EFFECTIVENESS OF VARIOUS SPICES IN INHIBITING
THE SPOILAGE RATE OF FOOD

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ABSTRACT

The effectiveness of locally used spices Zingiber officinale (ginger), Myristica fragrans (nutmeg), Allium sativum (Garlic), Thymus vulgaris (thyme), and Curry powder in inhibiting the spoilage rate of food were determined. The spoilage organisms isolated from control plates were Bacillus cereus and Staphylococcus aureus from jellof rice, Proteus mirabilis and Bacillus cereus from fish sauce, Enterobacter aerogenes, Klebsiella pneumonia and Escherichia coli from bean cake(moi moi). In the plates containing spices in all the food samples only Bacillus cereus was able to grow. The result of colony count expressed in cfu/ml showed that plates containing curry powder and thyme had no growth. Ginger had $1.0 \times 10^2$, garlic $2.0 \times 10^2$, and nutmeg $5.0 \times 10^2$, while control had $1.28 \times 10^4$ from jellof rice. In bean cake, curry powder had $4.0 \times 10^3$, thyme $1.2 \times 10^3$, ginger $9.0 \times 10^2$, and nutmeg $7.0 \times 10^2$ while control plate had $2.25 \times 10^4$. Curry powder and garlic had nil, thyme $9.0 \times 10^2$, ginger $3.0 \times 10^2$, nutmeg $9.0 \times 10^2$ while control had $1.15 \times 10^4$, from fish sauce. The plates containing curry powder in all the food samples showed highest inhibitory effect on the food spoilage organisms. Nutmeg, Garlic, Ginger, and Thyme also showed strong antimicrobial effect against food pathogens in all the food samples. There was a gross reduction in microbial load in the experimental plates (with spices) compared with control plates (without spices) as indicated by the number of colonies in each plate. The result of this study showed that spices have inhibitory effect on food borne pathogens, so they could be used in food preservation as main antimicrobial compounds in order to assure the production of microbiologically stable food.

Introduction

Spices are mixtures of several or dozens of phytochemicals from indigenous or exotic origin with the major bioactive compounds constituting up to 85%, while other components are found at trace levels (Burt, 2004 and Moreno et al., 2006), and according to Lamber et al., (2001) these bioactive compounds may evolve multiple modes of antimicrobial action including degradation of the cell wall, disruption of the cytoplasmic membrane, leakage of cellular components, alteration of fatty acid and phospholipid constituents, changes in the synthesis of DNA and RNA and destruction of protein
translocation (Shan et al., 2007). Spices include leaves (bay, mint, rosemary, coriander, laurel, oregano), flowers (clove), bulbs (garlic, onion), fruits (cumin, red chili, black pepper), stems (coriander, cinnamon), rhizomes (ginger) seed (nutmeg) and other plant parts (Smid & Gorris, 2005). Spices contain products of secondary metabolism such as phenolics, phenolic acids, quinones, flavonoids, tannins (Alvarez et al., 2008). Many of these phytochemicals are rich sources of antioxidants (Moreno et al., 2006). Many workers have reported a high correlation between antimicrobial efficacy and the level of phenolic components present in certain spice preparations. Indeed, compounds such as eugenol, carvacrol and carnosic acid present in clove, oregano and rosemary respectively, have been identified as being responsible for antimicrobial activity.

Spices have been currently used as a food additive for the purpose of flavoring and as a preservative by killing or preventing the growth of harmful bacteria in food (Agaoglu et al., 2007). Besides adding to the taste, spices have multifarious functions that include combating food borne microorganisms, reducing food poisoning, (Bajpai et al., 2008), antioxidant function, and antimicrobial activity (Alvarez et al., 2008). Spices are also known to possess a wide range of medicinal values, such as fight against cancer causing cells, reduction of cholesterol level in the blood and prevention of several skin diseases (Burt, 2004).

Despite the high degree of awareness of food preservation methods there is increasing occurrence of disease outbreaks caused by pathogenic and spoilage microorganisms in foods (Witkowska et al., 2011). Consumer awareness and concern that synthetic chemical additives may have some toxic or even carcinogenic effects, has increased the demand for high-quality, minimally processed foods with extended shelf-life, preferably free from or with a reduced level of added chemical antimicrobial agents (Desmond, 2006). There is growing interest in using natural antimicrobial compounds, including extracts of herbs and spices, as salt replacers or alternatives to synthetic compounds for food preservation (Smid & Gorris, 2005).

Food spoilage is a metabolic process that causes foods to be undesirable or unacceptable for human consumption due to changes in sensory characteristics such as changes in texture, smell, taste, or appearance (George, 2009).

Food borne diseases and food spoilage by microorganisms is responsible for food poisoning and is unhealthy for food producers, retailers, consumers and regulatory authorities(Lanciotti et al., 2004).

There are also increasing occurrence of food-borne disease outbreaks caused by pathogenic microorganisms. Recently in Bauchi state there was an outbreak of cholera - a food borne disease caused by *Vibrio cholera* which claimed many lives. Information on the effectiveness of locally used spices in inhibiting the spoilage organisms in food is scarce in Bauchi. Spices are abundantly available in Bauchi state all year round and are cheap. It is important to investigate the effectiveness of spices in inhibiting the spoilage organisms in foods commonly consumed in Bauchi metropolis.
Materials and Methods
Collection of Samples
*Zingiber officinale* (Ginger), *Myristica fragrans* (Nutmeg), *Allium sativum* (Garlic), *Thymus vulgaris* (Thyme) and Curry powder (Ducrose) used in this study were purchased from wunti market Bauchi state, likewise the rice, beans, fresh fish and other condiments. The samples were packed separately in cellophane bags to microbiology laboratory, Federal polytechnic Bauchi immediately and were kept in the cupboard.

Preparation of Culture Media
The media used were nutrient agar (oxoid) for isolation and enumeration of bacteria; MacConkey agar (Difco) as a differential media; potato dextrose agar (oxoid) for the isolation and enumeration of fungi and blood agar (oxoid) as a differential media. They were prepared according to the manufacturer’s instructions.

Processing of samples
Sample 1: Jellof rice
The rice were per boiled, washed and mixed with fried tomatoes and other condiments without salt, onions and pepper. 120g of the per boiled rice were weighed into five sterile beakers and 0.2g of each grounded spice was mixed one to each five beakers of rice and were cooked separately and then kept at room temperature for 36 hours.

Sample 2: Bean cake
The beans were soaked in water for one hour, washed and grounded without salt, onions and pepper. Other condiments were added and 120g of the grounded beans were weighed into five sterile beakers and 0.2g of each grounded spice were added one to each the five beakers and were cooked separately and then kept at room temperature for 36 hours.

Sample 3: Fish sauce
Fresh fish were washed thoroughly, pieced and 120g of the washed fish was weighed into five sterile beakers, 0.2g of each grounded spice was added to each of the five beakers and was cooked separately and kept at room temperature for 36 hours. One beaker of each of the food samples was prepared without any spice added to it and was kept at room temperature for 36 hours and this served as control.

Isolation of spoilage organisms
Isolation of food spoilage organisms from food samples was done using the standard method of (Teramota *et al.*, 2005). A tenfold serial dilution (10⁻¹-10⁻⁵) of food samples in each of the five beakers were prepared using distilled water. The test tubes containing 9ml of distilled water were autoclaved at 121°C for 15 minutes prior to serial dilution and 1ml from dilution 10⁻² and 10⁻⁴ of each samples were inoculated on nutrient agar and potato agar using spread plate method and incubated at 37°C for 24 hours for bacteria growth and at 28°C for 5 days for fungal growth. The growth and colony characteristics
of the colonies were observed on the Petri plates and the colonies were counted using colony counter. Each of the different colonies that grew on the plates was sub cultured by streaking onto a fresh nutrient agar, blood agar and mac Conkey agar separately. The plates were incubated for 24 hours at 37°C. The colony growth and its characteristics were macroscopically and microscopically observed.

Identification of bacterial isolates
Morphological examination of isolated bacterial colonies was done using microscopic and macroscopic methods as described by Sasidharan, (2011). Gram staining was done for primary morphological characterization of isolated bacterial colonies, for more precise identification of observed colonies various biochemical test viz; catalase, indole, coagulase, methyl red test were performed (Gaffa, 2005). Bacteria colonies that appeared on the plates were counted using a digital illuminated colony counter. The colony counts from the plates were obtained and expressed as colony forming unit per millitre (cfu/ml). Final identification of bacteria isolates was by comparison of results obtained with literature standard using Bergey’s manual of determinative bacteriology (Buchanan and Gibbons, 1975).

Gram Staining Reaction
A loopful of the bacteria culture was taken up with the inoculating wire loop and spread thinly on a clean grease free glass slide. The film was fixed by passing the dried slide with film upwards over a bunsen flame for a few seconds, the slide was then covered with crystal violet solution and allowed to act for 30 seconds. The stain was poured off by holding the slide at an angle downwards and pouring water to wash away the crystal violet. The slide was then covered with fresh Lugol’s iodine for one minute and washed off with water. The smear was decolorized rapidly with acetone and washed off with clean water. The smear was then counter stained with 30% safranin for 1 minute and washed with water, the slide was then air dried and was examined under the microscope with the oil immersion objective lens.

Biochemical tests:
For more precise identification of isolated organisms, the following biochemical test were carried out according the method described by (Gaffa, 2005).

Motility Test
A ring of plasticine was made on a glass slide and an inoculum of the organism was made at the center of the plasticine. The inoculums was emulsified with distilled water the slide was then observed using the microscope

Methyl Red Test
The isolates were inoculated in tubes of prepared and sterilized glucose phosphate peptone broth and incubated at 37°C for 48 hours. 5 drops of methyl solution was added to
each tubes and the color change was observed. The positive result gave bright red color while the negative gave yellow color with the indicator

**Voges Proskauer Test**
The isolates were inoculated in test tubes of prepared and sterilized methyl red Voges proskauer medium and incubated at 37°C for 5 days. 5ml of 40% potassium hydroxide was added to each tube. A bright pink color appearing within 5 minute showed a positive reaction.

**Oxidase Test**
A piece of filter paper was placed in a clean Petri dish and 2 drops of freshly prepared oxidase reagent was added using a wire loop. Colony of the isolates was smear on the filter paper. The development of a blue-purple color within a few seconds shows a positive result.

**Litmus Milk Test**
A sterile loop was used to inoculate 0.5 ml of sterile litmus milk medium with the isolate. Heavy inoculums of the isolates were used and the loop was scrapped three times across an area of heavy growth. It was then incubated at 37°C for 4 hours. It was examined half hour intervals for a reduction reaction as shown by a change in color from mauve to white or pale yellow which indicate positive while no color change or a pink color indicate negative.

**Indole Test**
Tubes of tryptone broth were inoculated with the test organisms and incubated at 35°C for 48 hours. After incubation, 2 ml of kovac’s reagent was added to each tube and shaken gently. The tubes were then kept on test tube rack and allowed to stand for 20 minute. A red color at the reagent layer indicates indole production.

**Citrate Utilization Test**
Citrate tablet was used, a dense bacteria suspension of the isolates was prepared in 0.25 ml of sterile physiological saline in a small tubes. A citrate tablet was added to it and the tubes were covered with a stopper. It was then incubated over night at 35-37°C. Appearance of red color shows positive reaction while yellow to orange color shows negative.

**Sugar Fermentation Test**
15 ml of peptone water was dispensed into ten test tubes with an inverted Durham tubes in each. The test tubes were capped and sterilized at 121°C for 15 minute. Then 1.5 g of each sugar were weighed and added into each test tubes containing peptone water and test tubes were shaken and a few drops of methyl red was added to test tubes. The cap was then replaced and sterilized at 100°C for 30 minute in water bath and was allowed to cool.
to about 45°C and was aseptically inoculated with the isolates and was incubated at 37°C for 48 hours. A positive result shows change in color to yellow while gas production was evidence by media displacement in the Durham tubes.

**Coagulase Test**

Nutrient broth was prepared and dispensed into test tubes and sterilized at 121°C for 15 minutes. 0.2 ml of blood plasma was added to each of the tubes and inoculated with the test organism. The tubes were incubated in a water bath for 6 hours and examined at intervals clothing shows a positive result.

**Result**

The effectiveness of the spice in inhibiting the spoilage organisms in food as shown by the reduction in microbial load as indicated by the number of bacterial colony counts from each food were presented in table 1. Bacterial load were higher in the controls while curry powder show the higher inhibitory action in all the food samples. The morphological, physiological and biochemical characteristics of the identified organisms were presented in table 2. As can be seen, only *Bacillus cereus* was isolated from all the food samples with spices while in the control *Staphylococcus aureus* and *Bacillus cereus* were isolated from jollof rice, bacillus cereus and *Proteus mirabilis* were isolated from fish sauce and *Enterobacter aerogenes, Klebsiella pneumoniae* and *Escherichia coli* were isolated from bean cake (moi moi).

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<th>Table 1: Result of Bacterial load after the addition of spices.</th>
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<td><strong>Food samples</strong></td>
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<td>Control</td>
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<td>Curry</td>
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<td>Bean cake</td>
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<tr>
<td>Jellof Rice</td>
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<td>Bean cake</td>
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<td>Fish Sauce</td>
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| Jellof Rice  | Yellow raised round colonies                  | +                  | -         | + | + | - | + | + | A | A |
| Fish sauce   | Creamy colored, rod in chain                  | -                  | +         | + | - | - | + | - | - | - |
| Bean cake    | Rod in chain                                  | -                  | +         | - | - | - | + | + | + | - |
|              | Rod raised circular colorless colonies        | -                  | +         | + | - | + | + | - | + | + |
|              | Rod in chain                                  | -                  | +         | - | - | - | + | + | + | - |

LMD – Litmus milk decolorization test  
VP – Voges proskauer test  
MR – Methyl red  
A – Acid production flowing fermentation of sugar
Discussion
From the result obtained, the colony count in the plates containing curry powder in all the food samples showed highest inhibitory effect on the food spoilage organisms. This high efficacy of antimicrobial activity of curry spice may be due to synergistic effect as curry powder contained turmeric, coriander, fenugreeks, salt, onion, fennel, garlic, cumin, caraway, paprika, chilipepper and black pepper. This agreed with the work carried out by Thomas and Isak (2006) who reported that this combination enhance the inhibitory activity of spice. Burt (2004) also reported the synergic effect of spices against B. cereus and Al-jedal et al., (2006) analyzed the action of combined spices on food pathogens count in fish sauce and their results showed that spice mixture were able to exert static effect on all assayed bacteria. nutmeg, garlic, ginger, and thyme also showed strong antimicrobial effect against food pathogens in all the food samples. There was a gross reduction in load in the experimental plates as compared with control plates as indicated by the number of colonies in each plate (table 1). This may be due to the antimicrobial effectiveness of their chemical compounds such as gingerone and gingerol in ginger, allicin in garlic, and thymol in thyme.
It was noticed that in plate where growth occurred, low colony counts were observed on the low dilution plates while higher counts were obtained on the higher dilution plates. This may be due to reduction in the concentration/strength of spice as effectiveness of spice is determined by its concentration.
The spoilage organisms isolated from control plates were Bacillus cereus and Staphylococcus aureus from jellof rice, Proteus mirabilis and Bacillus cereus from fish sauce, Enterobacter aerogene, Klebisella pneumonia and Escherichia coli from bean cake and in the plates containing spice in all food samples only Bacillus cereus that was able to grow, the rest of the organisms were inhibited by the spices .This may be due to their antimicrobial activity/effect on the microorganisms. This is in accordance with the work carried out by Gundogen et al., (2006) in six different spices which inhibited the growth of staphylococcus aureus. Al-jedal et al., (2006) also analyzed the action of combined spices on food pathogen count in fish sauce which shows that spice mixture were able to exert inhibitory effect on all assayed bacterial.

Conclusion
The result obtained in this study showed that spices are very effective in inhibiting food pathogens by exerting antimicrobial effect as proved by gross reduction in microbial load.

Recommendation
Proper hygienic should be maintained during harvesting, processing and storage of spices as spices could act as vehicles for micro for microbial contamination of foods. Spices should be washed before use. Further studies should be carried out on the mode of action of these spices.
References


