Advances in Biological Research 7 (4): 134-138, 2013
ISSN 1992-0067

DOI: 10.5829/idosi.abr.2013.7.4.73175

Laboratory Analysis of pH and Neutralizable Acidity of Commercial Citrus Fruits in Nigeria

[1]*Cornelius Tokunbo Bamise and* [2]*Elizabeth Obhioneh Oziegbe*

[1]Department of Restorative Dentistry, Faculty of Dentistry,
Obafemi Awolowo University, Ile-Ife, Osun-State, Nigeria
[2]Department of Child Dental Health, Faculty of Dentistry,
Obafemi Awolowo University, Ile-Ife,Osun-State, Nigeria

Abstract: This is an *in vitro* study to evaluate five different commercially available citrus fruits: *viz.,* Sweet orange [*Citrus sinences* (Osbeck)], Grape [*Citrus maxima* (J. Buruan) *Merrile*], Lemon [*Citrus limon* (Burn F.)], Tangerine [*Citrus reticulata* (Blanco)] and Lime [*Citrus aurantifolia* (Swingle)]. Evaluation of their pHs and neutralizable acidity were made. A 50 mL of each fruit was prepared by juice press. Initial pH was measured in triplicate for the five fruits. The neutralizable acidity was assessed by measuring the volume of 0.10 N sodium hydroxide required to raise the pHs of each fruit to a neutral pH of 7.0. Results showed that all the evaluated drinks were acidic with pHs that ranged from 2.4 to 4.1. Lime produced the lowest mean pH of 2.4 and tangerine yielded the highest of 4.1. The mean volume of sodium hydroxide to neutralize the fruits ranged from 5.1mL to 104mL. Regarding neutralizable acidity, Lime required the highest NaOH to neutralize thus possessing the strongest potential to erode enamel. Tangerine required the least base to neutralize. All the 5 evaluated citrus fruits are acidic with pHs well below the critical pH (5.5) of enamel demineralization. Inappropriate consumption of these fruits especially lime may incite toothwear.

Key words: Citrus · Acidity · Dental erosion

INTRODUCTION

Citrus fruits have long been valued as part of a nutritious and tasty diet. The flavours provided by citrus are among the most preferred in the world and it is increasingly evident that citrus not only tastes good, but is also good for people. It is well established that citrus and citrus products are a rich source of vitamins, minerals and dietary fibre (non-starch polysaccharides) that are essential for normal growth and development and overall nutritional well-being. However, it is now beginning to be appreciated that these and other biologically active, non-nutrient compounds found in citrus and other plants (phytochemicals) can also help to reduce the risk of many chronic diseases [1].

There has been a steady rise in citrus production globally due mainly to increase in hectarage, consumer preference for more health or convenience food and rising incomes [2]. Although about 140 counties are known to be involved in citrus production. Brazil and the United States alone were credited with contributing up to 45% of global orange production [3]. Year 2007 estimate indicates that the top 10 citrus producing counties are Brazil, China, United States, Mexico, India, Spain, Iran, Italy, Nigeria and Turkey. Citrus production in West Africa is on a small scale and unorganized, [4] the ranking of Nigeria on the 9th position among the world top 10 producers of the commodity signify that the situation might be changing. This is in consideration of the fact that previous statistics did not feature Nigeria or any of the West African countries [5]. In any case, most of the citrus fruit produced in the country is consumed locally [4] and does not seem to feature at all in international trade [2]. Sweet orange (*Citrus sinensis*) production in Nigeria is significant, with heavy direct consumption due primarily to few and small capacity processing industries to convert the fruit to juice, concentrate and canned fruit [6].

Corresponding Author: Cornelius Tokunbo Bamise, Department of Restorative Dentistry, Faculty of Dentistry, Obafemi Awolowo University, Ile-Ife, Osun-State, Nigeria.

Citrus fruits contain N (1-2 g/kg on a wet basis), lipids (oleic, linoleic, linolenic, palmitic, stearic acids, glycerol and a phytosterol), sugars (glucose, fructose, sucrose), acids (primarily citric and malic, but also tartaric, benzoic, oxalic and succinic), insoluble carbohydrates (cellulose, pectin), enzymes (pectin esterase, phosphatase, peroxidase), flavonoids (hesperidin, naringin), bitter principles (limonin, isolimonin), peel oil (d-limonene), volatile constituents (alcohols, aldehydes, ketones, esters, hydrocarbons, acids), pigments (carotenes, xanthophylls), vitamins (ascorbic acid, vitamin B complex, carotenoids) and minerals (primarily calcium and potassium). Citrus fruits are classified as acid fruits, since their soluble solids are composed mainly of organic acids and sugars [7].

The erosive capabilities of citrus juices have been demonstrated repeatedly on rat molar teeth. McClure [8] noted erosion with cranberry and grape-fruit juices. Bieri *et al.* [9] showed that rats fed orange juice for one week had tooth surface loss comparable with that seen with citric and acetic acids. Many animal studies have been carried out to evaluate the erosive properties of different juices [10-13]. Wynn and Haldil [14] reported that a wide range of juices, including grape juice, caused a similar effect to citric or acetic acid on rat molar teeth. Miller [15] noted similar results when he investigated several fruit juices. Holloway *et al*. [13] found diluted lemon juice diminished enamel in three weeks. Darby, [16] in what is apparently the first report of dietary erosion in humans, described changes in the teeth in two women who regularly sucked lemons; another two were reported to have undertaken the grape cure' in Switzerland; a practice that involved a high consumption of grapes. Many authors have since described the effect of citrus fruits and their juices on the human dentition [17-23].

In recent years, Citrus fruits are increasingly found in the daily diet of many people. Some of the more common being orange, lemon, tangerine and grapefruit and it is noteworthy that a number of these acidic fruit juices have consistently been implicated as important causes of erosion of tooth structure [24, 25]. Over the last decades, the decline in the prevalence of dental caries in the world population has been accompanied by a remarkable increase in the incidence of non-carious lesions, such as dental erosion [26]. Avalanche of current evidence has shown that dental erosion alone or in combination with attrition or abrasion is a predominant cause of toothwear [27] and the most important sources of erosive acids are those found in the diet.

MATERIALS AND METHODS

A 5 popular and commercially available citrus fruit were evaluated. Sweet orange [*Citrus sinences* (Osbeck)], Grape [*Citrus maxima* (J. Buruan) *Merrile*], Lemon [*Citrus limon* (Burn F.)], Tangerine [*Citrus retuculata* (Blanco)] and Lime [*Citrus aurantifolia* (Swingle)]. The erosive potential of each fruit was assessed by measuring its initial pH and neutralizable acidity *in vitro*.

Sample Preparation: Each sample of the citrus fruit was cut in half and the juice squeezed out with a juice-press. About 50mL of each sample was obtained and gently mixed. The skin and solids were not included; the solids being filtered out through a fine filter extracting as much juice as possible. A clean and dry safety 10ml pipette was used to draw up 10ml of juice and discharge it into a 250ml beaker.

Measurement of Initial pH: The initial pH of each fruit was measured using a pH meter (Hanna Instrument; Serial number S358236). A 20mL of each freshly prepared fruit juice was placed in a glass beaker on a thermostatically controlled electric hotplate at 37 °C. Before reading its pH, each sample was agitated (using a magnetic stirrer) for 1 min until a stable reading was obtained. Each fruit was tested three times to give a mean measurement. Between readings, the electrode was rinsed in distilled water to ensure that no cross-contamination occurred.

Measurement of Neutralizable Acidity: A 20 mL of the freshly prepared fruit juice was put in a glass beaker placed on a thermostatically controlled electric hotplate at 37 °C. This was titrated with 0.1N NaOH added in 0.2ml increments until the pH of 7 is reached. This was done by using a non heating magnetic stirrer until a stable pH reading was obtained after each increment (0.2ml) of NaOH. This was done to measure the total neutralizable. Titrations were repeated in triplicate for each fruit to check for reproducibility and to give a mean value for that fruit. Neuralizable acidity of a solution is usually measured by reacting the acids present with a base such as sodium hydroxide (NaOH) to a chosen end point, close to neutrality. The amount of NaOH required to raise the pH to 7 is then noted for each fruit juice.

RESULTS

Evaluation of the pH: The pH and standard deviation values of the fresh fruit juices were shown in Table 1.

Table 1: The pHs of the tested fruits.

S. No.	Citrus fruit	1st Reading	2nd Reading	3rd Reading	Mean	SD
1	Grape	3.2	3.2	3.2	3.2	.0000
2	Sweet orange	4.0	4.0	4.0	4.0	.0000
3	Tangerine	4.1	4.1	4.1	4.1	.0000
4	Lime	2.4	2.4	2.4	2.4	.0000
5	Lemon	3.1	3.1	3.1	3.1	.0000

Table 2: The amount of NaOH needed to raise the pH of the fruits to 7

S. N0.	Citrus fruit	1st Titration (ml)	2nd Titration (ml)	3rd Titration (ml)	Mean	SD
1	Grape	20.1	20.2	20.2	20.2	.0577
2	Sweet orange	11.5	11.4	11.4	11.4	.0577
3	Tangerine	5.3	5.3	5.3	5.3	.0000
4	Lime	103.8	104.0	104.1	104.0	.1528
5	Lemon	37.9	38	38.1	38.0	.1000

All the tested fruits were acidic and had pH well below 5.5. The mean pH was lowest for lime (2.4), followed by lemon (3.1) and highest for tangerine (4.1).

Evaluation of Neutralizable Acidity: Results showed that the mean volume of sodium hydroxide base needed to raise the pH of the fruits to 7.0 ranged from 5.3mL to 104mL (Table 2). Lime needed the most base (104mL) to raise its pH to 7.0 while Tangerine needed the least volume of base to raise its pH to 7.0. It was observed that Lime had the lowest pH and also the highest neutralizable acidity, signifying it to be the most acidic. Conversely, Tangerine had the highest pH (4.1) and the smallest amount of neutralizable acidity, making it the least acidic of the tested samples.

DISCUSSION

The initial pH of all the citrus fruit juices evaluated in the study ranged from 2.4 to 4.1, which is consistently below that considered to be the threshold for the initiation of enamel dissolution (pH 5.5). Lussi and Jaeggi in their review submitted that pH value of a drink or foodstuff among other factors is undoubtedly important in explaining erosive attack [28]. This was also pointed out by Jensdottir *et al*. that the pH of drinks determines their erosive potential within the first minutes of exposure [29].

On the other hand, acidity of a drink is believed to be the primary factor in the development of dental erosion; this total acid level (known as titratable acid), rather than the pH, is thought to be an important factor in erosion because it determines the actual hydrogen ion availability for interaction with the tooth surface [25, 30]. Subsequently, various workers have suggested that measurement of a beverage’s total acid content is a more realistic and more accurate method for predicting erosive potential [31-33]. This study showed that tangerine had the highest pH and required the least base to neutralize while lime had the lowest pH and needed the most base (104mL) to neutralize thereby having the strongest potential to erode teeth than the other citrus fruits evaluated. This is similar to that of Baldwin who reported that tangerines and oranges have the lowest acidity, while lemons, limes and sour oranges have the highest acidity among citrus fruits [34].

Erosive potentials of citrus have been demonstrated with animal experiments. In the rat molar teeth, McClure [8] noted erosion with cranberry and grape- fruit juices. Bieri *et al*. [9] showed that rats fed orange juice for one week had tooth surface loss comparable with that seen with citric and acetic acids. Many other animal studies have been carried out to evaluate the erosive properties of different juices [10-13]. Wynn and Haldil [14] reported that a wide range of juices, including grape juice, caused a similar effect to citric or acetic acid on rat molar teeth. Darby [16] was the first to report dietary erosion in humans, he described changes in the teeth in two women who regularly sucked lemons; another two were reported to have undertaken the grape cure’ in Switzerland; a practice that involved a high consumption of grapes. Many authors have since described the effect of citrus fruits and their juices on the human dentition [17-23].

As indicated by some researchers [35, 36] citric acid is the main organic acid found in citrus juices. Citric acid (2-hydroxy-1,2,3-propanetricar-boxylic acid) is a weak tricarboxylic acid that is naturally concentrated in citrus fruits. Among fruits, citric acid is most concentrated in lemons and limes, comprising as much as 8% of the dry fruit weight [37]. Lemon and lime juice, both from the fresh fruit and from juice concentrates, provide more citric acid per liter than ready-to-consume grape fruit juice, ready-to-consume orange juice and orange juice squeezed from the fruit [38].

In a study to determine the pH, ascorbic, citric and total organic acid content of some local fruits in Nigeria, Orange juice had the highest level of ascorbic acid but low in citric acid while lime juice is very rich in citric acid [39]. Meurman *et al'* reported that nine out of 13 sports drinks evaluated contained citric acid, two contained malic acid and two contained an unknown acid. Furthermore, the citric acid based drinks were more erosive than the malic acid containing drinks [40]. Pure fruit juice is now often advocated as a healthy drink which actually contains a lot of acid and can have a very low pH. Orange juice that is commonly served contains citric acid and its acidity is said to be comparable to that of soft drinks [31].

Etiology of dental erosion is multi-faceted; the results of the study would have to be applied with caution. This is also based on the fact that fruits generally have variable nutrient content depending on the stage of maturity. At the beginning of the ripening process the sugar/acid ratio is low, because of low sugar content and high fruit acid content, this makes the fruit taste sour. During the ripening process the fruit acids are degraded, the sugar content increases and the sugar/acid ratio achieves a higher value. Overripe fruits have very low levels of fruit acid and therefore lack characteristic flavour. Finally, this study will provide good baseline information on acidity of common citrus fruits in Nigeria and their potential to incite dental erosion. It also forms a good background data to formulation of further epidemiological and more advanced *in vitro* studies on these samples.

CONCLUSIONS

This study showed that all the commercially available citrus fruits evaluated are acidic with significant dental erosive potential. All the pHs were below the critical pH of enamel dissolution. Inappropriate consumption of these fruits especially lime could incite erosive toothwear.

REFERENCES

1. Economos, C.W.D. and W.D. Clay, 1999. Nutritional and health benefits of citrus fruits. a paper presented at the Twelfth Session of the Intergovernmental Group on Citrus Fruit, 22 to 25 September 1998, Valencia, Spain. Accessed on 12/03/2013 at http://www.fao.org/docrep/x2650T/x2650t03.htm.
2. UNCTAD., 2010: United Nations Conference our Trade and Development. Website: http://www. Unctad.org/infocomm.
3. Ortese, E., K.P. Baiyeri and F.D. Ugese, 2012. Demographic Features of Citrus Producers and Agronomic Management of the Crop in Benue State, Nigeria. PAT, 8(1): 180-190.
4. Opeke, L.K., 2005. Tropical Commodity Tree crops. Spectrum Books Ltd, Ibadan, pp: 503.
5. FAO Commodities and Trade Division, 1991. Citrus fruit fresh and processed annual production statistics. CCP: CI/91.
6. Hon, F.M., O.I.A. Oluremi and F.O.I. Anugwa, 2009. The Effect of Dried Sweet Orange (*Citrus sinensis)* Fruit Pulp Meal on the Growth Performance of Rabbits. Pakistan J. Nutr., 8: 1150-1155.
7. Kale, P.N. and P.G. Adsule, 1995. Citrus. In: Handbook of fruit science and technology. Production, composition, storage and processing. (Eds.: D.K. Salunkhe and S.S. Kadam), Marcel Dekker, New York, pp: 39-65.
8. McClure, F.J., 1943. The destructive action, *in vivo*, of dilute acids and acid drinks and beverages on the rats' molar teeth. J. Nutr, 26: 251-259.
9. Bieri, J.G., C.M. McCoy, J.S. Restarski and R.A. Gortner, 1946. Further studies on *in vivo* tooth decalcification by acid beverages. Arch Biochem. Biophys., 11: 33-40.
10. Gortner, R.A., J.S. Restarski, J.G. Bieri and C.M. McCay, 1945. Factors influencing the destructive effects of acidic beverages on the teeth of white rats and hamsters. Arch Biochem, 8: 405-415.
11. Gedalia, I., J. Anaise, V. Westeich and A. Fuks, 1975. Predisposition to caries in hamsters following the erosive effect of a commercial citrus beverage administered with and without supplemented fluoride. J. Dent Res., 54: 496-499.
12. Restarski, J.S., R.A. Gortner and C.M. McCay, 1945. Effect of acid beverages containing fluorides upon the teeth of rats and puppies. J. Am. Dent Assoc., 32: 668-675.
13. Holloway, P.J., M. Mellanby and R.J.C. Stewart, 1958. Fruit drinks and tooth erosion. Br. Dent J., 104: 305-309.
14. Wynn, W.J. and J. Haldi, 1948. The erosive action of various fruit juices on the lower molar teeth of the albino rat. J. Nutr., 35: 489-491.
15. Miller, C.D., 1950. Enamel erosive properties of fruit and fruit juices. J. Nutr., 42: 63-71.

16. Darby, E.T., 1892. Dental erosion and the gouty diathesis: are they usually associated? Dent Cosmos, 34: 629-6240.
17. Stafne, E.C. and S.A. Lovestedt, 1947. Dissolution of tooth substance by lemon juice, acid beverages and acids from some other sources. J. Am. Dent Assoc., 34: 586-592.
18. Hicks, H., 1950. Excessive citrus juice consumption: clinical observations of its effect on superficial and deep tissue of the oral cavity. J. Am Dent Assoc., 41: 38-44.
19. Finch, L.D., 1957. Erosion associated with diabetes insipidus. Br Dent J., 103: 280-282.
20. Thomas, A.E., 1957. Further observations on the influence of citrus fruit juices on human teeth. N.Y. State Dent J., 23: 424-430.
21. Allan, D.N., 1969. Enamel erosion with lemon juice. Br Dent J., 122: 300-302.
22. Künzel, W., M.S. Cruz and T. Fischer, 2000. Dental erosion in Cuban children associated with excessive consumption of oranges. Eur J. Oral Sci., 108(2): 104-109.
23. Bamise, C.T., V.E. Dinyain and K.A. Kolawole, 2009. Dental Erosion due to Lime Consumption; Review of Literature and case report. East Afr J. Public Health, 6(2): 141-143.
24. Lussy, A., T. Jaeggi and S. Jaeggi-Scharer, 1995. Prediction of the erosive potential of some beverages. Caries Res, 29: 349-354.
25. Zero, D.T., 1996. Etiology of dental erosion-extrinsic factors. Eur J. Oral Sci., 104: 162-177.
26. Torres, C.P., M.A. Chinelatti, J.M. Gomes-Silver, F.A. Rizóli, M.A.H. Oliveira, R.G. Palma-Dibb and M.C. Borsatto, 2010. Surface and Subsurface Erosion of Primary Enamel by Acid Beverages over Time. Braz Dent J., 21(4): 337-345.
27. Smith, B.G. and J.K. Knight, 1984. An index for measuring the wear of teeth. Br Dent J., 156(12): 435-438.
28. Bamise, C.T., E.O. Ogunbodede, A.O. Olusile and T.A. Esan, 2007. Erosive Potential of Soft Drinks in Nigeria. WJMS, 2(2): 115-119.
29. Jensdottir, T., P. Holbrook, B. Nauntofte, C. Buchwald and A. Bardow, 2006. Immediate erosive potential of cola drinks and orange juices. J. Dent Res., 85(3): 226-230.
30. West, N.X., J.A. Hughes and M. Addy, 2000. Erosion of dentin and enamel *in vitro* by dietary acids: The effect of temperature, acid character, concentration and exposure time. J. Oral Rehabil, 27: 875-880.
31. Edwards, M., S.L. Creanor, R.H. Foye and W.H. Gilmour, 1999. Buffering capacities of soft drinks: The potential influence on dental erosion. J. Oral Rehabil, 26: 923-927.
32. Davani, R., J. Walker, F. Qian and J.S. Wefel, 2003. Measurement of viscosity, pH and titratable acidity of sport drinks. J. Dent Res., 82(Special Issue A): Abstract No. 326.
33. Cairns, A.M., M. Watson, S.L. Creanor and R.H. Foye, 2002. The pH and titratable acidity of a range of diluting drinks and their potential effect on dental erosion. J. Dent, 30: 313-317.
34. Baldwin, E.A., 1993. Citrus fruit. In: Biochemistry of fruit ripening (Eds. G. Seymour, J. Taylor and E. Tucker). Chapman and Hall, New York, pp: 107.
35. Nour, V., I. Trandafir and M.E. Ionica, 2010. HPLC Organic Acid Analysis in Different Citrus Juices under Reversed Phase Conditions. Not. Bot. Hort. Agrobot. Cluj, 38(1): 44-48.
36. Scherer, R., A.C.P. Rybka, C.A. Ballus, A.D. Meinhart, J.T. Filho and H.T. Godoy, 2012. Validation of a HPLC method for simultaneous determination of main organic acids in fruits and juices. Food Chemistry, 135: 150-154.
37. Muller, M., U. Irkens-Kiesecker, B. Rubinstein and L. Taiz, 1996. On the mechanism of hyperacidification in lemon: Comparison of the vacuolar H(+)-ATPase activities of fruits and epicotyls. J. Biol Chem., 271: 1916.
38. Penniston, K.L., S.Y. Nakada, R.P. Holmes and D.G. Assimos, 2008. Quantitative Assessment of Citric Acid in Lemon Juice, Lime Juice and Commercially-Available Fruit Juice Products. J. Endourol., 22(3): 567-570.
39. Falade, O.S., O.R. Sowunmi, A. Oladipo, A. Tubosun and S.R.A. Adewusi, 2003. The Level of Organic Acids in Some Nigerian Fruits and their Effect on Mineral Availability in Composite Diets. Pakistan J. Nutr., 2(2): 82-88.
40. Meurman, J.H., M. Harkonen, H. Naveri, J. Koskinen, H. Torkko, I. Rytommaa *et al.,* 1990. Experimental sports drinks with minimal dental erosion effect. Scand J. Dent Res., 98: 120-8.