Bactericidal Effect of Selected Preservatives on Fresh-Cut Vegetables

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Abstract

The bactericidal effect of acetic acid, calcium chloride and sodium hypochlorite sanitizers on natural microbial flora of Telfeira occidentalis leaves, Corchorus olistorus leaves and Amaranthus hybridus was investigated. The reduction in the bacterial density of the selected vegetables showed that acetic acid was found to be most effective followed by calcium chloride then Sodium hypochlorite. A total of thirteen (13) bacteria genera were isolated from the decontaminated vegetables. The isolated genera were Klebsiella sp., E.coli, Bacillus sp., Proteus sp., Staphylococcus sp., Serratia sp., Citrobacter sp., Corynebacterium sp., Streptococcus sp., Enterobacter sp., Acinetobacter sp., and Salmonella sp., when their morphological, gram staining and biochemical characteristics were compared to known taxa. The use of chemical decontaminants/sanitizers could be preferred instead of sterile water as it showed the best and effective reduction rate in the microbial loads on the selected vegetables.

Keywords: Decontamination, Bacteria, Vegetables, Preservatives, Bactericidal, Sanitizer

Introduction

Fresh fruits jointly vegetables are an essential component of a healthy diet, able to decrease the risk of cardiovascular diseases and cancer (Allende *et al*., 2006). In the last years, their consumption has continued to grow rapidly linked to the increased public awareness of their health benefits, even if not remains below the recommended daily intake in many countries, due to barriers such as complacency and lack of willpower to change the diet [Ragaert *et al*., 2004].

In the last two decades, food scientists have attempted to develop new technologies that can improve the quantity and quality of fresh-cut products with the main objective of increasing their production without affecting quality and environment, while fulfilling consumer expectations. In the same time, consumers have also become more critical on the use of synthetic additives to preserve food or enhance its characteristics such as color and flavor [Corbo*et al*., 2009]. Once traditional processing technologies have been able to provide microbiologically safe food products with acceptable quality characteristics, the next step forward is to design mild but reliable new treatments in order to achieve fresh-like quality products with a high nutritional value. There are varieties of methods used to reduce the population of microorganisms on whole and fresh-cut produce. Each method has distinguished advantages and disadvantages depending upon the type of produce, mitigation protocol and other variables. It is important to use washing and sanitizing protocols that are efficient. Sanitization has been defined by FDA (2008) as the art of treat-cleaning the produce by a process that is effective in destroying or substantially reducing the numbers of microorganisms of public health concern as well as other undesirable microorganisms without adversely affecting the quality of the product or its safety for the consumer [FDA, 2008].

Chemical sanitizers such as sodium hypochlorite [Adams *et al*., 2001], chlorine dioxide [Kim *et al*., 2009], sodium bisulfite [Krahn*et al*., 1977], sulfur dioxide [Bolin *et al*., 1977], organic acids [Adams *et al*., 2001], calcium chloride [Izumi and Watada, 1994], acidified sodium chlorite [Allende *et al*., 2009] and ozone [Nagashima and Kamoi, 1997] have been shown to reduce microbial population on fresh produce. Among these sanitizers, chlorine-based chemicals particularly liquid chlorine and hypochlorite are probably the most widely used sanitizers for decontaminating fresh produce [Francis and O'Beirne, 2002].This work was carried out to assess the bactericidal effect of selected chemical preservatives on some fresh-cut vegetables.

Materials and Methods

Collection of Vegetables: The fluted pumpkin leaves, amaranthus leaves and jute plant leaves used in this work were purchased from Ojakoko in Owo, Ondo state, Nigeria.

Processing of Vegetable Samples: The purchased vegetables were taken to the processing laboratory of the Department of Food Science and Technology, Rufus Giwa Polytechnic, Owo. They were set on a sterilized clean stainless steel table and were cut into small pieces using a sterilized clean stainless steel knife. The cut vegetables were put into different clean sterilized bowls respectively and were taken to the microbiology laboratory for analysis.

Preparation of Water and Chemical Treatments: Three chemical treatments (at 5%v/v); acetic acid, calcium chlorite, sodium hypochlorite and Sterile Water were applied to determine their effectiveness in preserving the fresh-cut.

Microbiological analysis of the decontaminated vegetables: The method of Beuchat (2000) was used for the decontamination. The chopped vegetables were aseptically transferred to clean beakers containing 100 ml (5%w/v) each of acetic acid, calcium chlorite, sodium hypochlorite and Sterile Water as control. The chemicals were allowed to react with the vegetables for 10minutes

Result and Discussion

Table1 showed the bacteria density of freshly-cut vegetables. A total 743 colonies were found in all the samples. For *Telferia occidentalis* leaves, the total bacterial count, total coliform count and total *Salmonella/Shigella* count were $204x10^5$,$37x10^5$,$17x10^5$respectively;*Corchor usolitorius* had $132x10^5$, $62x10^5$ and $27x10^5$ respectively; while *Amaranthus hybridus* had $186x10^5$, $56x10^5$ and $22x10^5$cfu/g respectively.

Table 2 showed the bacteria density of washed vegetables using sterile water. A total 449 colonies were found in all the samples. For *Telferia occidentalis* leaves, the total bacteria count, total coliform count and total *Salmonella/Shigella* count were $117x10^5$, $21x10^5$ $08x10^5$ respectively; *Corchorus olitorius* leaves had $98x10^5$, $36x10^5$ and $10x10^5$ respectively. There was a decrease in the total colonies from the samples from $743X10^5$to $449X10^5$. This could be attributed to the efficacy of the sterile water on the vegetables in reducing the bacteria density of the washed vegetables.

Table 3 showed the efficacy of chemical decontamination on bacteria density of the selected vegetables. The decontamination assay revealed that acetic acid had the potential in reducing the bacteria load in the three selected vegetables However, Calcium chloride and Sodium hypochlorite showed to have the same effect in reducing the bacteria load of the vegetables. In total, acetic acid had the best decontamination potential followed calcium chloride and sodium chloride .There was a decrease in the bacteria density when compared to that of table1 and 2. This indicates that the chemical decontaminants were more effective in reducing the bacterial density of the vegetables.

Table 4 showed the biochemical characterization of bacteria isolates from the decontaminated vegetables. The isolates were characterized and thirteen (13) proposed organisms of both gram positive and gram negative were identified. They include; *Klebsiella sp., E.coli, Proteus sp., Staphylococcus sp., Bacillus sp., Pseudomonas sp., Citrobacter sp., Streptococcus sp., Corynebacterium sp., Enterobacter sp., Salmonella sp., Acinetobacter sp and Serratia sp.*

Table 5 showed the percentage occurrence of isolates from the decontaminated vegetables. It showed the occurrence pattern of bacterial isolates and their frequency .The isolated bacterial genera were *Klebsiella sp.*, (16%, 21% and 13%) for *T. occidentalis*, *C.olitorius* and *A.hybridus* leaves respectively. *E.coli* (09%, 12%, and 08%) for, *T. occidentalis C.olitorius* and *A.hybridus* leaves respectively. *Salmonella sp.*, (05%, 06%, and 13%), *Pseudomonas sp.*, (12%, 18%, and 08%), *Staphylococcus sp* (18%, 23%, and 09%), *Citrobacter sp.* (ND, ND, and 07%), *Serratia sp.*, (06%, ND, and 08%), *Streptococcus sp.*, (04%, 07%, and ND), C*orynebacterium sp.*, (ND, 04%, and 05%), *Enterobacter sp.*, (08%, ND, and 06%), and *Acinetobacter sp.*, (ND, ND, and 05%) for *T. occidentalis*, *C.olitorius* and *A.hybridus* leaves respectively.

Staphylococcus sp. has the highest occurrence in *T. occidentalis* and *C.olitorius* leaves with the values of 18% and 23% respectively, while *Klebsiella sp.*and *Bacillus sp.* had the highest occurrence in *A.hybridus* with the value 13% each. However, *Citrobacter sp.* was not detected in *T. occidentalis* and *C.olitorius*, *Serratia sp.* was not detected in *C.olitorius*, *Streptococcus sp.* was not detected in *A.hybridus*, *Enterobacter sp.* was not detected in *C.olitorius* leaves, *Acinetobacter sp.* was not detected in *T. occidentalis*, *C.olitorius* leaves. Fresh-cut vegetable is one of the hottest growing convenience foods in history because it offers freshness, nutrition and convenience. However, contamination of fresh vegetables with human pathogens can occur anywhere in the journey from farm to table. Given that fresh-cut vegetables are marketed as pre-washed and ready to eat and not subjected to further microbial killing steps, the need for effective disinfecting agents during vegetable washing is clearly evident to ensure produce safety [Beuchat, 1996]. Washing vegetables with sterile water is the most used decontamination method in household level, although its decontamination efficacy is very low when compared to chemical decontaminants as shown in this study when table 2 and 3 were compared, this supported the studies reported previously by Allende *et al*.[2004]; Lukasik *et al*.[2003]. The researchers also confirmed that the population of pathogenic bacteria on vegetable is limited.

However, chemical decontaminants such as sodium hypochlorite, calcium chloride, organic acids and Sodium chloride have been shown to reduce microbial population of fresh-cut vegetables to depreciable amount as reported by Francis and O'Beirne, [2002]. Calcium chloride has been widely used as preservative and firming agent in the fruit and vegetable industry for whole and fresh-cut commodities. Saftner and lee [2003] studied the effect of calcium chloride treatment on fresh-cut honeydew chunk and reported that vegetables treated with calcium chloride generally is effective in reducing and keeping the microbial load and also remain firmer than controls during storage.

Francis and O'Beirne, [2002] reported that sodium hypochlorite used at levels of 50e200ppm with typical contact times of less than 5 minutes is very effective for decontaminating fresh-cut produce. On the other hand, peroxyacetic (peracetic) acid is a strong oxidizing agent that has been approved by FDA as a disinfectant for fruits and vegetables and has showed to be effective against listeria monocytogenes, *salmonella sp.*,and *E. coli*. When these chemical decontaminants (acetic acid, calcium chloride and sodium hypochlorite) were used on the vegetables (fluted pumpkin leaves, jute plant leaves and amaranthus) a rapid decrease on the bacterial density was obtained as shown in table 3 proofing that this study is in accordance with these other research studies mentioned earlier in reducing the microbial loads on fruits and vegetables.

In comparing table 1, 2, and 3 to know which chemical decontaminant that is more effective in reducing the microbial loads on the selected vegetables. The initial bacterial density on fluted pumpkin leaves under total bacteria counts were 204, 37 and 17 colonies respectively, while on jute plant leaves under total bacteria count, total coliform count and total *salmonella/Shigella* counts were 132, 52, and 27 colonies respectively, and on *A. hybridus*, total bacteria count, total coliform count and total *salmonella/Shigella* count were 186, 56, and 22 colonies respectively. Although the bacteria density of the selected vegetables reduced after washing with sterile water, a rapid decrease in the bacteria density after washing the vegetables in chemical decontaminant was observed. It showed that calcium chloride was more effective on fluted pumpkin leaves under total bacteria count (48x10^5*cfu/g*) while acetic acid was more effective under total coliform count (03x10^5) and total *Salmonella/Shigella* counts (Nil) indicating that nothing was found. For *C.olitorius* leaves; it showed that acetic acid was more effective under total bacteria count, coliform and *Salmonella/Shigella* counts (28x10^5, 12x10^5 and Nil) respectively. For *A.hybridus;* calcium chloride was more effective under total bacteria and coliform counts (56x10^5 and 09x10^5) respectively, while acetic acid was more effecting under total Salmonella/Shigella counts (Nil) as no colonies was found. From this it could be deduced that acetic acid showed to be the most effective followed by calcium chloride in reducing the bacterial density of the selected vegetables.

The outbreak of infections and communicable diseases in tropical parts of the world is primarily as a result of food poisoning due to microbial contamination [Jay, 2005]. They are often responsible for acute gastroenteritis, abdominal discomfort, pain and diarrhea in infants and young adults [WHO, 2010]. A total of thirteen (13) bacteria genera were isolated and obtained from decontaminated vegetables. They include; *Klebsiella sp., E.coli, Salmonella sp., Staphylococcus sp., Bacillus sp., Proteus sp., Citrobacter sp., Serratia sp., Enterobacter sp., Corynebacter sp., Streptococcus sp., Pseudomonas sp. and Acinetobacter sp.* Table5 showed that on *T.occidentalis and C.corchorus* leaves; *Staphylococcus sp* has the highest occurrence (23%) and (18%) respectively, followed by *Klebsiella sp.*,(21%) on jute plant leaves, *Pseudomonas sp* (18%) on *C. corchorus* leaves and *Klebsiella sp.*, (16%) on fluted pumpkin leaves. For *A. hybridus*, *Klebsiella sp.*, and *Bacillus sp.*,have the highest occurrence (13%) each, followed by *proteus sp.*, *Salmonella sp.*,and *Staphylococcus* sp. (09%) respectively. The high frequency of these organisms could be as a result of contamination through the use of contaminated water used for irrigation, manure, natural soil flora, human pathogens (during harvesting and postharvest process like washing) which have many health implications. [Ray and Bhunia, 2007].*Staphylococcus sp.* is known to cause illnesses ranging from pimples and boils to pneumonia as reported by Karabay*et al*., 2007. The incidence of *E.coli* was fairly high on the vegetables. The presence of *E.coli* is commonly used as an indicator of fecal contamination in foods and its existence indicates the likely presence of pathogens such as *salmonella* and *Shigella*. Thus determination of *E.coli* is important in the food safety point of view. [Meldrum *et al*., 2009]. Altogether the presence of all these isolated organisms in the vegetables creates a public health concern.

Conclusion

The results obtained from this study showed that the level of microbial contamination of the selected vegetables was fairly high and sterile water had limited effect in reducing these microbial loads. The use of chemical decontaminants/sanitizers could be preferred instead of sterile water as it showed more effective reduction rate in the microbial loads on the selected vegetables. This study also demonstrated the strong efficacy of the chemical sanitizers on microbial load reduction on the selected vegetables.

Table 1: Bacteria Density of Fresh-cut Vegetables

Bacteriological Analysis (10^5CFU/g)			
Vegetable Samples	Total bacteria	Total coliform	Total Salmonella/Shigella.
Telferia occidentalis	204	37	17
Corchorus olistorius	132	62	27
Amaranthus hybridus	186	56	22

Table 2: Bacteria Density of Washed Vegetables Using Sterile Water

Bacteriological Analysis (10^5CFU/g)			
Vegetable Samples	Total bacteria	Total coliform	Total Salmonella/Shigella.
Telferia occidentalis	117	21	08
Corchorus olitorius	98	36	10
Amaranthus hybridus	124	24	11

Table 3: Effect of Decontamination on Bacteria Density of selected vegetables

Vegetable samples	Chemical sanitizers	Bacteriological Analysis (10^5CFU/g)		
		Total bacteria	Total Coliform	Total Salmonella/Shigella
Telferia occidentalis	Acetic acid	53	03	Nil
	Calcium chloride	48	08	02
	Sodium hypochlorite	62	08	04
Corchorus olitorius	Acetic acid	28	12	Nil
	Calcium chloride	34	18	04
	Sodium hypochlorite	32	13	04
Amaranthus hybridus	Acetic acid	62	12	Nil
	Calcium chloride	56	09	04
	Sodium hypochlorite	76	16	04

Table 4: Biochemical Characterization of Bacteria Isolates from the decontaminated Vegetables

	Distinct isolates												
Tests	A	B	C	D	E	F	G	H	I	J	K	L	M
Gram stain	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve	+ve
Citrate	+ve	-ve	+ve	+ve	-ve	+ve	-ve	+ve	+ve	-ve	-ve	+ve	+ve
Oxidase	-ve	-ve	–ve	-ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Catalase	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve
Coagulase	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve
Urease	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve
Indole	-ve	-ve	-ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve
Methyl red	-ve	-ve	-ve	+ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	+ve	-ve
Motility	-ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	-ve	-ve	-ve	-ve
Glucose	-ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Sucrose	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve
Manitol	-ve	-ve	+ve	-ve	-ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	-ve
Lactose	-ve	+ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve	-ve	+ve	-ve
Proposed microbes	Kleb	E.coli	Salm	Prot	Bac	Pseu	Stap	Citro	Serra	Stre	Cory	Ent	Acin

Key: Kleb=*Klebsiella sp*.,*E.Coli=Escherichia coli*; Salm=*Salmonella sp*.,Prot=*Proteus sp*.,Bac=*Bacillus sp*.,Pseu=*Pseudomonas sp*.,Stap=*Staphylococcus sp*.,Citro=*Citrobacter sp*., Serra=*Serratia sp*., Strep= *Streptococcus sp*., Cory=*Corynebacterium sp*., Ent=*Enterobacter sp*., Acin= *Acinetobactersp*.

Table 5: Percentage occurrence of isolates from the Decontaminated vegetables

Microbes	*Telferia occidentalis*	*Corchorus olitorius*	*Amaranthus hybridus*
Klebsiella sp.	16	21	13
E.coli	09	12	08
Salmonella sp.	05	06	09
Proteus sp.	08	ND	09
Bacillus sp.	14	09	13
Pseudomonas sp.	12	18	08
Staphylococcus sp.	18	23	09
Citrobacter sp.	ND	ND	07
Serratia sp.	06	ND	08
Streptococcus sp.	04	07	ND
Corynebacterium sp.	ND	04	05
Enterobacter sp.	08	ND	06
Acinetobacter sp.	ND	ND	05

Key: ND = Not Detected.

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