Influence of sugar content in soft bread on pH of human dental plaque

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Quantitative determination of monosaccharides, disaccharides and sorbitol by use of gas-liquid chromatography (GLC) was performed on thirty-three samples of different commercial soft bread. Maltose was found in all the bread samples. Fructose and glucose were found only in samples of sweetened bread. Sucrose was detected in 5 samples, lactose in 2, and sorbitol in 2. Up to 20 per cent of the fresh bread weight was found to be low-molecular weight carbohydrates. Plaque pH-changes were studied in 18 persons following a 30-second mouth rinse with each of 3 solutions: (1) 50 % sucrose, (2) water extract of sweetened bread, and (3) water extract of unsweetened bread. Mouth rinsing with the extract of sweetened wheat bread (sucrose 7.7 per cent of the dough weight) caused pH-decreases in plaque which were significantly more pronounced than those induced by the water extract of unsweetened wheat bread.

Key-words: Dental plaque; hydrogen-ion concentration; bread; food-analysis

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Bread plays an important role in present-day diet and its consumption varies greatly in different countries. In Sweden, for example, the per capita consumption of soft bread is about 30 kilograms per year, which approximates 8 per cent of the total calorie intake (*Statens Jordbruksnämnd, Stockholm, Sweden*, 1974).

Sweetened soft bread is generally considered to be only moderately cariogenic in comparison to products containing high concentrations of fermentable sugars, especially sucrose (*Gustafsson et al.*, 1954). However, *Neff* (1967) observed that an extract of sweetened whole-meal bread markedly lowered the pH of human dental plaque *in situ* in comparison to an extract of unsweetened dark bread.

The present investigation was carried out in order to determine the occurrence of low-molecular weight carbohydrates in commercial samples of soft bread, and to compare the pH-changes in human dental plaque *in vivo* in 18 persons following mouth rinsing with water extracts of sweetened and unsweetened wheat bread, baked with identical ingredients except for sugar content.

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MATERIAL AND METHODS

Bread samples

Bread samples were obtained from 33 different commercial products. In addition, two wheat breads (»test breads»), which contained identical ingredients (wheat-flour, powdered milk, lard, salt, baker's yeast and water) except for the sugar content, were prepared. One type (sweetened »test bread») contained 7.7 per cent sucrose by dough weight and the other type (unsweetened »test bread») contained no sugar. All samples were analysed for low-molecular weight carbohydrate content.

Gas-liquid chromatography (GLC) of low-molecular weight carbohydrates

Determination of low-molecular weight carbohydrates in the bread samples was carried out by use of GLC. The method has been described in detail in a previous publication (*Fuchs, Gawell & Lidhem*, 1974).

Water extracts of sweetened and unsweetened »test bread»

110 g of the »test bread» was suspended in 125 ml distilled water (4° C) and homogenized, by use of an electric homogenizer, for 60 seconds. The suspension was centrifuged at 10,000 *g* for 20 minutes at 4° C and the supernatant was recentrifuged. The homogenization and centrifugation procedures were repeated with five other weighed samples of the »test bread» and the recentrifuged supernatants were pooled and stored at —20° C in 10-ml aliquots until tested.

Changes in pH of dental plaque

Changes in pH of dental plaque were studied in 18 persons after a mouth rinse for 30 seconds with a 50 per cent (w/v) solution of sucrose, with the water extract of the sweetened »test bread» and with the water extract of the unsweetened »test bread» respectively. The method has been described previously in detail (*Frostell*, 1970).

The volunteers, aged 15—50, had at least 20 natural teeth, moderate to marked predisposition to dental plaque formation, and moderate to high caries frequency.

The subjects were instructed not to clean their teeth for two days before the experiment and not to eat or drink anything, except tap water, the morning of the examination. The examinations were always carried out in the forenoon. Each subject rinsed his mouth with tap water to remove loose debris before rinsing for 30 seconds with 10-ml of the specific test solution. A blunt scaler was used to remove small amounts of dental plaque from at least twenty different areas on the teeth. The material was pooled in a drop ($\sim$ 0.06 ml) of distilled water in a one drop glass electrode connected to a Beckman Expandomatic pH-meter, and the pH in the material was determined. The measurements were carried out at room temperature (22° C). The error of pH measurement was determined to be $\pm$ 0.06 units at pH 6.00, and $\pm$ 0.12 at pH 5.00 when the pH meter was calibrated against a standard buffer at pH 6.00. Before and during each experiment the pH-meter was checked against buffers at pH 6.00 and 5.00. Samples for pH-determinations were taken immediately before the rinse and 2, 5, 10, 20 and 30 minutes after the rinse. The results were evaluated statistically in the manner described by *Frostell* (1970). Analysis was carried out using the pH-values, or the differences between the pH-values, instead of the corresponding H-ion concentration (*Frostell*, 1973).

Table I. *Carbohydrate content of bread expressed as per cent of fresh weight*

Bread No.	Fructose	Glucose	Maltose	Sucrose	Lactose	Sorbitol	Total
Unsweetened bread							
1	0.3		2.8		1.0		4.1
2			2.6				2.6
3			1.7				1.7
4	0.3	0.1	4.3				4.7
5			2.3				2.3
Sweetened bread							
6	0.6	0.2	2.3				3.1
7	1.1	0.8	3.8				5.7
8	0.9	0.3	2.1		0.8		4.1
9	1.8	1.1	3.8				6.7
10	1.8	1.2	1.9				4.9
11	2.4	1.7	3.3				7.4
12	2.3	1.8	3.4				7.5
13	2.6	1.8	4.1				8.5
14	2.3	2.0	5.6				9.9
15	3.0	2.2	1.6				6.8
16	3.0	2.6	2.6	0.6			8.8
17	3.6	2.9	2.9				9.4
18	3.3	2.6	2.3				8.2
19	3.5	3.2	4.5			0.5	11.7
20	3.1	2.8	4.5			0.8	11.2
21	4.1	3.7	3.0				10.8
22	4.0	3.5	2.5	0.8			10.8
23	4.2	3.8	3.4				11.4
24	4.1	3.6	2.5				10.2
25	4.7	4.5	3.7	2.8			15.7
26	4.4	4.1	5.9				14.4
27	4.7	4.1	3.0				11.8
28	4.3	3.8	2.9				11.0
29	4.6	4.2	3.0				11.8
30	4.7	4.2	3.1				12.0
31	5.1	4.7	2.0	1.4			13.2
32	5.6	4.8	2.0				12.4
33	5.9	5.2	6.1	2.5			19.7
»Test bread»							
34			1.8		0.9		2.7
35	3.5	3.1	1.7		1.0		9.3

RESULTS

The results of the quantitative determination of monosaccharides, disaccharides and sorbitol by use of GLC in the commercial bread samples is given in Table I. Samples numbers 1—5 represent unsweetened, and numbers 6—33 sweetened commercial products. The unsweetened and sweetened »test bread» are shown as samples numbers 34 and 35, respectively. Maltose was found in all bread samples

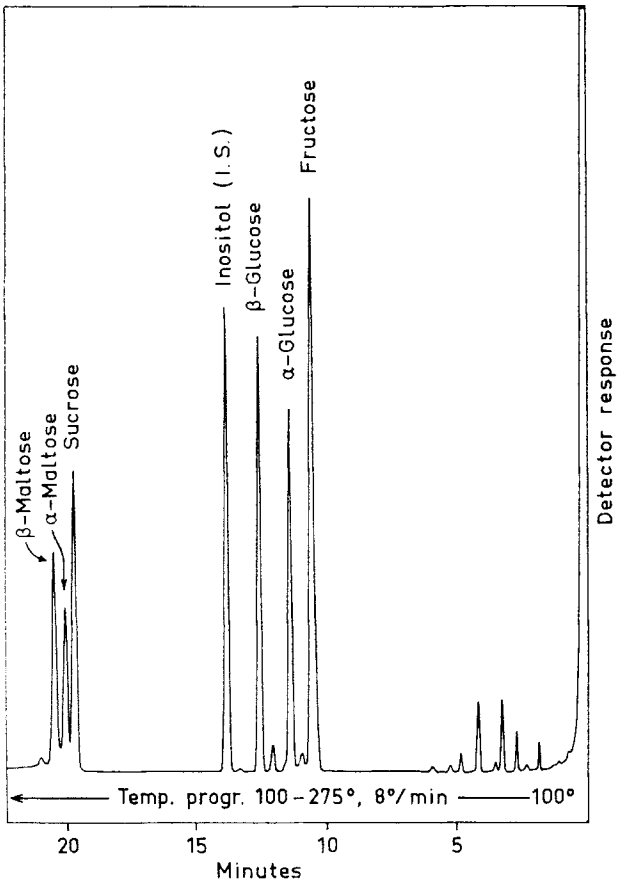

Fig. 1. Gas chromatogram of the TMS ethers of carbohydrates obtained from bread No 25 (Table I) on a 3 % OV-1 column.

and the concentration ranged between 1.6 and 5.9 per cent of the fresh weight. In the unsweetened bread samples only small quantities of fructose and glucose were found. In the sweetened bread the fructose and glucose content varied between 0.6 and 5.9 g and between 0.2 and 5.2 g per 100 g bread (fresh weight), respectively. The concentration of fructose was higher than that of glucose in all samples. Sucrose was found in only 5 of the samples and lactose and sorbitol were found in 2 samples of the commercial products.

Figure 1 shows a representative gas chromatogram of trimethylsilyl (TMS) ethers of carbohydrates obtained from a sample of bread (No 25, Table I) on a 3 % OV-1 column. Inositol was used as the internal standard (I.S.). The peak areas were measured as integrator counts.

As expected (*Frostell*, 1970; 1973), rinsing with the 50 % solution of sucrose markedly lowered the pH in the dental plaque tested (Fig. 2; Table II).

The effect of rinsing with sweetened wheat bread extract on the pH in dental plaque was less than that of sucrose at 10, 20 and 30 minutes (Fig. 2; Table II). The sweetened »test bread» caused pH-changes which were significantly more pronounced than those induced by the unsweetened »test bread», especially at 2, 5, 10 and 20 minutes (Fig. 2; Table II).

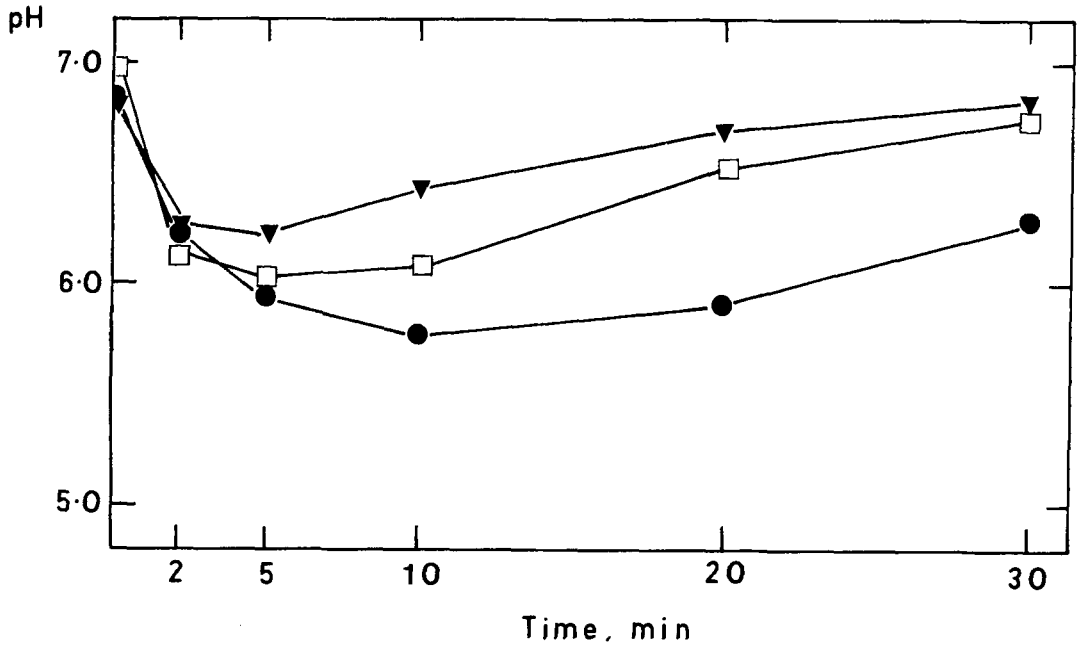

Fig. 2. Changes in the pH of dental plaque after a mouth rinsefor 30 seconds with (1) a 50 per cent sucrose solution (●—●), (2) a water extract of sweetened wheat bread (□—□), and (3) a water extract of unsweetened wheat bread (▼—▼). Mean values from experiments on 18 persons.

DISCUSSION

Separation of trimethylsilyl (TMS) derivates of carbohydrates by GLC is a well established technique in food analysis (see review by *Fuchs et al.*, 1974). The method used in this study was earlier found to be suitable for quantitative determination of monosaccharides, disaccharides and sorbitol in bread (*Fuchs et al.*, 1974).

The sugars in bread doughs arise from three sources: (1) the sugars originally present in the flour, (2) sugars produced from oligosaccharides or polysaccharides by the action of flour and yeast enzymes, and (3) those intentionally added as dough ingredients (*Atkin, Schultz & Frey*, 1946). Paper partition chromatography has been applied to the separation and determination of the amounts of individual sugars present in bread doughs and bread undergoing fermentation with a commercial baker's yeast (*Koch, Smith & Geddes*, 1954). Sucrose was hydrolysed very rapidly. Glucose was fermented at a uniform and rapid rate, either alone or in mixtures with other sugars. Glucose exerted a sparing action on the fermentation of fructose. As a consequence, bread made with sucrose contained a higher percentage of fructose than glucose. Maltose was fermented at a slow rate until the glucose and fructose were exhausted.

The sugar content of bread reported in this study (Table I) is in agreement with earlier investigations (*Rice*, 1938; *Koch et al.*, 1954; *Sihlbom*, 1959; *Malm & Liljamaa*, 1973; *Fuchs et al.*, 1974). Sucrose was detected in 5 of the 33 types of commercial bread subjected to analysis. However, the sucrose concentration (0.6—2.5 per cent of the fresh weight) was somewhat higher than that found by *Winholt* (1970).

Dietary sucrose plays an important role

Table II. *Comparison between plaque pH values in 18 subjects at different time intervals after a mouth rinse for 30 seconds with a water extract of sweetened wheat bread and a water extract of unsweetened wheat bread*

	pH-differences										
	Initial mean pH	2 min mean pH	mean pH decr.	5 min mean pH	mean pH decr.	10 min mean pH	mean pH decr.	20 min mean pH	mean pH decr.	30 min mean pH	mean pH decr.
Sweetened »test bread»	6.98	6.11	0.87	6.02	0.96	6.07	0.91	6.53	0.45	6.74	0.24
Unsweetened »test bread»	6.83	6.26	0.57	6.21	0.62	6.44	0.39	6.70	0.13	6.81	0.02
Difference	0.147		0.294		0.333		0.522		0.319		0.200
t-value	2.016		4.339		4.526		6.482		5.244		2.490
Level of significance	—		***		***		***		***		*
Sucrose	6.84	6.23	0.61	5.95	0.89	5.77	1.07	5.91	0.93	6.28	0.56

*** $p < 0.001$
** $p < 0.01$
* $p < 0.05$

in the etiology of dental caries (see review by *Mäkinen*, 1972). First, the consumption of this carbohydrate is very frequent. Second, the acid production rate in suspensions of dental plaque in sucrose solutions is rather high (*Frostell*, 1964). Third, sucrose induces the synthesis of specific bacterial polysaccharides such as glucans and fructans (for review, see *Chritchley*, 1971). These polymers contribute to the adhesion of microorganism to the teeth. However, other carbohydrates found in the human diet, such as glucose, fructose, lactose, maltose and starch, also induce acid production in dental plaque material *in vitro* (*Frostell*, 1964), lower the pH in dental plaque *in vivo* (*Neff*, 1967; *Frostell*, 1972; 1973; *Birkhed, Wickholm & Frostell*, 1975) and induce polysaccharide production (*Critchley et al.*, 1967). Soft bread, especially sweetened bread, contains different concentrations of these sugars (Table I) and it was therefore thought worthwhile to study the influence of sweetened and unsweetened bread on pH of dental plaque.

The low concentration of sucrose in bread and its influence on dental caries has been discussed by several authors (*Gustafsson, et al.*, 1954; *Lanke*, 1957; *Sihlbom*, 1959; *Mühlemann*, 1965; *Winholt*, 1970; *Winholt & Wånge*, 1973). *Lanke* (1957) found that unsweetened bread gave an enhanced salivary level of sugar for as long a period of time as a sweetened bread baked according to the same recipe but with sugar added. However, *Lanke* (1957) proposed that bread with a high sugar content can be expected to give an initially higher sugar level in saliva than the corresponding bread without sugar. This assumption is supported by the findings of this study when comparing the pH-changes in dental plaque after mouth rinses with extracts of unsweetened and sweetened bread (Fig. 2, Table II). The importance of the initial sugar concentration to the pH-decreases in dental plaque

in vivo has earlier been demonstrated by *Mühlemann & de Boever* (1969). They found, when using 0.1, 0.5, 1.0 and 10 per cent sugar solutions, that plaque pH decreased more with increasing concentration of sugar after mouth rinsing. These results indicate that sugar concentration in food is a factor of great importance to the degree of initial acid attack in dental caries.

Low-molecular weight carbohydrates are not the only carbohydrates which induce pH-decreases in dental plaque *in vivo*. High molecular weight carbohydrates, for example starch, can have similar effects. During the process of baking bread the starch is gelatinised (*Pomeranz*, 1973). *Frostell* (1972) demonstrated that a cooked starch solution induced pH-decreases in dental plaque *in vivo* which were significantly more pronounced than those caused by rinsing with an uncooked solution of starch. This fact may to some extent explain the pH-changes induced by the unsweetened »test-bread» (Fig. 2; Table II). However, the unsweetened »test bread» contained maltose (Table I), which also could have an influence on the pH-values (*Birkhed et al.*, 1975).

Many methods are available for comparing the cariogenicity of foodstuffs (*Bibby*, 1970). Acid production from foods incubated in saliva, acid formation in chewed food expectorate, acid formation in plaque, food retention and decalcification by fermenting foods, »artificial mouth» apparatus, *in vitro* caries, *in vivo* enamel demineralisation, *in vivo* »natural» caries and animal caries are some examples. The method used in this study (*Frostell*, 1970) provides information on pH-changes in superficial dental plaque following exposure to different types of foods and other substances. The results may be different if interdental plaque pH was measured. The method gives information on the primary acid production caused by different foods on accessible tooth surfaces. It does not, however, give complete information on the cariogenicity of a certain foodstuff, since cariogenicity is influenced also by other factors. Examples of other influences to be considered are: (1) effect on oral bacterial populations (*Bowen*, 1970), (2) effect on bacterial metabolic processes (*Critchley*, 1970), (3) enamel protective factors in food *i.e.* phytate, other organic phosphates, fat and others (*Jenkins*, 1970), and (4) different physical properties of foods *i.e.* adhesion, wettability, physiologic cleansing actions (*Caldwell*, 1970).

Caution must be exercised in drawing conclusions as to the cariogenicity of unsweetened and sweetened soft bread only on the basis of the results of this study. However, for persons with high predisposition to dental plaque formation and high caries frequency, it would seem to be of importance to avoid foodstuffs containing fermentable carbohydrates. Sweetened soft bread, which here has been found to contain up to 20 per cent low-molecular weight carbohydrates (fresh weight) is an example of such a food (Table I).

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