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Use of a Bacterial Mutagenicity Assay as a Rapid Method for the Detection of Early Stage of Maillard Reactions in Orange Juices

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ABSTRACT

Freshly pressed orange juice was heated up to 30 min at 93°C and assayed for mutagenicity by the Ames test with Salmonella typhimurium *strain TA 100 modified to include a 4 h preincubation time at 37°C and pH 7·4. Commercial juices of presumably different heat treatments were assayed in the same way. It was noticed that the heat treatment induced mutagenicity which was dose related and related to the time of heating and to the presumed heat load of the commercial juices. The mutagenic response must be ascribed to Maillard intermediary products as the heat treatments were mild and not sufficient to produce brown pigments. This effect may become the basis of a rapid and inexpensive method for measuring heat load and the inherent quality of fruit juices.*

INTRODUCTION

Fruit juices contain constituents such as sugars, amino acids and ascorbic acid which can undergo Maillard reactions, resulting in off-flavour and browning. Maillard reactions are therefore an important factor for the shelf-life of fruit juices. Their reaction rate in a prepackaged juice depends, at a given composition, on the temperature of storage and the concentration of Maillard intermediary products (MIP) which are not sensorially perceived. Such MIP can be formed during heat processing (evaporation, pasteurization) and storage of (concentrated) juices. A

rapid method, suitable for the fruit juice technologist, of judging the extent of formation of MIP is therefore desirable as a quality check on juices and concentrated juices to be reprocessed in consumer packages and also as a simple research tool with which to measure the heat load imparted to juices during processing. Methods used so far are loss of ascorbic acid, appearance of hydroxymethylfurfural (HMF) and changes of colour and flavour (Ranganna *et al.*, 1983).

Maillard reaction products have been reported to posses mutagenic activity in the Ames bacterial mutagenicity assay. Using model systems of amino-carbonyl reaction mixtures with equimolar concentration of sugars and amino acids, Shinohara *et al.* (1980) and Powrie *et al.* (1981) found positive mutagenic responses of *Salmonella typhimurium* strain TA 100 after intensive heat treatment (100 °C–121 °C; 1–10 h) which caused browning of the test solutions. Mutagenicity of foods showing Maillard browning, i.e. bread crust (Pariza *et al.*, 1979; Spingarn *et al.*, 1980; van der Hoeven *et al.*, 1982), fried meat (Dolara *et al.*, 1979; Pariza *et al.*, 1983) and dried fruits (Stich *et al.*, 1981), has also been described.

In this study with orange juice, we wanted to see whether mutagenicity was also provoked by lighter heat treatments which did not cause detectable colour changes and whether these mutagenic properties could be used as a measure of such treatments.

EXPERIMENTAL

Preparation of samples

Laboratory-prepared orange juice (code A) was obtained from Israeli Jaffa oranges using an electric household juice extractor and finishing the juice by squeezing through cheese-cloth. Commercial samples were directly bottled juice (code B), juice reconstituted with distilled water, according to label instructions, from canned frozen concentrated juice (code C) and juice in Brik-Pack made from concentrated juice (code D).

Samples of juice A were filled in capped Kimax tubes and held for various times at 93 °C by immersion in a boiling water-bath and cooling under running tap water. A slight change of colour was detected after 30 minutes' heating. The commercial juices were not heat treated. All juices were then further treated according to Fig. 1.

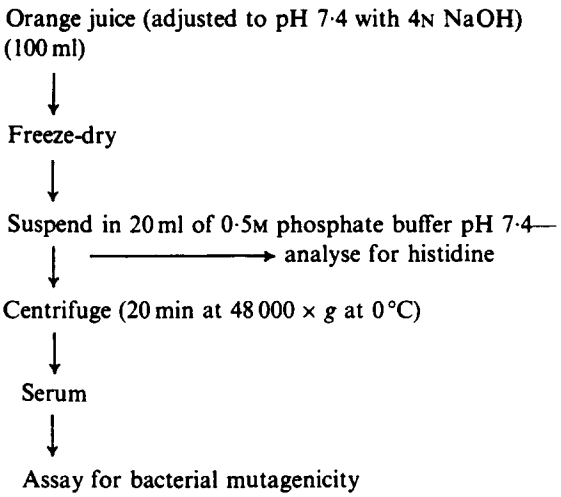

Fig. 1. Scheme of sample preparation.

Mutagenicity assay

Salmonella typhimurium strain TA 100 was used for the mutagenicity assay according to the method of Maron & Ames (1983) with minor modifications described by van der Hoeven *et al.* (1983). On the basis of preliminary experiments, a preincubation time of 4 h at 37 °C and pH 7·4 was applied in all tests. The procedure followed was: add 0·1 ml of 0·5M phosphate buffer, pH 7·4, in sterile 13 × 100 mm capped culture tubes, add 0·1 ml bacterial culture and then the test samples (pH 7·4) varying from 0·1 to 1 ml, add distilled water to give a final volume of 1·2 ml, mix gently on Vortex mixer and incubate in a shaking water-bath at 37 °C for 4 h. As a negative control, 1 ml of distilled water and, as a positive control, 0·1 ml of DMSO containing 0·1 μg NQO (4-nitroquinoline-*N*-oxide) were used. After 4 hours' preincubation, add 3 ml of molten top agar (45 °C), mix on a Vortex mixer and pour onto bottom agar plates. The plates are then incubated at 37 °C for 48 h.

The numbers of induced revertant colonies were counted manually and subtracted from the number of spontaneous revertants (negative control) which were in the range 93–174 revertant colonies per plate. Revertants induced by the positive control used (NQO) were $\geq 10\,000$ revertant colonies per microgram. Each experiment was done at least twice and each concentration of the sample was tested at least in triplicate.

Data interpretation

A result is considered positive when a reproducible dose–response curve with a mutation ratio (induced revertant colonies per plate divided by spontaneous revertant colonies per plate) of at least 2·0 is obtained.

RESULTS AND DISCUSSION

There was no positive response observed in unheated juices. Heated samples of juice A showed a positive mutagenic response. A reproducible linear relationship of the dose–response curves was observed up to a sample concentration of 0·2 ml per plate for heat treatment of 2 min and longer and of 0·3 ml per plate for heat treatment up to 1 min (Fig. 2). At higher concentrations, there was a steady decrease in the number of revertants, interpreted as a toxic effect of the sample. Microscopic examination showed a reduced background lawn of the corresponding samples, confirming this interpretation.

Figure 3 shows the positive linear correlation between the mutagenic response of juice A and heating times up to 2 min. The concentration of MIP mutagenic compounds increased with time of heating. It is

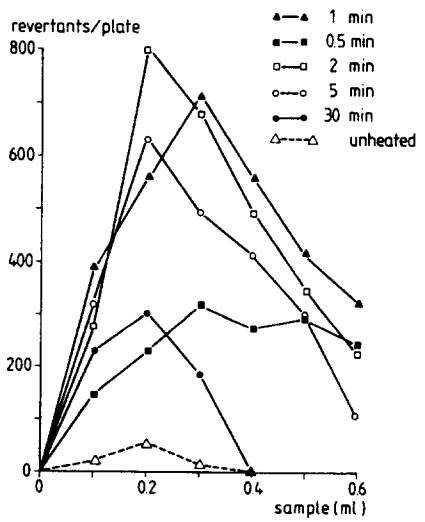

Fig. 2. Dose–response curves of the mutagenic effect of laboratory-prepared orange juice heated for various times at 93 °C on *Salmonella typhimurium* TA 100. For preparation of samples see Fig. 1.

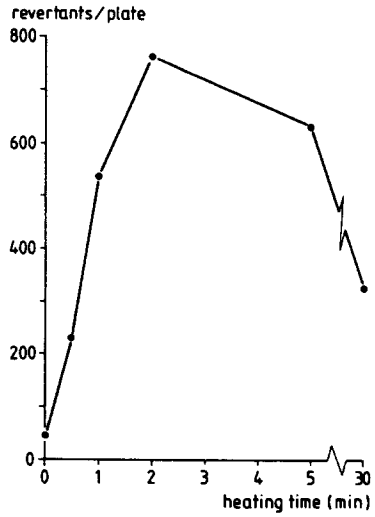

Fig. 3. Effect of heating time on the mutagenicity of orange juice. Data obtained from the slope of the linear portion of dose–response curves (Fig. 2) at a sample concentration of 0·2 ml.

interesting to note that, on longer heating, the mutagenic activity decreased and this may be due to the formation of toxic compounds.

For the commercial samples we may suppose that B has received the least heat treatment (one pasteurization treatment), followed by C (heat treatment during concentration) and then D (heat treatment during concentration and pasteurization before packing). Figure 4 shows that all three samples gave a positive dose-related response. All three dose–response curves show peaks at concentrations of 0·2 ml sample assayed per plate. At this point, the mutation ratio was calculated to be 2·7, 4·7 and 5·8 for juices B, C and D, respectively. Again, a toxic effect of higher sample concentrations was clearly seen.

Orange juice has a natural content of histidine with an average value of 5 μg/ml. According to van der Hoeven *et al.* (1983) the addition of HIS of more than 20 μg/plate, in addition to the normal content of 15 μg/plate in the bottom agar plate, depending on the type of mutagen, can potentiate the mutagenic effect. The additional average quantity of HIS from orange juice samples in our experiments was considerably lower than 20 μg/plate at the maximum doses of the non-toxic range; namely, 5 μg for 0·2 ml and 7·5 μg for 0·3 ml, according to direct analysis of the suspended freeze dried samples (Fig. 1). Interface of an HIS effect with the mutagenicity data is therefore improbable.

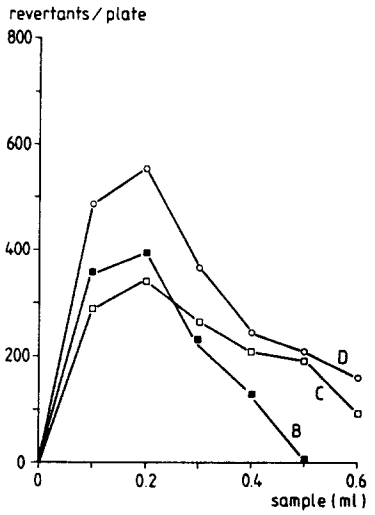

Fig. 4. Dose–response curves of the mutagenic effect of commercial orange juices on *Salmonella typhimurium* TA 100. B = Bottled juice. C = Canned frozen concentrated juice. D = Brik-Pack packed juice from concentrated juice.

CONCLUSIONS

The results show that freshly pressed orange juice has no mutagenic properties but that mutagenicity is induced by heat treatment. The mutagenicity is reproducible and dose-related and also related to the duration of heat treatment in laboratory prepared samples and to the supposed heat load of commercial samples with different histories. At doses higher than the peak doses (Fig. 2) the number of revertants decreases, indicating a toxic effect. A reduction of mutagenic activity is also illustrated at the same dose level of 0·2 ml/plate but at increasing heating times (Fig. 3). At concentrations and heating times which caused toxicity, a reduced background lawn was observed microscopically, confirming this explanation. Shinohara *et al.* (1980) and Powrie *et al.* (1981) also described toxic effects of amino-carbonyl Maillard reaction products.

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