# EFFECTS OF WAXING MATERIALS, STORAGE CONDITIONS ON PROTEIN, SUGAR AND ASH CONTENTS OF CITRUS FRUITS STORED AT ROOM AND REFRIGERATED TEMPERATURES 

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#### Abstract

A research was carried out on five different species of citrus fruits namely: Citrus paradise (grapefruit); $C$ aurantium (sour orange); $C$ auratifolia (lime); $C$ reticulate (tangerine) and $C$ sinensis (sweet orange). The fruits were immersed in a mixture of detergent solution and petroleum jelly and wrapped with aluminium foil and stored both at room and refrigerated temperatures for a period of 12 weeks.Fruits not treated and stored at room temperature served as control.The fruits that were immersed in a mixture of detergent solution and petroleum jelly had the lowest changes in protein and sugar contents.This was followed by fruits immersed in a mixture of detergent solution and petroleum jelly and put in the refrigerator without wrapping with aluminium foil.And the fruits immersed in a mixture of detergent solution and petroleum jelly and wrapped with aluminium foil and stored at room temperature. In terms of performance all the fruits given the above treatment were better when compared to the control stored at room temperature with no treatment applied Irrespective of the kind of treatment applied,sweet orange had the highest protein contents followed by tangerine, grapefruit, lime and sour orange. As for the sugar contents, sweet orange also had the highest contents followed by tangerine, grapefruit, sour orange and lime in that order.


Key Words: Research, Protein, Sugar, Detergent, Waxing, Citrus fruits.

## INTRODUCTION

The most important characteristic of freshly harvested fruits and vegetables is that, they are still alive and respiring (Snowdown, 1988). On the basis of respiration pattern, horticultural products
can be divided into two main groups. Vegetables and some fruits such as citrus, cherries, grapes, pineapples, that have a respiration rate which at a given temperature, remains fairly constant, or even fall slightly, during the post harvest period (Hardenburg et al., 1986) and the second group which includes fruits such as apples, pears, plums, tomatoes, avocadoes, mangoes, banana and many others; they are characterized by a sudden surge in respiration rate (usually after harvest) known as the climacteric rise. This is triggered by ethylene gas, produced in minute quantity by the fruits themselves (Blanpied, 1985).
(Wardowski et al., 1986) reported citrus fruits to be non - climacteric and do not undergo a ripening process. As the fruit matures on the tree, the pulp becomes juicy and sweet (or acid in lemons and limes), and remain so for a long time. In tropical growing areas, the peel remain green, even after the pulp has become edible, citrus fruits are marketed green.

Transpiration is one of the major processes that affect commercial and physiological deterioration of fruits and vegetables, it induces wilting, shriveling and loss of firmness, crispness and succulence all components of freshness. The desiccation resulting from moisture loss reduces the commercial value of the product adversely affecting its appearance, texture, flavour and weight (Ben and Rodov, 1999). (Shimshon et al., 1994) showed water loss during storage is a factor in the postharvest weight loss. It causes accelerated softening and loss of attractive appearance of fruits. The resultant water stress enhances senescence. Weight loss as low as 5\% renders oranges unsalable and shrinkage is visible at half this value. Loss of weight involves mainly the peel, not the pulp of the fruits. As the peel is a major marketing feature, peel appearance is as important economically, if not more than the flavour of the pulp.

Another method of preventing postharvest decay of citrus fruits is by curing. This is done by immersing sealed fruits in water at a high temperature of $34-36^{\circ} \mathrm{C}$ for 3 days. This practice accelerates healing of fruit injuries and increases the resistance of fruits to decay. Curing reduces decay of citrus fruits by thermic inhibition of pathogens. It also aids the activity of the enzyme phenyl alanine ammonialyase that catalyses lignifications and wound healing. This enzyme builds a better physical barrier against invading pathogens, thus raising and maintaining higher levels of antifungal materials in the flavedo of the fruits (Shimshon et al., 1994; Baldwin et al., 1996; Murata, 1997).

An investigation carried out by (Kawada and Kitagawa, 1992) showed the use of wilting treatment, a traditional practice in the far East, where fruits is kept at ambient temperature condition, immediately after harvest until it loses at least $3 \%$ of the initial weight, and is transferred to cold storage. This treatment not only extended the postharvest life, by reducing decay and storage injury, but result in eventual total weight loss of the fruits at the end of 3 to 8 months of storage.The aims of the research were (i) To determine the effectiveness 0f combining a mixture of
detergent solution with petroleum jelly and wrapping with aluminium foil on the changes in protein and sugar contents (ii) To know the effects of treatment and period of storage on the protein and sugar contents of the different citrus fruits.

## MATERIALS AND METHODS

## Determination of Crude Protein

The micro-kjeldahl method of (AOAC., 1980) as described by (Ranjhan. and Krishna., 1980) was used. Tem millilitres of juice was measured accurately and transferred to Kjeldahl flask. This was digested with concentrated 25 ml tetraoxosulphate VI acid $\left(\mathrm{H}_{2} \mathrm{SO}_{4}\right), 1.0 \mathrm{~g}$ of copper II sulphate (CUS04) using selenium as a catalyst. The digest was titrated with 0.1 N sodium hydroxide solution using boric acid indicator. The mixture was digested on a heating mantle until the solution was clear. The digest was made up to 250 ml with distilled water and distilled. The distillate was titrated against 0.1 N Hydrochloric acid $(\mathrm{HCl})$. The following formula was used to calculate percentage nitrogen.

$$
\% \text { Nitrogen }=\frac{\text { Titre value X } 0.0014 \times 250 \times 100}{\text { Weight of sample X } 5}
$$

Crude protein $=\%$ Nitrogen X 6.25

## Determination of Total Sugar

This was done according to (Dubois et al., 1956). To ten ml of sample, one ml of $5 \%$ phenol was added followed by 5 ml of concentrated tetraoxosulphate VI acid $\left(\mathrm{H}_{2} \mathrm{SO}_{4}\right)$ from a burette. The acid jet was directed to the liquid surface rather than the wall of the tube to ensure thorough mixing. The hot mixture was allowed to stand for ten minutes, the tube was shaken and placed on a water bath at $70^{\circ} \mathrm{C}$ for 30 minutes. Thereafter, the colour that developed was read with a spectrophotometer at 490 nm . The blank consisted of 1 ml distilled water. The standard curve was prepared from optical density readings of glucose containing various concentrations.

## Determination of Total Ash

This was determined as described by (AOAC., 1990). Fifty grams of oven dried pulp of citrus fruits was placed in a pre weighed crucible and then heated in an oven at $530^{\circ} \mathrm{C}$ for 4 hours. When the sample had turned into white ash, the crucible and its contents were cooled in a desiccator, before they were weighed again. The percentage of total ash was calculated using the formula
$\%$ Total ash $=\underline{\text { Weight of crucible }+ \text { ash }- \text { weight of empty crucible }}$
Initial weight of sample

## RESULTS

The protein content of different citrus fruits stored at room and refrigerated temperatures for 12 weeks

There were marked differences in the protein contents of the different citrus fruits .Sweet orange had the highest protein contents followed by tangerine, grapefruit, lime and sour orange.The results are depicted in Figure 1.
The sugar contents of different citrus fruits stored at room and refrigerated temperatures for 12 weeks
The sugar contents showed considerable variation from one citrus fruit to the other ,with sweet orange having the highest value followed by tangerine, grapefruit, sour orange and lime in that order. The results are shown in Figure 2.
The ash contents of different citrus fruits stored at room and refrigerated temperatures for 12 weeks
There were variations in the ash contents in all the citrus fruits, with tangerine having the highest value,this was followed by lime, sweet orange, sour orange and grapefruit.
The effects of period of storage on protein and sugar contents of the different Citrus species stored at room and refrigerated temperatures for 12 weeks are presented in Tables 1-2.
There was decrease in the protein contents in all the citrus fruits as the period of storage increased.
The effects of treatment on protein and sugar contents of the different Citrus species stored at room and refrigerated temperatures for 12 weeks are presented in Tables 3-4.
There was variation in the rate of decrease of protein and sugar contents in all the Citrus species depending on the type of fruit and the treatment applied the results are presented in Tables

## DISCUSSION

There were significant differences in the protein and sugar contents in all the citrus fruits as the period of storage increased , as well as in the treatment applied at ( $\mathrm{p}<0.5$ ). Sweet orange had the highest value of protein of $1.14 \mathrm{mg} / \mathrm{g}$, followed by sour orange, grapefruit, lime and tangerine. There exists a significant difference ( $\mathrm{P}<0.05$ ) in the protein contents in the period of storage in all the citrus fruits. As for the treatment, the fruits refrigerated with preservatives gave the better result in minimizing loss in protein contents. This was followed by fruits refrigerated without preservatives (control), except for grapefruit where there was no difference between the refrigerated fruits without preservatives (control) and its control. The fruits with preservatives followed and finally the control. Though minor variation exists in the level of significant difference ( $\mathrm{P}<0.05$ ) within each fruit, as regards the treatment applied. The observed decrease in protein values as the period of storage increased might be due to increased utilization of the nutrient by associated microflora and the kind of treatment applied.

Sweet orange also had the highest sugar content of $6.9 \mathrm{mg} / \mathrm{g}$, followed by tangerine, grapefruit, sour orange and lime. The amount of sugar present in the different citrus fruits reflects their degree of tartness, that is, sweetness to acidity ratio. There was a significant difference $(P<0.05)$ for all the fruits throughout the storage period. In case of treatment, the fruits refrigerated with preservatives
showed the least deterioration in sugar content in all the citrus fruits; this was followed by the fruits refrigerated without preservatives (control) except for grape fruit where there was no significant difference between the refrigerated fruits without preservatives (control) and the control. The fruit with preservatives stored at room temperature was third in the loss of sugar, and finally the control which showed the highest loss of sugar. There was small variation in the differences within each citrus fruit. In all the citrus fruits there was decrease in the sugar as the storage period lengthened. This might be due to the treatment applied and microbial attack, as well as the inherent nature of each fruit. The reports of other workers (Sowumi et al., 1982; Baiyewu and Amosa, 1999; Ladapo, 2002) corroborate the above, with the findings that decrease in the sugar of sweet orange and pawpaw as the duration of storage increased, depended on the kind of treatment applied, such as wrapping in aluminum foil, polyethylene bag, putting the fruits in baskets in open shelf, storing the fruits in evaporated cooler structure and in the refrigerator.

The ash content of the different citrus fruits varied from one fruit to the other, with tangerine having the highest value of $1.07 \%$, followed by lime, sweet orange, sour orange and finally grapefruit. There was decrease in the ash content for the control, which had the highest value when compared to the fruits refrigerated with preservatives which had the lowest values tangerine and sour orange. The reverse was noticed for lime and grapefruit with the control having the least value and the fruits refrigerated with preservatives having the highest values. These differences between the different citrus fruits might be due to their chemical composition as well as the types of cultivars used for planting. (Onwuzulu et al., 1987) found a decrease in the ash content of sweet orange as the period of storage increased.

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## REFERENCES

AOAC., 1980. Official methods of analysis . . 12th Edn., Washington D.C.: Association of Official Analytical Chemists.
AOAC., 1990. Official methods of analysis. . 15th Edn., Washington D.C.: Association of Official Analytical Chemists.
Baiyewu, R.A. and N. Amosa, 1999. Biochemical changes in pawpaw fruits infected with fungi Bio-Science Research Communications., 9(2): 257-261.

Baldwin, E.A., M.O. Nisperos Carriedo and A.D. Hagemmaier, 1996. Improving storage life of cut apple and potato with edible coatings. Postharvest Biol. Technol., 9: 151-163.
Ben, Y., S. and R. Rodov, 1999. Post harvest physiology and pathology of vegetables. . 2nd Edn., New York: Jerry, A.D. and Jeffry, K.B Eds Mercel Bekker, Inc.
Blanpied, G.D., 1985. Symposium: Ethylene in post harvest biology and technology of horticultural crops. Hort science(20): 39-60.
Dubois, N.I., J.K. Hamilton, P.A. Roberts and E. Smith, 1956. Colorimetric method for the determination of sugars and related substances. Analyst Chemistry(28): 350356.

Hardenburg, R.E., A.E. Watada and C.Y. Wang, 1986. The commercial storage of fruits, vegetables and florist and nursery stock. Agricultural Handbook of the United States Department of Agriculture(66): 30.
Kawada, K. and H. Kitagawa, 1992. Storage of sudachi (citrus sudachi hort. Ex shirai) in japan. Proceedings of the International society for citruculture: 1084-1085.
Ladapo, L.A., 2002. Assessment of postharvest losses of sweet orange (citrus sinensis) in oyo state of nigeria. Phd Thesis, University of Ibadan, Nigeria.
Murata, T., 1997. Citrus in: Postharvest physiology and storage of tropical and subtropical fruits New York: Mitral S.K. Ed. Cab International
Onwuzulu, O.W., O.W. Obani and W.F. Okoye, 1987. Storage of fruits in waxed / grease proofed wrappers. Nig. stored prod. Res. Inst. Tech.(10): 73-77.
Ranjhan. and Krishna., 1980. Laboratory manual for nutrition research,. New Delhi: Vicas Publishing House Ltd.
Shimshon, B.H., E.G. Eliezer and B.H. Moshe, 1994. Citrus fruits. In encyclopeadia of agric. Sci. by Charles, J.A. and Ellen, M.R. Eds. vol 1. Academic press, New York.
Snowdown, A.L., 1988. A review of the nature and causes of post harvest deterioration in fruits and vegetables, with especial refrence to those in international trade. Biodeterioration(7): 585-602.
Sowumi, O.O., O.R. Akinnusi, S.C. Nwangwa and J.O. Oyeniran, 1982. The effects of storage container on the preservation of quality of sweet orange. In: Nig. Stored Prod. Res. Inst. Tech Report. pp: 107-114.
Wardowski, W.E., S. Nagy and W. Grierson, 1986. Fresh citrus fruits. Avi Publishing Co. Westport.: 571.

## Appendix




Fig.-1. Changes in protein content of citrus fruits stored at room and refrigerated temperatures for 12 weeks



Fig. 1 continued.

> Preservatives
> Control (room temperature)
> Refrigeration + Preservatives
> Control (refrigeration)


Fig. 1 : Continued.



Fig-2. Changes in sugar content of citrus fruits stored at room and refrigerated temperatures for 12 weeks



Fig-2. Continued.


Fig-2. Continued.


Table-1. Ash content of different citrus fruits

| Fruit | Treatment | Ash content |
| :---: | :---: | :---: |
| Sweet Orange |  |  |
|  | 1 | 0.69 |
|  | 2 | 0.64 |
|  | 3 | 0.54 |
|  | 4 | 0.14 |

Table 2 continued.
Tangerine
1
1.07

|  | 2 | 1.02 |
| :---: | :---: | :---: |
|  | 3 | 0.39 |
| Sour Orange | 4 | 0.42 |
|  | 1 | 0.58 |


|  | 2 | 0.46 |
| :--- | :---: | :---: |
| Lime | 3 | 0.34 |
|  | 4 | 0.22 |
|  | 1 | 0.18 |
| Grapefruit | 2 | 0.14 |
|  | 3 | 0.61 |
|  | 4 | 0.70 |
|  | 1 | 0.36 |
|  | 2 | 0.42 |
|  | 3 | 0.46 |

1, Control (room temperature); 2, preservatives (petroleum jelly + detergent solution, with aluminium foil); 3, refrigerated (control); 4, refrigerated + preservatives (petroleum jelly +detergent solution, with aluminium foil)

Tabl-1. Effects of period of storage on protein of different citrus fruits

|  | Period (weeks) |  |  |  |
| :--- | :---: | :---: | :---: | :---: |
| Fruit | 1 | 4 | 8 | 12 |
| Sweet Orange | $1.0^{\mathrm{d}}$ | $0.72^{\mathrm{c}}$ | $0.59^{\mathrm{b}}$ | $0.22^{\mathrm{a}}$ |
| Tangerine | $0.44^{\mathrm{d}}$ | $0.36^{\mathrm{c}}$ | $0.29^{\mathrm{a}}$ | $0.23^{\mathrm{a}}$ |
| Sour orange | $0.89^{\mathrm{c}}$ | $0.63^{\mathrm{b}}$ | $0.47^{\mathrm{b}}$ | $0.36^{\mathrm{a}}$ |
| Lime | $0.54^{\mathrm{d}}$ | $0.42^{\mathrm{c}}$ | $0.38^{\mathrm{b}}$ | $0.06^{\mathrm{a}}$ |
| Grapefruit | $0.55^{\mathrm{d}}$ | $0.43^{\mathrm{c}}$ | $0.30^{\mathrm{b}}$ | $0.17^{\mathrm{a}}$ |

Means with different superscripts across a row are significantly different $(\mathrm{P}<0.05)$

Table-2. Effects of period of storage on sugar of different citrus fruits
Period (weeks)

| Fruit | 1 | 4 | 8 | 12 |
| :--- | :---: | :---: | :---: | :---: |
| Sweet Orange | $6.6^{\mathrm{d}}$ | $4.9^{\mathrm{c}}$ | $4.2^{\mathrm{b}}$ | $3.8^{\mathrm{a}}$ |
| Tangerine | $1.3^{\mathrm{d}}$ | $0.96^{\mathrm{c}}$ | $0.50^{\mathrm{a}}$ | $0.28^{\mathrm{a}}$ |
| Sour orange | $6.1^{\mathrm{d}}$ | $4.6^{\mathrm{b}}$ | $3.2^{\mathrm{b}}$ | $2.7^{\mathrm{a}}$ |
| Lime | $4.0^{\mathrm{d}}$ | $2.4^{\mathrm{c}}$ | $0.98^{\mathrm{b}}$ | $0.13^{\mathrm{a}}$ |
| Grapefruit | $3.1^{\mathrm{d}}$ | $2.2^{\mathrm{c}}$ | $1.2^{\mathrm{b}}$ | $0.60^{\mathrm{a}}$ |

Means with different superscripts across a row are significantly different $(\mathrm{P}<0.05)$

Table-3. Effects of different treatments on protein of different citrus fruits Period (weeks)

| Fruit | 1 | 2 | 3 | 4 |
| :--- | :---: | :---: | :---: | :---: |
| Sweet Orange | $0.87^{\mathrm{c}}$ | $0.61^{\mathrm{b}}$ | $0.62^{\mathrm{b}}$ | $0.53^{\mathrm{a}}$ |
| Tangerine | $0.40^{\mathrm{d}}$ | $0.33^{\mathrm{b}}$ | $0.36^{\mathrm{c}}$ | $0.29^{\mathrm{a}}$ |
| Sour orange | $0.72^{\mathrm{d}}$ | $0.55^{\mathrm{b}}$ | $0.70^{\mathrm{c}}$ | $0.46^{\mathrm{a}}$ |
| Lime | $0.58^{\mathrm{d}}$ | $0.39^{\mathrm{b}}$ | $0.48^{\mathrm{b}}$ | $0.27^{\mathrm{a}}$ |
| Grapefruit | $0.49^{\mathrm{d}}$ | $0.43^{\mathrm{b}}$ | $0.35^{\mathrm{b}}$ | $0.26^{\mathrm{a}}$ |

Means with different superscript across a row are significantly different ( $\mathrm{P}<0.05$ )
1, Control; 2, preservatives; 3 , control (refrigeration); 4, refrigeration + preservatives

Table-4. Effects of different treatments on sugar of different citrus fruits treatment

| Fruit | 1 | 2 | 3 | 4 |
| :--- | :---: | :---: | :---: | :---: |
| Sweet Orange | $5.3^{\mathrm{d}}$ | $4.9^{\mathrm{c}}$ | $4.8^{\mathrm{b}}$ | $4.5^{\mathrm{a}}$ |
| Tangerine | $1.1^{\mathrm{d}}$ | $0.55^{\mathrm{a}}$ | $0.91^{\mathrm{b}}$ | $0.53^{\mathrm{a}}$ |
| Sour orange | $5.0^{\mathrm{d}}$ | $4.4^{\mathrm{c}}$ | $4.1^{\mathrm{b}}$ | $3.5^{\mathrm{a}}$ |
| Lime | $3.8^{\mathrm{c}}$ | $0.98^{\mathrm{a}}$ | $2.9^{\mathrm{b}}$ | $0.93^{\mathrm{a}}$ |
| Grapefruit | $3.3^{\mathrm{c}}$ | $0.44^{\mathrm{b}}$ | $2.9^{\mathrm{c}}$ | $0.35^{\mathrm{a}}$ |

Means with different superscript across a row are significantly different ( $\mathrm{P}<0.05$ )
1, Control; 2, Preservatives; 3, Control (refrigeration); 4, Refrigeration + preservatives

