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RESEARCH ARTICLE

MICROBIOLOGY OF FRESHLY HARVESTED AND MARKET RETAILED CUCUMBER

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ABSTRACT

Some fresh minimally processed fruits and vegetables when eaten raw are sources of most foodborne disease outbreaks world wide. Cucumber (*Cucumis sativus*) is sold along major highways, streets and markets in Nigeria. This vegetable is in very high demand and is eaten raw. The microbial flora and contamination level of the freshly harvested and market retailed produce was investigated. Some of the produce were aseptically harvested in farms in Odukpani area of Cross River State, while others were purchased in are markets in Calabar. A total of 14 species of bacteria were isolated from market retailed cucumber as against 4 species from the freshly harvested ones. Among the market retailed isolates, 4 (40%) were foodborne pathogens, 4(40%) were lactic acid bacteria (LAB) and 2(20%) were contaminants. Only one probable foodborne pathogen was isolated from the freshly harvested ones. Among the 10 molds isolated, only 4 species were isolated from the freshly harvested cucumber. The average microbial load for the market retailed cucumber was 1.73×10^4 cfu/g as compared to 1.32×10^2 cfu/g for the freshly harvested produce. Results showed that market retailed produce had higher microbial load and foodborne pathogens than freshly harvested ones.

Key words: Ingestion, intoxication, fodborne, microflora

INTRODUCTION

Fruits and vegetables are ecological riches for a diverse and changing microflora which usually does not include types pathogenic to humans (Cantwell, 2002). The microflora of vegetables is dominated by soil organisms. Changes in the environmental conditions surrounding a product can result in significant changes in the microflora (Sapers and Annous, 2004). Cucumber, (*Cucumis Sativus*) falls under fruits and vegetables. It is an edible fruit or vegetable. *Cucumis sativus* belongs to the gourd family cucurbitaceae. It grows near the ground or on the ground. *Cucumis sativus* are usually green-skinned, roughly cylindrical, smoother, elongated with tapered ends and maybe as large as 30cm long and 5cm in diameter. The smoothness of the fruit makes it difficult for bacteria to adhere to the surface. After washing, the adhesion of bacteria to the fruit in wash water was less extensive at lower temperatures and shorter exposure times (Reina *et al.*, 2002).. Despite these characteristics, microorganisms are isolated from their surfaces and within the tissues. Knowledge of the microbial flora of these produce which maybe, spoilage, pathogenic, normal, transit and indicator organisms is very important (Fraizer and Westhoff, 1981; Banawrt, 1983). It is assumed that all fruits and vegetables get their natural flora from their environments such as soil, water and air but this is not always the case. Enterobacteriaceae, lactic acid bacteria (LAB), pseudomonads, *Erwinia carotovora* and some fungi species are frequently isolated from cucumber. This is to say that

fresh fruits and vegetables are never totally free of microorganisms. The number and kinds of normal flora vary with the differences in the forms of the fruits and vegetables (Oie, *et al.*, 2008). The most common or predominant types of spoilage varies not only with the kinds of vegetables and fruits but also to some extent with the variety. The spoilage organisms are those which result in the deterioration of food quality by the action of their enzymes altering certain food components and alterations in the appearance, texture, color, odor, or flavor or by slime formation. Another group of microorganisms isolated from fruits and vegetables are the foodborne disease causing pathogens which include all organisms that cause either food-borne infection or food intoxication after the ingestion of the organisms themselves or their toxigenic products with the food (Buck, *et al.*, 2005; Altekruze and Swerdlow 1996; Bean, *et al.*, 1997). The need to understand the ecology of fruits and vegetables cannot be underestimated.

In this part of the world, there is little or no knowledge about the microbiology of fruits and vegetable. This is why the incidence of food poisoning resulting from eating raw fruits and vegetables is high. Most of these produce are eaten raw or cooked half done. These produce after harvest go through unsanitary processes upto the market. At the open markets, these produce are displayed in open dirty environments with flies perching on them. Flies of course are vectors of foodborne pathogens and therefore can contaminate these produce with these organisms. Cucumber is freely hawked and sold in open markets in the southern and eastern parts of Nigeria and are mostly eaten raw with groundnut or in salads. As a consequence of the increasing market and the lack of

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information relating to surface contamination of cucumber and the possible outbreaks of foodborne diseases resulting from the consumption of the produce, it is pertinent to investigate the microbial contamination level and foodborne pathogens of cucumber.

MATERIALS AND METHODS

The microbial loads of the samples were determined using the methods of Sharp, (1966) and Von Schelhorn, (1980). Fifty grams of each sample type was weighed separately and placed in two different sterile blenders. This sample size was chosen in order to have good representation of the organisms. These were blended aseptically. Twenty five portion of each produce type was pooled placed in 225ml diluent of sterile maximum recovery diluent(peptone/saline) in two separate sterile flasks. In both cases, the homogenates were allowed to stand for 30min and vigorously shaken for 2 to 3min (Mosupye and von Holy, 1999). From these dilutions, further ten-fold dilutions up to 10^{-4} were prepared. Pour plate method was employed using plate count agar (PCA). In order to have 30-300cfu, 0.5ml from the 10^{-3} and 10^{-4} dilutions from each of the mixtures was used according to Sharp (1966). The plates were swirled gently to mix the contents properly (Geyid, *et al.*, 1991). These were incubated at 37°C for 18-24 hrs. At the end of the incubation period, discrete colonies (30-300cfu) were counted and the average of each taken. Microbial loads per gram for each sample type was determined using the dilution factor.

Microbial Flora Of The Freshly Harvested Produce

The vegetables were swabbed while still on the stems in the farms. The swabs were replaced into their jackets, placed in cool box at 4°C and transported to the laboratory (William, *et al.*, 2003}. The swabs were allowed to stand for one hour in their jackets. They were then streaked on nutrient agar, plate count agar, sabouraud dextrose agar, SSA, MacConkey agar and MRS agar and incubated at 37°C and 27°C for 24hrs and 72hrs respectively for bacteria and fungi. The plates were observed for growth after incubation period.

Isolation And Identification

The isolates were sub-cultured into fresh media to purify and incubated for 24hrs. The isolated colonies were culturally examined and stock cultures prepared. Wet mounts using lactophenol in cotton blue were prepared for the fungal isolates. The bacterial isolates were gram stained and viewed under the microscope. Also, biochemical tests such as citrate, urease, oxidase, catalase, sugar fermentation, and MR-VP were performed on the bacterial isolates. The method of William *et al.*, (2003) was used for the market retailed cucumber. The total surface area of the cucumbers was swabbed with moistened sterile swabs which were then placed into 50ml of maximum recovery diluents (peptone/saline). The suspensions were cultured on plate count agar, MacConkey agar, SSA and sabouraud dextrose agar using 0.5ml of the suspensions in each case. The plates were incubated at 37°C for 24hr for bacteria and 27°C for 48hrs for fungi.

RESULTS

The microbiological analysis of the freshly harvested cucumber samples resulted in the identification of *Micrococcus* spp, *Streptococcus* spp, *Bacillus* spp, *Lactobacillus* spp, *Rhizopus stolonifer*, *Yeast* spp, *Fusarium verticillioides*, and *Penicillium brevicompactum*. The microbial load was 1.32×10^2 cfu/g. On the other hand, a total of 10 species of bacteria and 4 species of fungi were isolated from the market retailed cucumber. These were *Leuconostoc* spp, *Salmonella* spp, *Pseudomonas* spp, *Staphylococcus aureus*, *Streptococcus* spp, *Bacillus* spp, *Pedococcus* spp, *Lactobacillus* spp, *Enterobacter* spp, *E. Coli*, *Rhizopus stolonifer*, *Yeast* spp, *Fusarium verticillioides* and *Penicillium brevicompactum*. Among these, 4(40%) foodborne pathogens, 4(40%) lactic acid bacteria, and 2(20%) contaminants. The average microbial load for the market retailed produce was 1.73×10^4 cfu/g.

DISCUSSION AND CONCLUSION

The isolation of different microorganisms from the freshly harvested produce shows that cucumber is usually not free from microbial contamination. Among the bacterial isolates from the freshly harvested cucumber, only *Bacillus* spp is a possible soil borne organism. *Erwinia* spp, a common plant flora was not isolated from the cucumber samples. This shows that not every plant is inhabited by all microorganisms. This is in line with the finding of Azcon (1989) who stated that plant growth and nutrient content was related to the bacterial association. The non-isolation of indicator organisms and foodborne pathogens could have been due to the farm locations and season. Usually, the microflora on the surface of freshly harvested vegetables include soil microorganisms, water and normal flora and these are distributed on various parts of the plant (Geyid *et al.*, 1991; Andrews and Harris, 2000).

The bacteria isolated from the market retailed cucumber included pathogens, LAB, and other contaminants. This is in line with the findings of Alzamora, *et al.*, (2000) and Nahaisi, *et al.*, (2003). The more microbial species and higher microbial load of the market retailed could be because of the unhygienic conditions of the markets and containers during transportation and also improper storage conditions. Thunberg *et al.*, (2002) and Sagoo *et al.*, (2001) found similar results. The common pathogens isolated from the market retailed samples were *S. aureus*, *Salmonella* spp, and *E. coli*. Abadias *et al.*, (2007) also isolated similar organisms from minimally processed fruits and vegetables. The results of this study has revealed that cucumber sold in the markets or hawked on the streets are not safe for consumption if not properly washed or disinfected. The level of contamination of the produce after harvest all the way to the market is of great concern. It is therefore highly advisable that the produce should be properly washed by the consumer or disinfected before eating it raw. It can be seen also from this work that fresh cucumber regularly or transiently host a variety of microorganisms, some of which are pathogenic. Knowledge of this is essential in an effort to prevent food poisoning resulting from eating raw cucumber. Since there are no agencies responsible for reporting foodborne

outbreaks, there are no reported cases of foodborne outbreaks in this country. This is not to say that there are no such cases.

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