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The ability of fruit teas to remove the smear layer: an in vitro study of tubule patency

Jeremy S. Rees[a,*], T. Loyn[a], W. Rowe[a], Q. Kunst[b], R. McAndrew[a]

[a] *Adult Dental Health Department, Dental School, Wales College of Medicine, Heath Park, Cardiff, CF14 4XY, UK*
[b] *Undergraduate student, ACTA, Louwesweg 1 1066 EA Amsterdam*

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Summary *Objectives*: Fruit teas are know to have an erosive effect on enamel, but the effects on dentine are unknown. Lesions of dentine hypersensitivity have numerous patent dentinal tubules and the aim of this paper was to examine the ability of various fruit teas to remove the smear layer.
Methods: The erosive potential of a variety of fruit teas was assessed in the laboratory by measuring their pH and neutralisable acidity. Smeared dentine specimens were prepared from extracted teeth and the ability of each tea to remove the smear layer was assessed by measuring the diameter and area of the opened tubules and counting the number of patent tubules seen in a unit area using scanning electron microscopy. A 0.2% citric acid solution was used as a positive control.
Results: The pH of the fruit teas ranged from 2.98 to 3.95 and the neutralisable acidity ranged from 10.63 to 33.0 ml of 0.1 M NaOH. All the fruit teas tested were able to remove the smear layer. The mean diameter of the tubules ranged from 0.61 to 1.14 μm and the mean area ranged from 0.31 to 1.03 μm^2. The number of patent tubules per specimen ranged from 13 to 121.
Conclusion: All the fruit teas tested were found to be highly acidic and able to remove the smear layer.

Introduction

Dentine hypersensitivity or sensitivity (DS) is 'characterized by a short, sharp, pain arising from exposed dentine in response to stimuli, typically thermal, evaporative, tactile, osmotic or chemical and which cannot be ascribed to any other form of dental defect or pathology'.[1] It is a commonly encountered as a clinical problem where dentine has become exposed following either gingival recession or loss of enamel as part of the tooth wear process. Various studies have suggested an average prevalence value for DS of around 15%

* Corresponding author. Tel.: +0044 2920 746 557; fax: +0044 92920 743 120.
E-mail address: reesjs1@cardiff.ac.uk (J.S. Rees).

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Table 1 Summary of prevalence studies on dentine hypersensitivity.

Authors	Country	Setting	Study type	Prevalence
Jensen[19]	USA	University	Clinical	30%
Graf and Glase[20]	Switzerland	Practice	Clinical	15%
Flynn et al.[21]	UK	University	Clinical	18%
Orchardson and Collins[22]	UK	University	Clinical	74%
Fischer et al.[23]	Brazil	University	Clinical	17%
Murray and Roberts[24]	Indonesia	Not stated	Questionnaire	27%
Murray and Roberts[24]	USA	Not stated	Questionnaire	18%
Murray and Roberts[24]	Japan	Not stated	Questionnaire	16%
Murray and Roberts[24]	France	Not stated	Questionnaire	14%
Murray and Roberts[24]	Germany	Not stated	Questionnaire	13%
Murray and Roberts[24]	Australia	Not stated	Questionnaire	13%
Chabanski et al.[25]	UK	University	Clinical	73%
Irwin and McCusker[26]	UK	Practice	Questionnaire	57%
Liu, Lan and Hsieh[27]	Taiwan	University	Clinical	32%
Rees[2]	UK	Practice	Clinical	4%
Rees and Addy[3]	UK	Practice	Clinical	4%
Rees et al.[28]	Hong Kong	University	Clinical	68%

(Table 1). However, many of these early studies were carried out on patient cohorts with significant periodontal disease, attachment loss and gingival recession. More recent general practice based studies have suggested a prevalence value of around 5%.[2-3]

The hydrodynamic theory of dentine sensitivity that was originally described by Gysi[4] but popularized by Brannstrom[5] and is accepted by most workers as the mechanism to explain dentine sensitivity. Briefly, dentine is a composite structure composed of collagen and mineral, principally hydroxyapatite. The dentine is permeated by many millions of dentinal tubules that extend from the pulpal aspect of the tooth to the external surface. The odontoblast bodies line the pulpal aspect of the dentine and the odontoblast processes only extend a short distance into the pulpal end of the tubule, the remainder of the tubule being filled with fluid. If the dentine becomes exposed to the oral environment, thermal or osmotic stimuli will cause movement of the tubular fluid and if this movement is large enough the Aδ nerve fibres found in the pulp periphery, just below the odontoblast cell bodies, are stimulated resulting in the short, sharp pain response characteristic of DS.

Although the majority of patients over 40 years of age have some evidence of gingival recession, only a small percentage will experience problems with dentine sensitivity. Clinical and laboratory studies have shown that patients with clinical sensitivity have lost the 'protective smear layer' and show many more wider dentinal tubules; these tubules are open at the external tooth surface and are patent to the pulp.[6-8]

Patients who suffer from DS have been shown to consume a more erosive diet that contains food and drinks high in fruit acids, such as citric acid.[9] Foods and drinks that contain high levels of fruit acid have been shown in vitro to remove the protective smear layer, exposing the underlying dentinal tubules.[7,8,10]

More recently, some in vitro studies have shown that fruit teas are erosive; being comparable to orange juice as far as enamel erosion is concerned.[11,12] Fruit teas continue to increase in popularity and within the UK they now account for 3-4% of all tea consumed.[13]

The aim of this study was to examine the effect that fruit teas had on dentine. The null hypothesis used in the study was that these drinks would have no effect on the dentinal smear layer.

Materials and methods

The fruit teas used in this study are listed in Table 2. In addition to the fruit teas that were 'bag based' products one 'loose tea' product was included; Lift Instant Lemon tea. This is a dehydrated powder product for consumption directly from the jar.

All the 'bagged teas' were produced using a standard method.[12] A single teabag was added to 250 ml of fresh boiled water and stirred at 0, 2, 4 and 5 min and the bag was removed. A volume of 250 ml was used as this represented the average

Table 2 Tea contents according to manufacturer's information.

Tea	Contents
Twining infusions exotic selection	
Pear and guava	Hibiscus, rosehips, apple pieces, blackberry leaves, pear flavouring, liquorice root, guava flavouring, pear pieces, guava pieces
Pink grapefruit, mandarin and lime	Hibiscus, rosehips, orange peel, apple pieces, blackberry leaves, grapefruit flavouring, mandarin flavouring, liquorice root, lime flavouring, lemon flavouring, lemon and mandarin pieces
Orange, mango and cinnamon	Hibiscus, orange flavouring, rosehips, apple pieces, blackberry leaves, orange peel, cinnamon, mango flavouring, liquorice root, passion fruit flavouring, cinnamon flavouring, mango pieces
Mandarin and lychee	Hibiscus, rosehips, orange peel, apple pieces, blackberry leaves, mandarin flavouring, liquorice root, jasmine flavouring, orange flavouring, lychee flavouring, lychee and mandarin pieces
Twining infusions fruit selection	
Raspberry, strawberry and loganberry	Hibiscus, rosehips, orange peel, blackberry leaves, apple pieces, strawberry flavouring, liquorice root, raspberry flavouring, loganberry flavouring, strawberry, raspberry and loganberry pieces
Lemon burst	Lemon peel, apple pieces, orange peel, lemon flavouring, lemon grass, citric acid, linden, cinnamon bark, cherry stems, orange leaves, hibiscus
Peach and passion fruit	Hibiscus, apple pieces, rosehips, orange, peel, lemon peel, blackcurrant pieces, peach flavouring, passion fruit flavouring, liquorice root, elderberry pieces, cinnamon bark, peach and passion fruit pieces
Blackcurrant burst	Hibiscus, rosehips, orange peel, apple pieces, blackberry leaves, blackcurrant flavouring, liquorice root, citric acid, blackcurrants
Lift instant tea	
Lemon	Dextrose, soluble solids of tea, citric acid, maltodextrin, sodium citrate, flavourings, vitamin C

1. R Twinning and Co, South Way, Andover, Hampshire UK. 2. Lift lemon tea, Premier International Foods Ltd, Bridge Rd, Long Sutton, Spalding, Lincolnshire, UK.

volume of a typical tea mug. The tea solution was allowed to cool to 37 °C before testing.

The Lift Instant lemon tea was prepared by following the manufacturer's instructions. Ten grammes of the powder, which was equivalent to two heaped teaspoons of the product, was added to 250 ml of fresh boiling water and stirred until dissolved.

Initial pH

The pH of each of the teas was tested using an electronic pH meter (Model 701A, Orion Research, Inc., Boston, USA) at 37 °C. The pH meter was calibrated using test solutions of known pH (Fisher Scientific International, Loughborough, UK) prior to testing each of the teas. Each tea was tested using four different samples.

Neutralisable acidity

The neutralisable acidity of each tea was tested by placing 20 ml of tea solution in a glass beaker, which was then placed in a thermostatically controlled water bath held at 37 °C. 0.1 M sodium hydroxide solution was then gradually added to the tea sample and the pH rise continuously monitored until it reached neutrality. Each tea solution was stirred continuously as the sodium hydroxide was added. The volume of sodium hydroxide required to increase the pH of the sample to neutrality was noted and this was repeated four times for each tea.

Dentine specimens

Dentine specimens were prepared using the technique of Banfield and Addy[14] from recently extracted, caries free human premolar and molar teeth. The crowns of the teeth were removed by sectioning horizontally using a diamond disc in a slow speed hand piece. The root portion was then sectioned longitudinally to give samples approximately 1.5 mm thick. From these sections specimens of root dentine approximately 5 mm square were polished using 320-grit silicon carbide paper (Kemet International Ltd, Maidstone, Kent, UK). One face, the test surface, was chosen and the

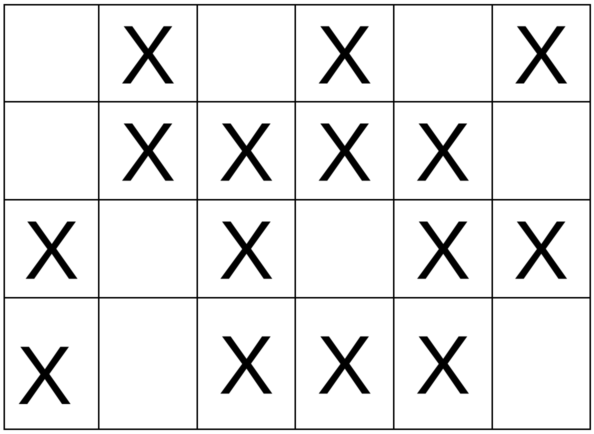

Figure 1 Counting grid used in the study (All tubules in the clear areas were counted and measured).

opposite face marked with indelible ink. The test face was then finally polished with 1000 grit. This reduced the specimen thickness to approximately 1 mm and also created a smear layer.

Each dentine specimen was then sectioned in half using wire cutters. One half was immersed in each tea solution for 15 min. The other half acted as the control specimen and was immersed in distilled water for 15 min. Two dentine specimens were immersed in each of the teas. Following immersion, specimens were carefully removed, dried and mounted on aluminium stubs. They were then sputtered with gold to a thickness of 20–40 nm.

Each half of the specimen was examined near its fractured edge to minimise any variations in tubule density. SEM photomicrographs were obtained as near as possible under the same working conditions (HT = 15 kW, working distance of 9.3 and a magnification of 1000).

All measurements of the number of opened tubules and the width and diameter of all of the opened tubules were made using Scion Image for Windows 4.0.2b (Scion Corporation, Frederick, Maryland, USA) An area of 100 μm^2 was chosen and the number of open tubules in this field of view was counted manually using the grid system described by Absi et al.[7] The grid (Fig. 1) was placed over the image and only those tubules in the clear areas were counted. The tubule diameter and area for each patent tubule was also measured and where the tubule deviated from a circular shape, the largest width was measured.

Results

The pH and neutralisable acidity values results for each fruit tea tested, together with the results for the 0.2% citric acid positive control are given in Table 3. The pH values ranged from 2.98 to 3.95 and the neutralisable acidity ranged from 10.63 to 33.0 ml of 0.1 M NaOH.

Table 4 shows the values for the mean tubule diameter, mean tubule area and number of patent tubules. For the fruit teas the tubule diameter ranged from 0.61 to 1.14 μm and the tubule area ranged from 0.31 to 1.03 μm^2. The number of visible tubules ranged from 13 to 121.

The majority of the negative water control specimens (14/20 specimens) were completely covered by a smear layer with no visible tubules. However, for three of the teas tested (orange, mango and lychee/mandarin and lychee/Lift lemon tea) a small number of tubules were visible. For these tubules the diameter ranged from 0.32 to 0.67 μm and the tubule area ranged from 0.18 to 0.37 μm^2. The number of visible tubules ranged between 2 and 4. An example of a control dentine specimen showing the smear layer is shown in Fig. 2(a) and a dentine specimen following immersion in blackcurrant tea and showing the exposed dentinal tubules is shown in Fig. 2(b).

Table 3 pH and neutralisable acidity.

Fruit tea	pH	Neutralisable acidity (ml)
Pear and guava	2.98 (0.04)	24.50 (0.35)
Pink grapefruit, mandarin and lime	3.03 (0.04)	20.75 (0.25)
Orange, mango and cinnamon	3.13 (0.04)	15.90 (0.23)
Mandarin and lychee	3.10 (0.07)	19.38 (0.39)
Raspberry, strawberry and loganberry	3.08 (0.04)	17.30 (0.41)
Lemon burst	3.53 (0.04)	18.10 (0.12)
Peach and passion fruit	3.33 (0.04)	10.63 (0.41)
Blackcurrant burst	2.98 (0.05)	33.0 (1.22)
Lift lemon tea	3.95 (0.05)	26.63 (0.82)
Citric acid (0.2%)	2.56 (0.04)	5.38 (0.05)

Table 4 Mean tubule diameter (μm), man tubule area (μm^2) and number of patent tubules visible.

Pink grapefruit	Active	Control
Specimen 1		
Diameter	1.02 (0.46)	None visible
Area	0.54 (0.30)	None visible
Tubule no.	58	0
Specimen 2		
Diameter	0.76 (0.32)	None visible
Area	0.55 (0.29)	None visible
Tubule no.	56	0
Orange, mango and lime	**Active**	**Control**
Specimen 1		
Diameter	0.88 (0.39)	0.67 (0.27) [NS]
Area	0.48 (0.25)	0.37 (0.29) [NS]
Tubule no.	76	2
Specimen 2		
Diameter	0.67 (0.38)	None visible
Area	0.50 (0.38)	None visible
Tubule no.	64	0
Mandarin and lychee	**Active**	**Control**
Specimen 1		
Diameter	0.83 (0.42)	0.38 (0.07) [$p<0.0001$]
Area	0.50 (0.29)	0.18 (0.04) [$p<0.0001$]
Tubule no.	59	3
Specimen 2		
Diameter	0.66 (0.42)	0.59 (0.25) [NS]
Area	0.31 (0.20)	0.26 (0.08) [NS]
Tubule no.	13	7
Peach and passion fruit	**Active**	**Control**
Specimen 1		
Diameter	0.63 (0.36)	None visible
Area	0.59 (0.32)	None visible
Tubule no.	52	0
Specimen 2		
Diameter	0.85 (0.43)	None visible
Area	0.43 (0.26)	None visible
Tubule no.	72	0
Blackcurrant burst	**Active**	**Control**
Specimen 1		
Diameter	1.14 (0.52)	None visible
Area	0.75 (0.45)	None visible
Tubule no.	57	0
Specimen 2		
Diameter	1.18 (0.51)	None visible
Area	0.98 (0.73)	None visible
Tubule no.	46	0
Pear and guava	**Active**	**Control**
Specimen 1		
Diameter	0.62 (0.31)	None visible
Area	0.60 (0.36)	None visible
Tubule no.	92	0
Specimen 2		
Diameter	0.99 (0.49)	None visible
Area	0.66 (0.39)	None visible

(*continued on next page*)

Table 4 (*continued*)

Pear and guava	Active	Control
Tubule no.	112	0
Lift lemon tea	**Active**	**Control**
Specimen 1		
Diameter	0.83 (0.31)	0.32 (0.21) [$p<0.01$]
Area	0.87 (0.46)	0.25 (0.17) [$p<0.001$]
Tubule no.	89	4
Specimen 2		
Diameter	0.61 (0.26)	0.36 (0.11) [$p<0.01$]
Area	0.68 (0.37)	0.35 (0.17) [$p<0.01$]
Tubule no.	24	4
Lemon burst	**Active**	**Control**
Specimen 1		
Diameter	0.77 (0.38)	None visible
Area	0.91 (0.50)	None visible
Tubule no.	68	0
Specimen 2		
Diameter	0.88 (0.45)	None visible
Area	0.88 (0.59)	None visible
Tubule no.	52	0
Raspberry, strawberry, and loganberry	**Active**	**Control**
Specimen 1		
Diameter	0.83 (0.43)	None visible
Area	0.96 (0.60)	None visible
Tubule no.	121	0
Specimen 2		
Diameter	0.91 (0.41)	None visible
Area	1.03 (0.53)	None visible
Tubule no.	113	0
Citric acid (0.2%)	**Active**	**Control**
Specimen 1		
Diameter	0.73 (0.27)	None visible
Area	0.36 (0.17)	None visible
Tubule no.	15	0
Specimen 2		
Diameter	0.64 (0.33)	None visible
Area	0.47 (0.33)	None visible
Tubule no.	25	0

Statistical analysis

For the six control specimens with visible tubules, the tubule diameter and area were compared with the corresponding samples immersed in tea using Student's t test with the level for statistical significance set at $p<0.01$. One of the orange, mango and lime specimens was not significantly different from its test partner and similarly one of the mandarin and lychee specimens was not significantly different from its test partner. Both of the Lift lemon tea specimens were significantly different from their controls.

The results for the mean tubule diameter and area for each of the tea immersed specimens and the citric acid positive control were compared with each other using analysis of variance and Turkey's test with the level for statistical significance set at $p<0.01$. The results of these comparisons are shown in Table 5.

The variables of pH and neutralisable acidity were independently compared with the data for mean tubule diameter, mean tubule area and number of patent tubules using linear regression. This found a weak relationship between neutralisable acidity and tubule diameter ($r^2=0.3$) and

(a)

(b)

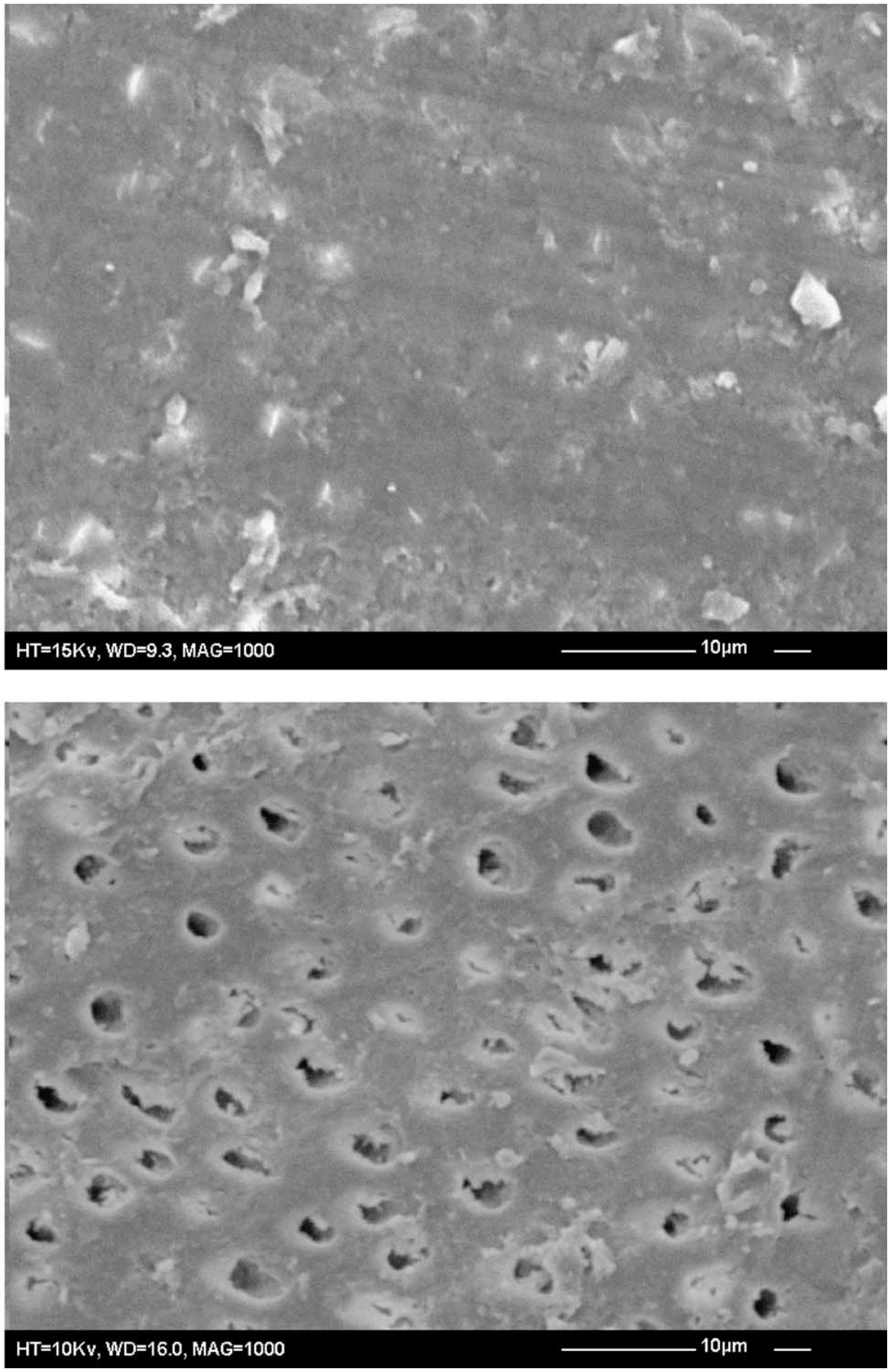

Figure 2 (a) Dentine specimen following immersion in water showing smear layer. Fig. 2(b) Dentine specimen following immersion in blackcurrant tea showing exposed dentinal tubules.

between neutralisable acidity and tubule area ($r^2=0.2$)

Discussion

The pH of the fruit teas tested ranged from 2.98 to 3.95 and the neutralisable acidity ranged from 10.63 to 33.0 ml of 0.1 M sodium hydroxide. These values are similar to the results of Brunton and Hussain[11] who reported a pH value of 3.2 and Phelan and Rees[12] who reported a pH range of 3.1–7.1 and a neutralisable acidity range of 3.5–60.3 ml. As the smear layer is an acid soluble structure measurement of the pH and neutralisable acidity will give some indication of the ability of a drink, such as a fruit tea, to remove it.

The neutralisable acidity value is a measure of the freely available acid in a given volume of drink, so that the higher this value is, the more readily it is able to remove the smear layer. A fruit tea with an acidic pH and a high neutralisable acidity level is therefore likely to remove the smear layer more completely and open up the dentinal tubules more fully, potentially making exposed dentine more sensitive.

Orange juice is known to be erosive and is often consumed in large volumes by patients with DS.[9] It has a pH of approximately 3 and a neutralisable acidity value of around 25 ml of 0.1 M sodium hydroxide.[15] Considering the data in this study, a number of the teas tested had a combination of an acidic pH of 3 or less, together with a high neutralisable acidity of 20 ml or greater. These included the following teas:

- Pear and guava
- Pink grapefruit, mandarin and lime
- Lift lemon tea
- Cranberry and raspberry
- Blackcurrant burst

The 'bag based' fruit teas tested seemed to be based on a similar recipe (Table 2) containing hibiscus, fruit peel, fruit leaves and liquorice root in addition to their stated fruit content that was either in the form of the dried fruit or added fruit flavorings. As many of these fruit products contain a complex mix of organic acids, such as citric, malic or oxalic acid[16] it is not surprising that these products are acidic once brewed. Lift Instant Tea is a dehydrated powder product containing both citric acid and Vitamin C (ascorbic acid), although the actual amounts present in these products was not stated on the label. Since both citric and ascorbic acids are relatively strong organic acids they probably account for the high neutralisable acidity value recorded.

As far as the dentine specimens were concerned, all the fruit teas tested were capable of removing the smear layer and exposing dentinal tubules. The mean diameter of the tubules measured in this study ranged from 0.61 to 1.14 μm. The diameter of a dentinal tubule normally ranges from 3 μm at the pulpal surface reducing to 1 μm at the external surface. In comparison to these values, the data from this study suggests that the tubules were at least partially opened by the teas and possibly fully opened by some of the more aggressive teas with a high neutralisable acidity value. It is also interesting to note that the clinical data of Absi et al.,[7] showed that the mean tubule diameter in patients with sensitive dentine was 0.83 μm, which falls within the middle of the data reported here.

As far as DS is concerned the tubule diameter has a critical effect on the rate of flow of fluid within the tubule and hence the ability to stimulate the pulpal nerve fibres. The flow rate of fluid within a tube is given by Poiseuille's Law[17]

$$\text{Fluid flow rate} = \frac{\pi r^4 \Delta P}{8\eta L}$$

where

r=radius; ΔP=pressure across tubule; η=viscosity; L=length of tube.

The important point is that the rate of flow is related to the radius of the tube by a 4th power relationship. This means that if the diameter of the tubule doubles, the rate of flow will increase by a factor of 16. This means that even small increases in

Table 5 Tubule diameter comparisons (red star = $p<0.001$; black star = $p<0.01$).

	P	PG	O	ML	R	LB	PP	B	L	C
C										-
L									-	
B								-		
PP							-	★ (black)	★ (black)	
LB						-	★ (red)			
R					-		★ (red)			
ML				-	★ (red)	★ (red)		★ (red)	★ (red)	
O			-		★ (red)	★ (red)		★ (red)	★ (red)	
PG		-			★ (red)	★ (red)		★ (red)	★ (red)	
P	-				★ (red)	★ (red)		★ (red)	★ (red)	

P = pink grapefruit; PG = pear and guava; O = orange, mango and cinnamon; ML = mandarin and lychee; R = raspberry, strawberry and loganberry; LB = lemon burst; PP = peach and passion fruit; B = blackcurrant burst; L = Lift lemon tea.

Table 6 Tubule area comparisons (red star = $p < 0.001$).

C										-
L									-	
B								-		
PP							-	*	*	
LB						-	*			
R					-		*			
ML				-	*	*		*	*	
O			-		*	*		*	*	
PG		-			*	*		*	*	
P	-				*	*		*	*	
	P	PG	O	ML	R	LB	PP	B	L	C

P, pink grapefruit; PG, pear and guava; O, orange, mango and cinnamon; ML, mandarin and lychee; R, raspberry, strawberry and loganberry; LB, lemon burst; PP, peach and passion fruit; B, blackcurrant burst; L, Lift lemon tea.

the diameter of an exposed dentinal tubule can potentially lead to dramatic increases in flow rate and clinical sensitivity.

The vales for the tubule area followed a similar pattern to the tubule diameter data, but this was not too surprising as the two are linked. The number of open dentinal tubules counted in the tea groups ranged from 13 to 121. This is again similar to the findings of Absi et al.,[10] who reported a range of open dentinal tubules of 12-101 with beverages such as apple juice and lemonade.

It was suggested earlier that the fruit teas with an acidic pH and a high neutralisable acidity level would result in tubules with a larger diameter and surface area. The tubule diameter and area data was compared using analysis of variance (Tables 5 and 6) and these show that overall the teas such as blackcurrant, Lift and cranberry and raspberry had tubule diameters and areas that were highly significantly different from the other teas. When the effect of Poiseuille's Law is taken into account, then it can be seen that this information would be particularly useful when counselling patients with DS.

In spite of the findings of this study, the results must be interpreted with a certain amount of caution. Firstly, only four dentine specimens were included per test group (two active and two control specimens). However, examining each individual specimen and counting and measuring each tubule was a time consuming process. This study was carried out in vitro rather than in vivo, so the flushing and neutralising effect of the saliva, which could partially neutralise the acidic teas, was not simulated.[18] Furthermore, the acidic teas when introduced into the oral cavity may also stimulate salivary flow thereby producing a greater salivary clearance rate.

In conclusion, within the limitations of this study, it was found that all the fruit teas tested in this study were capable of removing the smear layer and opening dentinal tubules.

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