

Erosive effect of energy drinks alone and mixed with alcohol on human enamel surface. An *in vitro* study.

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Abstract: Objective: To assess the erosive effect of energy drinks (ED) alone and mixed with alcohol on the human enamel surface *in vitro*. Methods: Twenty non-erupted human third molars were vertically sectioned in half. Specimens were exposed to 5mL of ED plus 5mL of artificial saliva or 5mL of ED plus 5mL of artificial saliva plus 5mL of alcohol (Pisco). Exposure times were set at 30min and 60min. Erosive assessments were made using scanning electron microscopy (SEM) and energy-dispersive x-ray spectroscopy (EDS). The ED analyzed were Mr. Big, Kem Extreme, Red Bull, and Monster Energy. ED pH measurements were performed at 25°C and titration was done with NaOH 0.1mol/L. Results: The pH ranges were: ED alone 2.55 to 3.46, ED mixed with artificial saliva 2.60 to 3.55, ED mixed with Pisco 2.82 to 3.70, and ED mixed with both 2.92 to 3.86. The pH of Pisco was 6.13, and Pisco mixed with artificial saliva had a pH of 6.23. Titration showed a pH range from 3.5 to 5.7. SEM-EDS analysis showed that Mr. Big and Monster led to clear demineralization at 30 min and remineralization at 60m in. Pisco slightly decreased the erosive effect of these ED. Kem Xtreme and Red Bull led to no demineralization in the first hour. Conclusion: According to the pH, acidity and EDS analysis, the ED of the present study likely caused enamel erosion in human teeth surface dependent on exposure time.

Keywords: energy drinks, dental enamel, tooth erosion, alcohols.

INTRODUCTION.

The consumption of energy drinks (ED) is attractive to students and workers to cope with long and exhausting days. It is assumed/believed that ED will improve performance and stamina and allow for longer periods of study or work.¹ ED contain stimulants such as caffeine, guarana, taurine, ginseng, L-carnitine, creatinine, glucuronolactone, and others.² EDs may contain tartaric, malic, lactic, ascorbic, citric or phosphoric acid, which cause erosion and increase the erosive activity of other EDs components.⁴ However, the erosive potential of drinks does not depend only on the type of acid. There is a complex interplay of various factors such as concentration, temperature, contact time with the teeth, and saliva buffer capacity.⁵ Additionally, alcohol consumption has been associated with dental erosion and tooth discoloration.⁶

Despite the increasing consumption of ED mixed with alcohol, especially in young people, there is a scarcity of reports regarding the erosive effects on human enamel. In order to test the above hypothesis, the aim of this study was to assess the erosive effect of ED alone and mixed with alcohol on the human enamel surface *in vitro*.

MATERIALS AND METHODS.

Study design and ethical considerations

This was an *in vitro* study. The Universidad Andrés Bello (UNAB) School of Dentistry Ethics Committee approved the study. Twenty non-erupted human third molars were collected from the UNAB Oral Surgery Clinic. All patients gave informed consent to use their teeth in this study.

Selection of ED and alcoholic beverage to test

A survey was made amongst 441 UNAB students to determine the most consumed ED, as well the alcohol commonly mixed with ED. The most consumed ED were: Mr. Big (Big Company, Chile), Kem Extreme (CCU, Chile), Red Bull (Red Bull GmbH, Austria) and Monster Energy (Corona, USA); the most commonly used alcoholic beverage for mixing was Pisco.

Preparation of enamel specimens

After extraction, the teeth were placed in 0.9% w/v sodium chloride solution. Later, teeth underwent ultrasound cleaning (Cavitron Bobcat Pro, Dentsply, USA), and were treated with rubber-cup polishing using fine powders in order to remove organic debris from the surface.

The root was cut with a high-speed diamond burr, and the intracameral contents were completely removed with hand instruments and profuse irrigation with 0.9% sodium chloride solution. Then, the molar crown was vertically sectioned into halves, one with the vestibular face and the other with the lingual one.

Each specimen was placed on an acrylic base (1.5x2.5cm) for better handling, leaving the vestibular/lingual face of the tooth exposed. After this, half of the tooth face was covered with a light-curing barrier resin (Top Dam, FGM, Brazil).

Preparation of artificial saliva

The artificial saliva was prepared according the following formula: hydroxypropyl methyl cellulose 1g, xylitol (70%) 4.3g, potassium chloride 0.1g, sodium chloride 0.1g, sodium fluoride (2ppm) 0.2g, magnesium chloride 5mg, calcium chloride 15mg, potassium sulfate (adjusted to pH 7) 40mg, and excipients q.s. in 100ml of distilled water.

Then, all components were mixed by agitation to ensure solubility.

Acid content evaluation

The pH of ED (15ml) was measured at 25°C (pH meter 111 HI, HANNA®, Italy). Each ED was then titrated, in duplicate, with standard 0.1mol/L sodium hydroxide solution, using phenolphthalein as the endpoint indicator.

Erosion challenge

Specimens were immersed in 20mL glass beakers with each ED (5.0 mL of ED plus 5.0mL of artificial saliva). Exposure times were set at 30min and 60min for each ED. To assess erosion caused by the ED mixed with alcohol; the specimens were immersed in 20mL glass beakers with each ED plus Pisco (5.0mL of ED plus 5.0mL of artificial saliva plus 5.0mL of Pisco).

The exposure time was set at 60min for each mixture. Following exposure, each specimen was washed with a strong jet of 0.9% sodium chloride solution and air-dried. Then, the resin barrier was removed and the specimens were placed in test tubes with serum and stored at 4°C.

Scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS) analysis

The analysis of enamel samples, by SEM and EDS, was performed using a Vega 3 LMU SEM (Tescan, Czech Republic), equipped with an electron gun. Each sample was dried and coated with carbon. The levels of calcium, oxygen, and phosphorus were measured on the exposed and covered enamel surfaces, and in the deep eroded enamel surface.

Statistical analysis

Descriptive measures of EDS analysis were summarized for each ED alone or mixed with Pisco, according to the exposure time.

RESULTS.

The pH value and volume of NaOH used in titration for each energy drink alone or mixed with alcohol (Pisco) and with the artificial saliva are shown in Table 1. The results of EDS analysis are summarized in Table 2 and Fig. 1 shows the effects on tooth enamel of 30min exposure to ED.

Table 1. pH and acidity values of energy drinks and alcohol beverage.

Drink	pH ED	pH ED+AS	pH ED+AB	pH ED+AS+AB	pH AS	Vp(mL) NaOH 0.1mol/L	Acidity in H ₂ CO ₃ (mg)
Monster	3.46	3.55	3.70	3.86	7.00	5.5	34.12
Red Bull	3.23	3.30	3.43	3.50	7.00	5.7	35.36
Kem Xtreme	2.55	2.60	2.86	2.93	7.00	3.5	21.71
Mr Big	2.59	2.66	2.82	2.92	7.00	3.9	23.88
Pisco	6.13	6.23	--	--	7.00	--	--

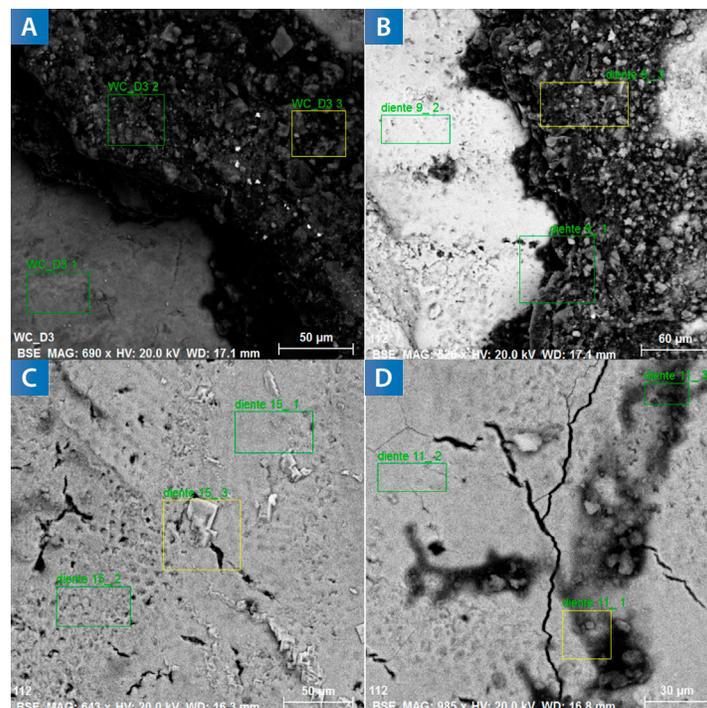
ED: energy drink. AS: artificial saliva. AB: alcohol beverage (Pisco). Vp: average volume of NaOH.

Table 2. EDS analysis of the exposed and covered enamel surfaces.

		30 min. Exp (%)	ED alone Cov (%)	60 min. Exp (%)	ED alone Cov (%)	60 min. Exp (%)	with Pisco Cov (%)	60 min. Ext (%)	Deep erosive zone Inn (%)
Mr. Big	O	74.50	68.73	64.98	56.66	72.31	71.94	79.61	57.44
	Ca	1.25	19.32	22.17	30.90	16.77	17.04	13.13	27.58
	P	0.83	10.84	11.96	12.08	9.70	9.87	7.26	14.98
Monster	O	71.32	67.69	64.46	64.44	68.48	67.51	59.43	48.78
	Ca	1.51	20.36	22.59	22.67	19.48	20.18	21.59	36.67
	P	0.98	10.84	12.08	12.18	1.95	11.28	10.39	14.57
Kem Extreme	O	60.03	59.70	67.25	63.95	68.23	66.07	74.14	64.62
	Ca	22.84	24.14	19.78	22.72	19.67	20.41	17.72	23.15
	P	11.86	12.80	11.22	13.33	11.76	13.03	8.14	12.24
Red Bull	O	70.0	70.0	65.60	65.00	72.04	69.97	56.81	62.46
	Ca	18.89	18.89	21.96	22.11	17.99	17.55	3.45	23.80
	P	10.84	10.84	11.84	12.07	9.80	10.08	1.63	13.04

Exp: Exposed surface. Cov: Covered surface. Ext: External zone. Inn: Internal zone.

Figure 1. SEM of the teeth submerged in ED for 30 min. Left side of each image displays tooth surface covered with Top Dam, while the the right side was exposed to the beverage.



A:Mr. Big. B: Monster. C:Kem Xtreme. D:Red Bull.

DISCUSSION.

Excessive consumption of ED and alcoholic drinks has increased the risk of teeth and restoration erosion. Different kinds of alcoholic (wine and beer) and no alcoholic beverages (sodas) can erode the teeth and resin composite materials as shown in *in vitro* studies.⁷ The degree of erosion is higher at lower pH, and the resin composites (methacrylate and silorane based materials) displayed significant decreases in weight with all kind of beverages evaluated. In the present study, Kem Xtreme appeared to be the most acidic ED, closely followed by Mr. Big. However, after mixing with alcohol, Mr. Big was more acidic than Kem Xtreme. However all the ED have a pH under the critical value for enamel to suffer dissolution.⁷ Although the liquor used in this work, Pisco, does not present an acidic pH, when it is mixed with ED, the pH drops to values under the critical pH for enamel erosion.

If we look at titratable acidity, the order is not the same as the pH. The titratable acidity considers those substances present in solution with the ability to accept electrons, such as some dyes and/or other chemical species. The importance of these acidity measurements is to show that all ED have a high content of acidic substances, and these substances can erode the enamel. A study by Wang *et al.*,⁸ reported the pH values of several soft drinks, with values very similar to those of our study, which ranged from 2.42 to 3.46. An important consideration is that acidity values are not specified on the ED packaging in order to warn consumers.⁹

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The EDS analysis showed that when calcium decreased on the exposed surface, phosphorus also decreased. In contrast, oxygen increased when calcium decreased. This confirms that ED disrupted the dental apatite structure in terms of its elemental composition. In the case of Mr. Big and Monster, when exposure time was considered, there was clear demineralization observed at 30 minutes, followed by remineralization at 60 minutes.

The irregularities on the surface (Figure 1) show the erosion action of each beverage on human enamel, and this erosion is major when acidity increases. This situation differs from that reported by Seong *et al.*,⁹ who determined that remineralization starts 2 hours after acid challenge (fruit juice). This difference may be explained by the acidic beverage used (different chemical and physical properties), and that Seong *et al.*,⁹ did not measure remineralization within the first hour.

When Mr. Big was mixed with Pisco, the erosive effect seems to have decreased in comparison to ED alone. In the case of Monster mixed with Pisco, there was a clear decrease in phosphorus, probably due to a specific chemical interplay with this beverage. This fact reinforces the idea that pH is just one of many factors to be considered in enamel erosion.¹⁰

CONCLUSION.

According to the pH, acidity and EDS analysis, the ED of the present study likely caused enamel erosion in human teeth surface dependent on exposure time.