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EFFECTS OF DIFFERENT PRETREATMENTS ON THE MICROBIOLOGICAL QUALITIES OF CARROTS (*DAUCUS CAROTA* L).

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ABSTRACT
Daucus carota (Subsp. sativus) is a vegetable that is normally eaten raw and since ready to eat vegetables do not need further preparation before consumption they could contain pathogens that can pose serious health concerns. In this study therefore the effects of various pretreatments on the surface microflora of carrots (*Daucus carota* L.) was investigated using standard microbiological techniques. Fresh carrots bought from different markets in Akure, were grouped into 8 groups. Each group was washed with different pretreatments while the last group was not washed at all. At different contact time (10minutes, 20minutes, and 30minutes), the microbial types and load were determined. *Klebsiella ozaenae, Micrococcus luteus, Staphylococcus epidermidis, Enterobacter aerogenes, Escherichia coli, Bacillus pasteurii, Citrobacter freudii* were the bacterial species while only four fungi; *Mucor mucedo, Sacharomyces cerevisiae, Peacilomyces viriotii,* and *Aspergillus flavus* were isolated. The study showed that Vinegar had the greatest growth inhibitory effect on all the microbial types present on the surface of carrots. Some of the organisms inhibited were *Staphylococcus aureus, Bacillus cereus, B. pasteurii, E. coli, M. luteus, M. mucedo, P. viriotii and Rhizopus megricans*. Therefore vinegar should be exploited in washing the vegetable before consumption to prevent food borne illnesses.
Keywords: Fresh carrot, *Daucus carota* L., Pathogens, Pretreatments.

INTRODUCTION

Carrot (*Daucus carota* L.) is a vegetable that is consumed all over the world. It is rich in β-carotene which is a powerful antioxidant that helps in maintaining a healthy skin and also keeps one away from many diseases. Carrots are rich in alkaline elements which purify, revitalize the blood and also balance the acid alkaline ratio in the body. Moreover, it is rich in potassium which helps to maintain a healthy electrolyte balance and fluid level in

the cells of the body and also balance the high levels of sodium associated with hypertension thereby keeping the body's blood pressure under control. It is considered a low – calorie food, high in rich in fibers, providing a great variety of vitamins, minerals and other phytochemicals (Verbeyst *et al.*, 2012). Carrots are rich in falcarinol and facarindiol which have anticancer properties, therefore reducing the risk of various types of cancer (Strube and Lar, 1999).

Carrots like other fresh vegetables can become contaminated by pathogens at any point during pre-harvest or after harvesting, handling, contact with contaminated irrigation water, soil, manure, or fecal matter of wild animals. Therefore, consumption of such contaminated vegetables can cause severe outbreaks of food borne diseases (Maatt *et al.*, 2013) more so that it is normally consumed raw. This study therefore is carried out to investigate the effects of different pretreatments (vinegar, brine, sugar solution, sodium hypochlorite, distilled water and moringa seed aqueous extract) on the microbial qualities of carrots in order to checkmate the consumption of microorganisms laden carrots that may predispose one to food borne illnesses.

MATERIALS AND METHODS

Sample collection

Carrots: Fresh carrots were purchased from various markets located at different areas of Akure (Oja-Oba, Isinkan, FUTA gate and Shasha), Ondo State, Nigeria. Each sample was kept in different clean polythene bags and labeled appropriately according to the place of purchase and then taken to the Microbiology Research Laboratory at the Federal University of Technology, Akure, Nigeria(FUTA) for analyses.

Preparation of different solutions used to rinse the carrots

Brine preparation: This was prepared according to Greger (2015)

Sugar solution preparation: The method of Davies (2006) was strictly followed.

Vinegar: the vinegar used was a product of Food condiments Nigeria Limited, Ogun state, Nigeria (NAFDAC approved).

Sodium hypochlorite: This was prepared according to the method of Rotola (1996).

Moringa seed aqueous extract: This was prepared according to the method of Beth (2005) with a slight modification.

Determination of the microbial quality of carrot after exposure to different pretreatments: Fresh carrots were grouped into 8 groups. The first group was washed with potable water, second group was washed with sterile distilled water, third group with brine (6%), the fourth group with vinegar (5% acetic acid), fifth group with sodium hypochlorite (0.05%), sixth group with sugar solution (5%), the seventh group with moringa seed aqueous solution (468g per 250ml (w/v)) while the last group was not washed at all. At different contact time (10minutes, 20minutes, and 30minutes), the microbial types and loads were evaluated using standard microbiological methods to know the effect of the pretreatments on the microbial quality. This procedure was repeated for the carrots bought from different markets.

Isolation and identification of microorganisms present on the surface of carrot: All the microbiological analyses were carried out using standard microbiological methods. Bacteria isolated were identified according to Holt *et al.* (1994) while the fungi isolated were identified according to Samson *et al.* (2010).

RESULTS

Effects of different pretreatments on the bacterial load on the surface of carrots

The different pretreatments used exerted varying degrees of effect on the bacterial load of the carrots bought from different markets that were used in this investigation. Carrots washsed with either water or the different solutions showed a great reduction in the bacterial population as compared with the unwashed carrots (fig. 1). The bacterial load reduced from 1.64 x 10^6 cfu/g to 4.5 x 10^3 cfu/g for the carrots bought from location A, from 3.9 x 10^5 cfu/g to 2.5 x 10^4 cfu/g for carrots bought from location B, from 9.1 x 10^5 cfu/g to 1.5 x 10^3 cfu/g for the ones bought from location C and 3.1 x 10^5 cfu/g to 4.0 x 10^2 cfu/g for the ones bought from location D. Carrots purchased from location D had the least bacterial load while the highest load was observed in carrots bought from location A.

Effects of contact times on the effectiveness of the different pretreatments on the bacterial load on the surface of carrots: The different pretreatments on carrots used exerted varying degrees of reduction of the bacterial load of carrots on its surface based on contact time (Figs. 2-6). The greatest reduction was observed after 30 minutes for all the pretreatments used while appreciable reduction was observed after 20 minutes.

Effects of the different pretreatments on the fungal load on the surface of carrots.

Carrots washed with either water or the different solutions showed a great reduction in the fungal population as compared with the unwashed carrots (Fig. 1). The fungal load reduced from 5.5 x 10^2 sfu/g to 4.0 x 10^0 sfu/g for the carrots bought from location A, from 4.5 x 10^3 sfu/g to 2.0 x 10^2 sfu/g for the carrots bought from location B, 7.2 x 10^2 sfu/g to 3.0 x 10^0 sfu/g for the carrots bought from location C and from 1.1 x 10^2 sfu/g to 2.0 x 10^0 sfu/g for the ones bought from location D. Carrot purchased from location D had the least fungal load while carrot purchased from location B had the highest load.

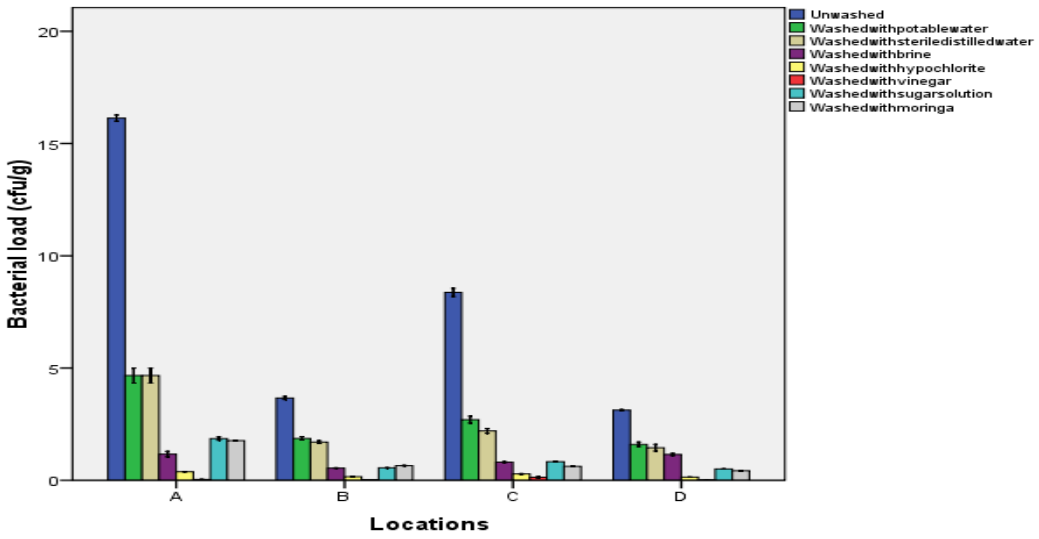

Figure 1: Effects of different pretreatments on the bacterial load (x10^5) on the surface of carrots purchased from selected markets in Akure Ondo State, Nigeria.

Key: A = Oja-Oba market ;B = FUTA area;C = Shasha market; D = Isinkan market

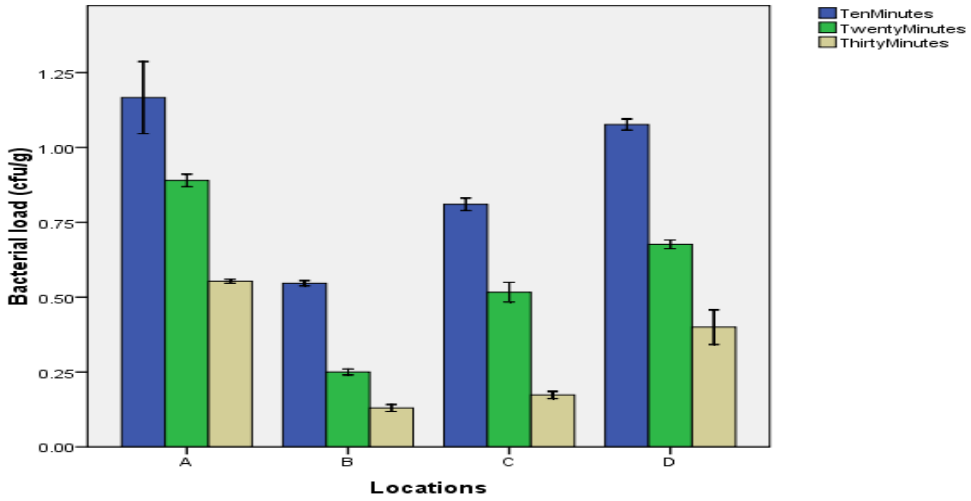

Figure 2: Effects of contact time with brine on the bacterial load (x10^5) on the surface of carrots purchased from selected markets in Akure, Ondo State, Nigeria.

Key: A = Oja-Oba market; B = FUTA area;C = Shasha market;D = Isinkan market

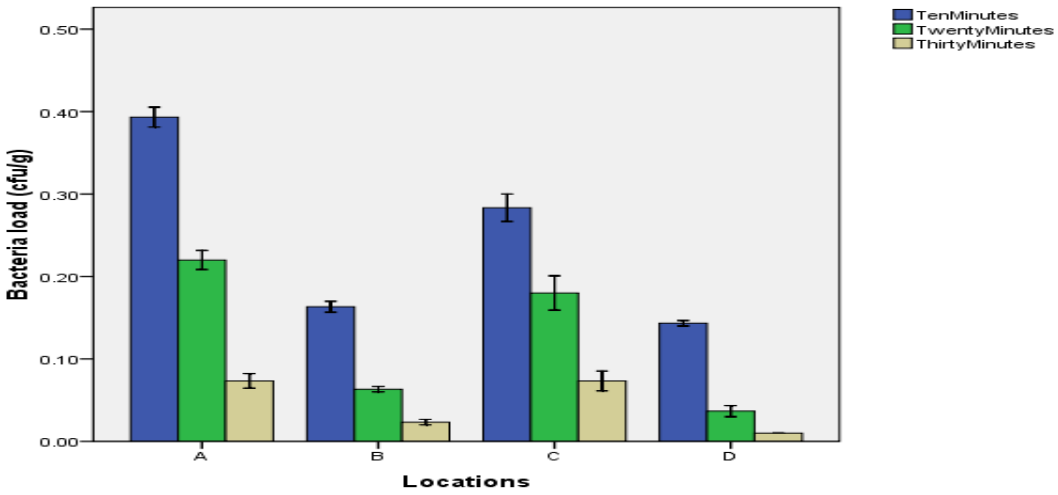

Figure 3: Effects of contact time with sodium hypochlorite on the bacterial load (x10^5) on the surface of carrots purchased from selected markets in Akure, Ondo State, Nigeria.
Key: A = Oja-Oba market; B = FUTA area; C = Shasha market; D = Isinkan market

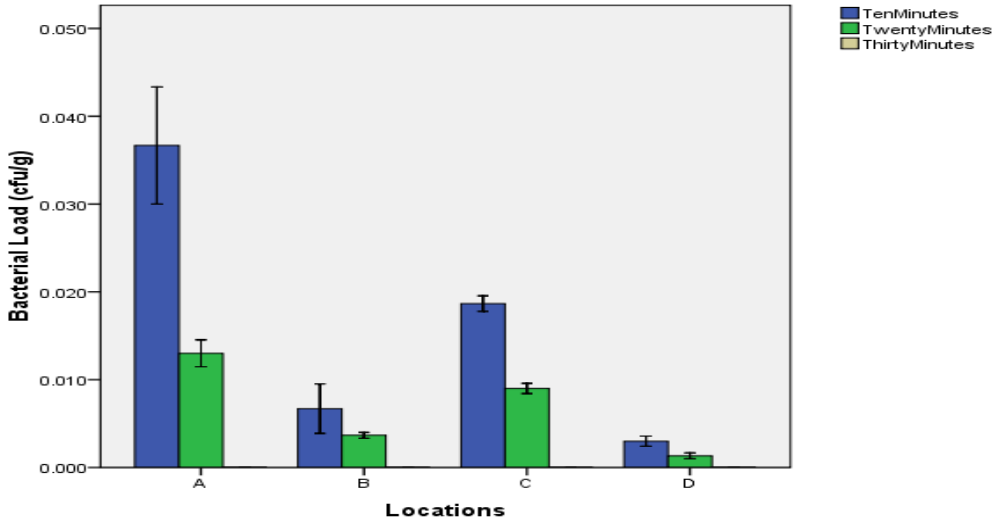

Figure 4: Effects of contact time with vinegar on the bacterial load (x10^5) on the surface of carrots purchased in selected markets in Akure, Ondo State, Nigeria.

Key: A = Oja-Oba market; B = FUTA area;C = Shasha marke;t D = Isinkan market

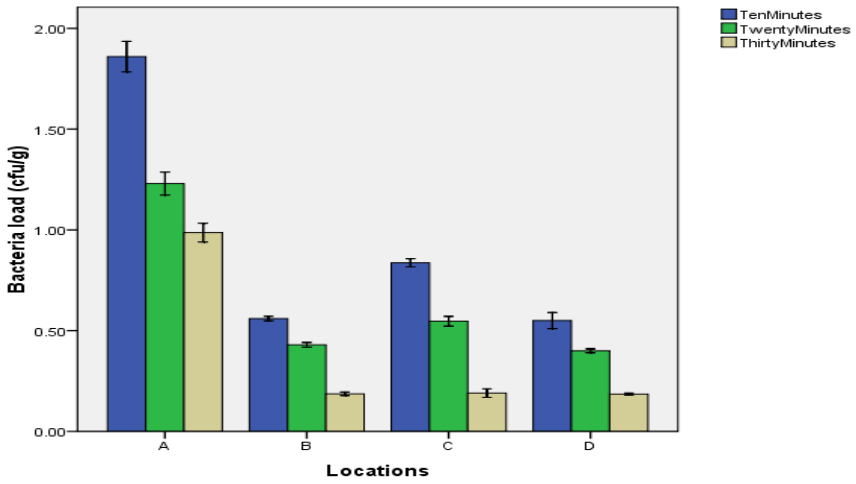

Figure 5: Effects of contact time with sugar solution on the bacterial load (x10^5) on the surface of carrots purchased in selected markets in Akure, Ondo State, Nigeria.

Key: A = Oja-Oba market; B = FUTA area; C = Shasha market; D = Isinkan market

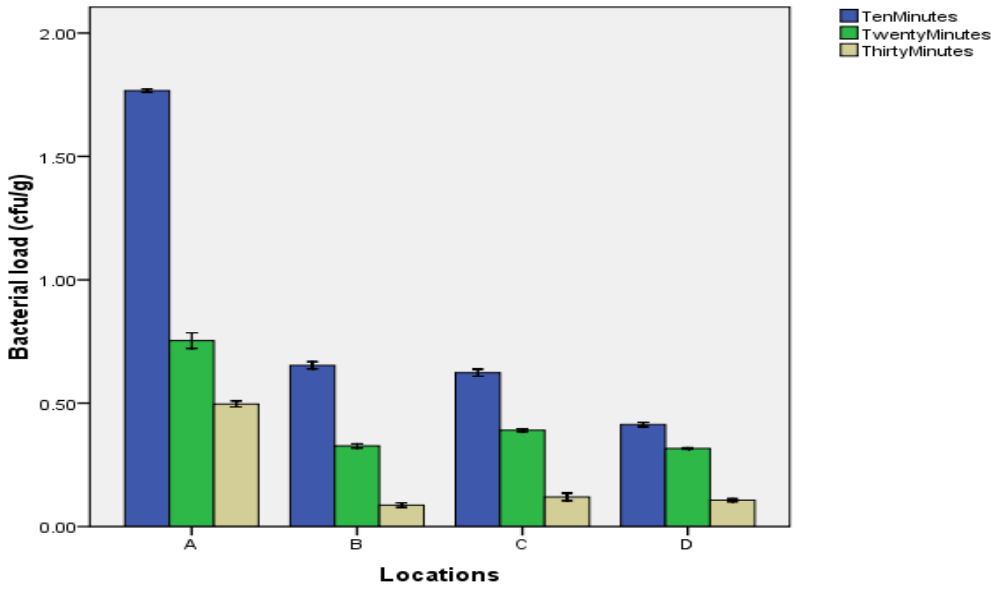

Figure 6: Effects of contact time with moringa on the bacterial load (x10^5) on the surface of carrots purchased in selected markets in Akure, Ondo State, Nigeria.

Key: A = Oja-Oba market;B = FUTA area; C = Shasha market; D = Isinkan market

Effects of contact times on the effectiveness of the different pretreatments on the fungal load on the surface of carrots: The different pretreatments on carrots used exerted varying degrees of reduction of the fungal load of carrots on its surface based on contact time (Figs. 8-12). The greatest reduction was observed after 30 minutes for all the pretreatments used while appreciable reduction was observed after 20 minutes.

Effects of different pretreatments on the microbial types on the surface of *carrots*: Seven different types of bacterial species were isolated from the carrot samples that were not washsed at all. These are: *Klebsiella ozaenae*, *Micrococcus luteus*, *Staphylococcus epidermidis*, *Enterobacter aerogenes*, *Escherichia coli*, *Bacillus pasteurii*, *Citrobacter freudii* while four fungi; *Mucor mucedo*, *Sacharomyces cerevisiae*, *Peacilomyces viriotii*, *Aspergillus flavus* were isolated. The types of microorganisms isolated however gradually reduced after 30 minutes contact time with the different pretreatments and in some cases, for example, for the ones washed with vinegar, no microbial growth was observed (Table 1). Similar trends were also observed in Tables 2 to 4.

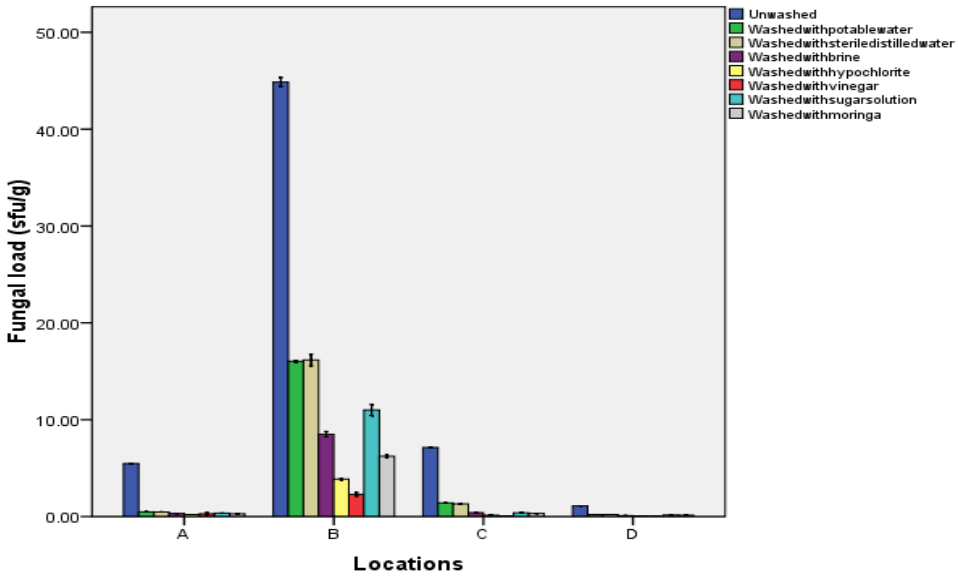

Figure 7: Effects of the different pretreatments on the fungal load (x10^2) on the surface of carrots purchased from selected markets in Akure, Ondo State, Nigeria.

KEY: A = Oja-Oba market ; B = FUTA area; C = Shasha market; D = Isinkan market

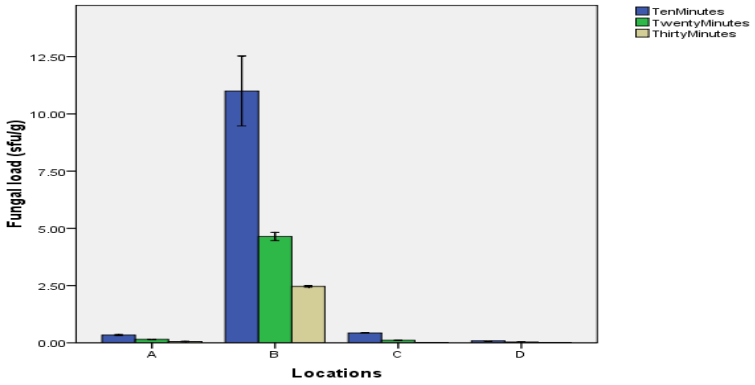

Figure 8: Effects of contact time with brine on the fungal load (x10^2) on the surface of carrots purchased in selected markets in Akure, Ondo State, Nigeria.

Key: A = Oja-Oba market ;B = FUTA area; C = Shasha market; D = Isinkan market

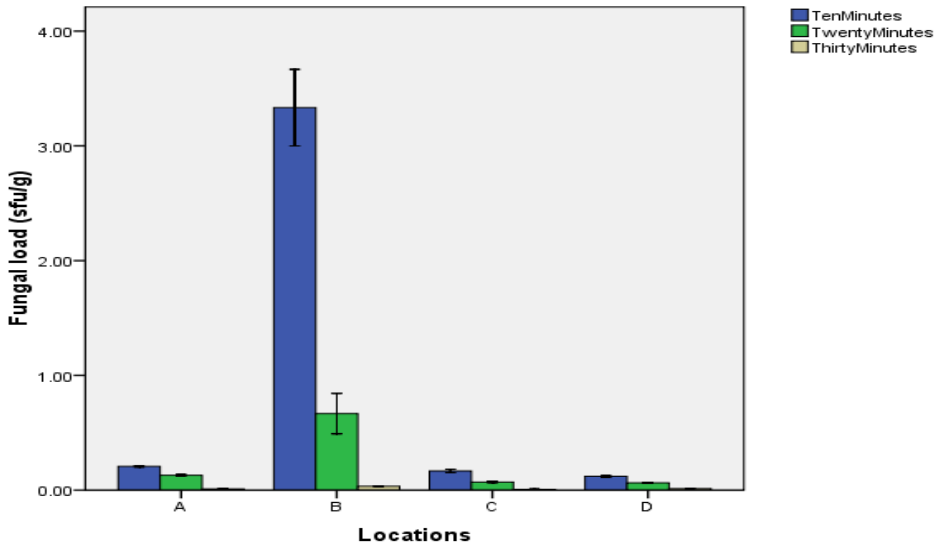

Figure 9: Effects of contact time with sodium hypochlorite on the fungal load (x10^2) on the surface of carrots purchased in selected markets in Akure, Ondo State, Nigeria.

Key: A = Oja-Oba market; B = FUTA area; C = Shasha market; D = Isinkan market

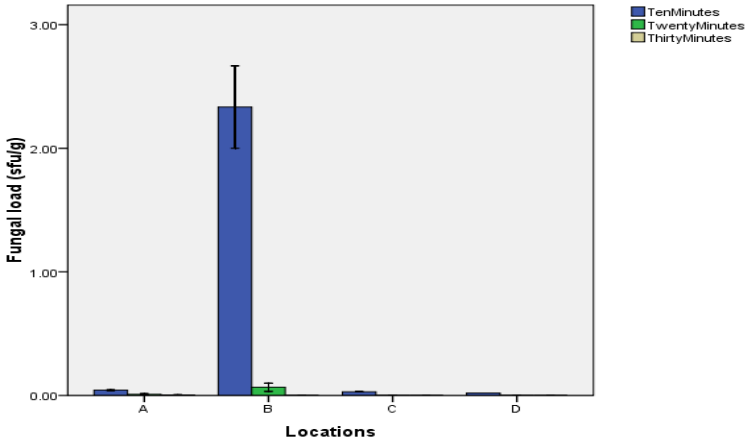

Figure 10: Effects of contact time with vinegar on the fungal load (x10^2) on the surface of carrots purchased in selected markets in Akure, Ondo State, Nigeria.
Key: A = Oja-Oba market;B = FUTA area; C = Shasha market; D = Isinkan market

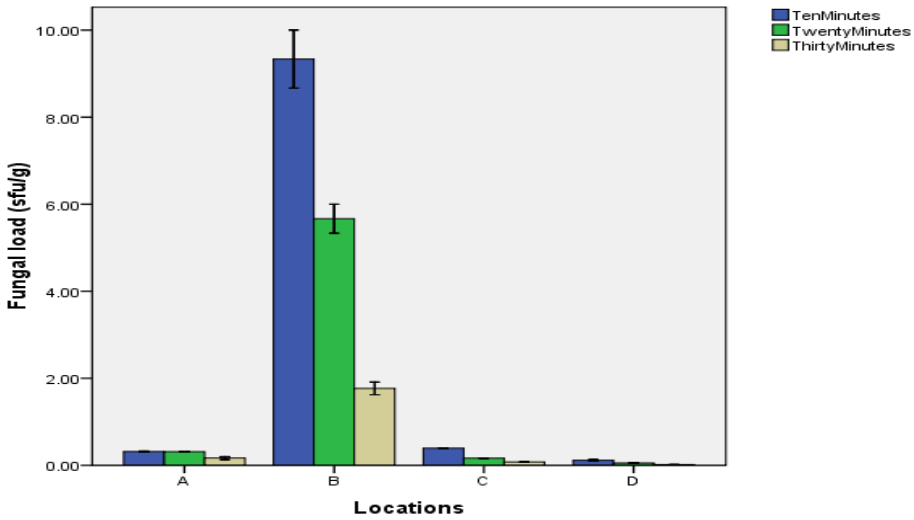

Figure 11: Effects of contact time with sugar solution on the fungal load (x10^2) on the surface of carrots purchased in selected markets in Akure, Ondo State, Nigeria.

Key: A = Oja-Oba market ; B = FUTA area; C = Shasha market; D = Isinkan market

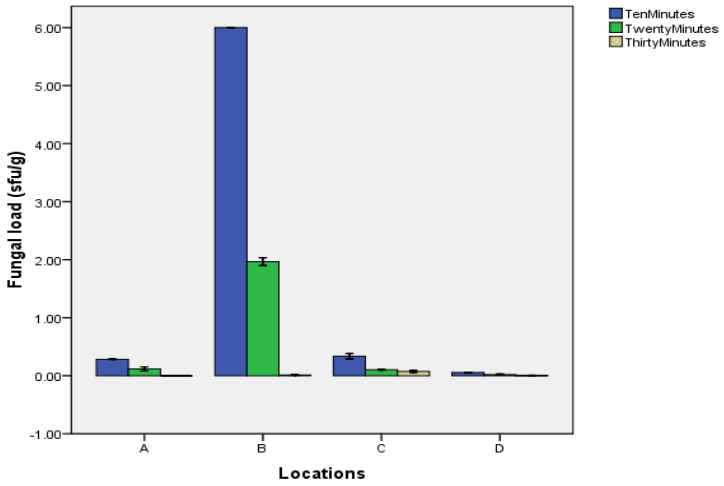

Figure 12: Effects of contact time with moringa on the fungal load ($x10^2$) on the surface of carrots purchased in selected markets in Akure, Ondo State, Nigeria.

Key: A = Oja-Oba market ; B = FUTA area; C = Shasha market; D = Isinkan market

Table 1: Effects of different pretreatments on the microbial types on the surface of carrots purchased from Oja-Oba market at 30 minutes contact time.

TYPES OF MICROORGANISMS (BACTERIA/FUNGI)	UNRINSED (CONTROL)	RINSED WITH POTABLE WATER	RINSED WITH VINEGAR (5%)	RINSED WITH BRINE (6%)	RINSED WITH SODIUM HYPOCHLORITE (0.05%)	RINSED WITH SUGAR SOLUTION (5%)	RINSED WITH MORINGA AQUEOUS EXTRACT (468G/250ML)
BACTERIA	*Klebsiella ozaenae, Micrococcus luteus, Staphylococcus epidermidis, Enterobacter aerogenes, Escherichia coli, Bacillus pasteurii Citrobacter freudii.*	*Staphylococcus epidermidis. Micrococcus luteus, Enterobacter aerogenes, Bacillus pasteurii Citrobacter freudii.*	Nil	*Staphylococcus aureus,Citrobacter freudii.*	*Bacillus pasteurii*	*Bacillus pasteurii, Citrobacter freudii.*	*Bacillus pasteurii*
FUNGI	*Mucor mucedo, Sacharomyces cerevisiae, Peacilomyces viriotii, Aspergillus flavus.*	*Sacharomyces cerevisiae, Peacilomyces viriotii, Mucor mucedo.*	Nil	*Aspergillius flavus.*	Nil	*Aspergillius flavus.*	Nil

Table 2: Effects of different pretreatments on the microbial types on the surface of carrots purchased from FUTA area at 30 minutes contact time.

TYPES OF MICROORGANISMS (BACTERIA/FUNGI)	UNRINSED (CONTROL)	RINSED WITH POTABLE WATER	RINSED WITH VINEGAR (5%)	RINSED WITH BRINE (6%)	RINSED WITH SODIUM HYPOCHLORITE (0.05%)	RINSED WITH SUGAR SOLUTION (5%)	RINSED WITH MORINGA AQUEOUS EXTRACT (468G/250ML)
BACTERIA	*Klebsiella ozaenae, Micro-coccus luteus, Staphylococcus aureus, Enterobacter aerogenes, Staphylococcus epidermidis, Escherichia coli.*	*Staphylococcus aureus, Streptobacillus moniliforms, Staphylococcus epidermidis.*	NIL	*Staphylococcus aureus, Klebsiella ozaenae Micrococcus luteus.*	*Klebsiella ozaenae*	*Staphylococcus aureus, Micrococcus luteus.*	*Staphylococcus aureus.*
FUNGI	*Mucor mucedo, Sacharomyces cerevisiae, Rhizopus megricans*	*Mucor mucedo, Sacharomyces cerevisiae, Rhizopus megricans*	NIL	*Sachraromyces cerevisiae*	*Sachraromyces cerevisiae*	*Sachraromyces cerevisiae, Mucor mucedo*	*Sachraromyces cerevisiae.*

Table 3: Effects of different pretreatments on the microbial types on the surface of carrots purchased from Shasha market at 30 minutes contact time.

TYPES OF MICROORGANISMS (BACTERIA/FUNGI)	UNRINSED (CONTROL)	RINSED WITH POTABLE WATER	RINSED WITH VINEGAR (5%)	RINSED WITH BRINE (6%)	RINSED WITH SODIUM HYPOCHLORITE (0.05%)	RINSED WITH SUGAR SOLUTION (5%)	RINSED WITH MORINGA AQUEOUS EXTRACT (468G/250ML)
BACTERIA	*Bacillus subtilis, Bacillus cereus, Staphylococcus epidermidis, Aerobacter aerogenes, Micrococcus luteus.*	*Proteus mirabilis, Aerobacter aerogenes Bacillus subtilis.*	Nil	*Staphylococcus aureus, Micrococcus luteus*	Nil	*Staphylococcus aureus, Micrococcus luteus, Klebsiella ozanae*	*Staphylococcus aureus, Aerobacter aerogenes*
FUNGI	*Sacharomyces cerevisiae, Peacilomyces viriotii, Penicillium italicum.*	*Sacharomyces cerevisiae, Peacilomyces sp,*	Nil	*Sachraromyces cerevisiae*	*Sachraromyces cerevisiae*	*Sachraromyces cerevisiae, Pleurothecium recurvatum.*	Nil

Table 4: Effects of different pretreatments on the microbial types on the surface of carrots purchased from Isinkan market at 30 minutes contact time.

TYPES OF MICROORGANISMS (BACTERIA/FUNGI)	UNRINSED (CONTROL)	RINSED WITH POTABLE WATER	RINSED WITH VINEGAR (5%)	RINSED WITH BRINE (6%)	RINSED WITH SODIUM HYPOCHLORITE (0.05%)	RINSED WITH SUGAR SOLUTION (5%)	RINSED WITH MORINGA AQUEOUS EXTRACT (468G/250ML)
BACTERIA	*Micrococcus luteus, Staphylococcus aureus, Proteus mirabilis, Staphylococcus epidermidis, Bacillus subtilis.*	*Staphylococcus aureus, Proteus mirabilis, Staphylococcus epidermidis.*	Nil	*Staphylococcus aureus.*	Nil	*Staphylococcus aureus, Micrococcus luteus.*	*Bacillus subtilis.*
FUNGI	*Sacharomyces cerevisiae, Aspergillus nidulans, Pleurothecium recurvatum.*	*Aspergillus nidulans, Pleurothecium recurvatum.*	NIL	*Sachraromyces cerevisiae, Rhizopus megricans.*	Nil	*Sachraromyces cerevisiae, Rhizopus megricans.*	Nil

DISCUSSION

In this study, the effect of different pretreatments on the microflora of carrot was investigated. The high microbial load found on unwashed carrot can be as a result of the fact that carrot is a root vegetable and therefore can easily be contaminated on the field during application of fertilizer, irrigation water, manure or faecal matter of wild animals (Jorge *et al*., 2015). Carrots can also become carrier of different bacterial species and or their toxins due to processing and storing errors (Kimberly, 2015). High fungal load on carrots purchased in FUTA area can be as a result of storing error or nearness to the main road where dust is raised from time to time by moving vehicles. Some of the microorganisms isolated in this investigation (*Staphylococcus aureus, Escherichia coli, B. cereus, Aspergillus flavus*) are known to be pathogenic. For example, *E. coli* is known to cause gastroenteritis, a food borne infection (Madappa, 2016). Bacterial like *B. cereus and S. aureus* can cause significant food-borne illness (Ahmad, 2016) while *A. flavus* can also lead to food poisoning (Machida and Gomi, 2010). Washing with potable water was observed to remove some of these microorganisms while vinegar was able to totally eliminate the microflora found on some carrots after contact time of 30minutes. The contact time played a major role in the inhibition of the microflora found on carrot. The microbial load reduced as the contact time increased this agrees with the findings of Adebolu and Ifesan (2001). Vinegar exerted the highest effect in reducing the microbial flora of carrots followed by sodium hypochlorite then followed by moringa seed aqueous extract and then the other pretreatments. The inhibition mediated by moringa agrees with the findings of Priadarshini *et al*. (2013) who worked on petroleum ether leaf extract of *Moringa oleifera* which showed maximum inhibition on the growth of *E. coli, B. subtilis, P. aeruginosa* and *Proteus vulgaris.*

CONCLUSION

Since some of the microflora found on unwashed carrots are pathogenic and pose serious health problem, it is advisable to always wash carrot with potable water prior to consumption. For best result however, vinegar should be used to wash carrot and a contact time of 30 minutes should be allowed before consumption. However in order to reduce the consumption of chemicals like vinegar and sodium hypochlorite which may have adverse effect due to overdose or long time ingestion, for example, hypokalemia, osteoporosis or cause tooth decay (Hill *et al.*, 2005), carrots pretreated with this chemicals therefore should be rinsed again with potable water before consumption. However to be on the safe side, moringa seed aqueous extract which is a natural product with no obvious side effect can be used instead since it has the potentials of reducing and also eliminating pathogens on carrots as observed in this study.

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