, milk, cheese, rice, corn and other compounds with carcinogenic, hemorrhagic, neurotoxic and uterotrophic properties have been isolated from food stuff and identified as metabolites of fungi common to a variety of agricultural commodities (24).

Heating the samples reduced the microbial load which in addition to controlling fungal toxin production can assist in the adjustment of pH, Water activity and temperature control in the product (25). It was also reported that some strains may be more toxigenic at low temperatures than at optimum growth temperatures, thus proper cooking of spices serves as a means of controlling the growth of microorganisms in foods (8).

5. Conclusion

Good hygiene practices, proper handling, storage and retail of spices in a clean environment cannot be over-emphasized to ensure good quality and safe spices.

It is important to handle food in such a way that the microorganisms present do not have a chance to multiply and to prevent food from becoming contaminated with other microorganisms. The presence of these microorganisms in spices may be unavoidable due to the production, storage and handling but they can drastically be reduced to a safe limit by good storage facilities.

In all the brands, cold samples had higher microbial counts, while a decrease in microbial load was observed when the samples were heated. The International Microbiological Standard recommended limit for bacteria contaminants in spices/ seasonings is in the range of 10 to 10^3 cfu/g; total microbial plate count 10 to 10^5 cfu/g and 10 to 10^3 cfu/g for moulds and yeasts, thus, most of these seasonings must be heated to reduce the microbial load. Many people globally consume food with spices daily due to the flavour, taste, aroma, color and pungency it added to the food, thus, to safeguard public health, Government and regulatory authorities should create public awareness of the danger inherent in consuming seasonings products of poor quality. Based on this study, consumer awareness of the dangers of eating undercooked (raw) spices and the need to insist on the

consumption of properly cooked spices should be reawakened.

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Authorship contribution

Kuforiji responsible for conceptualization, data curation, methodology, supervision, review and editing. Umar responsible for investigation, visualization, formal analysis and writing. Adetoyinbo in charge of project administration, methodology and validation.

Declaration of competing interest

the authors declare that the research was conducted without any financial and personal relationships with other people or organization that could inappropriately influence or bias the work.

Data availability

The original contributions presented in this study are included in the article, further inquiries can be directed to the corresponding author.

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