Food Additives And Their Mutagenicity

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Citation

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Abstract

Sweet and flavours, vital constituents of food are indispensable to the modern day consumer as a means for the rapid identification and ultimate acceptance of food. A large number of natural or synthetic food additives have been removed from both national and international lists of permitted food additives because of their mutagenic or carcinogenic activity. To the majority of the food additives, JECFA/FAO has assigned “Admissible Daily Intake Dose”-ADI, which are often temporary and emphasized the need for further genotoxic evaluation, since a number of them are reported to be genotoxic below the ADI dose. In India, the problem is severe because in spite of regulation and restrictions by the prevention of Food Adulteration Act of 1954, use of non-permitted food additives are prevalent. In the present study, the mutagenic effects of sweeteners, saccharin (sweetex) and aspartame (sugar free) and flavouring agents such as vanilla essence, soy sauce, chilly sauce, worcestershire sauce, ice cream essence and rose syrup were tested utilizing Ames Salmonella microsome assay. Salmonella typhimurium strains such as TA 98 and TA 100, with and without metabolic activation were used. The results showed that all the food additives were found to be mutagenic except vanilla essence and ice cream essence.

INTRODUCTION

Artificial sweeteners and flavoring agents are added to a wide variety of food drinks/ drugs and hygiene products. The cancer inducing activity of one of these substances would mean a health risk to an entire population. Therefore scientific assessment of genotoxicity of food additives is of utmost importance. Genetic abnormalities arise as a result of mutation at chromosomal level. The genetic damage expressed by Salmonella assay represents a class of DNA damage called gene or point mutation. The use of bioassays is an essential part of the hazard assessment and control procedures for toxic chemicals (Derelanko and Hollinger, 1995). The Salmonella/microsome assay is based on the premise that bacterial assay systems provide an efficient way to detect agents, which could interact with DNA and cause mutations. Such agents would probably also be capable of causing mutations in other species, including man.

Thus in the present study, the currently used food additives namely, the artificial sweeteners like saccharin (sweetex), aspartame (sugar free), and flavoring agents such as vanilla essence, soy sauce, chilly sauce, worcestershire sauce, ice cream essence and rose syrup were screened for their mutagenic activity by Ames assay.

Saccharin is three hundred times sweeter than sugar, so diet makers find it perfect for use in diabetic food products. Aspartame is found in more than 6000 products including health products as well as in pharmaceutical products. Studies of the carcinogenicity of aspartame performed by its producers have been negative. Ice cream essence is used as a flavoring agent in ice creams and its ingredients are propylene glycol and other natural and artificial ingredients (Davidson, 1999). Vanilla is used as a flavor enhancer in 48% of dairy industries. Soy sauce is a salty brown liquid condiment made by fermenting soy beans and roasted wheat or barley in brine with Aspergillus oryzae or Aspergillus soyae. Worcestershire sauce is a flavoring agent and rose syrup is used for making rose flavored ice cream. Chilly sauce are prepared from a mixture of chilly paste or chilly solids derived from clean and wholesome ripened chilies, sugar , vinegar and edible salt. It contains finely ground, clean and wholesome garlic, ginger, onion and other suitable spices or their extracts.

MATERIALS AND METHODS

SAMPLE PREPARATION

Saccharin (sweetex), aspartame (sugar free) and flavouring agents were obtained from commercial stores. Solutions of these sweeteners were made by dissolving 100mg /100ml in

distilled water and five different concentrations were tested. . Undiluted solutions of soy sauce, worcestershine sauce, chilly sauce and rose syrup were used at a concentration of 10, 20, 30, 40, 50 µl /plate. Undiluted solutions of ice cream essence and vanilla essence at a concentration of 1, 5, 10, 50 and 100 µl / plate were used for the assay.

AMES /MICROSOME MUTAGENICITY ASSAY

The Salmonella/microsome reversion assay was conducted using the pre incubation procedure described by Maron and Ames (1983). TA 98 (detects frame shift mutations) and TA 100 (detects base pair substitution mutations). The strains of Salmonella typhimurium were obtained from Microbial Type Culture Collection and Gene Bank (MTCC) IM Tech, Chandigarh, INDIA.

The samples were analyzed with and with out the hepatic S9 fraction. Based on the recommendations of Czygan et al., 1973, phenobarbitol induced sparague – Dawley male rats weighing approximately 200gm were used. Phenobarbital at a concentration of 500 mg/kg was administered intraperitoneally for 3 days before sacrifice. On the 3rd day of induction the rats were killed by cervical dislocation. To insure a clean S9 preparation, the liver was removed aseptically using sterile surgical tools.

PREPARATION OF LIVER HOMOGENATE S9 FRACTION

For the preparation of liver S9 fraction all steps were carried out at 0-4°C, using cold sterile solutions and glassware. The freshly excised liver was placed in a pre weighed beaker containing approximately 1 ml of chilled 0.15M KCl per gram of wet liver. After weighing, the liver was washed several times in fresh, chilled KCl.

Successive washings in KCl were done to ensure a sterile preparation and to remove hemoglobin, which can inhibit the activity of the cytochrome P450 enzymes. The washed liver was transferred to a beaker containing three volumes of 0.15M KCl (3ml/gm wet liver) and was minsed and homogenized. The homogenate was centrifuged for 10 minutes at 10,000 rpm and the supernatant (S9) was decanted and stored immediately at -20°C. The S9 required for the mutagenicity assay was thawed at room temperature and placed in container of crushed ice.

The S9 mix or S9 hepatic fraction was made as soon as S9 thawed. The standard S9 mix was prepared following the method of Maron and Ames (1983). Sterility of the S9 mix was determined by plating on minimal glucose plates. The protein concentration of S9 was determined by the procedure of Lowry et al., 1951. 50 µl of S9 mix was added to each plate to get a final concentration of 0.03mg/plate.

PRE INCUBATION ASSAY

In sterile test tubes, added 0.1 ml of overnight culture of tester strains (TA 98 /TA 100), appropriate concentration of test chemicals, 0.1 ml of sodium phosphate buffer (without S9) and 0.05ml of S9 (with S9). After incubation, each tube was mixed with 2 ml of top agar (0.6% agar, 0.6% Nacl) and plated onto minimal agar plates. Each chemical was tested at five different concentrations both in presence and absence of S9. Both TA 98 and TA 100 were used separately for all the test chemicals. Positive controls used were sodium azide (0.1µg/ plate) for TA100 and daunomycin (0.5µg/ plate) for TA98. Solutions of these chemicals were prepared in distilled water. Sterile distilled water was used as negative control. After 48 hours the revertant colonies on the test plates and on the control plates were counted, and the presence of the background lawn on all plates were confirmed.

RESULTS AND DISCUSSION

MUTAGENIC EFFECTS OF ARTIFICIAL SWEETENERS – SACCHARIN AND ASPARTAME

In both sweeteners, the number of revertant colonies in the strain TA 98 and TA 100 was high compared to the negative control. There was a dose related increase over the control set (Fig 1 & 2). The positive control compounds for the respective strains gave mutagenic responses as expected. A significant triple fold increase in the number of revertant colonies over negative control plates (40g concentration and above) was observed in TA 100. TA 98 showed a significant increase in the number of revertants at 50g concentration to that of negative control. A compound is considered as mutagen in Ames assay if it produces a reproducible dose related increase in the number of revertant colonies in one or more strains of Salmonella typhimurium and doubling of spontaneous reversion rate at one or two test chemical concentrations (Mathur et al., 2005).

Metabolic activation with S9 showed slight increase in the number of revertants in saccharin whereas with aspartame, multiple fold increase in revertants was observed. This may be due to metabolization of this compound into the potent, cumulative, mutagenic toxicants, formaldehyde and formic acid (Jeffrey and Williams, 2000). When Oyama (2002) tested formaldehyde directly on rat cells, 1mM/L

formaldehyde was found to cause damage. When comparing both the sweeteners, aspartame was found to be highly mutagenic.

MUTAGENIC EFFECTS OF FLAVOURING AGENT'S

There was no dose related increase over the control set, in both vanilla and ice cream essence (Fig 3& 4). This indicates that in the in vitro Ames Salmonella mutagenicity tests, these agents failed to induce point mutations as basepair mutation in TA 100 and frame shift mutation in TA 98. There are only a few reports on these flavouring agents indicating as mutagens (Durant and Peter Karrran, 2003). Our study reports the presence of only a few revertants (less than the negative control). Yet a consistent characterization of genotoxicity has not emerged and it status as a carcinogen remains equivocal.

Figure 1

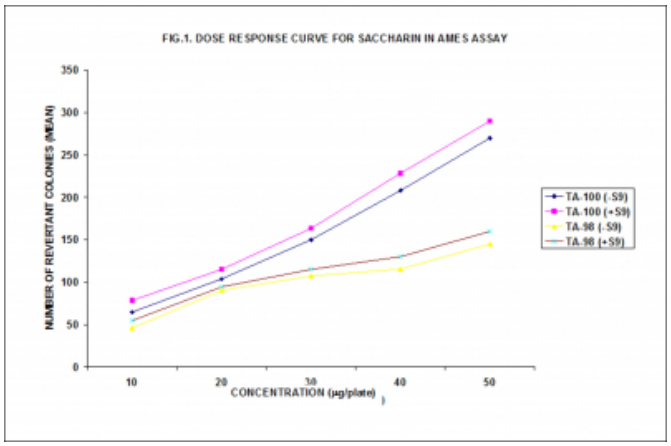
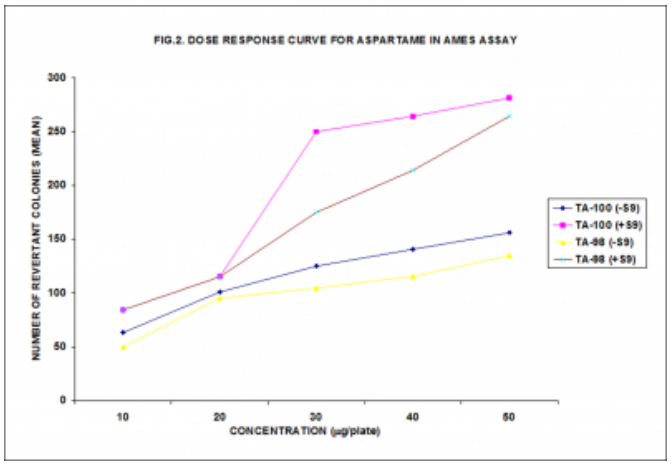


Figure 2



Soy sauce in Ames assay, with and without metabolic activation showed a dose related increase in the number of revertants (Fig 5). The mutagenic potential can be clearly seen from 20µl concentration onwards in TA100. Metabolic activation with S9 does not show significant differences. The present findings are consistent with the previous genotoxicity tests performed on soy sauce

In both TA 98 and TA 100, two-fold increase in the number of revertants was observed showing the mutagenic potential of worcestershire sauce (Fig 6). This may be due to the contaminants or the fermentation end products. Metabolic activation with S9 does not show significant differences.

Rose syrup, with TA 100 and TA 98 showed significant increase in the number of revertants (Fig 7), but when compared to soy sauce, the number of revertants is less. This shows that rose syrup can induce frame shift and base pair mutations. Chilly sauce also produced more revertants both in presence and absence of S9 in TA 98 and TA 100 (Fig 8).

From this study, the commonly used artificial sweeteners saccharin and aspartame may cause mutations. The flavoring agents soy sauce and worcestershire sauce possess mutagenic responses and therefore should be used only under conditions of absolute necessity.Vanilla and ice cream essence were found to be non mutagenic. Chilly sauce and rose syrup in the presence and absence of S9 showed significant increase in the number of revertants indicating their mutagenic potential. Further studies to confirm the presence of DNA lesions are warranted in these food additives.

Figure 3

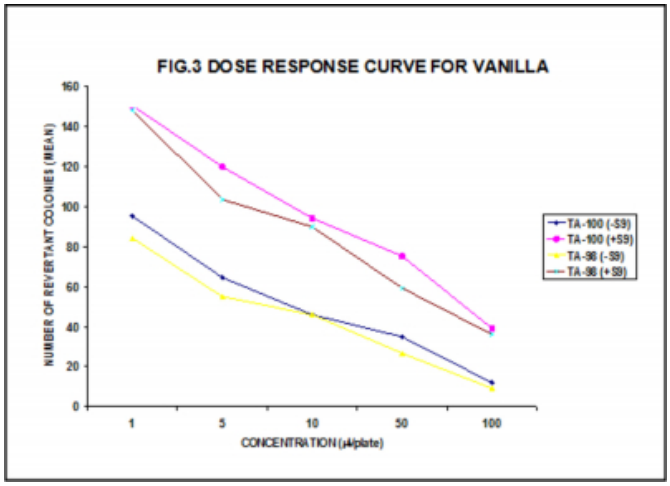


Figure 4

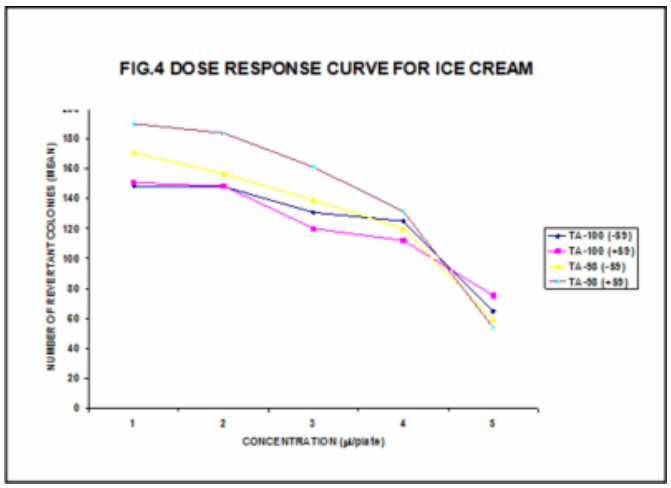


Figure 5

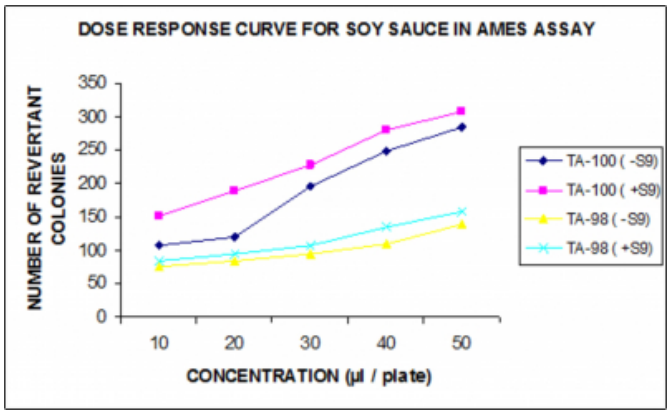
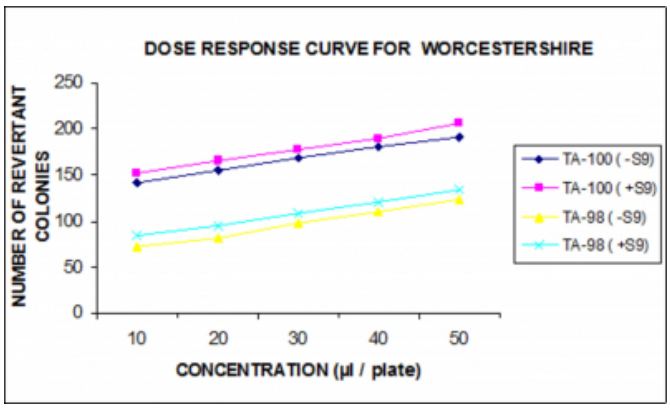


Figure 6



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