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Original Research Article

Quantitative Evaluation of Demineralizing Effects of Citrus Fruit Juices on Human Enamel - An *In-Vitro* Study

Ranjana Y. Bansod[1], R. S. Sastry[2], Prasad Y. Bansod[*3]

[1]*Associate Professor, Department of Dentistry, Indira Gandhi Govt. Medical College, Nagpur, Maharashtra, India*
[2]*Professor & Head, Department of Operative Dentistry, Govt. Dental College and Hospital, Nagpur, Maharashtra, India*
[3]*Assistant Professor, Department of Surgery, Govt. Medical College & Hospital, Nagpur, Maharashtra, India*

Abstract

Background: Excessive consumption of citrus fruits apparently results in destructive effects on the teeth which cause perplexing problems. Patients with loss of tooth surface due to attrition, abrasion and erosion have been increasingly seeking for help. Attention has been drawn to the effect of erosion on dental enamel due to excessive intake of fruit juices.
Materials & Methods: Sample of one hundred extracted adult human central incisors was used to estimate the demineralizing effect of lemon and orange fruit juices on human enamel. Levels of calcium and phosphorus were evaluated. The pH of fruit juice, weight of the tooth before and after the observation period, quantitative estimation of calcium and phosphorus in the fruit juices were estimate. Gross changes on the enamel were studied and the results tabulated.
Results: There is a gradual and significant loss of weight of the tooth due to demineralization as the period observation increases. The rate of demineralization is rapid in the first 30 minutes in both orange and lemon juices. Statistical analysis suggested that lemon and orange juice have definite demineralizing effect on human enamel at various intervals
Conclusion: Human enamel has the tendency to demineralize in the presence of lemon as well as orange juice. Overall the lemon juice has marked demineralizing effect on human enamel as compared to orange juice.
Keywords: Human enamel, Demineralization, Tooth erosion, Citrus fruit juices, Rate of demineralization.

***Correspondence Info:**
Dr. Prasad Y. Bansod
Assistant Professor,
Department of Surgery,
Govt. Medical College & hospital,
Nagpur, Maharashtra, India

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1. Introduction

Diet is a major factor in the preservation and maintenance of an individual's health. The qualitative and quantitative consumption of diet depends on various factors such as economic status, food habits, religious & cultural habits, food abuse, beliefs, diverse geographic locations of food production, the general status of an individual as well as the condition of one's masticatory apparatus. Increased production and wider distribution of lemon and other citrus fruits have led to marked increase in their consumption; now they are available at almost all times. This has brought a highly beneficial improvement in the diet. The citrus fruits are relatively cheap, rich and convenient source of vitamin C. However, some habits and methods related to use of citrus fruits apparently result in destructive effects on the teeth.[1]

Recently, patients with loss of tooth surface due to attrition; abrasion and erosion have been increased who have been seeking dental consultations. Attention has been drawn to the effect of erosion on dental enamel due to excessive intake of fruit juices and other acidic beverages. These recent dietary changes pose a frequent and perplexing problem to most dental practitioners who lead to ambiguous etiology, diagnosis, and treatment. Hence it is necessary to understand the various wasting processes that are involved in destruction of the dental tissues.[2]

The cause of erosion may be extrinsic due to influences outside the mouth or intrinsic from sources in the mouth. Extrinsic erosion may be industrial or due to demineralizing foods such as citrus fruits and acid beverages. Intrinsic erosion is the result of habitual regurgitation of gastric contents and may be a symptom

associated with anorexia nervosa, hiatus hernia or recurrent vomiting due to some abnormality of the gastrointestinal tract.[3, 4]

Dental demineralization is common in adults between 30 years and 40 years with a slight female predilection. At first it presents no problem except the loss of tooth substance. There is no softening but simply wasting of the substance leaving a perfectly smooth polished surface. The dentin wastes away in a similar manner and becomes hypersensitive, a characteristic of erosion in teeth with vital pulps. The lesions are usually bilateral involving buccal or labial surfaces with fewer tendencies for proximal or lingual surfaces.[5-8] Natural lemon and orange juices have a pH between 2.0 and 3.5. These fruits contain organic acids such as ascorbic and citric acids. Citric acid dissolves enamel more readily than other organic acids. In addition to the effect of its acidity, it also forms complex calcium Citrate [3,6]

Literature survey shows several reports indicating that citrus fruits are one of the common etiological agents causing erosion of enamel. Numbers of studies have been conducted to observe the enamel decalcification both in vivo in animals and in vitro on human dentition.

The results of these studies showed that there is loss of calcium from the enamel surface due to decalcification. Although most of the studies carried out were related to the erosion of teeth, considerable less research was undertaken regarding calcium and phosphorus estimation method. As these citrus fruits are taken daily in various forms in the diet it was decided to take up the comparative study of enamel demineralization by using lemon and orange fruit juices.[9-11]

2. Materials and Methods

For the estimation of demineralizing effects orange and lemon juice on human enamel and its comparison a study was formulated and was carried out in the Govt. Dental College and Hospital, Nagpur for a period of one year. The biochemical investigations were performed in the Clinical Biochemistry Department of Govt. Medical College, Nagpur.

2.1 Collection of specimen

Normal, mobile and healthy central incisors were selected for the study. The teeth with surface defects, cervical lesions, attrition, abrasion & caries were excluded from the study.

At the time of extraction adequate care was taken by keeping the cotton swab on the tooth surface to prevent the surface damage of the tooth structure. The teeth were scrubbed and washed under running water and examined under magnifying lens. In order to reduce the experimental error, teeth were selected to match in size as closely as possible. Then they were preserved in distilled water to prevent dehydration. Out of one hundred specimens, two batches, each containing fifty teeth were randomly selected.

2.2 Preparation of fruit juice

The fruit juice was extracted from fresh lemons and oranges by employing usual fruit juice extraction methods and kept ready for the experiment. The pH of lemon and orange juice was determined by Orion™ pH metre and combination electrodes. The calcium estimation of extracted juices was done by Baron and Bell Titration method and the phosphorus estimation was done by Fiske and Subbarow colorimetric method. The obtained readings were used as control reading (Normal value).

Batch A: In this batch the demineralizing effect of Lemon juice was studied.

Batch B: In this batch the demineralizing effect of Orange juice was studied.

It was decided to study the demineralizing effect of lemon and orange fruit juices on human enamel with estimation of calcium and phosphorus in vitro at regular intervals i.e. Zero minute to one minute, fifteen, thirty, forty-five and sixty minutes respectively. Subsequently each batch was made into five groups (Group I to V) containing ten specimens in each group to study the demineralizing effect at regular time intervals. The time intervals were fixed.

2.3 Preparation of the tooth and its suspension in the fruit juice

The root portion of the tooth including apical foramina was coated with the clear nail varnish. The individual tooth was weighted with the help of (oriental) electric balance before experiment. The specimens were suspended in the prepared fruit juice of 2.5 ml, so that only the enamel portion of the tooth was completely immerse into it. The bottles were closed with a rubber cork and sealed with a sticky wax. Then they were placed in incubator at 37°C and subjected to various observation periods.

The calcium and phosphorus estimation were performed in the respective fruit juices. At the end of the observation period the tooth was removed from the fruit juice, washed with distilled water, dried with filter paper and weight of the tooth was done.

2.4 Statistical data for demineralizing action of lemon and orange juice

The observations of demineralizing action were statistically analysed using students “t” test for the analysis. “P” value <0.05 was considered statistically significant.

3. Results

In total 100 teeth were observed and analysed for the effect of demineralisation by lemon and orange juices. Fifty teeth were categorised in lemon and orange group respectively and following observations were found. The weight loss of fifty specimens which were subjected for demineralizing action in lemon & orange juice at various intervals is shown in Table 1 & 2. It shows there is gradual weight loss in lemon juice while there is weight gain in orange juice.

Table 1: Showing Mean Loss of Tooth Weight at various intervals in Lemon Juice

Time interval in minutes	Weigh of tooth (grams)		Difference of weight loss (Milligrams)
	Before study	After Study	
0-1	1.0009	0.9999	1.0
0-15	1.024	1.021	3.0
0-30	1.0082	1.0030	5.2
0-45	0.964	0.957	7.0
0-60	1.1687	1 .1586	10. 1

Table 2: Showing difference of mean weight at various intervals in orange juice.

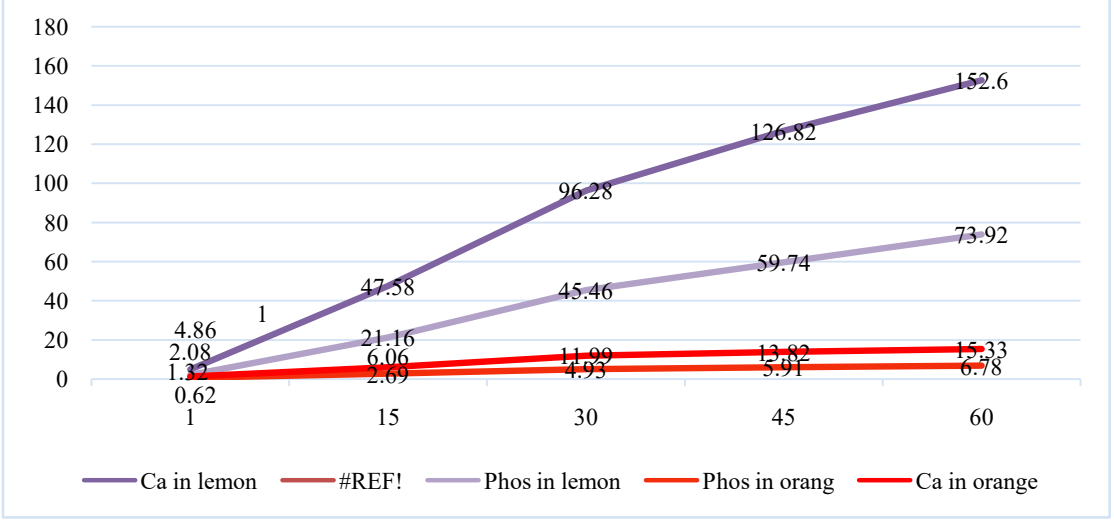
Time interval in minutes	Weight of tooth (grams)		Difference of weight gain (Milligrams)
	Before Study	After Study	
0-1	0.961	0.961	0
0-15	1 .0106	1. 0122	+1.6
0-30	0.9883	0.9915	+3.2
0-45	1 .013	1.018	+5.0
0-60	0.9805	0.9859	+5.4

On comparing the dissolution of calcium and phosphorous from human enamel in lemon & orange juice, it is found that there is more demineralizing action in lemon juice compared to orange juice Table 3 and Figure 1.

Table 3: Showing comparison of dissolution of calcium & phosphorous from human enamel in lemon & orange juice at various intervals

Time interval in minutes	Dissolution of calcium (mg %)		Dissolution of phosphorous (mg %)	
	Lemon	Orange	Lemon	Orange
0-1	4.86	1. 32	2.08	0.62
0-15	47.58	6.06	21. 16	2.69
0-30	96.28	11.99	45.46	4.93
0-45	126.82	13.82	59.74	5.91
0-60	152.6	15.33	73.92	6.78

Figure 1: Showing Dissolution of Calcium & Phosphorous in lemon & orange juice



The rate of dissolution of minerals in lemon and orange juice is shown in Table 4 which clearly indicates a decreasing trend in rate of dissolution after first thirty minutes Figure 2.

Table 4: Showing rate of dissolution calcium & phosphorous in lemon & orange juice at various intervals

Time interval in minutes	Lemon juice		Orange juice	
	Dissolution rate of Calcium	Dissolution rate of Phosphorous	Dissolution rate of Calcium	Dissolution rate of Phosphorous
0-1	4.86	2.08	1. 32	0.62
0-15	3.17	1. 41	0.40	0.17
0-30	3.20	1. 51	0.39	0.16
0-45	2.81	1 .32	0.30	0.13
0-60	2.54	1. 2.3	0.25	0.11

Figure 2: Showing Rate of Dissolution of Calcium & Phosphorous in lemon & orange juice

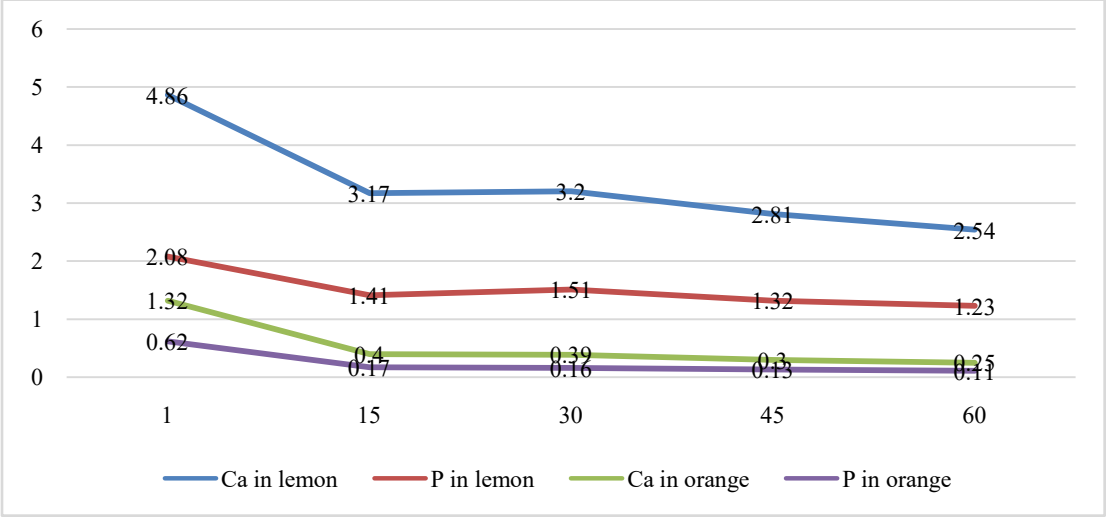


Table 5 & 6 shows the mean, standard deviation and "P" values of calcium and phosphorous at various intervals in lemon and orange juices respectively. It shows statistically significant difference on comparison as the "P" value is <0.05.

Table 5: Showing the statistical analysis of Calcium and Phosphorus Estimated values at various intervals in Lemon Juice

Time interval in minutes	N	Calcium			Phosphorous		
		Mean dissolution of "Ca" mg%	S.D. of Mean of "Ca"	P value	Mean dissolution of "P" mg%	S.D. of Mean of "P"	P value
0-1	10	4.86	0.92	<0.01	2.08	0.57	<0.01
0-15	10	47.58	5.23	<0.01	21.16	3.86	<0.01
0-30	10	96.28	5.053	<0.01	45.46	2.49	<0.01
0-45	10	126.82	11. 32	<0.01	59.74	4.57	<0.01
0-60	10	152.6	12.160	<0.01	73.92	6.23	<0.01

Table 6: Showing the statistical analysis of Calcium and Phosphorus Estimated values at various intervals in Orange Juice

Time interval in minutes	N	Calcium			Phosphorous		
		Mean dissolution of "Ca" mg%	S.D. of Mean of "Ca"	P value of Calcium	Mean dissolution of "P" mg%	S.D. of Mean of "P"	P value of Phosphorous
0-1	10	1.32	0.10	<0.01	0.62-	0.018	<0.01
0-15	10	6.06	1. 28	<0.01	2.69	0.94	<0.01
0-30	10	11.99	1.052	<0.01	4.93	0.85	<0.01
0-45	10	13.82	0.85	<0.01	5.91	0.78	<0.01
0-60	10	15.33	0.64	<0.01	6. 78	1.06	<0.01

4. Discussion

The citrus fruits lemons and oranges are highly acidic in nature when tested under laboratory conditions with a pH of 2 and 3 respectively. The pH does not change to alkaline side even after the maximum time interval of sixty minutes. Lemon juice is more acidic in nature compared to orange juice. On immersing the teeth for various observation periods, it shows decalcification increases as the time increases. However decalcification is more in case of lemon juice. This is confirmed by calcium & phosphorus estimation and by naked eye appearance of the human enamel. This suggests that high acidity definitely affects the enamel when such raw fruits are taken in different forms like fresh citrus fruits, juice extract, diluted form and addition with other additives.

Several clinical reports suggested that the sucking of fresh lemon and orange fruits, drinking of pure lemon and orange juice are the common etiological factors involved in erosion. Hay *et al* estimated that when half a pint of liquid fruit drink was taken slowly, the liquid was in contact with the teeth for more than 45second, the acidity of pure lemon and orange juice was sufficient to damage the teeth.[6] The evidence indicated that saliva does not cause erosion but rather protects the enamel because of its buffering action by diluting the acids.[12, 13]

It is well known that the incidence of caries and erosion increases when salivary secretion is greatly reduced or lost. However, experiments with saliva and buffer

solutions saturated with calcium phosphate have confirmed that the tooth substance dissolves in saliva at pH between 5.5 and 6.5. The pH of these fruits do not undergo marked change in saliva, suggesting that the dilution and buffering action of saliva is not sufficient enough to counteract the acidity.

The ability of citric acid to erode tooth enamel could be attributed to one or more of three factors

1) The citric acid has affinity for calcium,

2) Its high hydrogen ion concentration, owing to the presence of three carboxyl groups in each molecule,

3) The type of reaction that takes place when it is in contact with enamel.

Elsbury *et al* said that the erosion of enamel by mineral acids was self-limiting because of formation of insoluble end products. According to him two processes take place simultaneously when citric acid comes in contact with enamel. The first is dissolution of the enamel to form a calcium citrate salt which is inversely proportional to the pH of the solution, and the second is the withdrawal of the calcium from the tooth to form complex calcium citrate ion independent of the pH of the solution.[14]

Although this citrus fruit stimulates the salivary secretions still the pH remains acidic. The dissolution also depends upon the calcium and phosphate content of saliva, where the content of stimulated saliva is less than unstimulated saliva. In the presence of calcium and phosphate ions at salivary concentrations, calcium phosphate is insoluble at neutral pH but if saliva becomes acidic with critical pH, solubility of calcium phosphate becomes high as PO_4^{3-}ions changed to HPO_4^{2-})when the concentration of ions in the solution will fail to saturate the inorganic material of teeth may dissolve in it.[12, 13]

When there is habitual and abusive use of citrus fruits, particularly the lemon, there may be direct and prolonged contact of fresh acidic juice or fruit pulp with the teeth. Although there may be a rapid neutralization due to buffering action of saliva, still the pH level of the mouth contents remains low. The areas of outer enamel which are in contact with the least buffered fruit acid would be dissolved at a faster rate than other areas, where the pH is not low. This could be the cause of typical but unusual erosive pattern, once the outer layers of enamel are removed, the process may continue with greater ease as it comes in contact with the less resistant inner layers. Clinically the areas of erosion that commonly occur around the gingiva or cervical areas of the teeth (often referred to as tooth brush or idiopathic erosion) could be affected by acid dissolution of the enamel and dentinal surfaces. As the regions of the teeth near the gingiva being less self-cleansing than other areas of the teeth, these areas could harbour unbuffered acid contents of food in close proximity to the tooth surface for a considerable period of time the results could be the areas of gingival erosion, that are clean and non-carious with slow progress.

The teeth which were immersed in lemon juice showed gradual loss of weigh as the period of immersion of the specimen in the juice increases. This could be due to the low pH of the lemon juice which was 2, and the presence of high organic acidic contents like ascorbic acid & citric acid in the juice. All these factors might be contributing in demineralization of the tooth (loss of weight) with respect to time factor. The tooth immersed in orange juice showed increase of weigh as the period of immersion of the specimen in the juice increases. However the weight remains unchanged from zero to one minute and subsequently there be an increase in weight 1.6mg to 5.4mg at the end of the experiment. Jenkins, G. N.[7, 8] has reported that the permeability of enamel is slightly less than dentin. However in certain circumstances the permeability of enamel increases particularly in presence of glucose or saline solution. The orange juice being sweeter than lemon, contain excessive amount of glucose is responsible to increase the permeability of enamel and increase of weight.

As duration of the immersion of the specimen in the juice increases the calcium and phosphorus dissolution estimated values also increased. However, the estimated values of dissolved calcium and phosphorus are found to be more in lemon juice than in orange juice. Holloway, P. J. *et al*[15] reported that lemon and orange juices are responsible for the erosive action due to presence of citric acid in the juice. Stafne, E. C. *et al*[1] mentioned that the lemon juice has more demineralizing action although it was used in dilute form. According to Allen, D.N [16] pure bottled lemon juice as well as raw lemon fruit has strong demineralizing action. Dilley, G. J. *et al*[17] reported that supplement of fresh orange juice to children might be responsible for erosion. The findings of the present study were correlated with the findings of the above studies. Thus the lemon juice has more demineralizing action than orange juice. This might be due to the high acidic contents and low pH of lemon juice.

From table 5 & 6 it has been observed that the estimated values of dissolved calcium is twice that of estimated values of dissolved phosphorus in lemon and orange juice. This ratio is approximately maintained throughout dissolution process. Similar findings were reported by Hay, D. I. *et al* using dental cleaning tablets, McDonald J.L. *et al* using various forms of carboxylic acids.[19-22]

We also notice that the rate of dissolution of calcium and phosphorous in lemon and orange juice at the end of thirty minute goes on decreasing. This might be due to supersaturation of the juice with mineral salts released from human enamel. As the duration of immersion of the specimen in the juice increases the demineralization process decreases (Figure 2). These findings were correlated with the findings of Theon *et al* [18] who reported that the rate of demineralization decreases with the increase in time due to saturation of the solution.[23-25]

When values of dissolved calcium and phosphorous from human enamel were statistically analysed it was observed that the lemon as well as orange juice have demineralizing action at various intervals. All the values of dissolved calcium and phosphorous at various intervals were found to be significant (P<0.01).

Although the ingestion of citrus fruit in moderate amounts as a source of Vitamin C in the diet is not to be condemned, it would seem that more than two or three oranges per week is excessive, as vitamin C is obtainable from other sources. In current popular belief is that ingestion of large quantities of citrus fruit juices is harmful and should be carefully appraised.

5. Conclusion

Human enamel has the tendency to demineralize in the presence of lemon as well as orange juice. There is a gradual and significant loss of weight of the tooth due to demineralization as the period observation increases. Initially there was rapid loss of tooth weight; however it was decreased after thirty minutes. In orange juice, although there is rise of weight of the tooth in the initial period of observationit was not consistent through the study. This may be due to increased permeability of enamel in presence of glucose and other factors. Overall the lemon juice has marked demineralizing effect on human enamel as compared to orange juice. Statistical analysis suggested that lemon and orange juice have definite demineralizing effect on human enamel at various intervals.

Ethical approval

The protocol for the study was approved by the institutional ethics committee.

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