A comparison of different kinds of European chocolates on human plaque pH

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ABSTRACT

Aim To investigate the acidogenic response of plaque with various European chocolates of varying cocoa contents. Methods 14 subjects participated in the study. On each test day plaque pH measurements were taken at baseline and at 2, 5, 10, 15, 20 and 30 minutes after challenge with the test chocolates or control foods. A plaque sample was removed from the buccal surfaces of posterior teeth representing all quadrants, within 30 seconds. The plaque pH was measured on an ISFET electrode connected to a Sentron 2001 pH system. The test chocolates were: Diet chocolate (DC), Plain European chocolate (PEC 70% cocoa), Plain English chocolate (PenC 34% cocoa), Milk English chocolate (MenC 20% cocoa), Milk European chocolate (MEC 30% cocoa), White chocolate (WC no cocoa), Milk chocolate with hazelnuts (MHC 20% cocoa). 15 g of each chocolate was consumed by the volunteers and 10 ml of 10% sucrose and sorbitol solutions were included as controls. Results The data were analysed for: minimum pH, area below baseline, area below “critical pH”, time spent below “critical pH” and Acidogenic Potential Index (API). DC was found to be significantly different to sucrose and all the other test chocolates, and similar to sorbitol for all the parameters studied. The area below baseline plaque pH was also significantly smaller for PEC (p<0.006) and MHC (p<0.028) as compared with sucrose. The mean area below the “critical pH” (5.7) for PEC and MHC was lower at 0.20±0.34 and 0.60±1.40 respectively, compared with sucrose at 1.38±1.03, but not statistically significant. The API of DC, PEC, PenC, MenC, MEC, WC and MHC was 0.03, 0.41, 0.66, 0.93, 0.94, 0.88 and 0.45 respectively compared with an API of 1.00 of sucrose. Conclusion Diet chocolate was found to have no acidogenic effect on dental plaque. Also, the PEC and MHC had a lower acidogenic potential compared with sucrose.

KEYWORDS: Chocolate, Plaque, pH.

Introduction

The interaction between chocolate and dental caries has been evaluated by using several methodologies available to assess the relationship between diet and dental caries development. The classic Vipeholm study [Gustafsson et al., 1954], the only human intervention study, revealed that the people who were included in the chocolate group developed the lowest number of caries lesions. Stephan [1966] found that milk chocolate was among those foods that induced or increased the number of caries in animals. Bowen et al [1980] used the Konig-Hofer automatic feeding system to find that chocolate had a moderate cariogenic potential in rats. However, Morrisey et al. [1984], with a similar experimental design, found milk chocolate to have low cariogenic potential. Reynolds and Black [1987] reported chocolate to be cariogenic in animals and Grenby and Mistry [1995] identified dark chocolate as more cariogenic than milk chocolate when an electronic system to feed animals was used.

Previously Bibby et al. [1951] had carried out an in vitro study and found chocolates, such as milk chocolate and milk chocolate with almonds, to produce significant amounts of acids. In addition, Linke et al. [1995] noted that milk chocolate produced a considerable amount of acids. Pearce and Hampton [1987], being among the few to describe the tests products with their commercial names, found milk chocolate (Cadbury’s Dairy Milk) to be of intermediate cariogenicity in vitro. Mörch [1961] assessed the effects of carbohydrates, or foods containing carbohydrates, on plaque pH. Milk chocolate was included to the study and gave a considerable effect in pH values within a
short period of time, but the values tended to return to normal more quickly than other tested products. Macpherson and Geddes [1990] supported these results.

Edgar et al. [1975] used the plaque sampling technique to measure the effects of 54 different snack foods on plaque pH. In their research milk and the dark chocolate were ranked among the least acidogenic foods. These latter results have been confirmed by Rugg-Gunn et al. [1975].

The majority of plaque pH studies have compared one type of chocolate with other commonly consumed products and assumptions made about their relative cariogenicity. Frostell [1970] carried out experiments in order to evaluate the effects of different types of chocolates on the pH of dental plaque. This study is among the few where the effects of different chocolates were compared. The criteria to select the tested products were the concentration of sucrose and cocoa substances. Stephan curves were created by using the plaque sampling technique and it was found that the chocolate with a high concentration of cocoa and low sugar concentration gave an increased plaque pH. The cocoa and sucrose poor chocolate gave less pronounced pH decrease than the sucrose rich chocolate.

Although chocolate is among the foods that are considered as cariogenic, this review of the literature has revealed that chocolate’s cariogenicity might be low to moderate. Also, there are only few studies where the different types of chocolate have been studied whereby mainly milk and plain (dark) chocolates have been compared with a number of other foods, mainly snacks, or were used as products with known cariogenic potential in order to compare other foods or to assess methodologies.

There are some other factors that should be considered regarding the cariogenic potential of chocolates. Different regulations regarding chocolate manufacturing, constituents, and definitions exist between various European countries [Beckett, 1999; Bixler and Morgan, 1999]. For instance, UK chocolate follows different regulations than the other countries of the European Union and chocolate constituents are in different proportions. Cocoa and its extracts, which have been reported to exhibit an anti-cariogenic action [Strålfors, 1967; Oosima et al., 1999], is one such constituent used in different proportions and only in some cases have these proportions been studied for any relationship to plaque acidogenicity. Additionally, cocoa levels in chocolate seem to be related to the percentages of the other constituents, such as carbohydrates that might also influence the cariogenic potential. Furthermore, chocolates containing polyols had never been studied.

It was concluded that different cocoa proportions in chocolate confectionery might be a factor influencing the cariogenic response of different type and their acidogenic potential was considered worthy of study. The purpose of this study was to assess the acidogenic response of plaque to chocolates with varying cocoa contents, those containing hazelnuts and diet chocolate.

Materials and methods

Subject selection. Fourteen volunteers were selected according to the criteria laid down by the plaque pH committee of Food, Nutrition and Dental Health of the American Dental Association [Harper et al., 1986]. Consentig subjects were included in the study if they were fit and healthy, aged between 16-50 years, with a DMFS ≥ 12 and with at least 20 teeth. Moreover, a drop in plaque pH below 5.5 on challenge for 1 minute with 10 ml of 10% sucrose solution had to be achieved.

Test products. Seven commercially available chocolates were selected for the study. Plain European and English chocolates were chosen to assess the effect of different cocoa levels. European chocolate has a high cocoa content (70%) as compared with English chocolate (34%). For the same reason a Milk European (30% cocoa) and Milk English (20% cocoa) chocolates were tested. In addition a Milk chocolate containing hazelnuts (20% cocoa) was included to examine any effect of addition of nuts on the plaque pH. A white chocolate, which did not contain any cocoa solids, was selected. Finally, in order to study the effect of chocolate with very low levels of fermentable carbohydrates on plaque pH, a diet chocolate was included in the study.

The compositional details of the test products were as follows (see also Table 1 with some nutritional, cocoa and milk content information):

1 - Swiss reduced fat, plain chocolate, no added sugar, Boots (Diet chocolate);
2 - Lindt, Excellence Dark, 70% cocoa Extra Fine Dark Chocolate, Chocolate Negro (Plain European chocolate);
3 - Cadbury’s, Bournville, The original plain chocolate (Plain English chocolate);
4 - Cadbury’s, Dairy milk, Milk Chocolate (Milk English chocolate);
5 - Lindt, Excellence Milk, Extra Creamy, Extra Fine Milk Chocolate, Chocolate Con Leche (Milk European chocolate);
6 - Nestle, Milkybar, Smooth Creamy-White Chocolate (White chocolate);
7 - Cadbury’s, Milk chocolate with Hazelnuts, whole nut (Milk chocolate with hazelnuts);
8 - 10% sucrose solution as a positive control;
9 - 10% sorbitol solution as a negative control.
The quantity of each chocolate tested was 15 g. A person not related with the study in any way used a portable top-loading digital battery powered electronic balance and weighed the chocolate samples. These were wrapped with aluminum foil in order to cover the chocolate samples and so that all looked identical. This ensured that neither the examiner measuring plaque pH nor any subjects knew which chocolate was being tested. The samples were also coded, therefore, double blind, randomized, Latin Square, cross–over in design was used.

Plaque Sampling (Harvesting) Technique and pH measurements. Plaque pH was measured using the technique of Fosdick et al. [1957], as modified by Frostell [1970] and Rugg-Gunn et al. [1975]. Subjects participating in the study were asked to refrain from tooth brushing at least for 48 hours and from eating or drinking anything apart from water at least 2.5 hours prior to each visit. On each test day pooled plaque samples of approximately 1 mg were removed from six buccal surfaces, of posterior teeth representing all the quadrants of the mouth, using a sterile blunt explorer. The collection time for each sample was standardized to be within 30 seconds. Each plaque sample was thoroughly mixed with 20 ml of distilled water, measured by a pipette into a disposable tray and carried with another clean pipette on an Ion Sensitive Field Transistor with a diameter of 10 mm connected to a Sentron 2001 pH system. The reading was recorded after 30 seconds and thereafter the electrode was cleaned with a steam of distilled water and dried gently with soft tissue. The electrode was calibrated before starting the tests and in between measurements by using two buffering solutions of pH of 4.0 and 7.0.

A plaque sample was taken before the test products were consumed and a baseline plaque pH was recorded. The subjects were then instructed to eat or drink the test and control products and to swish them around all the teeth for 1 minute before swallowing. Plaque samplings were taken 2, 5, 10, 15, 20 and 30 minutes thereafter for the measurement of the plaque pH. The time was kept with a timer (Tudor).

Statistical evaluations. The data were analysed for the minimum pH, area under the curve, area under the curve below the “critical pH” equal to 5.7 and spent below the “critical pH” 5.7. The Acidogenic Potential Index (API) was also calculated for each chocolate. The area under the curve for the sucrose was assigned as being equal to 1 and the relative API of the other foods was calculated as a ratio of this score. Finally, Stephan’s curves were plotted by using the mean pH values achieved by all 14 subjects at predetermined time intervals. The aim of the statistical analysis was to investigate how the above under investigation measurements for each chocolate compared with sucrose and sorbitol and between the chocolates themselves. Therefore, for each chocolate the following relationships were examined:
- difference between the test chocolates,
- difference between test chocolates and the sucrose and sorbitol controls.

For data analysis, multiple comparisons were carried out and because these were between many different substances, Tukey’s HSD test was used in order to correct for the increased chance of a false positive result. The SPSS 9.0 statistical program and Microsoft Excel 97 were used for the analysis of the data.

Results
Following the screening procedure, 14 subjects participated in the study with a mean age of 29.9 years, a DMFS ranging between 13 to 39. The gender ratio, female to male was 9:5.
The Stephan’s curves created by using the mean pH readings achieved in relation to the time are shown in Figure 1. The mean minimum pH achieved from all the tested products and the two controls are summarised in Table 2. It can be seen that use of the Plain European, Diet, and Milk chocolate with hazelnuts did not lead to a drop in the plaque pH below the “critical pH” that was defined as 5.7. Sorbitol and Diet chocolate were statistically significantly different from sucrose (p<0.001). Plain European chocolate and Milk chocolate with hazelnuts were also significantly different as compared with sucrose (p<0.003 and p<0.002 respectively) whereas the rest of the chocolates were not significantly different.

The results for the area below baseline plaque pH are also shown in Table 2. The mean area below baseline pH value was greatest for sucrose and smallest for sorbitol. The mean areas were also small for the Diet chocolate, Plain European chocolate, and Milk chocolate with hazelnuts. Multiple comparisons revealed that there were significant differences between the sucrose and the negative control, Diet chocolate (p<0.001), Plain European chocolate (p<0.006) and the E. VERAKAKI, M.S. DUGGAL

The mean areas below the “critical pH” (5.7) for each of the test products including the two controls are presented in Table 3. It can be seen that the area below 5.7 was the largest for sucrose with all the test chocolates showing a smaller area under pH 5.7 compared with sucrose. Statistical analysis, however, revealed that sorbitol and Diet chocolate differed significantly from sucrose (p<0.031), while none of the other test chocolates differed statistically from sucrose.

The mean time spent below the critical pH is shown in Table 3. It can be seen that the plaque pH stayed below 5.7 the longest with sucrose followed in order by Milk European chocolate, Milk English chocolate, Plain English chocolate, Milk chocolate with hazelnuts, White chocolate and Plain European chocolate. Diet chocolate did not depress plaque pH below 5.7 and therefore was found to be similar to sorbitol. However, results for none of the test chocolates, except Diet chocolate, was significantly different as compared with sucrose.

The Acidogenic Potential Index results are shown in Table 3. The APIs for Milk European chocolate and Milk English chocolate were very close to that of sucrose. The White chocolate and Plain English chocolate had moderate APIs and the rest of the chocolates below 0.5. Diet chocolate had an API very close to that of sorbitol.

**Discussion**

Chocolate has been associated with dental caries by various researchers both positively and negatively, although its role is uncertain. Rugg-Gunn et al. [1984] found in their study that each child consumed a mean of Milk chocolate with hazelnuts (p<0.028). However, no differences were observed between sucrose and the rest of the chocolates. Moreover, Diet chocolate did not differ significantly from sorbitol.

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35 g of chocolate/day with mean frequency of intake 0.9/day. They found that those children who ate more sweets and chocolates had more caries, but this difference was not statistically significant, and concluded that sweet (candy) consumption was not as strong a factor for the occurrence of caries as others, e.g. oral hygiene and fluoride [Sundin et al., 1983]. In this study adults were used as subjects while the results are reported for their applicability to children. Previously Tahmassebi and Duggal [1996] have compared plaque pH findings made on both adults and children when using the same test materials. They found that the response in children was less than in adults. Thus any results of plaque pH response in adults will be greater than in children. Suitable adult subjects are considerably easier to find than children. In this study the findings on adults are, therefore, directly applicable to children.

Data from plaque pH studies have been presented in many forms, such as using minimum pH, time and areas under given pH values. Clearly, a substrate that does not cause any drop in plaque pH will probably have no detrimental effects on teeth. The amount of time that pH remains depressed is important and time spent under different pH values is probably indicative of a food’s retentiveness and may have an effect on its cariogenicity. However, the chemistry of the tooth surface should be taken into account, as there are variations among the tooth surfaces and food retention or salivary access [Harper et al., 1986]. The pH value at which enamel begins to dissolve (“critical pH”) is not known, although widely assumed. According to Edgar and Geddes [1986], this probably varies among individuals and among oral sites in any one individual and Harper et al. [1986] mentioned that the dissolution of enamel is a result of what happens in the plaque, pellicle and enamel. Edmondson [1990] suggested that the varying and critical level of saturation with calcium and phosphate in plaque and saliva was important, while Larsen and Pearce [1997] added that due to these variations the “critical pH” of the plaque varies correspondingly.

Different estimates of “critical pH” have ranged from 5.7 to 5.5 or lower [Margolis and Moreno, 1992]. Prolonged pH drop is considered to be more harmful than that of short duration. In addition, the depth of the pH drop within a certain period of time is proportional to the potential dissolution of enamel. This proportionality, however, does not accord with the concept, as enamel apatite solubility is not proportional to pH. Moreover, the function of concentration of H+ is related closely to solubility of apatite in a sense that both solubility and concentration of H+ increase logarithmically with decreasing pH.

In this study it was found that Plain English, Milk English, Milk European, and White chocolates depressed the pH below pH 5.7 (not significantly different to sucrose). The rest of the chocolates (Diet, Plain European, Milk with hazelnuts) were found to give results statistically different to sucrose. There are only very few

<table>
<thead>
<tr>
<th>Tested products</th>
<th>Mean area below “critical pH” (±SD)</th>
<th>Mean time below pH 5.7 (min, ±SD)</th>
<th>API</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose</td>
<td>1.38 (1.03)</td>
<td>6.91 (3.29)</td>
<td>1.00</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>0.00* (0.34)</td>
<td>0.00* (2.79)</td>
<td>0.00</td>
</tr>
<tr>
<td>Diet chocolate</td>
<td>0.00* (0.20)</td>
<td>0.00* (2.15)</td>
<td>0.036</td>
</tr>
<tr>
<td>Plain European chocolate</td>
<td>0.88 (1.23)</td>
<td>3.98 (4.13)</td>
<td>0.66</td>
</tr>
<tr>
<td>Plain English chocolate</td>
<td>0.90 (1.32)</td>
<td>4.41 (5.15)</td>
<td>0.93</td>
</tr>
<tr>
<td>Milk English chocolate</td>
<td>1.12 (1.78)</td>
<td>4.83 (5.29)</td>
<td>0.94</td>
</tr>
<tr>
<td>Milk European chocolate</td>
<td>0.46 (1.12)</td>
<td>2.86 (3.55)</td>
<td>0.88</td>
</tr>
<tr>
<td>White chocolate</td>
<td>0.60 (1.40)</td>
<td>3.23 (6.71)</td>
<td>0.45</td>
</tr>
<tr>
<td>Milk chocolate with hazelnuts</td>
<td>0.46 (1.12)</td>
<td>3.23 (6.71)</td>
<td>0.45</td>
</tr>
</tbody>
</table>

*significantly different to sucrose

**TABLE 3 - Mean area below “critical pH” (5.7), mean time spent below “critical pH” (minutes), and API for all the test chocolates and controls (±SD).**
studies where comparisons between different types of chocolate have been carried out selected according to the cocoa level [Strålfors, 1966a; 1966b], therefore our results cannot be compared to that obtained from other studies. Edgar et al. [1975] reported only the presence of milk and semi-sweet flavours as chocolate constituents of interest. The cocoa and carbohydrate levels were not given. They found that milk chocolate and dark chocolate gave a minimum pH of 6.06 and 6.10 respectively, values that are higher than that obtained from our study. Bibby and Mundorff [1975] reported minimum pH values of 5.85 and 5.65 respectively for dark and milk chocolate (no constituent proportions reported) that are similar to those for Plain European chocolate and both Milk chocolates reported here. On the other hand, Jensen and Schachtele [1983a], using in-dwelling electrode telemetry, reported a minimum plaque pH of 4.54 with milk chocolate. Similar results were presented for milk chocolate of a minimum pH 4.6, recorded with touch electrode, from Harper et al. [1985a], while in another study done by the same authors [1985b] minimum plaque pH given from milk chocolate was 5.2 when measured with indwelling electrode and 6.0 with the touch electrode. Koparal et al. [1998] reported a minimum pH value of 5.50 when milk chocolate was tested in children with a touch electrode. However, there are differences in the research designs for these studies and the ways in which the pH measurements were carried out.

Furthermore, not all the studies reported the chocolate constituent proportions. For instance, differences in the values obtained by different plaque pH monitoring methods [Jensen and Schachtele, 1983b] or differences in plaque reactions following cariogenic challenge in children and adults [Tahmassebi, 1993; Koparal et al., 1998] might influence the results. It was clear from our study that the minimum pH value obtained with all the chocolates was higher than that with 10% sucrose solution. As far as the mean area below baseline is concerned, only Diet, Plain European and Milk chocolate with hazelnuts were significantly different from sucrose.

For the mean area below pH 5.7, only Diet chocolate was found to be statistically significant different to sucrose. It is not surprising that Diet chocolate and Milk chocolate with hazelnuts gave higher pH readings and therefore a smaller area under the curve. It is interesting to note that the Plain European chocolate also produced a smaller area under the curve as compared with sucrose. This might be attributed to a higher cocoa content in this chocolate. European chocolates have a higher cocoa content than the English chocolates and therefore this might have influenced the plaque pH. Previous studies had shown an anticariogenic action of cocoa [Strålfors, 1967].

For the time spent below critical pH it was found that only Diet chocolate was significantly different from sucrose. Mörch [1961] reported that the time at minimum pH spent with milk chocolate was short as the plaque pH values returned to normal very quickly. Because the time spent below pH values, and especially the “critical pH”, could characterise food retention, it should be more widely tested or used as an index of cariogenicity. Linke et al. [1991] found that the amount of acids produced from milk chocolate could hardly be detected two hours after consumption, while Kashket et al. [1991] reported that milk chocolate was not detectable at 2 minutes after consumption.

Bibby and Mundorff [1975] found milk chocolate to have a moderate retentiveness, and food retention is also dependant on the amount that is consumed. However, milk chocolate has shown high retention, independent of the amount, followed by rapid rates of clearance [Kashket et al., 1991] as a high sugar content results in rapid clearance due to stimulation of salivary [Bibby and Mundorff, 1975].

It was found that, with sucrose having an API of 1.00, the Milk English chocolate and Milk European chocolate had high scores, White chocolate and Plain English chocolate moderate scores. The rest of the chocolates had APIs equal or lower than 0.4. Although API has been previously calculated for a number of foods [Gardiner et al., 1997; Tahmassebi, 1993] it has not been estimated for chocolates. Nevertheless, in animal studies a CPI (Cariogenic Potential Index) was reported to be 0.72. Mundorff et al., [1990] ranked USA milk chocolate to have CPI equal to 0.8. In our study, the API of Plain English chocolate was 40% less than that of sucrose, while Plain European and Milk chocolate with hazelnuts had an API of less than half that of sucrose.

Diet chocolate was found to be significantly different to sucrose in all the parameters tested. The levels of cocoa were not available, however Diet chocolate contained aspartame and acesulfame K instead of sucrose, generally found to be anticariogenic [Das et al., 1997], to prevent the fall in plaque pH [Mishiro and Kaneko, 1977] and the adherence of plaque formed by Mutans Streptococcus [Olson, 1977]. Acesulfame K has also been found to have anticariogenic action [Ziesenitz and Siebert, 1986]. Moreover, Edgar and Dodds [1985] stated that the most probably beneficial action of these sweeteners is the stimulation of salivary flow and thus raising the plaque pH. Krasse [1985] reported from the literature that chocolate with sweeteners belonged to the group with low cariogenic potential.

Studies had shown the low cariogenicity of nuts [Mundorff et al., 1990] while others reported that if nuts were given after sugar, it could raise the plaque pH effectively [Geddes et al., 1977]. One of the chocolates
used in this study was Milk chocolate with hazelnuts (Milk English chocolate with added nuts). According to this study, Milk English chocolate was not significantly different to sucrose, however when nuts were added the effects on plaque pH could be attributed to the presence of the nuts in the chocolate.

White chocolate is made using all the conventional ingredients of chocolate except for cocoa solids. It contains whole milk powder that has a number of constituents that exhibit anticiariogenic action such as casein [Reynolds and Black, 1987], whey protein in bovine milk [Reynolds and Del Rio, 1984], calcium and phosphorus [Rugg-Gunn, 1993]. The chocolate that we tested contained cocoa butter and vegetable fats (31.3 g/100 g) and carbohydrates (57.5 g/100 g). The presence of fat in a diet seems to accelerate clearance [Brudevold et al., 1990] and therefore reduces the acidogenic potential of foods. Nevertheless, Brudevold et al. [1990] emphasised that the relationship between fat content and oral clearance rate (and plaque pH values consequently) depended on the type of fat and other procedures, for example cooking procedures.

As this study failed to show any differences between Milk English and Milk European chocolate it can be concluded that the difference in cocoa solids between the two chocolates was not of a significant level to influence their acidogenic potential. Moreover, it can be assumed that the concentration of sugar into Milk European chocolate does not differ significantly to that in the Milk English chocolate. If that is the case, our results are in agreement with the study by Frostell [1970] who found that cocoa rich and sucrose rich chocolate did not differ from cocoa poor and sucrose rich chocolate. The same applied to Plain English chocolate. It contains higher cocoa solids (34% minimum) than the milk chocolates, but lower than the Plain European chocolate (70% minimum). Also, it has a high carbohydrate concentration (59.6 g/100 g of chocolate) and no milk solids; therefore it was found to have a moderate acidogenic potential (API: 0.66) when compared with the other test chocolates. Plain European chocolate was found to be moderately acidogenic (API: 0.41), probably due to high concentration of cocoa solid, although the area below pH 5.7 and the time spent below this level was less than sucrose. However, the API was less than half that of sucrose.

**Conclusion**

Some chocolates, particularly the Plain European chocolate (high cocoa content) and the Milk chocolate with hazelnuts had a lower acidogenic potential than sucrose. However, it should be remembered that they should not be considered as entirely “safe for teeth” and frequent consumption by children of any food containing fermentable carbohydrates should be avoided. Diet chocolate was found to have a low API and was significantly different to sucrose, similar to sorbitol in its effect on plaque pH. Milk European chocolate, Milk English chocolate and White chocolate were found to be similar to sucrose and with high API. Moderate to low APIs were found for Plain English chocolate and Plain European chocolate. The area below and the time spent below ‘critical pH’ of 5.7 were not significantly different to sucrose. Milk chocolate with hazelnuts gave significantly lower area under the ‘critical pH’ and spent significantly less time below ‘critical pH’ as compared with sucrose with moderate API of less than half of that of sucrose.

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