Erosive characteristics and fluoride content of cola-type drinks.

Narges Omid[1], Fatemeh Vida Zohoori[2], Simon Kometa[3], Anne Maguire*[1]

[1] Centre for Oral Health Research, School of Dental Sciences, Newcastle University, UK

[2] School of Health and Social Care, Teesside University, Middlesbrough, UK

[3]Research Collaborative Services, Newcastle University Information Technology, Newcastle University, UK

Running title: Erosive potential and fluoride concentration of cola-type drinks

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***Corresponding Author:**

Professor Anne Maguire

Centre for Oral Health Research,

School of Dental Sciences,

Newcastle University,

NE2 4BW, UK,

Email: anne.maguire@ncl.ac.uk

Abstract

Aim: Excessive consumption of carbonated soft drinks is detrimental to general and oral health. This study determined endogenous pH, titratable acidity (TA) and fluoride (F) ion concentration of cola-type drinks available in the UK. Subsidiary aims were to compare; i) endogenous pH and TA of drinks upon opening (T0) and after 20 minutes (T20); ii) endogenous pH, TA and F ion concentration of diet v regular and plastic v canned drinks.

Methods: Endogenous pH, TA (mls 0.1M NaOH) and F ion (mg/L) of 71 products measured using pH meter and F-ISE. A Wilcoxon Signed Ranks Test compared pH and TAs at T0 and T20; a Mann-Whitney U test compared pH, TAs and F ion concentration for; a) regular v diet drinks; b) plastic v canned drinks.

Results: Mean (±SD) pH for regular and diet drinks was 2.44± 0.12 and 2.83± 0.33 respectively ($p=0.001$); mean NaOH (ml) to raise pH to 5.5 and 5.7 was 5.49± 0.76 and 6.40± 0.78 (regular drinks); 5.17±1.03 and 6.03±1.07 (diet drinks). Diet ($p=0.040$) and regular ($p=0.041$) drinks had higher TA to pH 5.7 at T0 compared with T20; at T20 regular drinks had higher TA to pH 5.5 ($p=0.026$) and pH 5.7 ($p=0.030$) than diet drinks. There was no difference in F ion concentration between regular v diet drinks ($p=0.754$) and no significant container effect.

Conclusion: Erosive characteristics were similar between manufacturers but higher erosive potentials were evident at T0 compared with 20 minutes later and for regular compared with diet drinks. F ion concentration of drinks was low.

Introduction

Dental erosion is recognised as an important cause of tooth tissue loss in all age groups [1] and is defined as a “surface dissolution of dental hard tissues in the absence of dental plaque” [2].

The significant growth in soft drinks consumption in recent decades has resulted in a market which is now very competitive as more generic drinks, including cola-type drinks, are produced by supermarkets at lower prices compared to proprietary drinks. Children start consuming soft drinks at an increasingly younger age and the volumes consumed increase through young adulthood [3]. The assessment of dental erosion in a group of 14 year old British children showed that over 80% consumed soft drinks on a regular basis [4]. In a review comparing data from the 1993 UK children’s dental health (CDH) survey [5], the National Diet and Nutrition Surveys (NDNS) of 1½ - 4½ year olds in 1992-3 [6] and 4-18 year olds in 1996-7 [7], Nunn et al [8] reported a trend towards a higher prevalence of dental erosion in children aged 3½ and 4½ years and in those who consumed carbonated drinks on most days. They also reported a greater prevalence of erosion in both primary and permanent incisors shown in the 1996-7 NDNS survey of 4-18 year olds compared with a similar age group in the 1993 UK CDH survey. Overall, 18% of the labial surfaces of primary incisors in 4-6-year-olds were affected with erosion in 1993, compared with 38% in 1996/7 and the prevalence of erosion in permanent incisors of 11-14 year olds was 11% in 1993 compared with 23% in 1996/7 [8]. Limited information is available on pH and titratable acidity of proprietary brand cola- type carbonated drinks produced in the UK and these data are no longer current, while no information is currently available on generic brand cola-type drinks produced by UK supermarkets.

The erosive characteristics of a beverage are linked to several factors including its endogenous pH, the total acid content (titratable acidity) and the type of acid (pKa) it contains. In addition, length of exposure to acid and concentration of phosphate, calcium and fluoride (F) in foods and drinks can have a modifying effect on the development of dental erosion [9]. A drink with higher titratable acidity will result in the dissolution of more apatite before neutral pH is approached and the dissolution is terminated. While some studies suggest that titratable acidity is a more important indicator than the endogenous pH value in determining the erosive potential of drinks [10, 11], in studies by Hemingway et al [12] on a group of fruit juices, and by Jensdottir et al on soft drinks,[13] the effect of endogenous pH was reported to be more important since endogenous pH only provides a measure of initial hydrogen ion concentration and gives no indication to the current and potential presence of dissociated acid [3]. In addition to these factors, it is unclear whether the type of drink; i.e. whether it is a “regular” sugars-containing beverage or a “diet” sugars-free alternative changes its erosive characteristics. Furthermore, information on the effect of container type (i.e. contained in a plastic bottle or aluminium can) on the erosive potential of these drinks is limited.

There is strong evidence that topical F use can strengthen teeth against erosive acid damage and high-concentration F agents and/or frequent applications of lower concentration F, eg. fluoridated waters [14] are considered potentially effective approaches in preventing dental erosion [15]. With recent trends towards increased consumption of carbonated soft drinks and juices as a substitute for healthier options such as optimally fluoridated tap waters, there is also a need to assess the impact of these drinks on the overall F exposure of children and information on the F ion content of commonly consumed products is required to make this assessment. Although the F ion concentration of soft drinks is primarily determined by the F ion concentration in water used to manufacture these products, no information is available on the actual resultant F ion content of these drinks which may constitute a significant proportion of daily fluid intake in some individuals.

This study aimed to determine the endogenous pH, titratable acidity (to critical pH for enamel and dentine) and F ion concentration, of cola-type drinks. The subsidiary aims were firstly, to compare the endogenous pH and the titratable acidity (TA) of the drinks upon opening (T0) and after 20 minutes (T20), and secondly, to compare the endogenous pH, titratable acidity and F ion concentration of diet compared with regular drinks, and plastic-bottle-contained compared with can-contained cola drinks.

Materials and Methods

Sourcing samples

A list of all available cola-type drinks on the UK market was prepared by searching supermarkets' websites as well as visiting some of them directly. Based on the product and container types available for generic and proprietary drinks, 71 cola-type drink products from 9 different supermarket companies were identified for purchase.

Samples were purchased from different supermarkets in the north-east of England and stored at room temperature prior to analysis. For the purpose of F ion analysis, 3 different batch numbers for each product type and container were purchased which resulted in a total of 213 samples. All drinks were purchased over a two month period between February and April 2012. Information concerning brand name, flavour, sugars content, type of container and expiry dates was recorded.

To help determine the impact of sugars content (i.e. regular (sugars-containing) v diet (sugars-free) drinks) on erosive potential and F ion content, the drinks were divided into two broad categories of diet (n=48) and regular (n=23) drinks. Within each category the regular group comprised flavoured and classic drinks while the diet group included diet-flavoured, "Zero", caffeine-free, "Max" and no added sugar drinks.

Regarding packaging, three types of containers were identified, with plastic bottles (65%, n=46) and aluminium cans (32%, n=23) accounting for 97% of the 71 products analysed. The only drinks distributed in glass bottles (n=2) were regular and diet Coca-Cola products.

Endogenous pH

At the start of each analysis session, the meter (model 720A, Orion, USA) and pH electrode (Model 68788 Thermo Orion, USA) was calibrated using standard buffers (Mettler Toledo, UK) at pH 2.0, 4.0 and 7.0. The manufacturer's reported precision for the pH electrode was

±0.01. The electrode was then rinsed and placed into de-ionised water before starting pH measurements of the drinks, allowing the pH electrode to stabilise for between 2-3 minutes before each measurement was recorded. From the three batch numbered items purchased for each drink, one batch number was selected randomly and 50 ml of the freshly opened drink was poured into a plastic beaker.

The pH of each drink sample was measured just after opening (T0). Plastic-bottled drinks were re-capped after initial opening while cans and glass bottles were left exposed after opening to simulate usual drinking conditions. A second pH reading was performed after 20 minutes (T20) with a second aliquot from the same sample. For each sample one pH measurement was performed at each time point.

Titratable Acidity

Fifty ml of each drink, kept at room temperature and for which the endogenous pH had been measured was titrated manually to pH 6 by adding 0.1 M NaOH in 1ml increments with continuous stirring. The volumes of titrant needed to raise the sample pH to 5.5 and 5.7, (critical pHs for enamel and dentine dissolution respectively), were then calculated from the plotted volume against the pH curve.

After 20 minutes (T20), a second volume of 50 ml of the same drink sample was poured from the container into the beaker and the same process of titration was undertaken. For each sample one titration was performed for each time point.

F ion analysis

For F ion analysis, equal volumes (5 ml) of drink from each of the 3 batch numbered items within each product type and container type were taken and mixed together in a plastic beaker. Due to the large number of samples, three aliquots (5 ml each) of the mixed batches for each sample were taken and stored at -20ºC prior to F ion analysis until preparation of all samples had been completed.

The F ion concentration of each sample was measured in duplicate by a direct method using an F ion-selective electrode (F-ISE) (Model 9609, Thermo Orion, USA) coupled to a meter (Model 720A, Orion, USA) after sample buffering with Total Ionic Adjustment Buffer (TISAB III) [16]. The F-ISE was reported by the manufacturers to have a limit of detection of ±0.02µg/ml (Orion Plus F Electrode, Thermo Electron Corporation, USA). Ten percent of samples (n=7) were re-analysed to confirm the reliability of the method.

Statistical analysis

The data were analysed using IBM SPSS Statistics software (Version 21 Chicago, IL, USA). Descriptive (summary) statistics were used to present endogenous pH, TA (to pH 5.5 and 5.7) at T0 and T20, and F concentration, according to sugars content and container type.

Normality testing indicated that the data were not normally distributed and consequently a Wilcoxon Signed Ranks Test was performed to determine any statistically significant differences in endogenous pH and TAs at T0 and T20. A Mann-Whitney U test was used to determine whether sugars content (regular v diet) or container type (plastic v can) had any effect on these same variables. A p value less than 0.05 was considered to be statistically significant.

Results

Endogenous pH

Table 1 shows the mean ±SD endogenous pH of drinks based on their sugars content at T0 and after 20 minutes (T20) and for all brands. Overall, there was no difference in pH between opening and after 20 minutes for the 23 regular drinks with a mean pH at T0 and T20 of 2.43, while for diet drinks it was 2.85. Marks and Spencer regular cola drinks had the lowest mean (SD) endogenous pH for all drinks (2.10±0.00) at both T0 and T20 time points, while ALDI diet cola drinks had the highest pH overall (3.30±0.20), measured at T20.

Based on container type (Table 2), the lowest mean (SD) endogenous pH (2.35±0.35) was observed for plastic bottles from Marks and Spencer at T0 and T20, the highest mean endogenous pH being recorded for ALDI canned colas at T0 (3.20±0.00).

Table 3 shows the results of the Wilcoxon Signed Rank Test which compared related samples between opening (T0) and 20 minutes after opening (T20). For the diet drinks there was no statistically significant difference in the endogenous pH values between the two time points [Z-score=-0.13, p=0.894] and this was also true for regular drinks [Z-score=-0.74, p=0.458].

To consider the effect of sugars content and container type at a given time point a Mann-Whitney U Test was used. As Table 4 shows, there was a statistically significantly higher endogenous pH in diet drinks compared with regular drinks at T0 and T20 [Mann-Whitney U=149, p=0.001 and Mann-Whitney U=132, p=0.001 respectively]. There was no significant difference in the endogenous pH values between can and plastic bottles at T0 or T20 (Table 5).

Titratable acidity

When the TA of 6 drink samples was measured 3 times during the preparatory phase of the study, there was an average of 3% difference in TA at T0 and T20 between the replicates, indicating that the method was reproducible. The mean (SD) volumes (ml) of 0.1 M NaOH required to raise the pH of 50 ml of each drink sample to 5.5 and 5.7 at T0 and T20 based on their sugars content and container type are presented in Tables 1 and 2. The 23 regular drinks had a mean TA (to pH 5.5) of 5.49±0.76 ml at T0 and 5.38±0.72 ml at T20, while the equivalent recordings were 5.27±0.96 and 5.18±0.99 ml for the 48 diet drinks. The greatest TA recorded for raising the pH to 5.5 was for Marks and Spencer and Morrison regular colas (7.00 ml of 0.1M NaOH); the Marks and Spencer regular cola also had the greatest TA to pH 5.7 (8.00±0.00ml at T0 and T20). As Table 2 shows, it was the Morrison cola in a plastic bottle which showed the highest TA to pH 5.5 (6.03±1.77ml) at T0, while to raise the pH to 5.7 took a mean of 6.92±0.59 ml of NaOH for the Tesco plastic-contained colas at T0. For both regular and diet drinks, the TA (to pH 5.7) was statistically significantly lower 20 minutes after opening compared with T0 ($p<0.05$) (Table 3).

When the effect of sugars content and container type on titratable acidity was considered, as Table 4 shows, regular drinks had a statistically significantly higher TA (to pH 5.5 and 5.7) compared with diet drinks at T20. [Mann-Whitney U=372, p=0.026 and Mann-Whitney U=376, p=0.030 respectively]. However there was no significant difference in the TA values between can and plastic bottles at T0 or T20 (Table 5)

F ion concentration (mg/L)

The % difference in F ion concentration between test- to re-test for 10% of the samples was 5% which indicated an acceptable level of reliability in measurement. The F ion concentrations of drinks (mg/L) based on their sugars content (i.e. regular or diet) and container type (i.e. plastic bottle or metallic can) are presented in Tables 1 and 2 respectively. The overall mean F ion concentration for both regular and diet drinks was 0.09±0.11 mg/L with only 3 (4%) of the 71 drinks having a F ion concentration $\geq$ 0.3mgF/L. The highest F ion concentration was found in a regular Pepsi cola group (mean 0.24±0.14 mg/L); the lowest mean being 0.02mg/L and found in 3 regular cola groups (ALDI, Morrison and Tesco) and 2 diet cola groups (Co-op and Tesco). Based on container type, Pepsi drinks distributed in cans showed the highest F ion concentration (mean 0.28mg/L) followed by Coca-Cola drinks distributed in glass (0.26mg/L), while the mean (SD) F ion concentrations of Coca-Cola drinks in cans (n=6) and plastic bottles (n=7) were 0.08(0.04) mg/L and 0.06(0.04) mg/L respectively.

No statistically significant differences in F ion concentration were found between either regular and diet type drinks (p=0.754) or between plastic bottle and canned drinks (p=0.726) (Tables 4 and 5).

Discussion

This study measured the endogenous pH of 71 cola drinks at baseline (upon opening at T0) and 20 minutes after opening. The 20 minute time interval was selected to simulate average drinking time. Endogenous pH and TA for Coca-Cola and Pepsi products have been reported in other studies [17, 18], however, this is the first study to consider the erosive characteristics of all cola-type drinks available in the UK market. All drinks had an endogenous pH below the critical pH (5.5) [19] for enamel dissolution. The inherent acidity of these drinks is mainly due to the addition of acids during their manufacturing process to improve their organoleptic properties since acidity is a key factor to stimulate taste and counteract sweetness. The strength of an acid is usually described by its pK_a values - negative logarithms of K_a values, i.e. their acid dissociation constants. According to the manufacturer's labelling, phosphoric acid (with pK_a values of 2.15, 7.20, and 12.35 – one for each of the hydrogen ions reversibly bound to the phosphate ion) was the main acid included in both regular and diet type drinks. However, citric acid (with pK_a values of 3.08, 4.74 and 6.42) was additionally included in regular ASDA and Marks & Spencer drinks. Phosphoric acid and citric acid were included in almost all the diet type drinks except for those from Tesco and the Co-op. Citric acid has been highlighted as having a particularly detrimental effect on dental enamel and can cause severe demineralisation due to its ability to chelate minerals of apatite such as calcium [20, 21], while malic acid, present in the diet cola drinks produced by ASDA, Co-op and Marks & Spencer, is slightly less detrimental [22]. In a study by Edwards and co-workers, [23] the higher TA for fruit juices compared with other carbonated drinks was reported to be due to citric acids derived from the fruit. However, in the current study, despite the presence of additional exogenous acids in the diet drink group their endogenous pH was higher and their TAs were lower compared with the regular group of drinks.

It is important that total TA [23, 24] and the relative strength of the acid which indicates how easily the acid will "actively" give up free H^+ ions (pK_a) [25] is considered when determining the erosive potential of drinks. While TA is responsible for maintaining the H^+ concentration available for the interaction with the tooth surface [10, 22], its importance in the clinically erosive situation is influenced by conditions such as exposure time and the ratio of the volume of eroding solution to the area of exposed tooth surface [18]. The *in vitro* conditions in which the drinks were tested in this study will impact on the clinical relevance of the results since biological factors such as salivary flow rate and its buffering capacity, as well as individual's drinking habits, are important modifiers in the erosion process clinically. However this study provides a simple method for identifying potentially erosive drinks *in vitro* and helps to inform clinicians and their patients when potentially erosive products are being discussed as part of dietary analysis and advice.

This study also investigated any potential differences in TA of plastic bottled versus canned drinks following opening since bottled drinks can be recapped while canned drinks are left exposed after opening. The decrease in TA after 20 minutes was found to be similar between plastic bottles (recapped) and cans (exposed) contained drinks. In contrast, for a group of UK flavoured sparkling waters the decrease in TA after 30 minutes exposure to air was reported to range from 26-48% [26]. According to the authors, this difference could be due to a variation in the degree of carbonation introduced during production between cola drinks and flavoured waters.

As one of the main aims of this study, F ion concentrations of drinks were also measured. Several studies have previously measured F ion concentrations of carbonated drinks, either in relation to measuring F exposure [27, 28], or in determining whether the F can mitigate against dental erosion [17, 29] and have reported a wide range in F ion concentrations from 0.02 mg F/L to 1.62 mg F/L. However, no study has reported on the F ion concentration of the different types of cola drinks available in the UK market. Overall, the median (range) F ion concentration of drinks in the present study at 0.03 (0.01-0.60) mg/L was lower than the 0.72 (0.02-1.28) mg/L and 0.91 (0.1-1.62) mg/L reported for the cola drinks from the US [27] and Mexico [28] respectively. In both those studies the wide range in F ion concentration was attributed to the variation in the F ion concentration of water sources used in the production sites which could also explain the range in values found in the present study. The substantial difference in the F ion concentration of identical products from different sites for the US study also highlights that variation in F ion concentration of water sources appears to be the main determinant of any variation seen in soft drink product F ion concentrations.

The current study found no statistically significant difference between F ion concentrations of diet versus regular drinks which is consistent with the results reported by Heilman and co-workers [27] in the US.

Although between-container differences in F ion concentration have been reported previously [28], the present study found similar F ion concentrations for drinks sold in cans and in plastic bottles (median: 0.03 mg/L). While no significant difference was reported for F ion concentrations of drinks sold in glass and cans in the work by Heilman et al.,[27] in the US, the F ion concentration of canned Mexican cola drinks was reported to be higher (0.74mg/L) than those drinks sold in plastic (0.37mg/L) and glass containers (0.34 mg/L) [28]. This may reflect the use of different containers in different parts of a country in which the F ion concentrations of the manufacturers' supply waters also differ.

Furthermore, the current study found that only the Coca-Cola Company produced drinks in glass containers. In laboratories, F solutions are not normally stored in glass containers as F ion has an affinity for glass, reducing the F ion concentration of its contents [28]. Despite this high affinity of F ion for glass the median F ion concentration of Coca-Cola drinks in glass containers was higher (0.26 mg/L) compared with the 0.1mg/L found for Coca-Cola drinks in cans and plastic bottles, suggesting that higher F ion concentration waters may have been used for the production of these drinks.

Overall, there was no difference in the F ion concentration of regular versus diet cola drinks, nor between plastic bottled and canned drinks. It is the water used in production of cola drinks which appears to be the main determinant of their F ion concentration. Overall, the F ion concentration of drinks in this study was low (Mean (SD) 0.09 (0.11) mgF/L), although it did appear to vary with water sources used in their production. Unfortunately, soft drinks may comprise a significant proportion of an individual's fluid intake and detract from the general and oral health benefits associated with drinking water. It is important for health professionals and consumers to be aware of this when supporting their patients to make good dietary choices. The inclusion of general and oral health information covering dental caries and erosion risk as well as including the F ion content on soft drinks packaging and labels would facilitate this.

The cola-type drink products tested in this study were representative of those available in the UK market. Their erosive characteristics were broadly similar between manufacturers but with significantly higher erosive potentials evident upon opening compared with 20 minutes later and for regular drinks compared with diet drinks. The F ion concentration of drinks was low; 96% of the 71 products contained <0.3 mgF/L, most probably due to the F ion concentration of water used by manufacturers to prepare them.

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Table Legends

Table 1 Mean (SD) endogenous pH, titratable acidity (TA -mls of 0.1M NaOH) to pH 5.5 and 5.7 at opening (T0) and 20 minutes after opening (T20) and F ion concentration (mg/L) of drinks based on brand and sugars content (Regular or Diet).

Table 2 Mean (SD) endogenous pH, titratable acidity (TA -mls of 0.1M NaOH) to pH 5.5 and 5.7 at opening (T0) and 20 minutes after opening (T20) and F ion concentration (mg/L) of drinks based on brand and container type.

Table 3 Comparison of endogenous pH and titratable acidity (TA -mls of 0.1M NaOH) to pH 5.5.and 5.7 upon opening (T0) and 20 minutes after opening (T20).

Table 4 Comparison of endogenous pH, titratable acidity (TA - ml of 0.1M NaOH) to pH 5.5 and 5.7 and F ion concentration (mg/L) of drinks based on their sugars content (Diet v Regular).

Table 5 Comparison of endogenous pH, titratable acidity (TA - ml of 0.1M NaOH) to pH 5.5 and 5.7 and F concentration (mg/L) of drinks based on container type (can v plastic bottle).

Table 1. Mean (SD) endogenous pH, titratable acidity (TA - mls of 0.1M NaOH) to pH 5.5 and 5.7 at opening (T0) and 20 minutes after opening (T20) and F ion concentration (mg/L) of drinks based on brand and sugars content (Regular or Diet)

Drinks (number of products)	Mean (SD)						
	Endogenous pH		TA (ml) to pH 5.5		TA (ml) to pH 5.7		F ion concentration (mg/L)
	T0	T20	T0	T20	T0	T20	
Regular							
ALDI (n=1)	2.30 (0.00)	2.30 (0.00)	5.50 (0.00)	5.50 (0.00)	6.50 (0.00)	6.50 (0.00)	0.02 (0.00)
ASDA (n=4)	2.53 (0.05)	2.55 (0.13)	5.63 (0.85)	5.21 (0.63)	6.75 (0.87)	6.13 (0.63)	0.14 (0.13)
Co-op (n=1)	2.30 (0.00)	2.40 (0.00)	5.33 (0.00)	5.66 (0.00)	6.00 (0.00)	6.50 (0.00)	0.03 (0.00)
Coca-Cola (n=5)	2.42 (0.04)	2.40 (0.10)	5.00 (0.00)	5.02 (0.48)	5.93 (0.15)	5.78 (0.65)	0.09 (0.08)
Marks & Spencer (n=1)	2.10 (0.00)	2.10 (0.00)	7.00 (0.00)	7.00 (0.00)	8.00 (0.00)	8.00 (0.00)	0.20 (0.00)
Morrison (n=1)	2.30 (0.00)	2.30 (0.00)	7.00 (0.00)	6.00 (0.00)	7.66 (0.00)	7.00 (0.00)	0.02 (0.00)
Pepsi (n=2)	2.50 (0.00)	2.40 (0.14)	4.66 (0.00)	4.66 (0.00)	5.50 (0.00)	5.50 (0.00)	0.24 (0.14)
Sainsbury’s (n=4)	2.55 (0.06)	2.48 (0.13)	5.33 (0.38)	5.15 (0.34)	6.17 (0.41)	5.92 (0.44)	0.03 (0.01)
Tesco (n=4)	2.45 (0.10)	2.48 (0.10)	5.83 (0.87)	5.93 (0.92)	6.67 (0.93)	6.54 (0.93)	0.02 (0.01)
All (n=23)	2.44 (0.12)	2.43 (0.14)	5.49 (0.76)	5.38 (0.72)	6.40 (0.78)	6.18 (0.76)	0.08 (0.10)
Diet							
ALDI (n=4)	3.23 (0.21)	3.28 (0.22)	4.08 (0.17)	4.00 (0.00)	5.00 (0.00)	5.00 (0.00)	0.03 (0.01)
ASDA (n=9)	2.71 (0.09)	2.68 (0.08)	5.11 (0.61)	5.14 (1.08)	5.92 (1.12)	5.92 (1.12)	0.16 (0.13)
Co-op (n=2)	2.45 (0.07)	2.55 (0.07)	5.21 (0.77)	4.97 (0.90)	5.63 (0.88)	5.63 (0.88)	0.02 (0.00)
Coca-Cola (n=10)	3.06 (0.11)	3.03 (0.12)	5.52 (1.46)	5.43 (1.44)	6.18 (1.44)	6.18 (1.44)	0.10 (0.08)
Marks & Spencer (n=1)	2.60 (0.00)	2.60 (0.00)	5.00 (0.00)	5.00 (0.00)	5.66 (0.00)	5.66 (0.00)	0.21 (0.00)
Morrison (n=4)	2.43 (0.34)	2.40 (0.35)	5.79 (1.94)	5.90 (2.08)	6.69 (2.17)	6.69 (2.17)	0.03 (0.01)
Pepsi (n=5)	3.12 (0.35)	3.06 (0.34)	5.20 (0.30)	5.06 (0.36)	5.97 (0.42)	5.97 (0.42)	0.22 (0.21)
Sainsbury’s (n=7)	2.53 (0.17)	2.99 (1.17)	5.05 (0.85)	4.90 (0.60)	5.56 (0.74)	5.56 (0.74)	0.05 (0.07)
Tesco (n=6)	2.93 (0.23)	2.97 (0.20)	5.14 (0.87)	4.89 (0.66)	5.72 (0.80)	5.72 (0.80)	0.02 (0.01)
All (n=48)	2.83(0.33)	2.89 (0.51)	5.17 (1.03)	5.08 (1.09)	6.03 (1.07)	5.87 (1.12)	0.09 (0.12)
All products (n=71)	2.71 (0.33)	2.74 (0.48)	5.27 (0.96)	5.18 (0.99)	6.15 (0.99)	5.97 (1.02)	0.09 (0.11)

Table 2. Mean (SD) endogenous pH, titratable acidity (TA - mls of 0.1M NaOH) to pH 5.5 and 5.7 at opening (T0) and 20 minutes after opening (T20) and F ion concentration (mg/L) of drinks based on brand and container type

Container type (number of products)	Mean (SD)						
	Endogenous pH		TA (ml) to pH 5.5		TA (ml) to pH 5.7		F ion concentration (mg/L)
	T0	T20	T0	T20	T0	T20	
Can							
ALDI (n=2)	3.20 (0.00)	3.15 (0.07)	4.00 (0.00)	4.00 (0.00)	5.00 (0.00)	5.00 (0.00)	0.04 (0.01)
ASDA (n=5)	2.66 (0.11)	2.68 (0.13)	5.13 (1.06)	5.13 (1.06)	6.17 (0.80)	6.07 (1.11)	0.03 (0.00)
Coca-Cola (n=6)	2.86 (0.37)	2.82 (0.38)	5.39 (1.31)	5.36 (1.35)	6.22 (1.41)	6.11 (1.35)	0.08 (0.04)
Pepsi (n=3)	2.96 (0.50)	2.93 (0.40)	4.99 (0.34)	4.77 (0.19)	5.83 (0.29)	5.61 (0.35)	0.28 (0.28)
Sainsbury's (n=5)	2.60 (0.10)	3.18 (1.36)	4.79 (0.65)	4.83 (0.50)	5.63 (0.77)	5.56 (0.62)	0.03 (0.00)
Tesco (n=2)	2.75 (0.49)	2.75 (0.49)	6.00 (0.71)	5.66 (1.41)	6.92 (0.59)	6.33 (1.41)	0.01 (0.00)
All (n=23)	2.79 (0.32)	2.90 (0.66)	5.09 (0.92)	5.03 (0.97)	5.98 (0.96)	5.84 (0.97)	0.07 (0.12)
Plastic bottles							
ALDI (n=3)	2.93 (0.60)	3.03 (0.66)	4.61 (0.79)	4.50 (0.87)	5.50 (0.87)	5.50 (0.87)	0.02 (0.00)
ASDA (n=8)	2.65 (0.13)	2.61 (0.09)	5.35 (0.68)	5.18 (0.92)	6.23 (0.88)	5.94 (0.96)	0.23 (0.09)
Co-op (n=3)	2.40 (0.10)	2.50 (0.10)	5.25 (0.55)	5.19 (0.75)	5.89 (0.51)	5.92 (0.80)	0.02 (0.01)
Coca-Cola (n=7)	2.84 (0.28)	2.81 (0.29)	5.55 (1.25)	5.52 (1.19)	6.48 (1.18)	6.33 (1.19)	0.06 (0.04)
Marks & Spencer (n=2)	2.35 (0.35)	2.35 (0.35)	6.00 (1.41)	6.00 (1.41)	6.75 (1.77)	6.83 (1.65)	0.21 (0.01)
Morrison (n=5)	2.40 (0.30)	2.38 (0.30)	6.03 (1.77)	5.92 (1.79)	6.79 (1.85)	6.75 (1.89)	0.02 (0.01)
Pepsi (n=4)	2.93 (0.42)	2.83 (0.49)	5.08 (0.42)	5.08 (0.42)	6.04 (0.67)	6.00 (0.41)	0.19 (0.10)
Sainsbury's (n=6)	2.48 (0.15)	2.48 (0.20)	5.44 (0.66)	5.12 (0.53)	6.25 (0.70)	5.79 (0.71)	0.05 (0.07)
Tesco (n=8)	2.74 (0.29)	2.78 (0.28)	5.27 (0.91)	5.22 (0.84)	6.17 (0.92)	5.97 (0.87)	0.02 (0.00)
All (n=46)	2.66 (0.32)	2.66 (0.34)	5.40 (0.98)	5.30 (0.99)	6.26 (1.02)	6.09 (1.04)	0.09 (0.10)
Glass							
Coca-Cola (n=2)	2.80 (0.57)	2.85 (0.49)	4.50 (0.71)	4.30 (0.06)	5.50 (0.71)	4.88 (0.18)	0.26 (0.06)
All containers (n=71)	2.71 (0.33)	2.74 (0.48)	5.27 (0.96)	5.18 (0.99)	6.15 (0.99)	5.97 (1.02)	0.09 (0.11)

Table 3. Comparison of endogenous pH and titratable acidity (TA - mls of 0.1M NaOH) to pH 5.5.and 5.7 upon opening (T0) and 20 minutes after opening (T20)

Product Type	Comparisons	Ranks	N	Mean Rank	Z-score	p value
Diet (n=48)	Endogenous pH at T20 - Endogenous pH T0	Negative	15	15.90	-0.133	0.894
		Positive	15	15.10		
		Ties	18			
	TA to pH 5.5 at T20 - TA to pH 5.5 at T0	Negative	21	14.33	-1.81	0.070
		Positive	8	16.75		
		Ties	19			
	TA to pH 5.7 at T20 - TA to pH 5.7 at T0	Negative	21	16.79	-2.05	0.040
		Positive	10	14.35		
		Ties	17			
Regular (n=23)	Endogenous pH at T20 - Endogenous pH at T0	Negative	6	8.00	-0.74	0.458
		Positive	6	5.00		
		Ties	11			
	TA to pH 5.5 at T20 - TA to pH 5.5 at T0	Negative	8	8.94	-1.20	0.232
		Positive	6	5.58		
		Ties	9			
	TA to pH 5.7 at T20 – TA to pH 5.7 at T0	Negative	10	6.50	-2.05	0.041
		Positive	2	6.50		
		Ties	11			
All (n=71)	Endogenous pH at T20 - Endogenous pH at T0	Negative	21	23.36	-0.523	0.601
		Positive	21	19.64		
		Ties	29			
	TA to pH 5.5 at T20 - TA to pH 5.5 at T0	Negative	29	22.72	-2.25	0.024
		Positive	14	20.50		
		Ties	28			
	TA to pH 5.7 at T20 - TA to pH 5.7 at T0	Negative	31	22.81	-2.83	0.005
		Positive	12	19.92		
		Ties	28			

Table 4. Comparison of endogenous pH, titratable acidity (TA - mls of 0.1M NaOH) to pH 5.5 and 5.7 and F ion concentration (mg/L) of drinks based on their sugars content (Diet v Regular)

Variables	Diet / Regular	N	Mean Rank	Mann-Whitney U	p value
Endogenous pH T0	Diet	48	44.40	149.00	0.001
	Regular	23	18.48		
Endogenous pH after T20	Diet	48	44.75	132.00	0.001
	Regular	23	17.74		
TA to pH 5.5 at T0	Diet	48	32.91	403.50	0.065
	Regular	23	42.46		
TA to pH 5.5 at T20	Diet	48	32.25	372.00	0.026
	Regular	23	43.83		
TA to pH 5.7 at T0	Diet	48	32.72	394.00	0.051
	Regular	23	42.85		
TA to pH 5.7 at T20	Diet	48	32.34	376.50	0.030
	Regular	23	43.63		
F ion concentration	Diet	48	36.52	527.00	0.754
	Regular	23	34.91		

Table 5. Comparison of endogenous pH, titratable acidity (TA - mls of 0.1M NaOH) to pH 5.5 and 5.7 and F ion concentration (mg/L) of drinks based on container type (can v plastic bottle)

Variables	Container type	N	Mean Rank	Mann-Whitney U	p value
Endogenous pH at T0	Can	23	40.89	393.50	0.083
	Plastic bottle	46	32.05		
Endogenous pH at T20	Can	23	41.26	385.00	0.065
	Plastic bottle	46	31.87		
TA to pH 5.5 at T0	Can	23	29.85	410.50	0.128
	Plastic bottle	46	37.58		
TA to pH 5.5 at T20	Can	23	29.30	398.00	0.093
	Plastic bottle	46	37.85		
TA to pH 5.7 at T0	Can	23	30.87	434.00	0.222
	Plastic bottle	46	37.07		
TA to pH 5.7 at T20	Can	23	30.46	424.50	0.180
	Plastic bottle	46	37.27		
F ion concentration	Can	23	36.17	502.00	0.726
	Plastic bottle	46	34.41		