African Journal of Biotechnology Vol. 6 (6), pp. 720-722, 19 March, 2007
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2007 Academic Journals

Short Communication

Antibacterial and antifungal effect of high pH and paraffin wax application on tomatoes, oranges and peppers

Magashi Abdulkadir M. and *Bukar Aminu

Department of Biological Sciences, Bayero University, P.M.B. 3011, Kano, Nigeria.

Accepted 31 January, 2007

The antibacterial and antifungal effects of high pH (9, 10) and paraffin wax were determined. Determination of antibacterial and antifungal activity of the combined treatments was achieved by aerobic mesophilic count of bacteria and fungi on the surface of the tomatoes, peppers and oranges using serial dilution and pour plate techniques and compared prior to and after 4 days of treatment with buffer (pH 9, 10) and wax for 3 min using dipping method. Reduction in bacterial and fungal count indicates antifungal and antibacterial activity. A bacterial count reduction of 84.3 (control), 63.4 (pH 9) and 78.2% (pH 10) and fungal count reduction of 53.6 (control), 43.4 (pH 9) and 73.5 (pH 10) were achieved after 4 days of treatment respectively. The study shows that the control (unwaxed) had similar antibacterial and antifungal effect as waxed fruits at pH 9 and 10, except for pH 10 that had higher reduction of fungal counts than the control, showing prospect of higher activity with wax at higher pH than 10.

Key words: Antibacterial, antifungal, paraffin wax, pH, fruits, vegetables.

INTRODUCTION

Fruits and vegetables are normally contaminated with microorganisms, since the entire environment in which we live is colonized by them (Ihekoronye and Ngoddy, 1985). Microbial population associated with food is generally specific depending on the type of food and particular condition of storage. The primary causative agents of microbial spoilage are the bacteria, yeasts and molds. While viruses have the capacity to damage both plant and animal tissues, these agents, along with mycoplasmas are not generally regarded as being important in fruit and vegetable spoilage (Jay, 2003).

Most important, however, is that microorganisms generally are not found within healthy living tissues – such as within the flesh of animals or the flesh of fruits or juice of plant. But they are always present to invade the flesh on the skins and peels of fruits and vegetables, or if the skin is weakened by disease or death. In this case they may digest the skin (by pecteolytic enzymes) and penetrate through it to the tissue below. In nearly all cases, the presence of spoilage organisms in fruits and vegetables is a result of contamination of their surfaces. Therefore, one of the major strategies in reducing food spoilage due to microorganisms is to reduce contamination by ensuring sanitization of fruits and vegetable surfaces (Potter and Hotchkiss, 1993).

All preservation techniques basically serve to prevent or control the growth and activities of spoilage microorganism in and around food. Wax application on surfaces of fruits and vegetables has been reported to serve as a means of reducing surface contaminants and spoilage causing organisms. Waxes that are applied on fruits are generally high in pH (>8.0) and after application dried at high temperature (50 – 55^{o}C) for 2 – 3 min during fresh fruit waxing (Grierson, 1986; Pao and Brown, 1998). The pH in wax is adjusted by incorporating buffer solutions to provide the desired pH.

Contamination of surfaces of fruits and vegetables has been reported to serve as vehicle for transmission of pathogens and food-borne infections (Kawo et al., 2005). This study is aimed at determining the efficacy of high pH and wax treatment in reducing the surface count of both spoilage organisms and potential pathogens with the

*Corresponding Author's E-mail: al_amsak2004@yahoo.com.

Table 1. Viable aerobic mesophilic bacterial count (cfu/ml) for paraffin waxed tomatoes, peppers and oranges at pH 9 and 10 prior to and after 4 days of treatment.

	Tomato			Pepper			Orange		
	Control	**pH 9**	**pH 10**	**Control**	**pH 9**	**pH 10**	**Control**	**pH 9**	**pH 10**
1st day	2.84 $x10^4$	2.76 $x10^4$	3.16 x 10^4	2.44 x 10^4	2.56 $x10^4$	$4.24x10^4$	2.88 $x10^4$	$1.24x10^4$	$2.84x10^4$
4th day	4.8 x 10^3	7.2 x 10^3	6.00 x 10^3	1.60 x 10^3	1.08 $x10^4$	6.8 x 10^4	6.40 $x10^3$	6.00 $x10^3$	2.00 $x10^3$
%reduction in bacterial count	83.0	73.9	81.0	93.4	57.8	83.9	77.7	51.6	92.9

Overall reduction by control fruits = 84.3%
Overall reduction by wax of pH 9 = 63.4%
Overall reduction by wax of pH 10 = 78.2%

hope that it will reduce or eliminate the possibility of food-borne infections.

MATERIALS AND METHODS

Wax application

Paraffin wax (Raymond Lamb, A. London) was purchased from Total Nigeria Limited, Kano. The procedure for wax preparations were carried out according to the manufacturers directions on the packets. Paraffin wax (Raymond A. Lamb, London) was prepared by melting about 200 g in a stainless steel container on a hot plate at 60°C. When the wax had melted completely, the container was left on the hot plate at 50°C prior to wax treatment on the fruits and vegetable fruits.

Wax treatment was carried out according to the method of Pao et al. (1999) with a slight modification in the buffer application. The fruits and vegetable fruits were divided into three sets with each set containing duplicate number of fruits and vegetable fruits. The first set was immersed into the beaker-containing buffer at pH 9 for 2 min. While the second set was immersed into buffer at pH 10 for 2 min also. The fruits and vegetables were removed from the buffer and dipped into the container containing the molten wax and allowed to stay for 3 min. The last set which was the control was kept unwaxed. The waxed fruits and vegetable fruits were then stored in open plastic containers placed in the laboratory cupboard for four days, after which they were again subjected to aerobic mesophilic count determination.

Determination of aerobic mesophilic count of fruits and vegetable fruits

Oranges, peppers and tomatoes were purchased at Kabuga market, Kano, Nigeria before they were washed. The fruits and vegetable fruits were not allowed to be washed as washing will reduce or eliminate the microorganism on the surface of the fruits and vegetables. Visually unbruised fruits and vegetable fruits were sorted out and transported in sterile polythene bags to the laboratory for investigation (Refai, 1979).

Determination of aerobic mesophilic count of fruits and vegetable fruits was achieved by using the serial dilution and pour plate techniques described by Cheesbrough, (2000) and Refai, (1979). 100 ml of sterilized distilled water was dispensed into sterile beakers. 2 sets of 100 ml capacity conical flasks were arranged containing 99 ml of sterile distilled water each. Using a hand glove, the fruits and vegetables were immersed into the beakers containing 100 ml of distilled water i.e. the stock solution. It was shaken for sometime and then 1 ml was transferred into the 2 sets of conical flasks containing 99 ml of distilled water using a sterile pippette. From the first set of conical flasks, 1 ml was dispensed into 2 petri dishes each labeled 10^{-2}. For bacterial count enumeration, already prepared molten Nutrient agar was dispensed into two (duplicate) petri dishes, swirled to spread the inoculums evenly on the surface of the agar medium. From the second set of conical flasks, 1 ml was dispensed into 2 other petri dishes each labeled 10^{-2}. While already prepared molten Potato Dextrose Agar (PDA) was added into the two other petri dishes for fungal count enumeration. The petri dishes containing Nutrient Agar were incubated at 37°C for 24 h, while the PDA petri dishes were incubated at room temperature (30 – 34°C) for 6 days. The bacterial and fungal colonies formed were observed after the incubation period and counted (Cheesbrough, 2000).

RESULTS AND DISCUSSION

Result of aerobic mesophilic count of wax treated fruits and vegetable fruits (Table 1) shows that after 4 days of treatment, buffer pH 9 and paraffin wax caused a reducetion in bacterial count of 73.9% for tomatoes, 57.8% for peppers and 51.6% for oranges, while buffer pH 10 and paraffin wax treatment resulted in reduction in bacterial count of 81.0% for tomatoes, 83.9% for peppers, and 92.9% for oranges. The control had a reduction of bacterial count of 83.0% for tomatoes, 93.4% for peppers and 77.7% for oranges.

Table 2 shows that tomatoes, peppers and oranges treated with buffer pH 9 and paraffin wax had a reduction in fungal count of 40.0% for tomatoes, 51.1% for peppers and 33.3% for oranges, while tomatoes, peppers and oranges treated with buffer pH 10 and paraffin wax had a reduction in fungal count from 50.0% for tomatoes, 90.0% for peppers and 50.0% oranges.

The control tomatoes, peppers and oranges had a reduction of fungal count of 52.3% for tomatoes, 54.3% for peppers and 55.5% for oranges. The present study has shown that buffer and paraffin wax application is a potential means of reducing both bacterial and fungal count on surface of tomatoes, peppers and oranges. However, the control (unwaxed) fruits and fruit vegetables show the same tendency after 4 days of treatment.

Table 2. Viable aerobic mesophilic fungal count (cfu/ml) for paraffin waxed tomatoes, peppers and oranges at pH 9 and 10 prior to and after 4 days of treatment.

	Tomato			Pepper			Orange		
	Control	pH 9	pH 10	Control	pH 9	pH 10	Control	pH 9	pH 10
1st day	2.10×10^3	1.00×10^3	8.00×10^2	1.10×10^3	7.00×10^2	2.00×10^3	9.00×10^2	6.00×10^2	6.00×10^2
4th day	1.00×10^3	6.00×10^2	4.00×10^2	5.00×10^2	3.00×10^2	2.00×10^2	4.00×10^2	4.00×10^2	3.00×10^2
% reduction in fungal count	52.3	40.0	50.0	54.5	57.1	90.0	55.5	33.3	50.0

Overall reduction by control fruits = 53.6%
Overall reduction by wax of pH 9 = 43.4%
Overall reduction by wax of pH 10 = 73.5%

This is in line with the report of Pao and Brown (1998) that waxing of citrus fruits with shellac wax at pH 9 and 10 for 3 min at 50°C has been shown to be effective means of reducing count of both aerobic bacteria and *Escherichia coli* on the surface of citrus. And the findings also corroborate the work of Pao et al. (1999), who reported a five log reduction in *E. coli* count on the surface of orange and glass slide attained by dipping in heated (50°C) alkaline (pH 10,11) wax for 4 min. But the two researches did not report on the effect of a control experiment as a basis for comparison.

The results (Tables 1 and 2) show that the control (unwaxed) fruits had equivalent reduction in counts of bacteria and fungi after 4 days of treatment. But in our previous report (Magashi and Bukar, 2006), it was observed that control (unwaxed) fruits started rotting at 6 – 7 days of storage which increased the counts of both bacteria and fungi, while paraffin waxed fruits at pH 9 and 10 stored for up to 25 – 30 days as a result of protection conferred by the wax. The buffer (pH 9, 10) treatment on the surface of tomatoes, peppers and oranges might have provided an environment cidal to the bacteria and fungi. Additional waxing enhanced that, as most waxes have been reported to contain compounds that are antimicrobial (Hall, 1981; Eckert and Brown, 1986). The unwaxed (control) tomatoes, peppers and oranges showed reduction in bacterial and fungal count (Tables 1 and 2) probably because they were kept in containers dry with no moisture, thereby reducing the water activity of the organisms on the microenvironment of the surface of test fruits and vegetable fruits, which has been reported to be very vital for their survival (Troiler, 1993).

The reduction or elimination of bacteria and fungi on the surface of fruits and vegetables by paraffin wax is good development, because it offers a means of reducing counts of potential pathogenic bacteria and fungi and spoilage causing organisms, which after long period of storage might be eliminated completely, thereby reducing the possibilities of food-borne infections and also aiding in the post-harvest storage of fruits. Waxing at pH 10 reduced fungal counts by 73.5% as compared with the control which had 53.6%. This shows the possibility that waxing at pH 11 and above has prospects for more antibacterial and antifungal activity on the surface of fruits and fruits vegetables

Conclusion

The present research has shown that a combination of high pH and paraffin wax treatment possess antibacterial and antifungal effect on bacteria and fungi on the surface of tomatoes, peppers and oranges compared to control test fruits and vegetable fruits. This method can be employed to decontaminate or sanitize the surfaces of fruits and vegetables as well as a means of preserving them.

REFERENCES

Cheesbrough M (2000). District Laboratory Practice in Tropical Countries. Part 2. Cambridge Low price edition. UK. Pp: 250 – 253.

Eckert JW, Brown GE (1986). Post Harvest Citrus Disease and their Control. Fresh Citrus Fruit. pp: 315–360.

Grierson W (1986). In: Pao S, Davis CL, Petrack PD (1999). Sanitizing Effects of Fruits waxes at high pH and temperature on orange surfaces inoculated with *Escherichia coli*. J. Food Sci. 64(2): 359–362.

Hall DJ (1981). Innovation in Citrus Waxing. An overview. pp: 258– 263.

Ihekoronye AI, Ngoddy PO (1985). Food Microorganisms and Food Spoilage. Integrated Food Science and Technology for the Tropics. Macmillan Publishers, London. pp. 106–107.

Jay JM (2003). Microbial Spoilage of Food. Modern Food Microbiology. 4th ed. Chapman and Hall Inc. New York. pp. 187– 195.

Kawo AH, Bassey SE, Aliyu YU (2005): Bacteriological Quality of Vegetables sold in some shops around Kano metropolis, Nigeria. Best J. 2(1): 145-148.

Magashi AM, Bukar A (2006). Preservative Effect of High pH and Paraffin Wax Application on Tomatoes, Oranges and Peppers. Best J. 3(3): 126–128.

Pao S, Brown GE (1998). Reduction in Microorganisms on Citrus Fruit Surface during packinghouse processing. J. Food Prod. 61: 903–906.

Pao S, Petracek PD, Knabel SJ (1999). Sanitizing Effect of Fruits Waxes at High Temperature and High pH on Orange Surfaces Inoculated with *E. coli*. J. Food Sci. pp: 102-110.

Potter NN, Hotckiss HH (1993). Food Deterioration and Its Control. 5th edition. Food Science. CBS Publishers and distributors, New Delhi, India. pp: 113 – 17.

Refai MK (FAO, 1979). Manuals of Food Quality Control 4. Microbiological Analysis. FAO of the United Nations Publications, Rome, Italy. FAO and Nutrition paper.

Troiler JA (1993). Sanitation in Food Processing In: Norman NP, Joseph HH (1996): Food Sciences. 5th ed. CBS Pub. & Dist. pp: 113–137.