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Microbial profile of cabbage (*Brassica oleraceae*) sold in markets within Ibadan metropolis

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Abstract

Cabbage is a dietary source of micronutrients, vitamins and fiber, vital for human health. Cabbage samples for this study were obtained from selected markets in Ibadan, Nigeria. Serial dilutions and membrane filter techniques were used to determine microbial load and profile of the samples. Appropriate culture media and techniques were used to detect for the presence of Fungi and Bacteria in samples. Fungi isolated included; *Sclerotinia sp, Fusarium sp, Cladosporium sp*, *Aspergillus sp, Mucor sp, Alternaria sp and Rhizopus sp*. Bacteria isolated included; *Staphylococcus sp, Pseudomonas sp*, *Xanthomonas sp, Micrococcus sp, Bacillus sp, Erwinia sp*, and *Streptococcus sp*. The fungi load was highest in cabbage samples collected from challenge having 158.0 X 103 cfu/ml, followed by cabbage purchased from Bodija having 67.6 X 103 cfu/ml, Monatan 63.6 X 103 cfu/ml. , Oje 27.0 X 103 cfu/ml, and Gbagi 24.0 X 103 cfu/ml. The bacterial load on the cabbage samples was observed to be the highest in cabbage samples from Bodija with 360.0 X 103 cfu/ml, followed by samples from Gbagi 188.6 X 103 cfu/ml, Monatan 171.3 X 103 cfu/ml, Challenge 133.6 X 103 cfu/ml and Oje 85.3 X 103 cfu/ml. Results also showed that Ascetic Acid lowered microbial load and thus researchers recommend that cabbage be treated with 2.5 - 5% acetic acid (vinegar) for 5-10 minutes to reduce microbial load then rinse with sterile water before consumption. Enlightenment campaigns are necessary for vendors and people purchasing cabbage for consumption on the dangers of not washing before selling and consuming.

Keywords: Cabbage; Micronutrients; Bacteria; Fungi; Microbial load; Ibadan

1. Introduction

Vegetables such as cabbage are an extraordinary dietary source of nutrients, micronutrients, vitamins and fiber for humans and are thus vital for health and wellbeing. Well balanced diets, rich in vegetables such as cabbage, are especially valuable for their ability to provide vitamins C, K, B1 (Thiamin), B2 (Riboflavin), B3 (Niacin), B5 (Pantothenic acid), B9 (Folate), B6 (pyridoxine) and vitamin A [1] . Vegetables including cabbage are widely exposed to microbial contamination through contact with soil, dust and water and by handling at harvest or during postharvest processing. They therefore harbor a diverse range of microorganisms including plant and human pathogens [2] Differences in microbial profiles of vegetables result largely from unrelated factors such as resident micro flora in the soil, application of non-resident micro flora via animal manures, sewage or irrigation water, transportation and handling by individual retailers and vendors etc. [2,3].

Food-borne bacterial pathogens commonly detected in fresh vegetables include coliform bacteria such as *E. coli*, along with *Staphylococcus aureus* and *Salmonella sp.* [4]. Enteric pathogens such as *Escherichia coli* and *Salmonella sp.* are also among the greatest concern during food-related outbreaks of diseases [5]. Several cases of typhoid fever outbreak have been associated with eating contaminated vegetables grown in or fertilized with contaminated soil or sewage [6]. These increases in vegetables-borne infections may have resulted from increased consumption of contaminated vegetables

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outside the home as most people spend long hours outside the home. In Nigeria for instance, street vending of handy ready-to-eat sliced vegetables like Cabbage has recently become very common and the market is thriving. Proper washing of vegetables is essential for decontamination. Water supplemented with varying concentrations of organic acids, such as acetic, citric and ascorbic acids, has been shown to reduce microbial populations on vegetables [7]. Previous studies revealed that a vinegar dip resulted in a decrease in the number of aerobic bacteria on parsley leaves, depending on vinegar concentration used and incubation time [8].

Microorganisms capable of causing human illness and others whose food-borne disease potential is uncertain, such as *Aeromonas hydrophila* , *Citrobacter freundii*, *Enterobacter cloacae* and *Klebsiella* sp. have been isolated in lettuce and salad[9]. Numerous food-borne molds can produce mycotoxins, and some yeasts and molds are responsible for human and animal infections [10]. This study intends to determine the microbial profile of samples of cabbage bought at select major markets within Ibadan Metropolis. This happens to be important as Ibadan is the city with largest black city in term of landmass in the whole of Africa with a very large human population.

2. Material and methods

Cabbage (*Brassica oleracea*) samples was collected aseptically in sterilized nylon bags from five different Markets (Challenge, Monatan, Bodija, Gbagi, and Oje) in Ibadan, Oyo State, Nigeria. Serial dilution was carried out on 25 g of cabbage from each of the samples using dilution factors of 10^{-2}, 10^{-4} and 10^{-5} before inoculating the samples to culture media. The samples were inoculated unto *Samonella-Shigella* Agar, Eosin Methylene Blue Agar, Nutrient Agar and Potato Dextrose Agar. All samples were then incubated at room temperature 37 °C for 24-48 hours. After incubation, the number of colonies was counted from each plate using a colony counter followed by morphological identification, Gram staining and Biochemical tests. To test for coliform bacteria, the membrane filter technique was used as five beakers into which 100 ml of distilled water containing the filtered cabbage sample each, was filtered using the membrane filter machine aseptically. The trapped residue on the filter membrane was innoculated into petri dishes containing Eosin Methylene Blue and Salmonella-Shigella Agar respectively and this was done for other four samples. These were also incubated at 37 °C for 24-48 hours.

2.1. Quality Control using Acetic Acid

To determine the efficacy of varying concentrations of acetic acid solution on the microbial load of Cabbage, 25 g of cabbage sample from five different locations was weighed and washed in 100 ml of 0.5, 1.5 or 2.5% acetic acid (vinegar) solutions respectively. Using 2 ml syringe, aliquot of 0.1 ml of each rinse solution was inoculated on nutrient agar and potato dextrose agar at the initial time of rinsing, then after 5 and 10 min exposure and was incubated at 37 °C for 24-48 hours. Number of colonies on each plate was counted using a colony counter. Normal household vinegar contains about 5% acetic acid. A concentration of 2.5% acetic acid was obtained by making a 1:1 dilution of household vinegar with distilled water and subsequently diluted accordingly to obtain other dilutions [11].

3. Results and discussion

3.1. Microbial load of cabbage (*Brassica oleracae*) samples

Results from the study showed that all the cabbages purchased from five different locations were contaminated with fungi. The fungi load was the highest in cabbage samples collected from challenge having 158.0 X 10^3 cfu/ml, followed by cabbage purchased from Bodija having 67.6 X 10^3 cfu/ml, Monatan having 63.6 X 10^3 cfu/ml. , Oje has 27.0 X 10^3 cfu/ml, and Gbagi has the lowest fungal load 24.0 X 10^3 cfu/ml (Table 1). These cabbages were probably from the same source, only sold in different parts of the city. They all had fungi count of x$10^{3.}$

The bacteria load (Table 1) on the cabbage samples was observed to be the highest in cabbage samples from Bodija having 360.0 X 10^3 cfu/ml, followed by cabbage from Gbagi 188.6 X 10^3 cfu/ml, Monatan 171.3 X 10^3 cfu/ml, Challenge 133.6 X 10^3 cfu/ml. Cabbage from Oje was observed to have 85.3 X 10^3 cfu/ml which is the lowest bacterial load (Table 1).

Table 1 Fungal load on cabbage from five different locations

Location	Microbial Load (cfu/ml) 1st Sampling	Microbial Load (cfu/ml) 2nd Sampling	Microbial Load (cfu/ml) 3rd Sampling
Gbagi	24.0 X 10^3	22.0 x 10^3	23.0 x 10^3
Oje	27.0 X 10^3	27.0 x 10^3	26.0 x 10^3
Monatan	63.6 X 10^3	60 x 10^3	63 x10^3
Challenge	158.6 X 10^3	140 x 10^3	145 x 10^3
Bodija	67.6 X 10^3	66.5 x 10^3	70 x 10^3

Table 2 Bacterial load on cabbage from five different locations

Location	Microbial Load (cfu/ml) 1st Sampling	Microbial Load (cfu/ml) 2nd Sampling	Microbial Load (cfu/ml) 3rd Sampling
Gbagi	188.6 X 10^3	180 x 10^3	185 x 10^3
Oje	85.3 X 10^3	86 x 10^3	85 x 10^3
Monatan	171.3 X 10^3	170 x 10^3	170 x 10^3
Challenge	133.6 X 10^3	135 x 10^3	136 x 10^3
Bodija	360.0 X 10^3	340 x 10^3	350 x 10^3

3.2. Occurrence of fungi and bacteria from cabbage purchased in five different locations

Table 3 Frequency of occurrence of microorganisms in cabbage samples

Organsims	Location														
Fungal isolates	**Gbagi**			**Oje**			**Monatan**			**Challenge**			**Bodija**		
Mucor spp	+	+	+	+	+	+	-	+	-	+	+	-	+	+	+
Aspergillus spp	-	+	+	+	+	+	+	+	+	-	+	+	+	-	+
Cladosporium spp	-	+	-	+	+	+	-	-	+	+	+	-	-	+	+
Rhizopus spp	-	-	-	+	-	-	-	+	-	+	+	-	-	+	-
Alternaria spp	-	+	-	-	+	-	+	+	-	-	-	+	-	-	-
Sclerotinia spp	-	-	+	-	-	-	-	+	-	+	+	-	-	-	-
Fusarium spp	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-
Bacterial Isolates															
Erwinia spp	+	-	+	-	-	-	+	-	-	+	-	-	-	-	-
Staphylococcus spp	+	+	-	-	-	+	-	-	+	+	-	-	-	+	+
Streptococcus spp	+	-	-	+	+	-	+	-	+	+	-	-	-	+	-
Bacillus spp	+	+	-	-	+	+	+	-	-	-	-	-	+	-	+
Micrococcus spp	-	+	-	+	-	-	+	+	-	-	+	+	+	+	-
Pseudomonas spp	-	-	+	-	-	-	+	-	-	+	-	-	+	-	-
Xanthomonas spp	-	-	-	-	-	-	-	+	-	-	+	+	-	-	-

Key: Absent is – Present is +

From this study, it was observed that fungi such as *Mucor sp.,Aspergillus sp., Cladosporium sp.,* were present in all the samples but with varying frequency, followed by *Rhizopus sp., Alternaria sp.,* which is followed *Sclerotinia sp.,* while *Fusarium sp.,* has the lowest frequency, present in cabbage samples from Gbagi and Monatan only (Table 3). For the bacterial isolates, *Staphylococcus sp., Streptococcus sp., and Micrococcus sp., Bacillus sp.,* and *Pseudomonas sp.,* were present in all the samples, followed by *Erwinia sp.,* and *Xanthomonas sp.* (Table 3)

3.3. Percentage of occurrence of microorganisms in cabbage, first sample.

Table 4 Percentage occurrence of microorganisms in cabbage during the first sampling

Location	Organisms	Percentage of Occurrence (%)
Gbagi	*Mucor sp.*	100
Oje	*Mucor sp.*	40
	Aspergillus sp.	20
	Cladosporium sp.	20
	Rhizopus sp.	20
Monatan	*Mucor sp.*	94
	Aspergillus sp.	4
	Alternaria sp.	2
Challenge	*Mucor sp.*	96
	Rhizopus sp.	2
	Sclerotinia sp.	1
	Cladosporium sp.	1
Bodija	*Mucor sp.*	93
	Aspergillus sp.	7
Nutrient Agar		
Gbagi	*Erwinia sp.*	43
	Staphylococcus sp.	25
	Bacillus sp.	7
	Streptococcus sp.	23
Bodija	*Streptococcus sp.*	46
	Pseudomonas sp.	11
	Micrococcus sp.	25
	Bacillus sp.	18
Monatan	*Bacillus sp.*	3
	Erwinia sp.	20
	Sreptococcus sp.	34
	Pseudomonas sp.	36
	Micrococcus sp.	6
Oje	*Micrococcus sp.*	89
	Streptococcus sp.	11
Challenge	*Pseudomponas sp.*	56
	Staphylococcus sp.	34
	Streptococcus sp.	3
	Erwinia sp.	7

Table 5 Percentage occurrence of microorganisms in cabbage during the second sampling

Location	Organisms	Percentage of Occurrence (%)
Gbagi	*Cladosporium sp.*	65
	Fusarium sp.	13
	Aspergillus sp.	9
	Alternaria sp.	13
Oje	*Mucor sp.*	51
	Aspergillus sp.	5
	Alternaria sp.	38
	Cladosporium sp.	5
Monatan	*Fusarium sp.*	45
	Rhizopus sp.	3
	Sclerotinia sp.	34
	Aspergillus sp.	3
	Alternaria sp.	14
Challenge	*Alternaria sp.*	40
	Rhizopus sp.	8
	Aspergillus sp.	4
	Sclerotinia sp.	30
	Cladosporium sp.	16
Bodija	*Mucor sp.*	71
	Rhizopus sp.	1
	Cladosporium sp.	27
Nutrient Agar		
Gbagi	*Staphylococcus sp.*	16
	Bacillus sp.	61
	Micrococcus sp.	23
Bodija	*Staphylococcus sp.*	68
	Micrococcus sp.	5
	Streptococcus sp.	27
Monatan	*Xanthomonas sp.*	81
	Micrococcus sp.	19
Oje	*Bacillus sp.*	20
	Xanthomonas sp.	80
Challenge	*Micrococcus sp.*	61
	Xanthomonas sp..	39

As shown in Table 4, *Mucor sp.* had the highest percentage of occurrence in all the cabbage samples purchased from five different locations having 100% in sample from Gbagi, 40% in sample from Oje, Monatan 96%, Challenge 96% and 93% in sample from Bodija. *Aspergillus sp.* also appeared in 3 different locations having 20% in cabbage sample from Oje, Monatan 4%, and Bodija 7% respectively. *Rhizopus sp.* appeared in two locations, Oje and Challenge with 20% and 1% respectively. *Alternaria sp.* occurred in cabbage sample from Monatan having 2%.Gram negative bacillus, *Erwinia sp.* has the highest percentage of occurrence in cabbage sample from Gbagi (43%), followed by *Staphyloccous sp.,* (25%), *Streprococcus sp.,* (23%), *Bacilus sp.,* (7%) which is line with the work of Eni *et al.,* 2010. In cabbage purchased from Bodija, *Streptococcus sp.* (46%), *Micrococcus sp.* (25%), *Bacillus sp.* (18%), *Pseudomonas sp.,* (11%). In cabbage from Monatan, *Pseudomonas sp.* (36%), *Streptococcus sp.,* (34%), *Erwinia* (20%), *Micrococcus* (6%), *Bacillus* (3%). In cabbage sample from Oje, *Micrococcus* (89%), *Streptococcus* (11%). Cabbage sample from Challenge, *Pseudomonas* (56%), *Staphylococcus* (34%), *Streptococcus* (3%), and *Erwilnia* (7%). (Table 4)

During the second sampling, the following fungi were isolated from Gbagi *Cladosporium sp.* (65%), *Fusarium sp. (*13%), *Alternaraia sp.* (13%), *Aspergillus sp.* (9%). From Oje, *Mucor sp.* (51%), *Alternaria sp.* (38%), *Cladosporium sp.* (5%), *Aspergillus sp.* (5%); from Monatan, *Fusarium sp.* (45%), *Sclerotinia sp.* (34%), *Alternaria sp.* (14%), *Rhizopus sp.* and *Aspergillus* 1% respectively; from Bodija, *Mucor* (71%), *Cladosporium* (27%), *Rhizopus* (1%) and from challenge, *Alternaria sp.* (40%), *Scleorinia sp.* (30%), *Cladosporium sp.* (16%), *Aspergillus sp.* (6%). *Aspergillus sp.*, *Alternaria*, *sp.* and *Cladosporium* sp., were isolated from cabbage samples purchased from Gbagi, Oje, Monatan, and challenge. *Fusarium sp.* occured only in samples from Gbagi and Monatan. *Rhizopus sp.* occurred in samples from Monatan, Bodija and Challenge. *Sclerotinia sp.* occured in Monatan and Challenge. (Table 5)

Bacillus sp. was isolated from samples of cabbage purchased from Gbagi and Oje, with the following percentage occurrence 61% and 20% respectively. *Staphylococcus sp.* occurred in Gbagi and Bodija having 16% and 68% respectively. *Micrococcus sp.* occurred in Gbagi, Bodija, Monatan and Challenge having 23%, 5%, 19% and 61% respectively. *Streptococcus sp.* occurred in Bodija samples and Oje having 27% and 80% respectively. *Xanthomonas sp.* occurred in Monatan and Challenge samples having 81% and 39% respectively.

During the third sampling, *Sclerotinia sp.* appeared only in samples from Gbagi with 60% of occurrence, *Aspergillus sp.* appeared in all samples (Gbagi 12%, Oje 8%, Monatan 5%, Challenge 15%, Bodija 3%. *Mucor sp.* appeared in samples from Gbagi 28%, Monatan 51%, and Bodija 29%. *Cladosporium sp.* occurred in samples from Oje 92%, Monatan 44%, Bodija 68%, making it the highest occurring fungi in all the samples. *Alternaria sp.* occurred only in samples from Challenge having 99 % (Table 6).

Pseudomonas and *Erwinia sp.* occurred only in samples from Gbagi having 35% and 65% respectively. *Bacillus sp.* occurred in samples from Bodija 72%, Oje 45%, and *Staphylococcus sp.*occurred in samples from Bodija, Monatan and Oje having 28%, 22% and 60% respectively making it the highest occurring bacteria in all the samples. *Streptococcus* appeared only in monatan samples having 78%, *Micrococcus sp.* and *Xanthomonas sp.* occurred only Challenge samples having 67% and 33% respectively (Table 6)

Table 6 Percentage occurrence of microorganisms in cabbage during the third sampling

Location	Organisms	Percentage of Occurrence (%)
Gbagi	*Sclerotinia sp.*	60
	Mucor sp.	28
	Aspergillus sp.	12
Oje	*Aspergillus sp.*	8
	Cladosporium sp.	92
Monatan	*Mucor sp.*	51
	Cladosporium sp.	44
	Aspergillus sp.	5
Challenge	*Alternaria sp.*	99
	Aspergillus sp.	1

Bodija	*Mucor sp.*	29
	Aspergillus sp.	3
	Cladosporium sp.	68
Nutrient Agar		
Gbagi	*Pseudomonas sp.*	35
	Erwinia sp.	65
Bodija	*Staphylococcus sp.*	28
	Bacillus sp.	72
Monatan	*Staphylococcus sp.*	22
	Streptococcus sp.	78
Oje	*Bacillus sp.*	45
	Streptococcus sp.	60
Challenge	*Micrococcus sp.*	67
	Xanthomonas sp.	42

3.4. Effect of Acetic Acid Concentration and Exposure Time on Microbial Load.

The increase in the concentration of acetic acid (vinegar) with exposure time has great influence on microbial load, but acetic acid has higher bactericidal activity and low antifungal activity. Increase in the concentration of acetic acid and exposure time the number of bacteria susceptible to acetic acid also increased but reduced in the case of fungi. The highest reduction rate was observed in 2.5% acetic acid for 10minutes in bacteria while the lowest was observed in 2.5% acetic acid for 10 minutes in fungi.

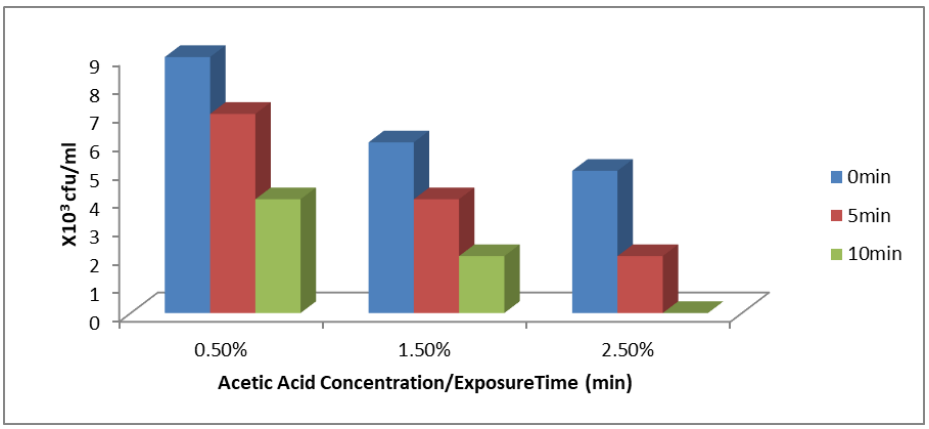

Figure 1 Effect of acetic acid concentration and exposure time on microbial load

Microbial contamination occurs as a result of poor quality of sanitation at all levels of cabbage production i.e. during the process of cultivation, watering, harvesting, transportation, storage and processing [12]. The results and microorganisms isolated in this state are comparable in evidence to results of other studies that isolated organism from cabbage vegetables both within and outside Nigeria [13]. The high microbial count observed for cabbage samples in this study are similar to those that were obtained in other studies [11]. The high microbial contamination observed in this study shows a reflection of storage and handling conditions and period of storage before they were obtained for sampling. Bacteria and fungi on storage materials may be transferred to the stored produce and cause cross contamination among produce kept together. The multiplication of microorganisms depend on the storage conditions especially those that are psychrophilic [14]. Some microorganisms isolated in this study may be part of the normal flora of cabbage or may be contaminant from soil, irrigation, water, environment during transportation, storage condition or/and handling condition [15].

Pseudomonas sp., Erwinia sp., Bacillus sp., are natural flora among most common vegetable bacteria while *Fusurium sp., Clalosporium sp., Sclerotinia sp.* are soil contaminant that contaminate vegetables. The presence of *Staphylococcus aureus* is of Public Health concern, while *Bacillus Sp.,* are capable of causing food-borne illnesses, and these present a need to safeguard the health of consumers by proper washing and decontamination of cabbage and other similar vegetables which are consumed without treatment.

The result obtained from this study confirms previous report of microbial load reduction observed in fruits and vegetables washed or rinsed in vinegar. The efficacy of method used for the reduction of microbial load is usually dependent on the type of treatment, type and physiology of target organism, characteristics of produce surfaces, exposure time and concentration of cleaner of sanitizer, pH & temperature [16]. Increase in the concentration of acetic-acid with exposure time, which reduced the microbial population in cabbage is in line with what was observed in their study. The reduction in microbial load of the cabbage when treated with acetic-acid can be attributed to the further reduction in pH resulting from increased vinegar concentration since most bacteria do not survive in acidic pH. The non-effect of acetic acid concentration and exposure time on the fungi associated with the cabbage samples suggests that fungi survive acidic pH [17].

Decontamination with 1.5% to 2.5% acetic acid is a non-toxic, simple and in-expensive means of reducing the risk of food-borne illness in Nigeria. A disadvantage of acetic acid is that it may change the taste of the vegetable but this can be overcome if the cabbage is rinsed in a clean portable drinking water.

4. Conclusion

Despite the high microbial load obtained from cabbage samples in this study, it is note- worthy that these cabbage samples showed no visible sign of spoilage and thus, physical appearance may be a poor criterion for analyzing the quality of cabbage and probably other vegetables too. The cabbage obtained from the five locations looked fresh but were heavily loaded with bacteria and fungi. It is thus necessary that cabbage be washed and well treated with vinegar or even table salt solution to reduce the microbial load of cabbage to a tolerable rate for human consumption. It is also necessary for the seller to observe strict hygienic measures to ensure they do not serve as a source of microbial contaminants.

Recommendation

Researchers within this study conclude that it is necessary for sellers and vendors in the different markets to observe strict measures and practices of hygiene to ensure they do not serve as a sources or reservoirs of microbial contaminants for their goods especially when it comes to vegetables and ready to eat foods. Government in Nigeria should also work harder to provide better facilities for the markets such as portable water, sinks and taps so that traders can improve their hygiene. Traders unions can also play the role of educating their members by bringing trained personnel to give talks and carry out campaigns to improve knowledge and awareness amongst them.

Compliance with ethical standards

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Disclosure of conflict of interest

All the authors disclose no conflict of interest.

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