The Effect of Some Carbonated Beverages on Enamel of Human Premolars (Scanning and Light Microscopic Study)

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Abstract: In modern societies, the increased consumption of soft drinks is becoming more important because of the concern for dental erosion. The aim of the present study is to reveal and compare the possible effect of some carbonated beverages on occlusal and cervical parts of enamel in the buccal surface of human premolars. Twenty sound (caries-free) human maxillary premolars extracted for orthodontic reasons were used in the present study. The teeth were then divided into: **Control group:** (before immersion in the beverages), in which the collected teeth were immersed at first in tap water and subdivided into 4 subgroups (5 teeth each) and named; Control Sprite, Control Mirinda Orange, Control Coca- Cola and subgroup R. **Experimental group:** (after immersion in the beverages) in which the first 3 subgroups which were used as control were then utilized as experimental after immersion in the corresponding beverage. They were named; Subgroup S (Sprite experimental Subgroup), Subgroup M (Mirinda Orange experimental Subgroup) and Subgroup C (Coca-Cola experimental Subgroup). The teeth were examined using SEM and light microscope. Morphometric study was performed, using computerized image analyzer for the assessment of affected band thickness. The SEM results of the experimental subgroups revealed that each beverage cause different pattern of erosion. In Subgroup S, the enamel surface was feather like and pitted. In Subgroup M, the enamel surface presented the honeycomb pattern. In Subgroup C, the enamel surface was nearly smooth with generalized structural loss. It was noticed also that the changes in enamel surface became more accentuated toward the cervical third. The light microscopic examination revealed that in both Subgroups S and C the outer enamel layer exhibited dark band of affection, while in Subgroup M this band was translucent. The affection of subsurface and deep enamel layers was noticed in all the experimental subgroups, in addition the changes in enamel became more accentuated toward the cervical third. The morphometric data revealed that the thickness of the affected band in the outer layer of enamel increased toward the cervical third in all experimental subgroups. This band was minimal in Subgroup S, and increased in Subgroup M, followed by Subgroup C. From the present study we can conclude that acidic beverages had deleterious effect on dental hard tissues. Among the investigated drinks of the present work, the Sprite had the least erosive potential, followed by Mirinda Orange, then Coca-Cola which had the most erosive potential. The erosive potential of a beverage was depended on its pH value, titratable acidity, type and concentration of the acid(s) present. Enamel affection for a given beverage was maximum at the cervical third and minimal in the occlusal.

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Key words: Carbonated beverages, enamel, premolars, scanning electron microscope, light microscope.

1. Introduction

In modern societies, the increased consumption of soft drinks is increasingly becoming more important because of the concern for dental erosion. Dental erosion is defined as the pathological, chronic, localized and painless loss of hard tooth tissue resulting primarily from non-bacterial chemical attack, and usually involving acid substances **(Litonjua et al., 2003).** Erosion is classified as extrinsic (i.e., diet) or intrinsic (i.e., gastro-esophageal) in origin. Erosion is typically progressive and results in the wearing away of the exposed tooth surface i.e., enamel or root surface **(Scheutzel, 1996; Lussi et al., 2004).**

Soft drinks are non-alcoholic, flavored, carbonated beverage, usually commercially prepared and sold in bottles or cans **(Lussi et al., 2004).**

In Sprite, high fructose corn syrup used as sweetener. Acids are added to soft drinks for extra bite, and mouth feel. The acid used in Sprite® is citric acid. Carbonated water is also mildly acidic. In addition lemon-lime flavor is used but there is no caffeine added **(Sprite® ingredients, The Free Encyclopedia, 2010).**

In Mirinda Orange the constituents are almost like those used in Sprite except for the addition of natural orange flavor. Ascorbic acid (Vitamin C) which is used as an anti-oxidant and yellow 6 color (sunset) are also added **(Mirinda Orange® ingredients, The Free Encyclopedia, 2010).**

In Coca-Cola the constituents are almost like those used in Sprite except for the phosphoric acid and caffeine which are added as flavors **(Coca-Cola® ingredients, *The Free Encyclopedia, 2010).***

Many studies support an association between acidic beverages (such as fruit juices, fruit teas, sodas and sports drinks) consumption and dental erosion. **Gedalia *et al.* (1991)** studied the softening effect of

Coca-Cola on human enamel. Their results showed that 1 hour exposure to Coca-Cola caused a decrease in enamel hardness and changes in surface structure visualized by the SEM.

Gray *et al.* (1998) studied the effect of immersing human unerupted lower third molar teeth in white wine (pH 3.3) for 24hrs using SEM. The scanning electron micrographs showed that the wine caused irregular areas of erosion. A similar pattern was described by **Meurman and Frank (1991)** for human enamel after its immersion in a phosphoric acid-containing beverage (pH 2.6) for 30min.

Von Fraunhofer and Rogers (2004) studied the effects of different beverages (Coca-Cola, Mountain Dew, Sprite of both regular and diet forms, A&W root beer, Brewed black tea, Brewed black coffee, and tap water which was used as control) on 20 sound (caries-free) human molars and premolars. Mean percentage weight losses and weight losses per unit area were calculated for each set of enamel specimens and beverages. The data reported in their study indicated that carbonated soft drinks cause significant long-term enamel dissolution, and they were markedly more aggressive toward the enamel than coffee, tea, and root beer which appeared to be more safe for the health of enamel.

Fathilah and Rahim (2008) studied the effects of different beverages (Coke®, Sprite®, Ribena®, and Chrysanthemum tea) as well as mineral water on the demineralization of the enamel surface. Demineralization was determined by the rate of calcium released from the enamel surface on exposure to the solutions. Calcium was determined using the EDTA titration method. The pH of these beverages was measured and found to be in the acidic range (2.43 to 5.79), while mineral water which served as a control has a pH of 7.00. It was found that calcium released from the enamel surface following exposure to the beverages. The highest rate of calcium released was exhibited by Coke™ this was followed in a descending order by Ribena™, Sprite™ and Chrysanthemum tea. However mineral water did not display any release of calcium over the study period.

Ehlen *et al.* (2008) (*in vitro*) studied the erosive potential of representative commercial beverages (100% apple juice, Coke®, Diet Coke®, Lemon-Lime Gatorade® and Red Bull®) on extracted human permanent teeth using polarized light microscope. Their results showed that lesion depths in both enamel and cementum were greatest in specimens exposed to energy drinks (Lemon-Lime Gatorade® and Red Bull®), followed in a descending order by regular soda (Coke®), diet soda (Diet Coke®), and then 100% apple juice.

Although many authors had studied the effect of some acidic beverages on dental hard tissues, none had compared the effect of these beverages on the occlusal and cervical enamel or their effect on subsurface and deep layers of enamel. So the aim of the present work is to reveal and compare the possible effect of some acidic beverages on the occlusal and cervical thirds of enamel using SEM and light microscope.

2. Materials and Methods

This experimental design was approved by the Research Ethics Committee of the Faculty of Dentistry, Ain-Shams University, Egypt.

Twenty sound (caries-free) human maxillary premolars extracted for orthodontic reasons were collected from the surgery department in Faculty of Dentistry, Ain-Shams University. The extracted teeth were cleaned gently of residual debris and washed thoroughly under running water and then they were examined under stereomicroscope to ensure the absence of caries, calculus, or surface defect. Teeth were then divided into:

A) Control group: (before immersion in the beverages)

This group contained the 20 premolars in which the collected teeth were immersed at first in tap water and they were randomly subdivided into 4 equal subgroups (5 premolars each) as follows: **Control Sprite, Control Mirinda Orange, Control Coca-Cola and subgroup R.** Teeth used as control were examined using SEM. The teeth of the first 3 subgroups were then utilized as experimental after immersion in the corresponding beverage and they were re- examined using SEM and to assess the internal extension of the changes by light microscope, bucco-lingual ground sections were prepared from each premolar**. Subgroup R** was utilized for light microscopic examination of the normal histology.

B) Experimental group: (after immersion in the beverages)

This group was subdivided into; **Subgroup S** (Sprite experimental subgroup), **Subgroup M** (Mirinda Orange experimental subgroup) and **Subgroup C** (Coca-Cola experimental subgroup). Beakers were filled with 330ml of beverages; and each tooth in each subgroup was given a number, placed in a separate beaker and immersed for 25hrs. The beverages were replaced every 5hrs, and the pH was measured at opening the cans then it was measured again at the end of the 5hrs, using Jen Way 3505 pH meter **(Ehlen *et al.*, 2008).**

For the scanning electron microscopic examination, the teeth were mounted on the SEM holder using removable adhesive. For each tooth, the buccal surface was adjusted to be examined at the occlusal and cervical thirds using FEI/ Inspect (S) scanning electron microscope (SEM Unit, Main Defense Chemical Laboratory, Cairo, Egypt). The collected teeth were examined at 30kV using the secondary electron LFD detector under the magnification (X1000) and (X 4000)

with a (spot size 4.7-5.3nm) in each magnification **(Rabertson and Nietzche, 2009)**.

For the light microscopic examination, the premolars were sectioned bucco-lingually into 2 halves using a diamond disc. From each half, at first 1mm thick section (nearest to the midline) was obtained, and then further thinning was carried out using carborundum abrasive disc paper. Polishing was performed using 600 or 800 grit silicon carbide paper under water lubrication. The specimens were then washed in water, dehydrated in ascending grades of alcohol, cleared in xylol, and mounted in Canada balsam **(Frost, 1958).** These sections were examined under the light microscope to study the buccal surface at the occlusal and cervical thirds of enamel.

For every premolar in the experimental group a morphometric study was performed, using computerized image analyzer (UTHSCSA Image Tool program) for the assessment of affected band thickness at the occlusal and cervical thirds of enamel. The measurements were performed in all sections and 2 measures were done in each third as the band was measured at the narrowest area and at the widest area as shown in figure 1.

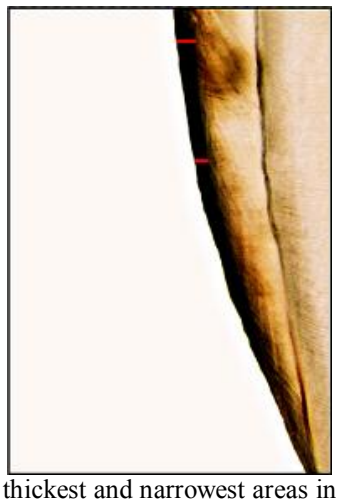

Fig. 1: The thickest and narrowest areas in the band of affection.

3. Results:

pH measurement of the different solutions showed that tap water had neutral pH (6.93), while Sprite, Mirinda Orange and Coca-Cola were acidic. The pH values of the different beverages are summarized in table 1.

Table (1): pH measurements of beverages utilized in this study

pH	Sprite	Mirinda Orange	Coca-Cola
On opening the cans	2.83	2.72	2.37
End of the 5 hours	2.77	2.69	2.41

Ultrastructural Results:

Control group:

On scanning electron microscopic (SEM) examination, enamel of the buccal surface of all specimens of the control subgroups presented more or less the same normal surface features. The occlusal third showed few perikymata grooves and ridges. There were plenty of enamel rod ends and narrow areas of rodless enamel were observed (Figs. 2a & 3a). In the most cervical part of the cervical third, there were plenty of perikymata grooves & ridges, few enamel rod ends, and wide areas of rodless enamel (Figs. 2b & 3b).

Experimental group:

Subgroup S:

SEM examination of the occlusal third of the buccal surface of premolars of this group revealed some areas in the enamel surface have fish scale appearance, while other areas were covered by rodless enamel (Figs. 2c & 3c). The most cervical part of the cervical third showed irregular, feather like, structurless, pitted enamel surface with some cracks, and few areas of exposed dentin (Figs. 2d & 3d).

Subgroup M: SEM examination of the crown occlusal third revealed eroded enamel surface with some areas had destructed prism cores but with definite prism boundaries giving the characteristic honeycomb structure, while other areas were covered by rodless enamel (Figs. 2e & 3e). The most cervical part of the cervical third showed irregular, structurless, undermined, and pitted feather like enamel with plenty of cracks. Some areas of dentin were exposed through holes in the enamel surface (Figs. 2f & 3f).

Subgroup C:

The occlusal third of the crown SEM examination revealed smooth enamel surface with fish scale appearance (Figs. 2g & 3g). The most cervical part of the cervical third showed irregular enamel surface with small deep depressions, the enamel surface exhibited an evident generalized structural loss (Figs. 2h & 3h).

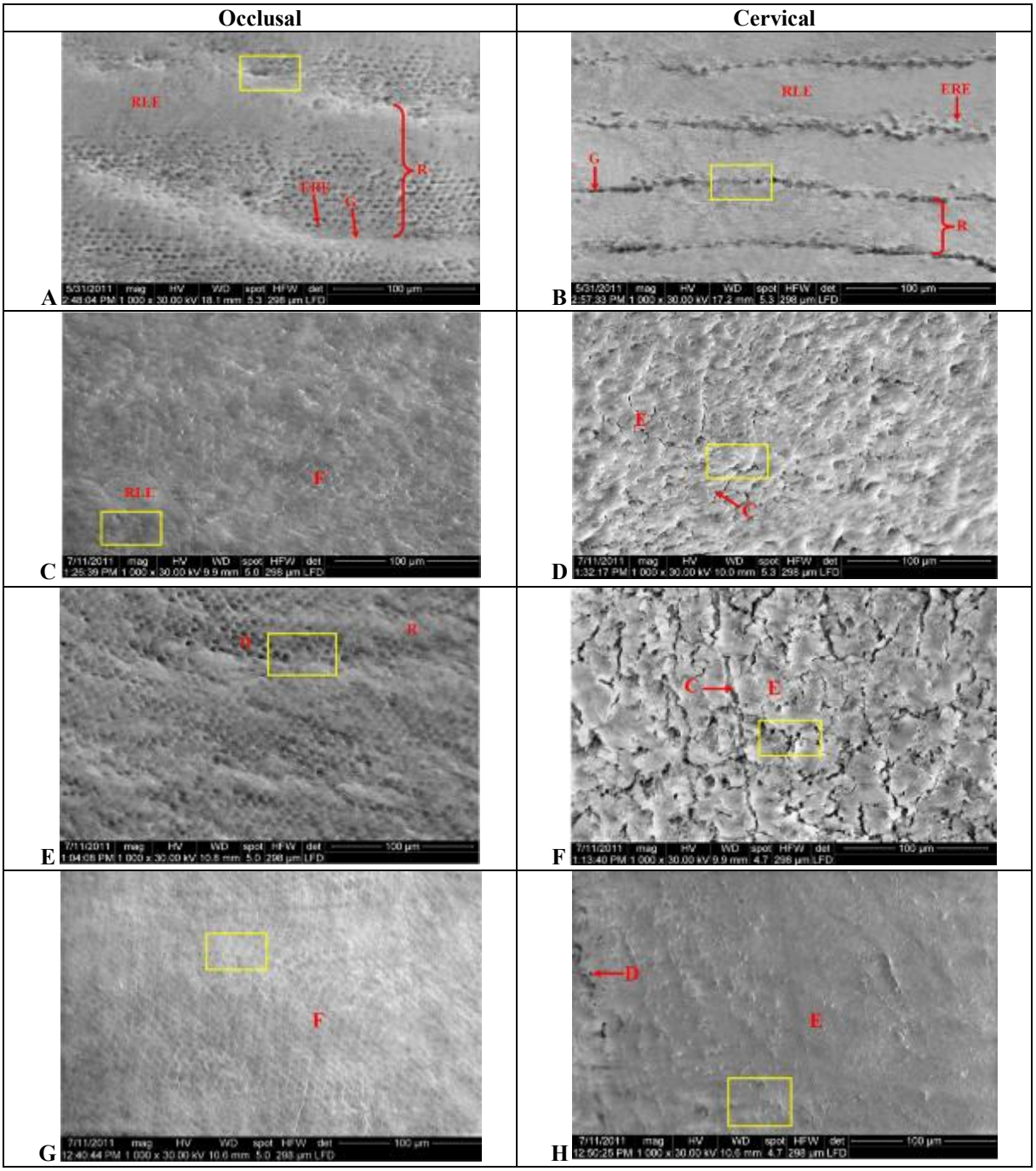

Fig. 2: Scanning electron micrographs for enamel showing:

A) Occlusal third of the control group, showing: Few perikymata grooves (G) & ridges (R), plenty of enamel rod ends (ERE), narrow areas of rodless enamel (RLE) (X 1000).

B) Cervical third of the control group, showing: Plenty of perikymata grooves (G) & ridges (R), few enamel rod ends (ERE), and wide areas of rodless enamel (RLE) (X 1000).

C) Occlusal third of the subgroup S, showing: Some areas in the enamel surface have fish scale appearance (F), while other areas were covered by rodless enamel (RLE) (X1000).

D) Cervical third of subgroup S, showing: Irregular structurless pitted enamel surface (E) with some cracks (C) (X1000).

E) Occlusal third of subgroup M, showing: Eroded enamel surface with some areas had the characteristic honeycomb structure (H), while other areas were covered by rodless enamel (R) (X1000).

F) Cervical third of subgroup M, showing: Irregular structurless pitted enamel surface (E) with plenty of cracks (C) (X1000).

G) Occlusal third of subgroup C, showing: Smooth enamel surface with fish scale appearance (F) (X1000).

H) Cervical third of subgroup C, showing: Irregular enamel surface with small deep depressions (D), and the enamel surface exhibited an evident generalized structural loss (E) (X1000).

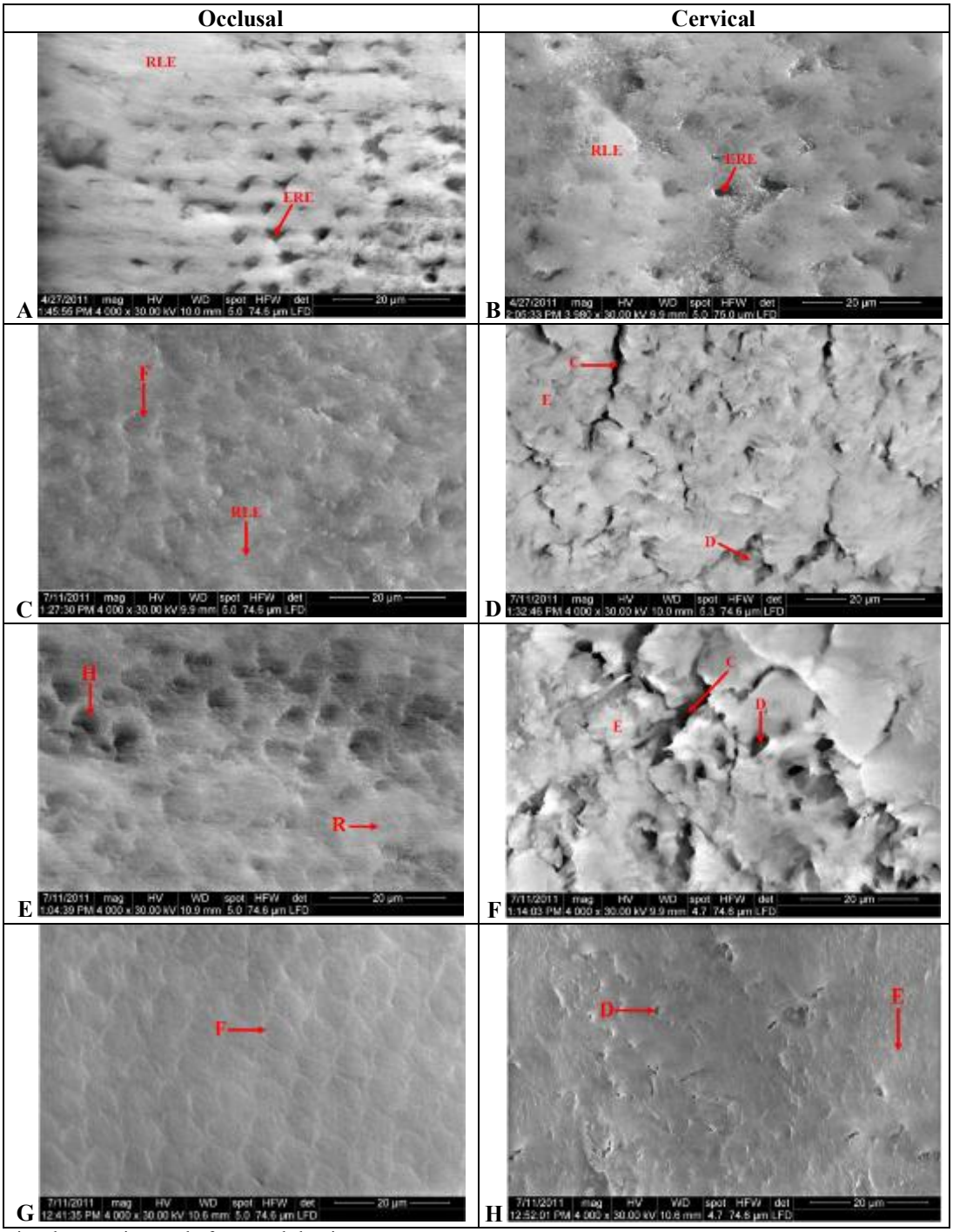

Fig. 3: Scanning electron micrographs for enamel showing:

A) A higher magnification of the inset (a) showing: Enamel rod ends (ERE), rodless enamel (RLE) (X 4000).

B) A higher magnification of the inset (b) showing: Enamel rod ends (ERE), and rodless enamel (RLE) (X 4000).

C) A higher magnification of the inset (c) showing: Some areas in the enamel surface have fish scale appearance (F), while other areas were covered by rodless enamel (RLE) (X4000).

D) A higher magnification of the inset (d) showing: Feather like, pitted (E) and cracked (C) enamel surface. Few areas of exposed dentin (D) were noticed (X4000).

E) A higher magnification of the inset (e) showing: Areas of eroded enamel had the characteristic honeycomb structure (H), while other areas were covered by rodless enamel (R) (X4000).

F) A higher magnification of the inset (f) showing: Undermined, feather like (E) and cracked (C) enamel with some areas of dentin were exposed through holes in enamel surface (D) (X4000).

G) A higher magnification of the inset (g) showing: Smooth enamel surface with fish scale appearance (F) (X4000).

H) A higher magnification of the inset (h) showing: Enamel surface with small deep depressions (D) and generalized structural loss (E) (X4000).

Histological results:

Control group (subgroup R):

Light microscopic examination of the ground bucco-lingual sections of the crown revealed the normal structure of enamel.

The occlusal third of the crown, showed the incremental lines of Retzius, the enamel rods that run at a right angle to the dentin surface, enamel lamella, enamel spindles, dentino-enamel junction (DEJ) and dentinal tubules (Fig. 4a). At a higher magnification in subsurface enamel, the enamel rods and interrod regions were clearly obvious and the enamel rods were parallel to each other and of even thickness (Fig. 5a). At a higher magnification in the enamel near to DEJ, the enamel rods and interrod regions appeared as those in subsurface enamel.

While the cervical third showed the incremental lines of Retzius, the enamel rods run in an apical direction, also showing enamel lamella, DEJ and dentinal tubules (Fig. 4b). At a higher magnification in subsurface enamel and in enamel near to DEJ, the enamel rods and interrod regions appeared as those in the occlusal third.

Experimental group:

Subgroup S:

During ground sections preparation, it was noticed that premolars of this subgroup exhibited less hard texture than those of the control group.

The occlusal third in ground sections of this subgroup showed that at enamel surface an uneven thin dark structureless band with nearly straight line of demarcation from the subsurface enamel could be noticed. In subsurface enamel, the rod structure was apparently different from that of the control group, while the enamel adjacent to the DEJ appeared as uneven thin light structurless band (Fig. 4c). At a higher magnification in subsurface enamel, the enamel rods had uneven thickness and an irregular course, also an apparent widening of interrod regions could be detected (Fig. 5b). At a higher magnification in the light band of enamel, the enamel rods and interrod regions were faintly appeared and had greyish colour. The enamel rods had uneven thickness and in some areas the enamel rods had irregular course while in other areas they were regularly arranged. Also an apparent widening of interrod regions could be detected (Fig. 5c).

The cervical third revealed at enamel surface an uneven thin dark structureless band with nearly straight line of demarcation from the subsurface enamel could be detected.This band was noticed in the full enamel thickness at cemento-enamel junction (CEJ). In subsurface enamel, the rod structure was apparently different from that of the control group, while the enamel adjacent to the DEJ appeared as uneven thin dark structureless band followed by uneven thin light structurless band (Fig. 4d). At a higher magnification in subsurface enamel and in the light band of enamel, the enamel rods and interrod regions appeared as those in the occlusal third.

Subgroup M:

During ground sections preparation, it was noticed that the premolars of this subgroup exhibited much less hard texture than those of subgroups R&S.

The occlusal third in ground sections of this subgroup showed that at enamel surface a nearly even, moderatly thick transleucent band could be detected. In this band enamel rods, interrod regions and incremental lines appeared faintly with scalloped line of demarcation from the subsurface enamel. The outer part of the subsurface enamel appeared as uneven thick light band with rod structure apparently different from that of the control group and the enamel rods become more accentuated in the enamel near to DEJ. The enamel adjacent to the DEJ appeared as uneven thin light structurless band (Fig. 4e). At a higher magnification in subsurface enamel and in the light band of enamel, the enamel rods and interrod regions appeared as those in the subgroup S.

The cervical third revealed that at enamel surface an uneven moderatly thick transleucent band could be detected. In this band enamel rods, interrod regions and incremental lines appeared faintly. A scalloped line of demarcation from the subsurface enamel could be identified. This transleucent band was noticed in the full enamel thickness at CEJ. In subsurface enamel erosive cavity was detected togather with rod structure apparently different from that of the control group, while the enamel adjacent to the DEJ appeared as uneven thick dark structureless band followed by uneven thin light structurless band (Fig. 4f). At a higher magnification in subsurface enamel and in the light band of enamel, the enamel rods and interrod regions appeared as those in the occlusal third.

Subgroup C:

During ground sections preparation, it was noticed that the premolars of this subgroup exhibited soft chalky texture.

The occlusal third in ground sections of this subgroup showed an apparent decrease in the enamel thickness. At enamel surface an uneven thick dark srtucturless band with straight line of demarcation from the subsurface enamel could be detected. In subsurface enamel, the rod structure was apparently different from that of the control group, while the enamel adjacent to the DEJ appeared as uneven thin light structurless band (Fig. 4g). At a higher magnification in subsurface enamel and in the light band of enamel, the enamel rods and interrod regions appeared as those in the subgroup S.

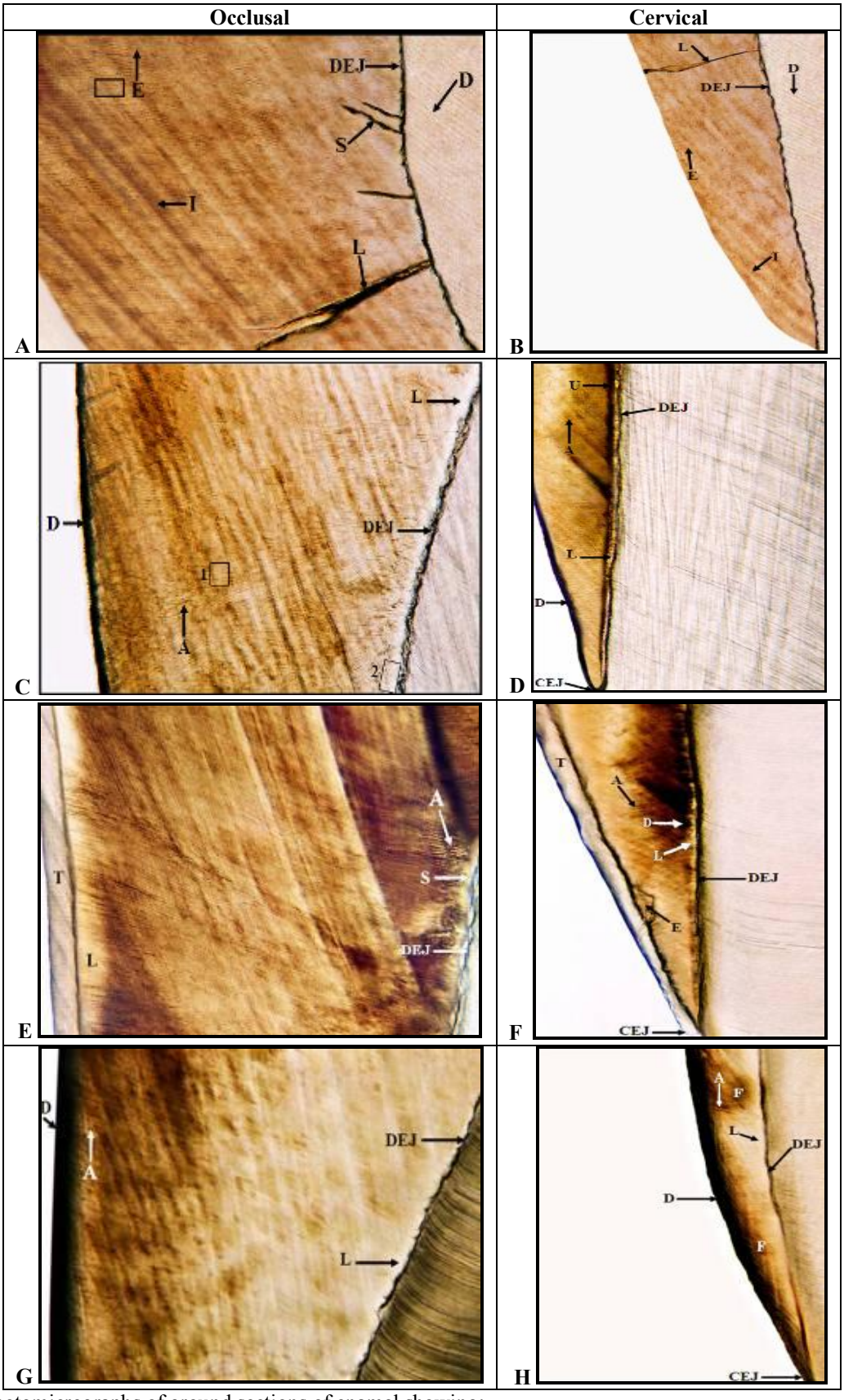

Fig. 4: A photomicrographs of ground sections of enamel showing:

A) Crown occlusal third of the control group (subgroup R), showing: Incremental lines of Retzius (I), enamel rods (E), enamel lamella (L), enamel spindles (S), dentino-enamel junction (DEJ) and dentinal tubules (D) (x100).
B) Crown cervical third of the control group (subgroup R) showing: Incremental lines of Retzius (I), enamel rods (E), enamel lamella (L), dentino-enamel junction (DEJ) and dentinal tubules (D) (x100).
C) Crown occlusal third of subgroup S, showing: Uneven thin dark structureless band (D). Apparently different rod structure (A). Enamel adjacent to the (DEJ) appeared as uneven thin light structurless band (L) (X100).
D) Crown cervical third of subgroup S, showing: Uneven thin dark structureless band (D) which was noticed in the full enamel thickness at cemento-enamel junction (CEJ). Apparently different rod structure (A). The enamel adjacent to (DEJ) appeared as dark structureless band (U) followed by light structurless band (L) (X100).
E) Crown occlusal third of subgroup M, showing: Transleucent band (T). Light band with apparently different rod structure (L), the enamel rods become more accentuated in the enamel (A) near to (DEJ). Enamel adjacent to the DEJ appeared as light structurless band (S) (X100).
F) Crown cervical third of subgroup M, showing: Transleucent band (T) which was noticed in the full enamel thickness at (CEJ). Erosive cavity (E). Apparently different rod structure (A). The enamel adjacent to (DEJ) appeared as dark structureless band (D) followed by light structureless band (L) (X100).
G) Crown occlusal third of subgroup C, showing: Dark srtucturless band (D). Apparently different rod structure (A). Enamel adjacent to (DEJ) appeared as light structurless band (L) (X100).
H) Crown cervical third of subgroup C, showing: Dark structurless band (D), which was noticed in the full enamel thickness at (CEJ). Apparently different rod structure (A). Enamel adjacent to (DEJ) appeared as light structurless band (L). Localized dark areas of affection extended from the surface toward the DEJ (F) (X100).

The cervical third showed an apparent decrease in the enamel thickness. At enamel surface an uneven thick dark structurless band with irregular line of demarcation from the subsurface enamel could be detected. This band was noticed in the full enamel thickness at CEJ. In the subsurface enamel, the rod structure was apparently different from that of the control group, while the enamel adjacent to the DEJ appeared as uneven thick light structurless band. In some parts of cervical enamel it was noticed that localized dark areas of affection were extending from the surface toward the DEJ (Fig. 4h). At a higher magnification in subsurface enamel and in the light band of enamel, the enamel rods and interrod regions appeared as those in the occlusal third.

Morphometric results:

The morphometric study was done to determine the thickness of the affected band in the outer enamel layer in the different experimental groups at the occlusal and cervical thirds of the crown. This band was measured at the narrowest area and at the widest area in each third. The readings revealed that, this band was narrowest in the Sprite subgroup then increased in thickness in Mirinda Orange subgroup followed by Coca-Cola subgroup, also increased in thickness from the crown occlusal third to the cervical third in each subgroup.
Data are summarized in table (2).

Table (2): Surface affected band thickness measured by micrometers (μm). Enamel (E)

Site	Sprite	Mirinda Orange	Coca-Cola
Crown occlusal third	E= 28-41	E = 85-113	E= 90-122
Crown cervical third	E= 36-58	E= 116-130	E= 135-220

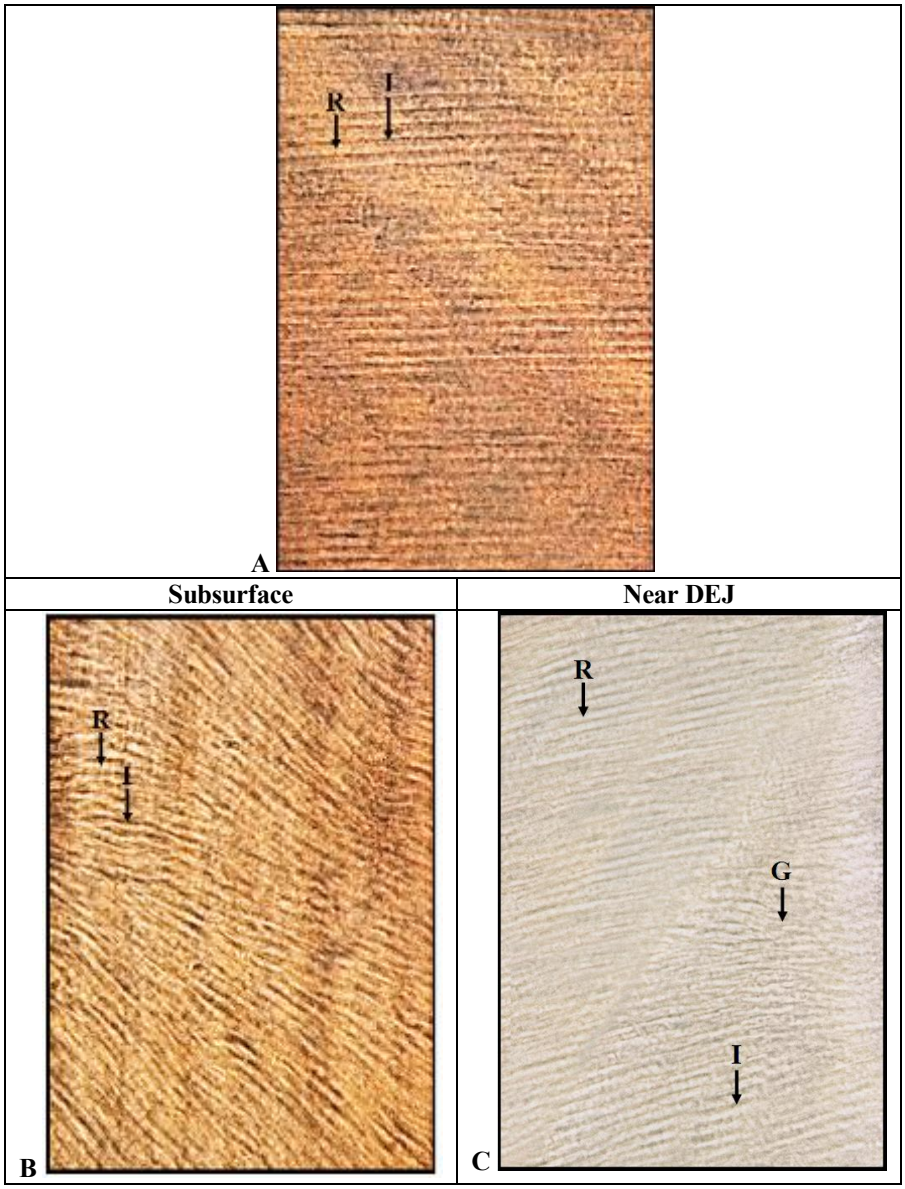

Fig. 5: A photomicrographs of ground sections of enamel showing:

A) A higher magnification of the inset (Q) showing: Enamel rods parallel to each other and of even thickness (R) and interrod regions (I) (X400).

B) A higher magnification of inset 1 (S) showing: Uneven enamel rods (R) and apparently wide interrod regions (I) (X400).

C) A higher magnification of inset 2 (S) showing: Uneven enamel rods with regular course (R), uneven enamel rods with irregular course (G) and apparently wide interrod regions (I) (X400).

4. Discussion:

The present work assumed an average daily consumption of one can (330 ml) of the most popular beverages over 10 minutes. The total exposure time to the beverages in 30 days would be 300 minutes (5 hrs), thus 25 hrs would be the cumulative effect of beverages consumption in 5 months. In the present study, the *in vitro* nature of the experimental design and artificial pattern of continuous exposure were applied according to **Ehlen *et al.* (2008)** protocol. However in **Attin *et al.* (2005)** protocol all samples were submitted to three alternating episodes of de- and remineralization in a so-called artificial mouth. Remineralization was accomplished by rinsing with artificial saliva. For demineralization the beverage was applied for 1 min through a chamber, immediately followed by a remineralization period (1 min). The specimens were cycled through this alternating procedure five times within 10 min. In the present study to standardize the enamel thickness factor, only maxillary premolars were utilized.

In the current study, the SEM examination results of the Mirinda Orange subgroup revealed that the erosive lesions had the characteristic honeycomb pattern. A similar pattern was reported by **Meurman and Vesterinen (2000)** on their SEM examination of human premolar teeth with erosive effect of white wine. The authors reported that, in prismatic enamel the erosive lesions take a honeycomb pattern. The authors attributed this pattern to the dissolution of the enamel prisms cores, leading to a honeycomb structure. In the current study, the results of the light microscopic examination of all the experimental subgroups revealed the affection of subsurface enamel. This could be explained according to **Downer (1995)** who stated that erosion attacks the enamel surface, so the acids find their way to the subsurface layers and cause prism destruction. In the present study, the apparent decrease in enamel thickness observed in the light microscopic examination of Coca-Cola subgroup comes in accordance with **Lussi *et al.* (2011)** who stated that dental erosion started by initial softening of the tooth surface followed by continuous layer-by-layer dissolution of the dental hard tissue crystals, leading to a permanent loss of tooth volume with a softened layer persisting at the surface of the remaining tissue. The authors added that in advanced stages of erosion, the dentine becomes increasingly exposed.

The pH measurements of the utilized beverages in this study revealed that the highest pH value was that of the Sprite, followed in a descending order by Mirinda Orange, then Coca-Cola which had the least pH value. In the current study, results of the SEM, light microscopic examination as well as morphometric data clearly revealed that the least erosive effect was observed in the Sprite subgroup, followed in an ascending order by Mirinda Orange subgroup, then Coca-Cola subgroup. These data clearly show the pH values as a determining factor for the intensity of erosion. This comes in agreement with **Hughes *et al.* (2000)** who stated that the decrease in pH had been associated with increase in dental erosion, as the critical pH values at which tooth decalcification occurs were well below 5.5. The results of the current study are also in agreement with those of other epidemiological and clinical studies by **Dugmore and Rock (2004)** which revealed that carbonated drinks especially cola drinks were associated with dental erosion. The authors attributed the greatest erosive potential of the cola drinks to their low pH in comparison to the other non cola carbonated drinks (Seven-Up, Mirinda Orange, Fanta Orange and Schweppes) utilized in their study. Although only permanent teeth were utilized in the current study, yet the present results could apply to deciduous teeth. This assumption is supported by the *in vitro* study of **Seow and Thong (2005)** who reported that the dental erosion intensity both in permanent and deciduous teeth increases with the decrease in the pH values of soft drinks.

Despite of the little difference in the pH values between the utilized beverages of the present work, yet their erosive potential varied markedly as expressed by the results of the SEM and light microscopic examination as well as the morphometric data. These results comes in agreement with those of **Lussi *et al.* (2004)** who studied the erosive potential of different commercial acidic beverages (Sprite, Mirinda Orange, Coca-Cola, Seven up and Schweppes) on the tooth surface. Their results revealed that despite of the little difference in the pH values between the acidic beverages found commercially, they affect the tooth surface in different degrees. The authors attributed this finding to the importance of a drink titratable acidity (the quantity of base required to bring a solution to neutral pH) than its pH value in determining its erosive potential. **Zero and Lussi (2005)** as well as **Jensdottir *et al.* (2005)** and **Bamise *et al.* (2007)** stated that the amount of base (mmol/L) needed to raise the pH value to 7.0 was: 13.6 for Sprite, 26.6 for Mirinda Orange and 34 for Coca-Cola. Moreover **Borjian *et al.* (2010)** utilized SEM to study the erosive potential of two different cola soft drinks (Coca-Cola and Pepsi Cola) on the dentin surface of unerupted extracted wisdom teeth. Their results revealed that the dentinal tubules appeared clearer, wider and more opened in Pepsi Cola than in Coca-Cola. The authors attributed their results to the fact that the Pepsi Cola has higher titratable acidity than that of Coca-Cola. **Ramalho *et al.* (2010)** in their study on bovine enamel surface reported an increase in erosive lesions associated with the increase of titratable acidity. The previous findings raise the importance of titratable acidity of the beverage as another factor highly affecting the erosion intensity.

The types of acids varied in the beverages utilized in the present work. While Sprite and Mirinda Orange contained organic acids, yet the inorganic acid was present in Coca-Cola. The organic acids are; citric acid (0.060%) in Sprite, citric and ascorbic acids (0.075%) in Mirinda Orange. The inorganic acid in Coca-Cola beverage is the phosphoric acid (0.30%), **(Corrêa *et al.*, 2004; Bamise *et al.*, 2007).** Variation of acid type and concentration in different beverages could also explain the difference in their erosive potentials. This is supported by **Lodi *et al.* (2010)** who studied the effect of different carbonated beverages on animals' teeth. Their results revealed that phosphoric acid was much erosive than citric, malic and tartaric acids, as the phosphoric acid has more ability to cheleate calcium. Moreover the authors attributed the erosive potential of a drink to the type and concentration of the acid present in that drink. However results of the study performed by **Attin *et al.* (2005)** on the erosive potential of some acidic beverages (Sprite and Coca-Cola) on enamel of bovine incisors, revealed that the erosive potential of Sprite is more than that of Coca-Cola. They attributed

this result to the severe demineralizing potential of citric acid than that of phosphoric acid.

In the current study, SEM examination results of the experimental subgroups revealed that each beverage cause erosive lesion with a pattern that differs from that of other beverages. This could be attributed to the type of the acid present in each beverage as explained by **Lussi *et al.* (2011)** who utilized the SEM to study the effect of citric and hydrochloric acids (gastric acids) on human enamel surface. The authors stated that each type of acid was associated with a characteristic pattern of erosive lesions. As their results revealed that eroded enamel surface by citric acid appeared loosely structured, while the enamel surface eroded by hydrochloric acid presented erosive lesions with a honeycomb pattern.

Results of the present work showed, variable grades of enamel affection with respect to its occlusal and cervical thirds. In all experimental subgroups, the cervical third of enamel was more affected than the occlusal third. This finding comes in agreement with that of **Gray *et al.* (1998)** who reported a case of an individual who had worked in the wine industry for ten years, and this occupation involved daily tasting of at least 20 wines or more. The authors stated that erosion was manifested as lesions that were usually bilateral, affecting mostly the labial and buccal surfaces of teeth specially those adjacent to the gingival margin. The authors attributed these lesions to increase of the contact duration of the acidic beverage with teeth in these areas which represent areas of isolation from the buffering effect of saliva. On the other hand the *in vitro* nature of the current study eliminated the effect of these factors reported by **(Gray *et al.*, 1998)**. So the current study results could be attributed firstly, to the hypocalcified nature of the perikymata which are most frequent and deep cervically, secondly to the decrease of enamel thickness cervically and finally, to the decrease in the level of Ca and P weight % cervically. These suggestions are supported by data of **Scott and Wyckoff (1959)** as well as **Huanga *et al.* (1998)** who stated that perikymata are the outer manifestation of enamel incremental lines of Retzius which are hypocalcified and becomes more concentrated toward the cement-enamel junction, while their concentration gradually decreases toward the occlusal. They added that enamel has variable thickness over the entire surface of the crown, as on the cusps the enamel attains a maximum thickness, thinning down to almost a knife edge at the neck of the tooth. Moreover, **Takahashi *et al.* (2008)** studied the difference in the elemental composition of the cuspal and cervical enamel among the human permanent teeth, using electron probe microanalyzer (EPMA) that quantitatively analyzed the contents of seven elements (calcium, phosphorus, oxygen, carbon, magnesium, sodium and fluorine). Their results revealed that the calcification level was lower at the cervical enamel than that at the cuspal enamel as the level of Ca and P weight % was less in the cervical enamel.

In the current study the light microscopic examination results revealed that the pattern of affection was different in the most superficial enamel layer than the layer near to the DEJ in all the experimental subgroups. These results could be explained according to **Lussi *et al.* (2011)** who stated that the enamel mineral content and hardness tend to decrease with increasing the distance from the surface. So the solubility of the enamel tends to increase toward the DEJ, therefore the hydrogen ions in acidic drinks complex easier with the calcium of the enamel nearer to the DEJ. The authors added that erosive demineralization of enamel is a centripetal process starting with the partial loss of surface mineral causing an increase in roughness and if the acid impact continues, bulk mineral loss occurs while the remaining surface still exhibits partial demineralization.

Finally, results of the present work were limited to buccal surface of enamel with continuous pattern of exposure to utilized beverages in this study for 25 hrs. However other investigations concerning the effect of intermittent pattern and longer periods of exposure to other carbonated beverages on other aspects of the tooth and on cementum are highly recommended to widen the scopes and knowledge in this research field.

Conclusions:

1. Acidic beverages had deleterious effect on dental hard tissues.
2. Among the investigated drinks of the present work, the Sprite had the least erosive potential, followed by Mirinda Orange, then Coca-Cola that had the most erosive potential.
3. The erosive potential of a beverage was depended on its pH value, titratable acidity, type and concentration of the acid(s) present.
4. Enamel affection for a given beverage was maximum at the cervical third and minimal in the occlusal.

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