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# EFFECTS OF MILK, FRUIT JUICES AND SWEETENED BEVERAGES ON THE pH OF DENTAL PLAQUES

*by*
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## INTRODUCTION

Animal and *in vitro* experiments support the opinion that milk and acid fruit juices are not very cariogenic, whereas artificial beverages to which sugar has been added may be strongly cariogenic (*Weiss and Bibby*, 1966; *Jenkins and Ferguson*, 1966).

However, clinical observations indicate that frequent consumption of fruit and fruit juices may be associated not only with dental erosion but also with active dental caries in man.

Since the available information on the cariogenicity of milk, sour fruit juices and sweetened beverages rely mainly on animal experiments and since the primary acid attack on the tooth surface is considered to be an important cariogenic factor, it was considered to be of interest to study the effects of milk, sour milk, sour fruit juices and some sweetened beverages on plaque pH in man.

## MATERIAL AND METHODS

Eighteen subjects at the age of 15—59 years participated. They had all a moderate or strong tendency to dental plaque accumulation and a moderate

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to high caries activity. Before the experiment the subject had to refrain from toothbrushing for 2 days and appeared in the laboratory in the morning without having eaten or drunk anything except tap water. Dental plaque material was collected with a blunt instrument from about 20 spots on or between the teeth. The material was immediately transferred to a one drop pH electrode and one drop of distilled water was added and the pH was determined electrometrically with a Beckman Zeromatic pH-meter.

The patient then rinsed his mouth for 30 seconds with a 25 per cent sucrose solution (10 ml) or a juice or a beverage. The pH of the dental plaques was then measured again as described after 2, 5, 10, 20 and 30 minutes (*Frostell*, 1969).

Control experiments showed that the standard deviation of the instrument at pH 6.00 (when calibrated against pH 6.00 buffer) was $\pm$ 0.025 pH units. At pH 5.00 (when calibrated against buffer pH 6.00) the result was 5.01 $\pm$ 0.054. These values were calculated on figures obtained at control experiments performed during every experiment with every subject in the investigation.

In the statistical analysis of the results, the pH-values or the differences between the pH-values have been used instead of the corresponding antilogarithm figures since it was found that they were distributed according to the normal curve and that they were more representative in this respect than the antilogarithms. The mean pH-values given in the tables and the diagrams are thus the arithmetric means of the actual pH-values or the differences between them.

The level of significance between the means for a certain time interval has been calculated with Student's t-test with the aid of an Olivetti Programma computer. A difference between the means of two curves at a certain time interval exceeding 0.3 pH-units was usually statistically significant at the 1 per cent level whereas a difference over 0.5 pH-units usually was highly significant at the 0.1 per cent level. The standard deviation of the method was determined at 0.13—0.26 pH-unit depending on the different time intervals.

## RESULTS

*Milk.* Standardized Swedish cow's milk (fat content 3 per cent) was used. The result is given in Fig. 1 and Table I. Usually only small changes in plaque pH occurred.

*Sour milk.* Standardized Swedish sour milk was used, pH slightly over 4.0. The result is given in Fig. 1 and Table I. A moderate initial pH-decrease usually occurred which was soon followed by an increase.

Table I.

*Comparison between the results of the different experimental series*

| | 2 | 5 | 10 | 20 | 30 min. |
|---|---|---|---|---|---|
| Milk | 0.13 | 0.18 | 0.37 | 0.25 | 0.08 |
| Sour milk | 0.60 | 0.65 | 0.45 | 0 26 | 0 08 |
| Difference | 0 47 | 0.47 | 0.08 | 0.01 | 0.00 |
| t-value | 4.34 | 4.36 | 0.98 | 0.11 | —0.22 |
| Level of significance | *** | *** | — | — | — |
| Milk | 0.14 | 0.20 | 0.37 | 0.28 | 0.10 |
| Apple juice | 1.42 | 1.31 | 0.91 | 0.54 | 0.28 |
| Difference | 1.28 | 1.11 | 0.54 | 0.26 | 0.18 |
| t-value | 10.73 | 10.41 | 4.70 | 2.47 | 1.83 |
| Level of significance | *** | *** | *** | * | — |
| Milk | 0.14 | 0.20 | 0.37 | 0.28 | 0.10 |
| Lemon juice | 1.79 | 1.04 | 0.49 | 0.05 | +0.14 |
| Difference | 1.65 | 0.84 | 0.12 | 0.23 | 0.24 |
| t-value | 9.86 | 4.19 | 0.90 | 2.73 | 3.27 |
| Level of significance | *** | *** | — | * | ** |
| Milk | 0.14 | 0.20 | 0.37 | 0.28 | 0.10 |
| Orange juice | 1.18 | 1.15 | 0.72 | 0.33 | 0.01 |
| Difference | 1.04 | 0.95 | 0.35 | 0.05 | 0.09 |
| t-value | 6.99 | 7.23 | 2.47 | 0.51 | 1.37 |
| Level of significance | *** | *** | * | — | — |
| Milk | 0.13 | 0.18 | 0.37 | 0.25 | 0.08 |
| Coca-Cola | 1.02 | 1.22 | 1.05 | 0.68 | 0.38 |
| Difference | 0.89 | 1.04 | 0.68 | 0.43 | 0.30 |
| t-value | 8.16 | 9.53 | 9.53 | 4.82 | 3.80 |
| Level of significance | *** | *** | *** | *** | ** |
| Milk | 0.14 | 0.20 | 0.37 | 0.28 | 0.10 |
| Limonade (»Sockerdricka») | 1.11 | 1.32 | 1.13 | 0.81 | 0.54 |
| Difference | 0.97 | 1.12 | 0.76 | 0.53 | 0.44 |
| t-value | 9.95 | 11.70 | 6.88 | 4.66 | 5.95 |
| Level of significance | *** | *** | *** | *** | *** |

| | 2 | 5 | 10 | 20 | 30 min. |
|---|---|---|---|---|---|
| Lemon juice | 1.79 | 1.04 | 0.49 | 0.05 | +0.14 |
| Orange juice | 1.18 | 1.15 | 0.72 | 0.33 | 0.01 |
| Difference | 0.61 | 0.11 | 0.23 | 0.28 | 0.15 |
| t-value | 4.55 | 0.84 | 2.40 | 2.75 | 1.82 |
| Level of significance | *** | — | * | * | — |
| | | | | | |
| 25 % sucrose solution | 0.95 | 1.35 | 1.33 | 0.98 | 0.61 |
| Lemon juice | 1.79 | 1.04 | 0.49 | 0.05 | +0.14 |
| Difference | 0.84 | 0.31 | 0.84 | 0.93 | 0.75 |
| t-value | 4.79 | 1.70 | 7.61 | 9.34 | 6.30 |
| Level of significance | *** | — | *** | *** | *** |
| | | | | | |
| 25 % sucrose solution | 0.95 | 1.35 | 1.33 | 0.98 | 0.61 |
| Orange juice | 1.18 | 1.15 | 0.72 | 0.33 | 0.01 |
| Difference | 0.23 | 0.20 | 0.61 | 0.65 | 0.60 |
| t-value | 1.68 | 1.64 | 4.87 | 11.68 | 7.61 |
| Level of significance | — | — | *** | *** | *** |
| | | | | | |
| 25 % sucrose solution | 0.95 | 1.35 | 1.33 | 0.98 | 0.61 |
| Apple juice | 1.42 | 1.31 | 0.91 | 0.54 | 0.28 |
| Difference | 0.47 | 0.04 | 0.42 | 0.44 | 0.33 |
| t-value | 4.51 | 0.41 | 4.61 | 4.53 | 2.55 |
| Level of significance | *** | — | *** | *** | * |
| | | | | | |
| 25 % sucrose solution | 0.95 | 1.35 | 1.33 | 0.98 | 0.61 |
| Limonade (»Sockerdricka») | 11.1 | 1.32 | 1.13 | 0.81 | 0.54 |
| Difference | 0.16 | 0.03 | 0.20 | 0.17 | 0.07 |
| t-value | 1.58 | 0.30 | 1.92 | 1.47 | 0.51 |
| Level of significance | — | — | — | — | — |
| | | | | | |
| Limonade (Sockerdricka») *) | 1.11 | 1.34 | 1.15 | 0.83 | 0.55 |
| Coca-Cola *) | 1.05 | 1.25 | 1.06 | 0.68 | 0.38 |
| Difference | 0.06 | 0.09 | 0.09 | 0.15 | 0.17 |
| t-value | 0.50 | 0.67 | 0.65 | 1.04 | 1.44 |
| Level of significance | — | — | — | — | — |

*) 17 subjects

| | 2 | 5 | 10 | 20 | 30 min. |
|---|---|---|---|---|---|
| Limonade (»Sockerdricka») | 11.1 | 1.32 | 1.13 | 0.81 | 0.54 |
| Lemon juice | 1.79 | 1.04 | 0.49 | 0.05 | +0.14 |
| Difference | 0.68 | 0.28 | 0.64 | 0.76 | 0.68 |
| t-value | 3.99 | 1.83 | 6.68 | 8.01 | 7.34 |
| Level of significance | *** | — | *** | *** | *** |
| | | | | | |
| Limonade (»Sockerdricka») | 1.11 | 1.32 | 1.13 | 0.81 | 0.54 |
| Orange juice | 1.18 | 1.15 | 0.72 | 0.33 | 0.01 |
| Difference | 0.07 | 0.17 | 0.41 | 0.48 | 0.53 |
| t-value | 0.49 | 1.41 | 3.00 | 4.13 | 5.98 |
| Level of significance | — | — | ** | *** | *** |
| | | | | | |
| Limonade (»Sockerdricka») | 1.11 | 1.32 | 1.13 | 0.81 | 0.54 |
| Apple juice | 1.42 | 1.31 | 0.91 | 0.54 | 0.28 |
| Difference | 0.31 | 0.01 | 0.22 | 0.27 | 0.26 |
| t-value | 2.61 | 0.17 | 2.48 | 2.95 | 2.76 |
| Level of significance | * | — | * | ** | * |

In Fig. 1 and Table I, a comparison is made between milk and sour milk. After 2 minutes and 5 minutes the pH was significantly lower after intake of sour milk than after intake of milk. At 10, 20 and 30 minutes there was no significant difference between the pH-changes caused by the two substances.

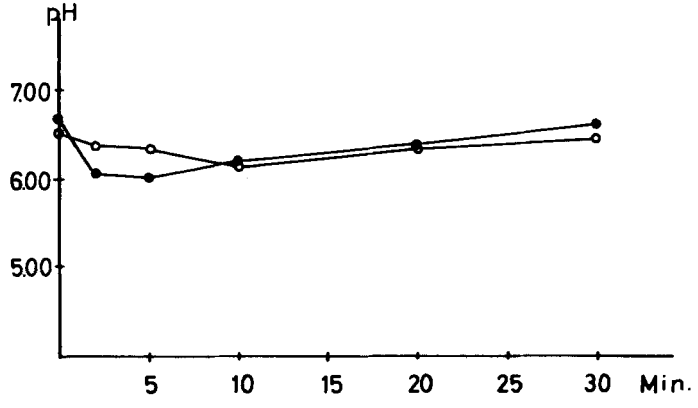


Fig. 1. Changes in pH of superficial dental plaques after a mouth rinse with milk (O———O) and sour milk (●———●). Means of 18 subjects.

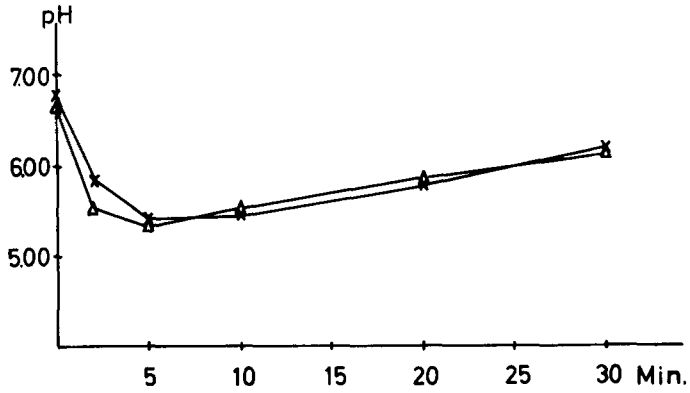


Fig. 2. Changes in pH of superficial dental plaques after a mouth rinse with a 25 per cent sucrose solution (×———×) and limonade (△———△). Means of 18 subjects.

*Limonade* (»*Sockerdricka*»). This beverage, which is commonly appreciated by children in Sweden, is carbonated and contains 10—15 per cent sugar.

The result is given in Fig. 2 and Table I. For comparison the result of an experiment with a 25 per cent sucrose solution is given, which was undertaken for another investigation. There was no significant difference between the results with these two solutions.

On comparison between milk and the limonade it was found that the pH of the dental plaques was significantly lower at all time intervals when the limonade was used than when milk was tested. Fig. 3 and Table I.

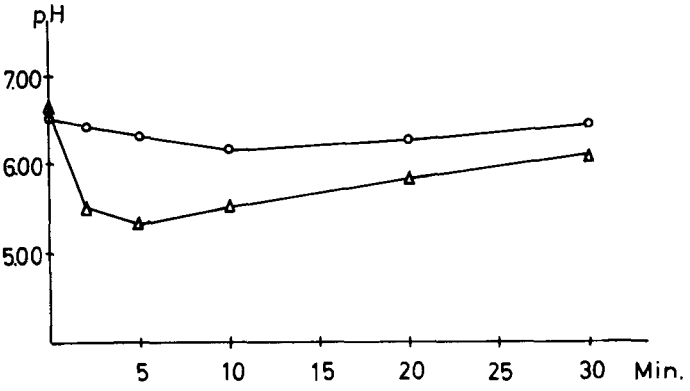


Fig. 3. Changes in pH of superficial dental plaques after a mouth rinse with milk (O———O) and limonade (△———△). Means of 18 subjects.

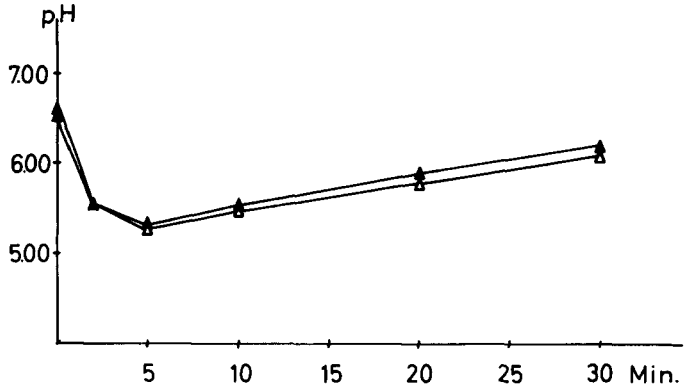


Fig. 4. Changes in pH of superficial dental plaques after a mouth rinse with limonade (△———△) and Coca-Cola (▲———▲). Means of 17 subjects.

*Coca-Cola.* The commercial product was bought on the market. The pH was 2.6—2.8. The result is given in Fig. 4 and Table I. The result was very close to those obtained with limonade and with the sucrose solution.

*Orange juice.* The orange juice was prepared from fresh oranges grown in Spain. The pH was about 3.50. The results are given in Fig. 5 and 6 and Table I. The largest pH-decrease was found at two minutes. This decrease, however, was not significantly greater than that obtained with a 25 per cent sucrose solution at 2 and 5 minutes. At 10, 20 and 30 minutes the mean plaque pH had risen again and there was a significantly less pH-decrease at these time intervals than with the sucrose solution and the limonade. On

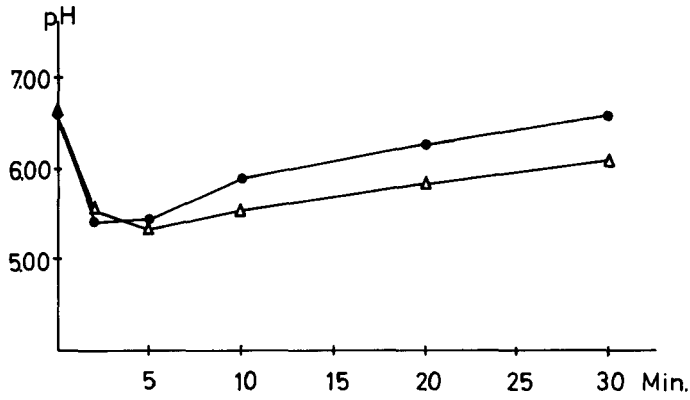


Fig. 5. Changes in pH of superficial dental plaques after a mouth rinse with orange juice (●———●) and limonade (△———△). Means of 18 subjects.

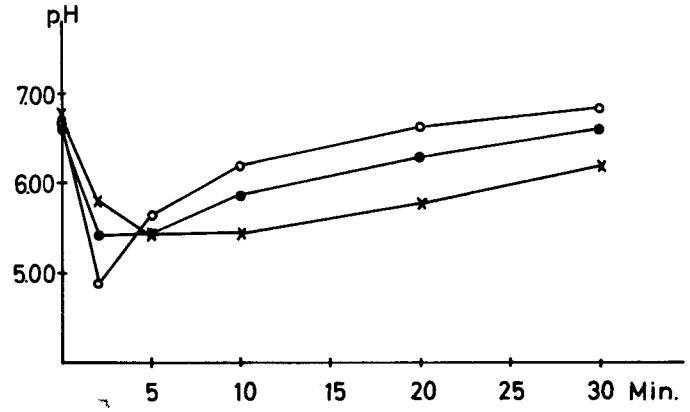


Fig. 6. Changes in pH of superficial dental plaques after a mouth rinse with lemon juice (O———O), orange juice (●———●) and a 25 per cent sucrose solution (×———×). Means of 18 subjects.

comparison with milk the pH-decreases were significantly greater at 2, 5 and 10 minutes but not at 20 and 30 minutes.

*Lemon juice.* This juice was prepared from yellow lemons. Its pH was about 3.5. The results are given in Fig. 6 and Table 1. The mean pH-decrease found in the plaques at 2 minutes exceeded 1.5 pH-units and was significantly greater than with the orange juice or the 25 per cent sucrose solution. However, the pH quickly rose again and at 10, 20 and 30 minutes the lemon juice had given a significantly less pH-decrease than the orange juice and the solution. Fig. 7 and Table 1.

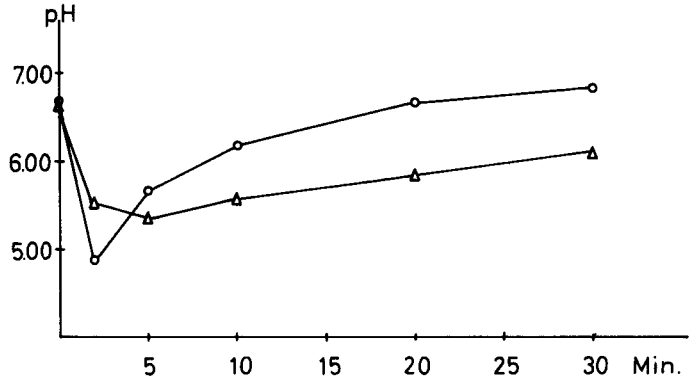


Fig. 7. Changes in pH of superficial dental plaques after a mouth rinse with lemon juice (O———O) and limonade (△———△). Means of 18 subjects.

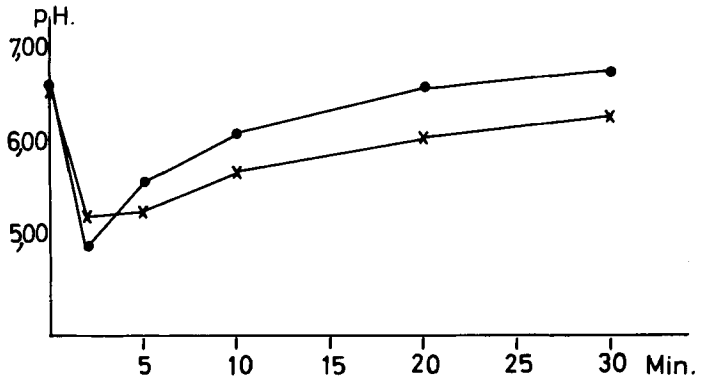


Fig. 8. Changes in pH of superficial dental plaques after a mouth rinse with lemon juice (●———●) and apple juice (×———×). Means of 18 subjects.

*Apple juice.* A commercial apple juice without any additives was used, the pH of which was 3.50. The results are given in Fig. 8 and 9 and Table 1. The resulting curve was very similar to that obtained with the orange juice but the mean pH-decreases were slightly greater with the apple juice at all time intervals than with the orange juice.

Apple juice, lemon juice and orange juice all caused a greater pH-decrease at 2 and 5 minutes than milk. Apple juice and lemon juice but not orange juice did cause a significantly greater pH-decrease at 20 and 30 minutes than milk.

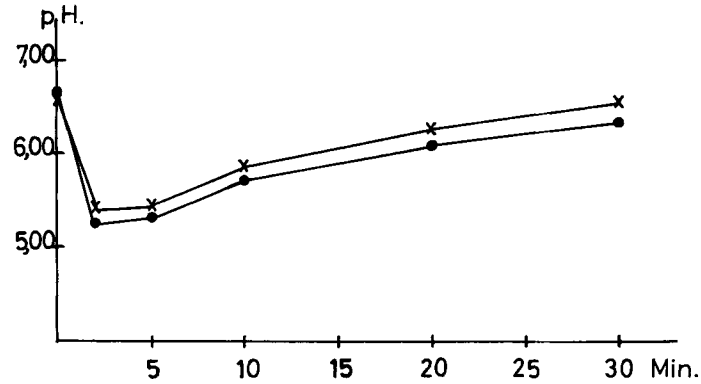


Fig. 9. Changes in pH of superficial dental plaques after a mouth rinse with orange juice (×———×) and apple juice (●———●). Means of 18 subjects.

## DISCUSSION

The method used has been discussed in some detail in previous publications (*Frostell,* 1965, 1970). It is considered that with this method the pH-changes of buccal and superficial proximal plaques may be determined but not the pH of plaques in fissures or at the proximal contacts of the teeth. (*Mühlemann,* 1969; *Graf,* 1969).

The results with milk and sour milk show that usually only slight pH-changes occur in superficial plaques in contact with these substances, in good agreement with the opinion that milk and sour milk are only slightly or not at all cariogenic.

The results with limonade and Coca-Cola show that these beverages give rise to a primary acid attack comparable to that of a 25 per cent sucrose solution. It must be kept in mind, however, that Coca-Cola contains phosphoric acid, the effect of which may be quite different from that of lactic or citric acids. The results are in good agreement with animal experiments showing that beverages of this type are rather cariogenic.

Possibly, it would have been more accurate to use a 10 or 15 per cent sucrose solution instead of a 25 per cent solution. The limonade contains 15 per cent sucrose, however, and was considered to represent a sucrose solution of that concentration.

The results with orange juice, apple juice and lemon juice show that they all give rise to an initial pH-decrease comparable to or exceeding that caused by a 25 per cent sucrose rinse, or of limonade (»sockerdricka»).

However, after 20 minutes the pH-differences caused by the juices were significantly smaller than those caused by a 25 per cent sucrose solution or by limonade. The reason for this may be that the acid juices are more rapidly washed away by an increased salivary flow or that the saliva secreted after consumption of acid solutions is more alkaline and has a greater buffering capacity than after consumption of a neutral sucrose solution or limonade. It may also be due to a combination of these factors and others. It is interesting to see that the very acid lemon juice gave — as expected — a very rapid and intense acidification of the plaques, but after 5 minutes the mean pH was higher than after the orange juice and remained significantly more alkaline for the rest of the experimental period.

Coca-Cola containing phosphoric acid gave a pH-curve resembling that previously obtained with a sugar-phosphoric buffer solution (pH 3.5) whereas the orange- and lemon juices gave curves resembling that obtained with a sugar-citric acid solution (*Frostell,* 1970). The difference between the two kinds of curves may to some extent be referred to the difference in buffering action between phosphoric and citric acids in this pH regions.

These results confirm the opinion that milk is very slightly if at all cariogenic, that natural acid fruit juices give an acid attack of short duration followed by a reactive period when the pH of the plaques is brought back to »resting value» more rapidly than after consumption of pure sucrose solutions and that artificial beverages are cariogenic owing to their sugar content and not to their content of acids. However, the aid attack in the plaque after rinsing with natural juices was significantly more intense and of longer duration than after milk.

The cariogenicity of these products cannot be evaluated by measurements of pH-changes in superficial plaques only. Further experiments on animals and/or humans are required or may be at least partly substituted by intraoral measurements of interdental pH, calcium ion concentration etc. Such investigations are in progress.

SUMMARY

The pH-changes of the buccal and superficial proximal plaques of groups of 18 persons were followed by an *in vitro*-method for 30 minutes after rinsing the mouth with milk, sour milk, limonade (»sockerdricka»), a 25 per cent sucrose solution or orange-, lemon- or apple juices.

Milk gave only slight pH-decreases during the experimental period. Sour milk gave significantly greater pH-decreases after 2 minutes and 5 minutes, but after 10, 20 and 30 minutes the mean pH-decreases were slightly smaller than after milk.

The sucrose solution and limonade gave the usual Stephan-curve and were very similar to each other.

Coca-Cola gave pH-changes which were similar to those obtained after the sucrose solution and after the limonade.

Orange juice, apple juice and lemon juice all gave an initial intense acid attack of short duration followed by a period when the pH tended to go back to resting values more rapidly than after a sucrose solution or limonade. These differences were highly significant for the lemon-, apple- and orange juices.

The results confirm the opinion that milk and sour milk are very slightly acidogenic in the plaques, that beverages containing sugar give a pH-curve very similar to that of sucrose solutions and that natural fruit juices give an acid attack of short duration followed by a period when the pH of the plaques return back to resting values more rapidly than after consumption of sucrose solutions of corresponding strength. However, the natural juices

gave pH-decreases which were significantly greater than those after milk during the whole experimental period.

The cariogenicity of these solutions, however, cannot be determined entirely by the pH-changes caused in superficial plaques. Animal and human experiments are required or may possibly be substituted by interdental measurements of pH, concentration of calcium and other divalent ions etc.


## RÉSUMÉ

### ACTION DU LAIT, DES JUS DE FRUITS ET DES BOISSONS SUCRÉES SUR LE pH DES PLAQUES MICROBIENNES SUR LES DENTS

Les modifications du pH des plaques microbiennes vestibulaires et des plaques proximales superficielles provenant de groupes de 18 personnes sont été observées par une méthode *in vitro* pendant 30 minutes après rinçage de la bouche avec du lait, avec du lait aigre, avec de la limonade (»sockerdricka»), avec une solution de saccharose à 25 %, ou avec des jus d'orange, de citron ou de pomme.

Le lait ne provoquait qu'un faible abaissement du pH pendant la période expérimentale. Le lait aigre donnait au bout de 2 minutes et au bout de 5 minutes des abaissements du pH significativement plus importants, mais au bout de 10, de 20, et de 30 minutes, les abaissements moyens du pH étaient légèrement plus petits qu'après le lait.

La solution de saccharose et la limonade donnaient la courbe de Stephan habituelle, et se ressemblaient beaucoup.

La Coca-Cola donnait des modifications du pH semblables à celles qu'on obtenait après la solution de saccharose et après la limonade.

Le jus d'orange, le jus de pomme et le jus de citron donnaient tous une attaque acide initiale intense et de courte durée, suivie d'une période où le pH tendait à revenir aux valeurs au repos plus rapidement qu'après une solution au saccharose ou après la limonade. Ces différences étaient hautement significatives pour les jus de citron, de pomme et d'orange.

Les résultats de cette étude confirment l'opinion selon laquelle le lait et le lait aigre sont très légèrement acidogènes dans les plaques, selon laquelle les boissons contenant du sucre donnent une courbe du pH très semblable à celle des solutions de saccharose et selon laquelle les jus de fruits naturels donnent une attaque acide de courte durée suivie d'une période où le pH dans la plaque microbienne revient aux valeurs au repos plus rapidement qu'après consommation de solutions de saccharose de force correspondante. Cependant, les jus naturels donnaient des abaissements de pH significative-

ment plus importants que ceux obtenus après consommation de lait pendant toute la période expérimentale.

Le pouvoir cariogène de ces solutions, cependant, ne peut être entièrement déterminé par les modifications du pH qu'elles provoquent dans les plaques microbiennes superficielles. Des expériences sur l'homme et sur l'animal sont nécessaires, ou peuvent éventuellement être remplacées par des mesures interdentaires du pH, de la concentration du calcium et des autres ions divalents, etc.


## ZUSAMMENFASSUNG

### EINWIRKUNG VON MILCH, FRUCHTSÄFTE UND GESÜSSTE GETRÄNKE AN DER PH DER ZAHNBELEGUNGEN

Die pH-Veränderungen der buccalen und superficialen Plaques von Gruppen von 18 Personen wurden mit einer *in vitro*-Methode für 30 Minuten nach Mundspülung mit Milch, Dickmilch, Limonade (»sockerdricka»), eine 25-Prozentige Saccharoselösung oder Apfelsin-, Citronen- oder Apfelsaft gefolgt.

Milch gab nur unbedeutende pH-Senkungen während der Testperiode. Dickmilch verursachte signifikant grössere pH-Senkungen nach 2 Minuten und nach 5 Minuten, aber nach 10, 20 und 30 Minuten waren die pH-Senkungen nur unbedeutend grösser als nach Milch.

Die Saccharoselösung und die Limonade gaben die gewöhnliche Stephankurve und waren einander sehr gleich.

Coca-Cola gab pH-Veränderungen die derjenigen nach Saccharoselösung und nach Limonade sehr ähnlich waren.

Apfelsinsaft, Citronensaft und Apfelsaft verursachten alle einen initialen intensiven Säureattack von kurzer Dauer, die von einer Periode gefolgt war, wenn die Plaque-pH zu einem eiligeren Rückgang nach Ruhewerte neigte als nach Mundspülung mit Saccharoselösung oder Limonade. Diese Verschiedenheiten waren stark signifikant für die Apfelsin-, Citronen- und Apfelsäfte.

Die Resultate bestätigen die Auffassung, dass Milch und Dickmilch sehr unbedeutend acidogen in der Zahnbelegungen sind, dass zuckerhaltige Getränke eine pH-Kurve geben, die derjenigen nach Saccharoselösungen sehr ähnlich ist, und dass natürliche Fruchtsäfte eine kurze Säureattacke verursachen, die von einer Periode gefolgt ist, wenn die Plaque-pH eiliger nach Ruhewerte zurückgeht als nach Konsumtion von Saccharoselösungen von korrespondierende Konzentrationen. Die natürlichen Säfte gaben doch pH-Senkungen die signifikant grösser waren als diejenigen nach Milch während der ganzen Testperiode.

Die Kariogenität von diesen Lösungen kann doch nicht nur durch Bestimmung von pH-Veränderungen in überflächigen Plaques beurteilt werden. Tiere- und Humanexperimente sind erforderlich oder können vielleicht durch interdentale pH-Bestimmungen von der Kalciumionenkonzentration und der Konzentration von divalenten Ionen substituirt werden.

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