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Microbial, chemical composition evaluation and development of a technological process for the production of compound spices in Nigeria

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Abstract

Spices are vegetable products derived from fruits, seeds, roots and tree barks. They are important mainly as additives to food because of the presence of essential oil having anti-microbial and fungicidal properties. These properties have been used to some degree as the basis for food preservation and as medicinal products for certain types of diseases in Nigeria. This paper takes a look at the microbial load and chemical compositions of some of the compound spices found in Nigeria. Results reveal that some compound spices are highly contaminated with microorganisms. Isolates from sample B were identified as *Salmonella, Bacillus* and *Escherichia coli* bacteria while those from sample A contained *Staphylococcus aureus* and *Streptococcus*. The fungi isolates were *Aspergillus niger*, *A. flavus* and *Microsporum caris*. The bio-load of the spices ranged from 1.64×10^5 to 2.09×10^7 cells per gram of spice. *Salmonella* species and *Bacillus* sp. were the most common microorganisms. It is recommended that higher sanitary conditions be employed during the processing, handling and storing of the spices. This paper proposes a technological process route for the production of more hygienic spices.

Introduction

The definition of spices, herbs and condiments is still the focus of much debate over the years. The International Organization for Standardization (ISO) defines spices and condiments as the natural vegetative products or mixture thereof without extraneous matter that are used for flavourings, seasonings and imparting aroma to food.[1] Spices and condiments are usually grown in tropical or semi tropical climates and comprise of one portion of the plant. Spices are the whole or ground seeds, fruits, bark or roots of a plant.

Spices have characteristic odours or flavours. The chemicals responsible for their distinctive tastes and smell are essential oils or volatile oils, which contains terpene, aldehydes, ketones and alcohol of various phenol based compounds. Most spices and condiments do not have any reasonable bacteriostatic effect on the concentration of the food material they are used on.[1] The United States, Food and Drug Administration (FDA) specifies that paprika, saffron and turmeric are colours and they must be labelled with common names on food labels.[1, 2]

Normally high concentrations of plant extracts are required before anti-microbial properties become apparent. It is well documented that vegetables like, onions, garlic and horse-radish provide anti-microbial extracts.[3,4] For centuries it has been known that certain foods especially spices possess naturally occurring preservative qualities but only recently has the technology been developed to extract, quantify and identify these substances.

Spices vary in their effectiveness depending upon the source, freshness and method of processing and storage. The inhibitory effect of spices differs with the kind of spice and the

microorganism being tested for. Mustard and the volatile oil of mustard have varying effectiveness against *Saccharomyces cerevisiae* but are not effective against most bacteria as cinnamon and cloves.[1,5,6] Cinnamon and cloves contain cinnamic aldehydes, which makes them more bacteriostatic than mustard. Thyme, bay leaves, marjoram saucy, rosemary, black pepper and others have weak inhibitory power against most organism.

Several studies have been conducted using different spices to inhibit the growth of pathogenic organism. Kivanc and Akgul[6] tested the bacteriostatic and bactericidal activities of twenty-two essential oils from Turkish spices and citrus against *Aerobacter aerogenes*, *Bacillus subtilis*, etc. The results showed that the essential oils tested varied in their antibacterial activity. Anise, celery, coriander and sage were inactive or had little activity while corn, mint, cumin, lemon peel and ziziphora were active against all tested bacteria to a variable extent. *Staphylococcus aureus* and *Pseudomonas aeruginosa* were the most resistant except towards thyme oil. Antibacterial and antifungal effectiveness of the compounds of the spice ***Aframomum melegueta*** have been carried out by Oloke *et al*[7] and the volatile oil showed considerable bactericidal activities against *Escherichia coli*, *Pseudomonas*, etc. and fungicidal activities against *Candida albicans*, *Trichophyton mentagrophytes*, etc.

These properties have been used to some degree as the basis for food preservation. However, certain spices are known to contain large number of microorganism despite their apparent anti-microbial effect. Some of these organisms and chemical content may cause spoilage or become pathogenic to man when introduced to food.[10] There is little information available on the microflora, volatile oil, production techniques and quality of local Nigerian spices. This paper presents results of some of the analyses carried out on the local spices and proposes a technological process for the production of more hygienic spices.

Methodology

The material used for the experiment include spice product such as garlic **(*Allium sativum* Linn.)**, ginger root **(*Zingiber officinale* Rosc.)**, allspice **[*Pimenta dioica* (Linn.) Merrill]**, mansoro, cloves **[*Syzygium aromaticum* (Linn.) Merrill & Perry]**, gyada muyan, thyme **(*Thymus vulgaris* Linn.)**, crayfish, alligator pepper **[*Aframomum melegueta* (Rosc.) K. Schum.]**, white pepper, African hot pepper and locust beans **(*Ceratonia siliqua* Linn.)**. The spices were gathered from the local farm and market, cleaned and sun dried at temperature of 45°C maximum, until a constant dried weight was attained. It was then passed through magnetic separator to remove the foreign material manually, sieved to remove the dirt, insects and ground to fine particles of size 0.05 μm. Two samples (A & B) as proposed by Djmen[11] for mixing by weight were used; the ratios are given in Table 1.

Table 1 : Mixing ratio by weight (wt.) for spices mixture (samples)

Sample A		Sample B	
10 % wt. Garlic	5%wt.Gyanda muyan	15 % wt. Garlic	10 % wt. Thyme
10 % wt. Ginger	10 % wt. Thyme	15 % wt. Ginger	10 % wt. Crayfish
5 % wt. Allspice	5 % wt. Crayfish	5 % wt. Allspice	10% wt.White pepper
10%wt. African hot pepper	10% wt. Alligator pepper	15%wt. African hot pepper	10% wt. Alligator pepper
5 % wt. Mansoro	20% wt.White pepper	5 % wt. Mansoro	
10 % wt. Cloves		5 % wt. Cloves	

Ceratonia siliqua

Syzygium aromaticum

Pimenta dioica

Determination of moisture and ash content

The moisture and ash contents were determined by estimating the difference in fresh and dry weight.

Determination of protein and carbohydrate

Protein content was measured by Kjedahl method and the total carbohydrate was determined by using estimation method.[8] If the total of protein, lipid acid content was subtracted from organic matter, the remainder accounts for carbohydrate and nucleic acid. Thus, % carbohydrate = 100 - (% moisture + % ash + % protein + % lipid + % fibre) contents.

Microbiological analysis of samples A and B

Dilute Solution Preparation Procedure

A sterile 1 ml blowout pipette was held vertically and the pipette tip was introduced to the surface of samples A and B and sucked up and down five times to the 1 ml mark. One ml of samples A and B was withdrawn. The pipette content was transferred to the first tube of dilution solution series with the tip touching the side above the level of the diluents. This pipette was then discarded and the first dilution tube was labelled 10^{-1}.

Taking a fresh sterile pipette, the content of the first dilution tube was mixed by sucking up and down to the 1 ml mark five times. Then 1ml of the first dilution was withdrawn and transferred to a second tube of sterile dilution expelling the contents of the pipette. This pipette was also discarded and the second dilution tube was labelled 10^{-2}. Taking fresh sterile pipettes in each case other specimen were prepared for 10^{-3}, 10^{-4} and 10^{-5}.

Plate preparation procedure

Taking a fresh sterile pipette the content of the final dilution tube (10^{-3}) was mixed by sucking up and down five times. One ml of the dilution was withdrawn by touching the tip of the pipette against the side of the tube to remove excesses adhering to the outside and the contents was transferred to a sterile petri dish. Allowing about 3 minutes to elapse the tip of the pipette was touched against the dish away from the previous inoculum and the remaining drops gently blown away. This same pipette was used to transfer 1 ml from the 10^{-2} dilution to a sterile petri dish but before taking the samples it was raised and lowered for about three times in order to rinse the sides of the pipette and also to give the dilution a final mixing. The same procedures were repeated for the 10^{-1} dilution.

To each plate 15 to 20 ml of molten compound spices agar (bacterial) and PDA medium at 45°C was added and immediately the medium and inoculum was mixed by a combination of to and fro and shaking for about 5-10 seconds. The plates were allowed to set, then inverted and incubated at the appropriate temperature of 25-30°C for fungi and 37°C for bacteria.[1, 8, 9, 12, 13, 14]

Fungi analysis

Preparation of compound spices broth

Thirteen grams of compound spices broth powders was weighed and dissolved in 1000 ml of distilled water. It was boiled, stirred, bottled and autoclaved at 121˚C for 15 minutes. The bottle tubes were kept in the refrigerator at 4˚C until required.

Preparation of nutrient agar

Twenty-eight grams of compound spices agar powder was weighed and dissolved in 100 ml of distilled water. It was boiled, stirred and autoclaved at 121˚C for 15 minutes. It was then cooled at 45˚C and powdered into sterile petri dish and allowed to set.

Preparation of potato dextrose agar

Potato tube was boiled in 500 ml of distilled water until thoroughly cooked. The suspension was filtered through cheese cloth and water added to make up to 1 ml. Dry ingredient was also added and agar was dissolved with heat. Sterilization was conducted in an autoclave at 121˚C for 15 minutes.

Results and Discussion

The results obtained from the various analysis carried out are presented in Tables 2-5.

Table 2 : Moisture, Ash, Protein, Lipid and Carbohydrate contents of the spice samples A and B

Spice sample	Moisture %	Ash %	Protein %	Lipid %	Carbohydrate %
A	7.00	21.40	7.50	19.70	44.47
B	10.80	15.00	8.25	19.78	46.17

Table 3: Bacterial count of the compound spices sample A and B

Sample	Dilution factor			Average Bacterial Count
	10^{-1}	10^{-2}	10^{-3}	
A	1.64×10^5	1.40×10^6	9.80×10^6	1.33×10^6
B	2.75×10^5	1.92×10^6	1.60×10^6	2.09×10^7

Table 4: Fungi count of the compound spices sample A and B

Sample	Dilution factor			Average Fungi Count
	10^{-1}	10^{-2}	10^{-3}	
A	7.80×10^3	5.60×10^4	3.60×10^6	5.67×10^6
B	1.20×10^5	9.80×10^4	7.60×10^6	9.80×10^6

Table 5 : Morphological characteristics and identification of fungi isolated from samples A and B

Serial No.	Sample	Description of species of fungi	Fungi species type
1	A	Upper side: blackish Under side: yellowish	*Aspergillus niger*
		Upper side: yellowish green Under side: brownish	*Aspergillus flavus*
		Upper side: light brown	*Microsporus caris*
2	B	Upper side: greenish Under side: yellowish	*Aspergillus parasiticus*
		Upper side: grey white Under side: brown	*Trichophyton verrucosum sp.*
		Upper side: pink Under side: deep red	*Fusarium sp.*
		Upper side: yellowish green Under side: grey black	*Rhizopus sp.*
		Upper side: blackish Under side: yellowish	*Aspergillus niger*
		Upper side: yellowish green Under side: brownish	*Aspergillus flavus*
		Upper side: white grey Under side: brownish	*Microsporum caris*

The laboratory tests were conducted to determine the physical composition and the microbial load of the compound spices and the fungi growth on these spices. The microbial analysis showed that the spices carried a fairly heavy load of microorganisms (Table 3). Table 2 gives the percentage estimation of the moisture and ash contents of the spices under investigation. The moisture content of spices sample A (7.0%) is lower than that of B (10.8%). The reverse was the case in the ash contents; that of A has 21.4% while that of B was 15.0%. These discrepancies are attributed to the differences in the composition of the spices mixture. Table 2 gives the percentage lipid content of the spices with A having 19.70% and B with 19.78%. The protein and carbohydrate contents for sample A were lower than that of B (Table 2). The percentage protein values for A and B are 7.5% and 8.25%, respectively while the carbohydrate was 44.47% and 46.17%, respectively.

Table 3 showed that the compound spices sample B seem to have more bacterial load than sample A. The total viable count of the spices ranged from 1.64×10^5 to 2.09×10^7 cells per gram of spice. The highest average counts were obtained from sample B with a value of 2.09×10^7, due to the higher moisture, content of B (crayfish, garlic, ginger and mansoro).[15, 16]

The level of contamination depended on the type of spices. The microfloras were heterogeneous and these data appear to agree with those described elsewhere for spices prepared for marketing.[13] The flora of these spices was mainly Gram negative rods, although Gram positive spore formers and spool were present. In this study, the spices examined contained *Salmonella, Bacillus cereus, Staphylococcus aureus* and *Pseudomonas aeruginosa*. Gilbert and Roberts,[3] and Laidley *et al*[15] examined samples of certain spices from military bases and reported that only a small proportion contained organisms of public health significance. Samples of herbs and

spices examined by several authors in some other countries have been shown to contain pathogenic organisms.[13, 14] Their microbial populations have been shown to be very high often exceeding 10^7 cells per gram. In this study the microbial count ranged from 1.5×10^5 to 9.0×10^7 (Tables 3 and 4). The presence of *E. coli* in some of the spices suggests the need for high sanitary procedure particularly during handling and storage.

The fungal species associated with the spices examined were *Aspergillus niger*, *A. flavus*, *A. parasiticus*, *Microsporum caris*, *Fusarium sp*. and *Rhizopus sp*. (Table 5). These fungi are often associated with mycotoxin production particularly the *Aspergillus*. It is however not known if the isolate obtained from these spices is toxigenic. Sanitary measures should therefore be introduced early in the storage of the spices to minimize excessive fungal growth. It is recommended that immediately after harvest, the spices should be cleaned by washing with potable water and kept dry during the handling process. Some form of packaging would be very useful to ensure reduced microbial growth.

Development of process technology

In order to minimize the contamination of the produced compound spices the following technological process was developed as shown in Fig. 1. The plant consists of a magnetic separator, sterilization tank, milling machines, drying machines, mixers and other ancillary equipment.

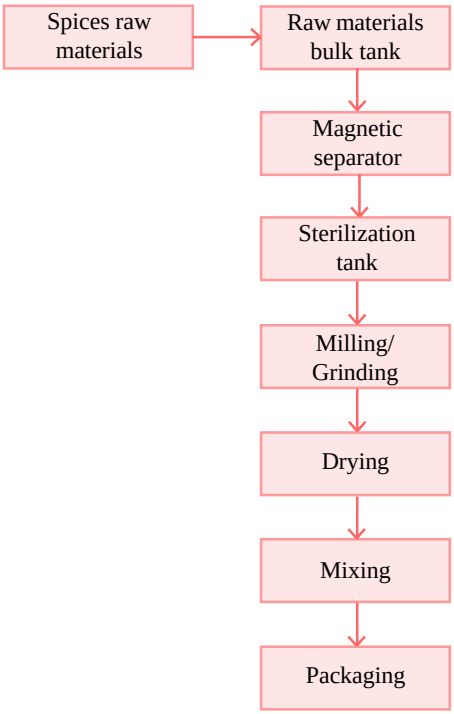

Fig. 1 : Flow diagram for the production of compound spices

Raw material offloads

The spices raw materials are offloaded from the tank into the conveyor, where they are passed on to the raw material storage warehouse.

Raw material storage warehouse

This consists of large cabinets with transparent cover and solar collector for pre-drying and conditioning. The spice materials are exposed to sunlight through transparent cover and the required energy for drying purpose is achieved as provided essentially by radiate heat transfer between the sun and the materials; this acts as a solar collectors in the warehouse. In this case the convective air acts only to carry away the removed vapour.

Magnetic centrifuge/ sterilizer

This comprises heating cylindrical container, with electro-magnetic properties capable of removing metallic material present in the raw material as it goes through it. The spices are then sterilized at a temperature of about 160˚C with a retention time of 120 minutes or 170˚C for 69 minutes. During this process the material loses about 2% of the moisture content.[9] The heat penetration time depends on the nature and volume of the spices. The contamination and infection of microbial load are reduced by 90% during this stage. The sterilized material is then passed on to the milling and grinding unit.

Milling/grinding

The mills consist of a specially designed rotors mounted vertically in a housing which acts as a stator and is equipped with a removable corrugated inner casing. A fan disc is attached to each of the rotor shaft. The spices are fed into the machine through an inlet in the wall of the housing at the level of the lowest grinding disc, being drawn onto the grinding zone by the air stream provided there. The rotation of the rotors causes strong

eddies of air to form in the chamber, which together with the impact of the particles on the walls of the grinding tools and the stator casing, grind the materials. The finer particles leave the mill as finished ground spices and are collected after passing through a sieve.

Drying

A vertical dryer is fitted with three heating elements arrayed in form of triangle on the dryer sidewalls. The dryer contains some drying trays. Here the grains are gradually heated to a maximum temperature of about 50°C. The spices are transferred into various bins and passed unto the mixer.

Mixing/packaging

The spices now in form of flour are mixed in desired proportions. Here complete homogenization takes place and the required consistency of spices is produced. The blended spices are packaged into smaller units of 20g nylon bags and are ready for marketing.

Conclusions

The formulated compound spices under study were fairly contaminated with some microorganism and were not suitable for consumption. The type of the spices composition also contributed to the level of contamination. The level of bio-load on these spices was greatly dependent on the level of moisture content. It is recommended that after harvesting, higher sanitary conditions should be employed during the processing, handling and storing of the spices. The proposed technological process will help in reducing the microbial growth of the produced compound spices.

References

1. Frazier WC and Westhoff DC, Food Microbiology, 3rd edition, (McGraw-Hill, New York), 1978, 1,191,273, 313-316.
2. Idris BM, Evaluation of technological processes for the production of compound spices, M. Eng Thesis, Federal University of Technology, Minna, Nigeria, 2002, 1-96.
3. Gilbert RJ and Roberts D, Food poisoning risks associated with food other than meat and poultry-outbreak and surveillance studies, *Health Hyg*, 1979, **3**, 33-40.
4. Purseglove JW and Brown EG, *Spices*, (Longman Publication, London), **Vol 1**, 1981, 524-528,
5. Bullerman LB, Lieu FY and Seier Sally A, Inhibition of growth and aflatoxin production by cinnamon and clove oils, cinnamic aldehyde and eugenol, *J Food Sci*, 1977, **42**, 1107-1109.
6. Kivanc M and Akgul A, Antibacterial activities of essential oils from Turkish spices and citrus, *Flavour Fragr J*, 1986, **1**,175-179.
7. Oloke JK, Kolawole DO and Erhun WO, The antibacterial and antifungal activities of certain components of *Aframomum melegueta* fruits, *Fitoterapia*, 1988, **59**,384-388.
8. Flick A S, Microbiology of foods, (W H Freeman Publication, San Francisco), 1976, 249-259.
9. Macrae S, Joshyn MA and Webster J, Methods in food Analysis, 2nd edition, (Academic Press, New York), 1997, 7-24.
10. Macrae S and Robinson J, Influence of the activity of water on spoilage of foodstuffs, *J Food Tech*, 1997, **3**,131-142.
11. Djmen K, Quality and uses of Nigerian spices, Home economics report, Dept. of Agr., Niger State, Nigeria,1997, 3-8.
12. Christopher O, The microbial content of some Nigerian spices, PhD Thesis, University of Port Harcourt, 1986.
13. Christensen G M, Fanse H A , Nelson G H and Bates F, Microflora of black and red pepper, *Appl Microbiology*, 1967, **15**, 626-662.
14. Kaferstein F K, The microflora of parsley, *J Milk Food Tech*, 1976, **39**, 837-840.
15. Laidley R, Handzel S, Sever D and Butler R, *Salmonella weltevreden* outbreak associated with contaminated pepper, *E Bull*, 1974, **18**, 62.
16. Oyebiodun F, The effect of extracts of some Nigerian spices on biodegradation of okro by fungi, PhD Thesis, Delta State University, Abraka, Nigeria, 1982. □