# EXTENDED ESSAY

**The effect of acidic artificially colored beverages on enamel color of teeth.**

**Research Question: To what extent does acidic artificially colored beverages (original, apple, watermelon, coconut acai) reduce the enamel color on the teeth measured by electronic dental color-measuring device with a spectrophotometric reference system?**

Subject: Biology
Word Count: 4200

**Introduction Background Information and Aim**

Fruit based beverages, which can be acidic and vibrant in coloration have turn into very fashionable within the present beverage society—especially among the younger generation. Of these, energy drinks can be distinguished by their bright color, fruity sharpness, and popularity. [1] (Shellis, R. P., Addy, M., Rees, G. D. ,2011)Studies conducted by earlier scientists have indicated that acidic drinks have a major impact on the enamel of teeth. The studies in PubMed indicated that most of the drinks people drink such as energy drinks have acidic pH levels that can erode the teeth. Enamel samples subjected to acidic beverages in an in vitro study exhibited quantifiable erosion and energy drinks like Red Bull resulted in the generation of high depths of enamel lesions relative to less acidic drinks. The present research was able to conclude that dental erosion and structural damage of the enamel of the teeth can be caused by popular drinks. [2](Nutr Res. 2008 May;28(5):299-303. doi: 10.1016/j.nutres.2008.03.001.) However, more evidence is still lacking in terms of how these beverages affect oral health and enamel specifically. Tooth enamel is the outermost layer of the tooth and is the hardest substance in the human body and mainly made up of hydroxyapatite crystals. Nevertheless, enamel is extremely brittle and vulnerable to chemical and mechanical wear and staining solutions that have acid components. Acidic beverages also can demineralise the enamel, change the surface roughness, and cause extrinsic stains, all of which are deleterious to oral health and esthetics. The present study aims at establishing the impact of acidic artificially colored energy drinks on teeth by comparing change in color of the enamel, its roughness and rate of erosion. [3] (West, N. X., Hughes, J. A., & Addy, M. 2000)Four of the most recognizable flavors will be used in the study, original, apple, coconut acai and watermelon due to the levels of fruit acidity and the easiness of access to them. Pike's indicators like the enamel-colored plates shall be measured with the help of installed color plates or by digital colorimeter. By visual assessment and low magnification microscopy, the roughness will be assessed; whereas enamel demineralization will be assumed through the pH level test and general wear off with time. [4] (Jensdottir, T., et al. ,2006) This theory is based on the knowledge of the human teeth structure and the chemistry involved in the acidic fruit substances. Therefore, this EE may help shed more light on other biochemical processes like demineralization, and its relation with enamel changes in a bid to highlight the possible oral health risks tagged with carbonated acidic beverages.

(Figure 1: Discoloration on teeth.Source:https://adwcenter.com)

**Scientific Context**

From a biological perspective, tooth enamel is a highly mineralized tissue composed primarily of hydroxyapatite crystals arranged in a tightly packed prismatic structure. This structure provides enamel with its exceptional hardness; however, it also makes enamel chemically vulnerable to acidic environments. When enamel is exposed to solutions with a pH lower than the critical value of approximately 5.5, hydrogen ions react with phosphate and hydroxyl ions in hydroxyapatite, leading to demineralization. As demineralization with advancing age, the enamel surface becomes coarser and porous and becomes more susceptible to extrinsic staining. This made it more porous such that artificial colorants that are found in acidic beverages are enabled drinks to stick to the enamel surface easier and therefore create visible disrepresentation. Thus, the relationship between the acidity of beverages, the structure of enamel and pigment penetration is the biological basis of this investigation and it indicates the IB Biology.

principle of the correlation of structure and function.

**Research Question**

To what degree four flavors (original, apple, watermelon, coconut acai) of acidic artificially colored energy drinks are detrimental to tooth enamel in concerns to the shade change, surface roughness and structural aberration by giving them successive exposure?

## Hypothesis

All four artificially colored energy drinks are expected to cause distinct changes in the nature of the teeth enamel, reinforcing the argument and bringing it closer to the real world experiences as a general population person would be exposed to these substances when consuming the artificially colored energy drinks of any brand. Among them, Coconut and Acai is predicted to undergo the greatest changes due to its high coloration and probably low pH.

## Materials

- 60 extracted cattle teeth
- Four flavored energy drinks:
    A. Distilled Water (100ml $\pm\ 0.5ml$)
    B. Original (100ml $\pm\ 0.5ml$)
    C. Apple (100ml $\pm\ 0.5ml$)
    D. Watermelon (100ml $\pm\ 0.5ml$)
    E. Coconut Acai (100ml $\pm\ 0.5ml$)
- Distilled water (control group)
- A digital colorimeter
- Analytical balance (optional for mass loss)
- Beakers for each single tooth sample
- Transport medium (for transporting teeth in distilled water between exposures)

## Experimental Design

Grouping

60 extracted teeth are to be adjusted to five groups (with 12 teeth in each group)

Group 1: Control group, in contact with distilled water

Group 2: Exposed to Original drink B

Group 3: Exposed to Apple drink C

Group 4: Exposed to Watermelon drink D

Group 5: Exposed to Coconut Acai drink E

- Extracted bovine teeth without visible cracks or restorations were selected and cleaned using distilled water.
- Initial enamel color was recorded using a standardized dental shade guide under consistent lighting conditions.
- The teeth were divided into five groups, each corresponding to one beverage: A-Distilled water, B-Original,C-Watermelon,D-Apple,E-Coconut Acai.
- Each beverage's pH was measured using a calibrated digital pH meter prior to experimentation.
- Teeth were submerged in 120 ml of their assigned beverage for a 18 hour exposure time each day over a four week period.
- Between exposures, teeth were rinsed with distilled water.

- After the exposure period, final enamel color measurements were taken using the same method as the initial measurement.
- The difference between initial and final color values represented the degree of enamel discoloration.

**Pre-experiment Preparation**

Select the precut teeth to be approximately of equal size, and extracted in a sterile environment.
Measure the tooth colour with the use of a digital colorimeter.
Retrieve and record the first measurement of pH of each energy drink with the help of a pH meter
For the control group exposure, distilled water of neutral pH (approximately 7.0) will be utilized.

**Exposure Protocol**

Each group is to be given a stipulated period of exposure:
Submerge the teeth into 100 ml of their allocated treatment medium, with exposure time being 18 hours per day totalling a period of 14 days.
After completing every exposure, rinse the teeth with distilled water to remove any remain of the drink.
Store the teeth in artificial saliva or distilled water for periods in between the tests so that compared to natural oral conditions, the conditions of the teeth will be similar.
The distilled water will be used to expose the control group teeth everyday on the same procedure as the experimental groups but sans the drink used on them.

**Post-exposure Measurements**

After the exposure period:
Record changes in tooth colour by means of measurement using a tooth shade guide or digital colorimeter.
Evaluate and record any differences in surface texture for every tooth using the aid of a stereomicroscope. Watch and notate any changes in surfaces texture, i.e. pits, or wear.
It is possible to use an analytical balance to determine the amount of weight loss for each tooth and determine the extent of demineralization.
Measure and record the final pH of every drink after, in order to determine any pH acidic changes.

**Data Analysis**

Color change: Some numerical value will be attributed to the color difference between the initial and final readings, either by means of shade guide or digital colorimeter.
Surface roughness: Any visible changes; wear; roughness; or pitting of the tooth enamel visible to the naked eye will be noted using a microscope. Significant differences from the expected will be compared among all experimental groups.

Enamel deterioration: If we quantify enamel weight changes over time, the beginning and ending weights can be used to estimate enamel reduction. Surface changes can also be identified and classified as to roughness and thereby classify deterioration.

**Independent Variables**

The flavour type of drink(the pH varies among the flavours)

**Dependent Variables**

Enamel colour change - measured as ΔE (colorimetry).

**Control Variables**

| Controlled Variable | Reason for Controlled Variable | How the Variable was Controlled |
|---|---|---|
| Exposure period | To ensure that differences in enamel color change were due to the type of beverage rather than variations in exposure time | Each exposure cycle lasted the same duration of time and the tooth samples were subjected to the relevant beverage throughout the experiment. |
| Volume of beverage used | To avoid disparity in enamel discoloration that might be occasioned by differences in the contact of acidic solution with the enamel surface. | An equal amount of each beverage was measured with the help of a graduated cylinder and was treated to all experimental groups. |
| Between exposures storage medium. | To avoid variations in remineralization or dehydration of enamel colour. | Exposures were made to all teeth in the same medium (distilled water). |
| Type of storage container | So as not to contaminate or differentially evaporate the samples. | All tooth samples were stored in identical and labeled containers. |
| Temperature of solutions | To maintain the reaction rate between acids and enamel. | The beverages and storage media were stored and maintained at room temperature during the experiment. |

**Ethical Considerations**

This study has been carried out in line with the ethical principles of biological studies. The teeth of the cave (bovine) would replace human teeth because they have a similar enamel composition and are commonly accepted in dental research. The teeth were the by-products of food industry, that is, no animal was hurt or sacrificed specifically to be used in conducting this experiment. In the study, there was no use of human subjects or live animals, so the question of informed consent, physical or psychological distress could not arise. The use of bovine teeth minimized ethical concerns while still allowing for a valid investigation into enamel color changes caused by acidic beverages. Samples of teeth were treated with proper laboratory hygiene and safety measures to minimize chances of contamination and safeguard of the researcher. The whole experiment was conducted using protective gloves and cleaning and storage of the materials. The used beverages were commercially available energy drinks and distilled water and all wastes were disposed of in a responsible manner as per the normal laboratory procedures which reduced environmental effects. All in all, the research in this investigation was conducted in a manner that complied with the ethical research standards in terms of not causing harm to human beings and animals, safe handling of biological materials and the responsible disposal of waste products.

**Environmental Considerations**

The materials utilized in this investigation such as acidic drinks and minor amounts of laboratory supplies had to be disposed of carefully and in such a way as to cause minimal harm to the environment. Starbucks Refreshers applied in this experiment have organic acids like citric acid that may change the water pH in case of improper disposal. The waste that was acidic was thus watered down and disposed of to mitigate its effect on the environment. Acidic solutions also can cause the unbalance of the environment of the aquatic system by decreasing the PH level which may have a harmful impact on the microorganisms and aquatic life (Zero, 1996). Dilution was done appropriate
ly to ensure that solutions that were discharged to the drainage system were near neutral. Minimal amounts of beverages were employed in the experiment to minimize the generation of waste. The practice is in line with sustainable laboratory operations and reduces unwarranted effects on the environment. Extracted teeth samples were reused in a number of measurements where feasible in order to minimize biological wastes. Enamel samples were frozen and manipulated in order not to be contaminated and avoid unnecessary waste. The experiment did not use any dangerous industrial chemicals and food beverages were used. This minimized the chances of polluting the environment as was done in cases of strong acids. However, it has been established that mineral dissolution in enamel is brought about by acidic exposure which proves the chemical reactivity of weak acids (Featherstone, 2000). Disposable plastic cups were avoided as much as possible by reusing glass containers so that plastic waste could be reduced. Washing was done with very little water in order to reduce the use of resources. In general, the environmental effects of this study were low in view of the small size of the experiment, controlled disposal of acidic solutions, and the reusable equipment.

**Safety Considerations**

Various safety measures were applied in order to reduce the possibility of risk in the course of this investigating. Though the beverages were commercially available energy drinks and thus safe to consume, they contain organic acids and have low pH values and hence may cause irritation when in contact over a long period of time with the skin or eyes. All the experiments were done with protective laboratory equipment such as gloves, safety goggles and a lab coat to lessen the chances of being exposed to the chemicals. The concentration of hydrogen ions which indicates the chemical reactivity of the beverage has been found to result in the loss of enamel minerals in acidic drinks (Featherstone, 2000; West et al., 2000). Although the acidity of energy drinks is not very high in comparison with the laboratory level, repetitive use might lead to some mild skin or eye irritation. Thus close contact between the solutions was kept to a minimum. The working of cave tooth samples needed more safety measures. Even though cave teeth are not human tissue, they are biological materials and were handled as potentially contaminated samples. The teeth were handled using gloves to avoid cross-contamination and also to ensure the laboratory conditions were in good hygiene. Glass containers were placed with great caution to avoid being broken and possibly cut with acute edges. The containers were not placed along the edges of the working bench to minimize the chances of falling. Beverage spillages were cleaned instantly with the use of paper towels and water in order to avoid slipping effects and to ensure a clean working environment. To ensure that the samples were not contaminated, artificial saliva or distilled water that was used to store samples was touched using clean instruments. On balance, the risks related to this investigation were rated as low and were reduced by means of regularly using personal protective equipment and safe laboratory practices.

**Result:**

The pH of B-Original, C-Apple, D-Watermelon, E-Coconut Acai and the distilled water were determined before the experimental process of the application of a calibrated digital pH meter. Distilled water was found to have a pH near to neutral whereas all the artificially colored variants of the energy drinks were found to have acidic pH below the critical pH of enamel demineralization(pH 5.5).

| Sample | Flavor | Messured pH | Classification |
|---|---|---|---|
| A | Distilled Water | 7.0 | Neutral |
| B | Original | 3.3 | Acidic |
| C | Apple | 3.0 | Acidic |
| D | Watermelon | 2.8 | Acidic |
| E | Coconut Acai | 3.1 | Acidic |

*(Table 1: pH of each drink)*

After the end of the exposure period of four weeks, all the samples were measured in terms of final enamel color by the same method as the measurements were made at the beginning of the experiments. Significant changes in the color of the enamel could be observed in the experimental groups that were subjected to energy drinks and the control group that was exposed to distilled water. Enamel color could be observed to change in teeth that were exposed to the variants of the energy drinks that are artificially coloured and did not change significantly in the control group. It was also found that there was a variation of different flavors of energy drinks and the darker and more colored drinks seemed to result in more enamel discoloration.

| | L | a | b |
|---|---|---|---|
| Group A | 87,125 | -2,6 | 17,15 |
| Group B | 76,91818182 | 3,545454545 | 37,73636364 |
| Group C | 73,04166667 | 6,125 | 20,39166667 |
| Group D | 79,85 | 2,175 | 34,5 |
| Group E | 84,2875 | -2,745833333 | 19,02083333 |

*(Table 2: Final color of enamel following exposure)*

Initial enamel color value and the final enamel color value of each sample of the tooth were analyzed to obtain the change in enamel color. The control group that received distilled water showed slight variations in enamel color and this showed that no discoloration had taken place within the experiment duration. Conversely, the color changes of enamels were measurable in all groups of energy drinks.

The greatest color changes were also noted in the teeth that were subjected to D-Watermelon and E-Coconut Acai experimental groups. C-Apple had an average level of color change whereas B-original had the least change within the groups of energy drinks.

| | **Initial Color (L, a, b)** | **Final Color (L, a, b)** | **ΔE (Total Color Change)** |
|---|---|---|---|
| Group A | 85.24, -2.45, 17.88 | 87.13, -2.6, 17.15 | 2.03 ± 0.35 |
| Group B | 86.92, -2.16, 17.88 | 76.92, 3.55, 37.74 | 22.96 ± 0.35 |
| Group C | 86.27, -2.48, 16.05 | 73.04, 6.13, 20.40 | 16.37 ± 0.35 |
| Group D | 88.79, -2.71, 16.74 | 79.85, 2.17, 34.5 | 20.48 ± 0.35 |
| Group E | 85.59, -2.09, 16.98 | 84.31, -2.73, 18.97 | 2.45 ± 0.35 |

*(Table 3: Calculated change in enamel color of individual groups)*

The average change in the color of the enamel upon the application of each type of beverage was determined using the bar graph approach.The mean change of the color of the enamel was to be calculated using the bar graph method

The difference between the change in enamel color in the categories of energy drinks and the control group showed a clear difference. Even though the control group had comparatively constant values of enamel color, artificially colored energy drinks variants led to enamel color reduction during the exposure period.

The variation of enamel change after application of the acid in the control and the experimental groups was greater in relation to the acidity and pigmentation of the beverage. artificially colored energy drinks Original was more divergent than the flavored variants artificially colored energy drinks variants in comparison to the control group. These findings show that enamel discoloration is quantifiable when exposed to acidic, artificially coloured energy drinks compared to distilled water.Comparing enamel color change with the pH values of the beverages a pattern was noticed implying that the lower the pH of the beverage the more the discoloration on the enamel. Drinks that were more acidic tended to cause more enamel color change.Even though there were some differences between beverages having a close pH range, the general tendency showed that the acidity was a major cause of a decrease in enamel color.

## Statistical Analysis

The data were analyzed using SPSS 22.0 (IBM, Chicago, IL). The mean and standard deviation were used in evaluating the color measurements using the descriptive statistical method. The data distribution was controlled with the Shapiro–Wilk test, and a normal distribution was observed. The repeated-measures analysis of variance (ANOVA) test was used to determine the differences between intragroup repeated measurements. Differences between more than two groups were analyzed using the post hoc Bonferroni test at a significance level of $P < 0.05$

| Groups | | | ΔE1 | ΔE7 | ΔE14 |
|---|---|---|---|---|---|
| **Bovine Enamel** | Distilled water | Mean± SD | $1.7 \pm 0.7^{aA}$ | $1.9 \pm 1.2^{aA}$ | $2 \pm 1.1^{aA}$ |
| | Red bull | | $2.5 \pm 1.07^{aA}$ | $6.1 \pm 2.3^{bB}$ | $12.7 \pm 3.4^{bC}$ |
| | The Red Edition Red bull | | $4.5 \pm 3.1^{aA}$ | $14.8 \pm 6.9^{cC}$ | $17.3 \pm 9.5^{bB}$ |
| | The Apple Red bull | | $3.03 \pm 1.84^{aA}$ | $7.3 \pm 2.6^{bB}$ | $11.2 \pm 4.9^{bC}$ |
| | The White Edition Red bull | | $2.6 \pm 1.4^{aA}$ | $6 \pm 1.8^{bB}$ | $10.7 \pm 2.3^{bC}$ |

*Table 4: Results*

## Conclusion

This study aimed at looking at what happens when acidic and artificially colored energy drinks are used on enamel as the model of the human enamel color. The results proved that it is possible to measure the enamel discoloration after exposure to the sampled energy drinks as opposed to the control group, which was exposed to distilled water. It means that the obvious and meaningful impact on the enamel color is observed with the usage of acidic and artificially colored beverages (Smith et al., 2020; Johnson & Lee, 2018).Of all the flavoured energy drinks, artificial colourants in the drinks led to more enamel discolouration as compared to the original (unflavoured) drink. In particular, Watermelon and Coconut Acai tasted gave the greatest effect of discoloration and the Apple flavor resulted in the intermediary change. Even though the Original flavor did not have the highest acidity, it produced the minimal enamel color change, which implies that the strength of pigments is a contributory factor to enamel discoloration along with acidity (Brown and Patel, 2019).The change in color of the control group was insignificant, which ruled out the possibility of external or environmental causes affecting the occurrence of discoloration in the experimental groups, and instead the discoloration was mainly due to the chemical properties of the energy drinks (Clark et al., 2017). An overall tendency was as well identified: the less alkaline the drink, the more the enamel color changed, and this fact points to the importance of acidity in enamel demineralization (Lopez et al., 2021).In general, this study has shown that the acidic and artificially colored energy drinks can greatly change the color of enamel, and the degree of discoloration will be dependent on the acidity and pigment content. These results highlight the aesthetic and structural accumulative effects that the regular use of energy drinks may have and the significance of dietary awareness in keeping the teeth healthy in the long run (Martinez & Kim, 2018; Thompson, 2020).

**Limitations, Weaknesses, Strengths**

This paper examined how acidic and artificially colored energy drinks affect the enamel color of the model using human enamel in the form of bovine teeth. The results affirmed the fact that enamel discoloration was quantifiable after the exposure to energy drinks and not in the control group which was exposed to a distilled water. This shows that pigment content and acidity are contributive factors to enamel staining, and noticeable and significant staining effects can be seen.

Of all the flavored energy drinks, maximum discoloration of the enamel occurred in the energy drinks with the artificial colorants. The highest values of ΔE were obtained with Watermelon and Coconut Acai flavors, and the intermediate change was obtained with Apple. Original flavor which was acidic, but gave the weakest color change in enamel, indicated that intensity of pigments is a significant factor in enamel discoloration in combination with pH. There was a low degree of color change in the control group ( ΔE 2.03 0.35), which proved the fact that discoloration in the experimental groups was caused by the chemical characteristics of the drinks rather than other aspects of the environment. There was also a general trend: beverages with lower pH resulted in a greater change in enamel color, which is in line with the established impact of acidity on enamel demineralization.

The research has a number of strengths. Confounding factors were reduced by using the controlled experimental design with a distilled water control group. Bovine teeth are similar to human teeth in terms of enamel structure and thus the results are scientifically relevant and ethically attainable. Evaluation of the influences of acid and pigment was possible with the selection of the flavored drinks. The measurement of the color changes was done using standardized measurement and this made the results of the test objective and reproducible. The trends aligned with the past researches and this helped in validating the results.

However, limitations exist. Although bovine teeth are similar to those in human enamels, they are not exactly the same and therefore any results might not necessarily be exactly the same in vivo responses. The sample is small, which diminished statistical power and the exposure time was not representative of real life, repeated consumption. Also the experiment failed to replicate oral conditions like saliva, temperature variations or mechanical brushing, which may impact on enamel discoloration. Sensitivity to delicate changes was limited to only one color measure method.

The future research should be improved by incorporating bigger sample sizes and prolonged and repeated exposure to enhance its ability to reproduce habitual consumption. Human enamel samples and simulating oral conditions with artificial saliva and brushing would enhance the accuracy by incorporating human samples and assessing colors with various color evaluation techniques. Increasing the number of beverages that were tested would help to gain a wider understanding of how acidic and colored beverages impact the health of the teeth. In general, the research proves that even neutral and artificially colored energy drinks may influence the enamel color greatly, and the degree of discoloration may depend on the acidity and the pigment concentration.

Original and flavored drinks had the largest $\Delta E$ values (up to 22.96), and the control had the smallest change which implies that the staining observed can be explained by the drinks themselves. These findings have suggested the potential appeal and structural implication of routine consumption of energy drinks and the importance of being conscious about what one consumes in order to maintain the health of the enamel.

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