

## IN VITRO pH CHANGES AND ACID PROFILE OBTAINED DURING METABOLISM OF DATES BY ORAL FLORA

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تمثل فاكهة التمر الغذاء الرئيسي لسكان دول الشرق الأوسط وشمال أفريقيا. وأظهرت التحاليل الكيميائية أنها غنية بالسكريات، المعادن، البروتينات، والفيتامينات. وعليه فإن تناول التمر سيؤدي إلى تغير في درجة حموضة اللعاب. هذا التغير سيكون معتمدا على كميات الحمض الناتجة من تحلل السكريات وكذلك على كميات المواد القاعدية الناتجة من تحلل المكونات النيتروجينية للتمر.

ونظرا للدورا الرئيسي الذي يلعبه السكروز والسكريات الأحادية كالجلكوز والفركتوز في بدء عملية النخر فقد أجريت دراسات مستفيضة عن تأثير السكريات على إنتاج الأحماض وعلى درجة الحموضة بواسطة مزيج من بكتريا الفم الموجودة في كل من الترسبات السنية واللعاب.

يمكن تلخيص الأهداف الرئيسة للبحث على النحو التالي:

١ - دراسة التغير في درجة الحموضة الذي يحدث أثناء تحلل أجزاء مختلفة من محلول التمر بواسطة مزيج من بكتريا الفم.

٢ - دراسة تأثير اللعاب على درجات الحموضة.

٣ - مقارنة التغير في درجات الحموضة عند استعمال أنواع مختلفة من التمر.

٤ - مقارنة التغير في درجات الحموضة للتمر عند مراحل مختلفة من النضج.

وقد وجد من خلال الدراسة أن:

- \* إن القدرة على إنتاج الحمض للأنواع المختلفة من التمر مشابهة لمثلتها في كل من الجلكوز والسكروز.
- \* وإن تحلل الطبقة العليا من محلول التمر ينتج عنها أكبر كمية من الحمض بينما يكون ناتج الحمض من الطبقة الوسطى أقل كمية عند مقارنتها بالمحلول غير المعامل بالطرد المركزي.
- \* ووجد في المراحل الأولى من التجربة أنه كلما كان التمر ناضجا كلما كان هناك انخفاض أكبر في درجة الحموضة. أما في المراحل النهائية من التحلل فيكاد يكون الاختلاف ضئيلا جدا.
- \* وإن إضافة المحلول الرائق للعباب لا يؤدي فقط إلى الانخفاض في الناتج الحمضي وإنما يعمل على حدوث زيادة طفيفة في درجة الحموضة وذلك بعد ٦٠ دقيقة من بداية التجربة.
- \* ووجد أن عملية تحلل التمر بواسطة بكتريا الفم ينتج عنها أنواع مختلفة من الأحماض والتي تشمل حمض اللبن، حمض الخلل، حمض البروبيونيك، وكذلك كميات صغيرة من حمض النمل.

Dates is a commonly eaten fruit in many Middle East and North African countries. Chemical analysis of this fruit by previous workers showed that it is rich in carbohydrates, proteins, amino acids and minerals. The acid-base changes, which occur when dates is metabolized by mixed oral bacteria was investigated. The results showed that despite the presence of nitrogenous

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substances in dates, a profound acidogenic responses was obtained with the different types of the fruit and at different ripening stages. A variety of organic acids, which included lactic acid, acetic acid and propionic acid, were produced during this process.

### Introduction

Because of the primary role of sucrose and other monosaccharides (glucose and fructose) in the initiation and progression of dental caries, sugars have been extensively studied for their effects on the production of organic acids and acidic pH by the mixed microbial flora in the dental plaque and saliva.<sup>1-5</sup> It has been amply demonstrated that rinsing, with a glucose or sucrose solution, results in a rapid decrease in the plaque pH followed by a slow return to the starting level.<sup>1</sup> This response to sugar challenge (Stephan's curve) has been related to the level of caries activity.<sup>6</sup>

In most of the Middle East countries, dates is a staple food for the population in the past. History reveals that many desert dwellers, both in Africa and Asia, used to live for months on dates and milk alone. Eating dates is said to prevent hunger and thirst for several hours in spite of a successive and stressful workload. Dates was not used as a food item only, but has also been recommended for the management of other conditions such as bone fracture, parturition, pregnancy, lactation and convalescence.<sup>7</sup> Dates has also been shown to be rich in carbohydrates, minerals, proteins and vitamins.<sup>8,9</sup> Chemical analysis shows that "dates" contain about 80% carbohydrates, most of which are fermentable sugars, fructose, glucose and sucrose.<sup>10</sup> Therefore, consumption of this fruit should produce a net pH change which is dependent on the amount of acid production from the carbohydrate component and base production from the nitrogenous components of the fruit.

The objectives of this study were to analyze the occurrence of pH changes during metabolism of date fruits by mixed oral bacteria, to examine the effect of saliva on the pH profiles, to compare the pH profiles of different types of the fruit, to compare the pH profiles at different ripening stages of the fruit, and to determine the type of acids being produced during the metabolic processes.

### Methods and Materials

#### Preparation of the Date Fruits:

The date fruits were purchased at the local

market in Riyadh. The seeds were removed and the fruit was grounded to a pulp with the aid of a commercially available blender. A 15% stock solution of the various types was then prepared with deionized distilled water. In one of the experiments, the prepared solution was centrifuged at 10,000 g for 15 minutes. This resulted in a 3-layer suspension. The topmost layer and the middle were carefully decanted separately and used in the appropriate experiment designed to grossly examine the glycolytic effect of the content of the fruit's different layers. Fresh stock solutions of the dates were prepared on the date of the experiment and were stored at room temperature before use.

#### Preparation of Salivary Sediment:

Salivary sediment was used for this study as it represents a very convenient method of obtaining mixed oral flora.<sup>11,12</sup> Wax stimulated whole saliva was collected into a test tube from subjects who had avoided all forms of oral hygiene for 24 hours and had not eaten for 8-10 hours prior to collection. The samples were pooled and centrifuged at 10,000 g for 15 minutes at 4°C. The supernatant was decanted and stored at 4°C until required which was usually within 1 hour. The salivary sediment was washed 2 times by re-suspending in distilled water and re-centrifuging at 1,740 g for 15 minutes each. A final stock concentration of 50% (v/v) of the sediment was prepared by adding equal volume of distilled water to the measured volume of the sediment.<sup>13</sup> Fresh salivary sediment was prepared on the day of each experiment.

#### Final Incubation Concentrations and pH Monitoring Procedure:

Five experiments were carried out to determine the pH changes during metabolism of the dates. In each of these experiments, the final incubation concentration of various components in the suspended salivary sediment (SSS) system were the: (1) suspended salivary sediment (16.7%), (2) glucose or sucrose (positive control - 5%), (3) different varieties of dates (5%), (4) salivary supernatant (33.3%), and (4) water (negative control).

The first experiment compared the acidogenic potential of dates with glucose and sucrose; the second experiment compared the dates' pH changes during metabolism at different centrifuge fractions; the third experiment compared the pH changes of dates in the presence or absence of salivary supernatant; the fourth experiment was on the comparison of pH changes obtained from the dates' different ripening stages (*Khalal* and *rutab* stages), while the final experiment was designed to evaluate the type of acids being produced from the fruit by oral bacteria.

The initial pH of the mixtures at the start of each experiment was adjusted to pH 7 with either 1M NaOH or 1M HCl. Each experiment was done in duplicate and incubated in a water bath at 37°C. The pH changes were then monitored with a pH meter every 5 minutes for 15 minutes then at 30, 60, 90 and 120 minutes, respectively. The mean pH at each time interval was plotted against time to obtain the pH profile for each experiment. For the acid profile, 100 $\mu$ L aliquot in duplicate were removed from the mixtures, at 15, 30, and 60 minutes. These were analyzed for different types of acid with the aid of a Varian Gas Chromatograph\* which is equipped with a dual flame ionization detector (FID) attached to a Varian linear recorder. The column used was a stainless steel column 2" x 1/8", 15% FFAP on Chromosorb\*\*. This was conditioned and operated at 155°C isothermal detector and injector temperature of 250°C. The carrier gas was pure nitrogen at a flow rate of 30 ml per minute.<sup>1</sup> The chart speed was set at 1 cm per minute while the attenuation was 10<sup>-9</sup> x 16. All the samples and standards were prepared in acetone BP (56  $\pm$  0.50°C) before analysis.

## Results

Figure 1 shows the pH profiles of different types of dates during metabolism by mixed oral bacteria. All fruit types tested produced a significant acidic pH profile comparable to glucose. In the first 15 minutes of the incubations, however, glucose and sucrose produced greater acidic response than any of the fruits. No rise in pH was evident during the period of monitoring.

Figure 2 shows the pH profiles obtained when

\* Model 3720, Varian, USA.

\*\* Chromosorb WAW 80/100, Varian, USA.

the different centrifuged fractions of the dates were incubated with oral bacteria. The topmost layer produced the greatest acidic response while the middle layer produced the least in comparison with the uncentrifuged solution.

The effect of different stages of ripening of the fruits on its acidogenic potential is shown in Figure 3. In the initial stages, the more ripened stage (*Rutab*) produced a greater pH decrease than the less ripened stage (*Khalal*). However, by the end of the monitoring period, the difference was minimal.

The result of the acid analysis showed that a variety of acids were being produced during the metabolic processes [Fig. 4], These acids include. lactic acid, acetic acid, propionic acid and, occasionally, some minute quantities of formic acid. Lactic acid and acetic acid were the two major acids produced during the process. The results also showed a greater accumulation of

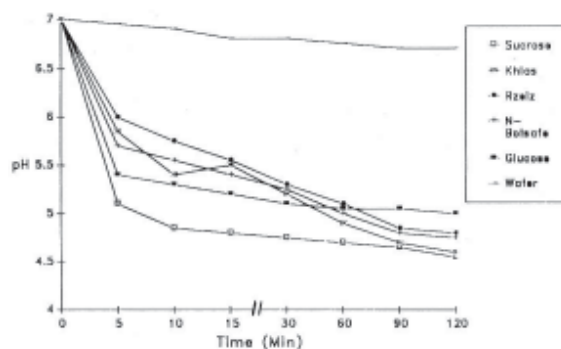


Figure 1. pH profiles produced by different types of dates during metabolism by mixed oral bacteria.

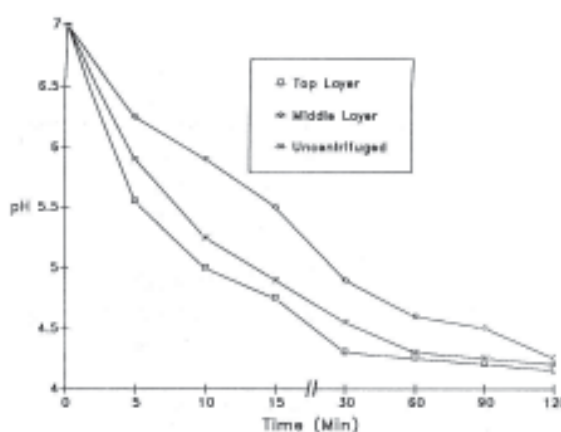


Figure 2. pH profiles produced by different centrifuged fractions of the date fruits during metabolism by mixed oral bacteria.

acetic acid with time compared to lactic acid.

The effect of saliva supernatant is shown in Figure 5. The presence of supernatant not only created a reduction in the acidic response but at 60 minutes produced a slight pH rise which continued till the end of the monitoring period.

**Discussion**

In this investigation, the various comparisons made on the different varieties of dates showed

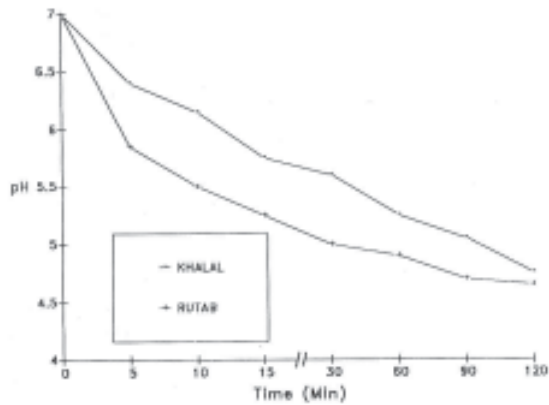


Figure 3. pH profiles produced by date fruits at different ripening stages.

acidogenic potential similar to glucose and sucrose. This, therefore, confirms that the different types of sugar in dates are easily metabolized by the oral flora. The results also show that these fermentable sugars are present in enough concentration at different ripening stages of the fruit to produce rapid pH fall when supplied to the oral bacteria.

When saliva supernatant was added to the incubation mixture, there was a reduction in the pH fall and a slight rise at the latter part of the

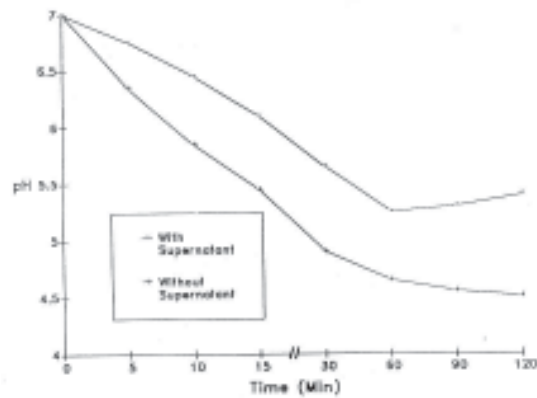


Figure 5. Effect of saliva supernatant on the pH profiles produced by date fruits during metabolism by mixed oral bacteria.

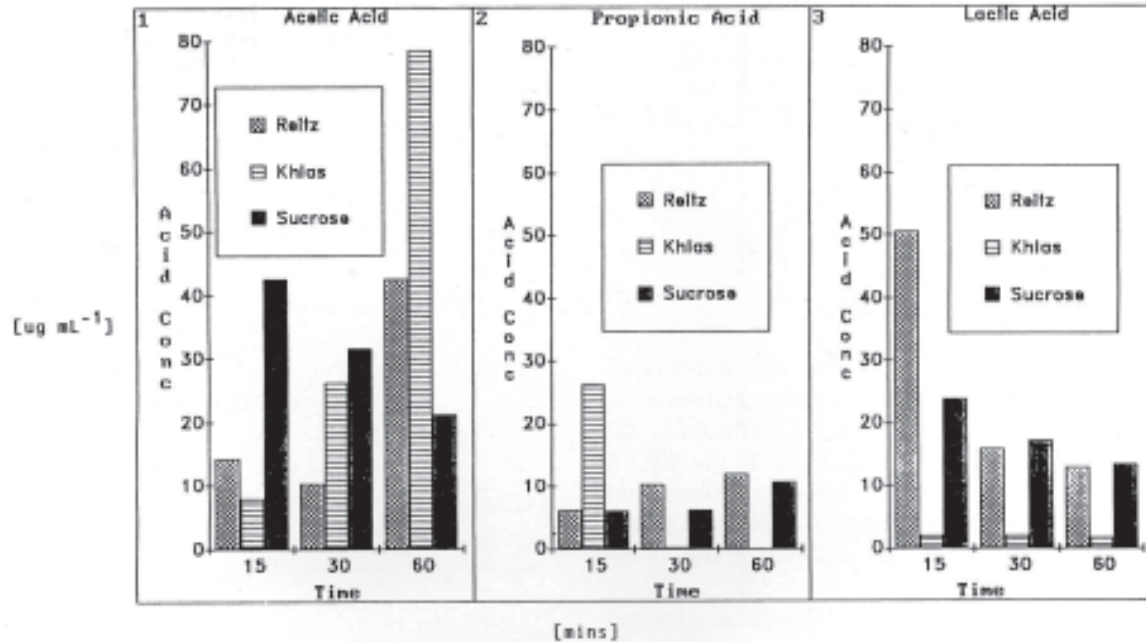


Figure 4. Types and amounts of different organic acids produced during metabolism of the date fruits by mixed oral bacteria.

incubation period. This response confirms earlier observations that saliva is capable not only of buffering the acid being produced during glycolysis as evident by the reduction in pH but also of raising the pH of the system.<sup>14</sup> This rise in pH is due primarily to the production of ammonia from urea and other amino acids notably arginine, present in saliva by the oral bacteria.<sup>15</sup> The buffering capacity of saliva has been shown to be dependent mainly on (a) its bicarbonate content which increases with increase in salivary flow rate and at acidic pH and (b) partly on the presence in saliva of a complex substance - "precipitin" (Calcium-phosphate-carbohydrate protein complex).<sup>16</sup>

The minimum pH observed when dates are metabolized in vivo therefore would be influenced by such factors as saliva flow rate and oral clearance of the fruit. While the two different ripening stages were shown to provoke similar acidogenic responses, the texture of the fruits at different types may play a role in determining how long the pH would remain low. It is opined that the crispy ripened type would be capable of stimulating more saliva than the very soft and sticky type and therefore would clear faster from the oral cavity. This would enhance a rapid return to baseline pH value.

Other non-carbohydrate components in the date fruits especially the various amino acids such as glutamic acid and aspartic acid, proteins and fats seemed unable to exert any effect on the pH changes observed. This may either be due to the fact that (a) they are not being metabolized in the presence of excess carbohydrates or that (b) they are in such small quantities that the base production was too small to counteract the level of acid being produced. Further investigation is required to evaluate whether by increasing the level of these non-carbohydrate components especially the amino acids and peptides the pH profile during metabolism of the fruits would be modified.

The acid analysis shows that both acetic and lactic acid are the prominent acids being produced during the metabolic processes. The fact that with time the proportion of the acetic acid continued to rise while the lactic acid was falling suggests that the lactic acid was being converted to acetic acid. The situation, however, would be expected in intraoral areas where clearance of substances is delayed due

to poor access to salivary action. These sites would include the interproximal areas. However, for easily accessible areas of the teeth, clearance would be faster and therefore, less time would be available for the conversion of lactic acid to acetic acid. All these aspects, however, warrant further in vivo investigation.

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### References

1. Stephan RM. Intraoral hydrogen ion concentrations associated with dental caries activity. *J Dent Res* 1944;23:257-66.
2. Stephan RM, Hemmens ES. Studies of changes in pH produced by pure cultures of oral microorganisms. *J Deni Res* 1947;26:15-41.
3. Stalfors A. Investigations into the bacterial chemistry of dental plaque. *Odoni Tidskr* 1950;24:217-26.
4. Denepitiya L, Kleinberg I. A comparison of the acid-base and acidogenic properties of various serotypes of *Streptococcus mutans*. *Arch Oral Biol* 1984;29:385-93.
5. Higham SM, Edgar WM. Human dental plaque pH and the organic acid and free amino acid profiles in plaque fluid after sucrose rinse. *Arch Oral Biol* 1989;34:329-34.
6. Mandel ID, Zengo AN. Genetic and chemical aspects in resistance. In: Mergenhagen SE, Scherp HW, eds. *Comparative immunology of the oral cavity*. Bethesda: HEW Publ, 1973:73-438.
7. Shinwari MA. Chemical composition of dates. *J Coll Science King Saud University* 1980; 18:5-12.
8. Hassan J. Dates: the fruit of ages. *Saudi Business* 1986;6:20-22.
9. Sawaya WN, Khalil JK. Growth and compositional changes during the various developmental stages of the date fruits. In: *Dates of Saudi Arabia* 1986:75-94.
10. Sawaya WM. Dates of Saudi Arabia: Research studies on compositional characteristics and product development, 1986.
11. Dolan MM, Murphy CV, Kavanagh B), Yankell SL. Development of an in-vitro plaque model from human salivary sediment suspensions. *Arch Oral Biol* 1972;17:147-54.
12. Mishiro Y, Kaneko H. Effect of a dipeptide, aspartane on lactic acid induction in human whole saliva. *J Dent Res* 1977;56:1427.
13. Singer DL, Chatterjee R, Denepitiya L, Kleinberg I. A

- comparison of the acid-base metabolisms of pooled human dental plaque and salivary sediment. Arch Oral Biol 1983;28:29-35.
14. Kleinberg I, Craw D, Komiyama K. Effect of salivary supernatant on the glycolytic activity of the bacteria in salivary sediment. Arch Oral Biol 1973;18:787-98.
  15. Kleinberg I. Control of plaque pH and caries with apH rise factor. Br Dent J 1981;127:94-5.
  16. Salako NO, Kleinberg I. Buffering capacity of human maxillary and mandibular incisor plaque and its relation to their acid-base pH responses with test carbohydrate and nitrogenous substrates. J Dent Res 1988;67, Abstr. #1930.