Int. J. Chem. Sci.: 10(4), 2012, 1943-1950
ISSN 0972-768X
www.sadgurupublications.com

RAPID RESPIROMETRIC TOXICITY TESTS FOR EVALUATING POSSIBLE CYTOTOXICITY OF PACKAGED FOOD

N. MATHUR*, S. PAREEK, P. YADAV, A. SINGH and R. ATRI

Environmental Molecular Microbiology Lab, Department of Zoology, University of Rajasthan, JAIPUR – 302004 (Raj.) INDIA

ABSTRACT

Over the last serval years the market of packaged and canned food has strategically grown and lack of time further is making people dependant on these food products. Packaged and Canned foods are known to contain food additives and chemicals in large amount. To the majority of food additives added Joint FAO/WHO expert committee on food additives and Food and Agricultural Organisation have imposed certain limitations. However, most of the food additives are being used which are either not safe or are being used in quantities exceeding the statutory limits. Therefore there is need for screening of these food products for cytotoxicity analysis.

The purpose of this study was to assess the cytotoxic effect of packaged food products and to develop a bio-assay using commercial dry yeast as as a test organism, which enables rapid screening of variety of packaged food available in the market.

Key words: Rapid respirometric test, Microbial toxicity bioassay, *S. cerevisiae*, Cyto toxicity, Packaged food.

INTRODUCTION

Vast population base and rapid lifestyle transformation, particularly among those living in urban areas, has resulted into a dramatic increase in demand for processed or packaged food and ready to eat food products. Unfortunately, most processed food are laden with artificial sweeteners, salt, artificial flavours, factory created fats, colourings and chemicals that alters textures and techniques of preservation in food industries to increase the shelf life of food. Many food additives have been reported to have carcinogenic and genotoxic effects[1-3]. Moreover coatings on the canned food which is of bisphenol A

*Author for correspondence; E-mail: nupurmathur123@rediffmail.com; Ph.: +91-141-2707927

diacylether was also found, to have genotoxic effect[4]. Thus evaluation of harmful side effects of packaged food is considered as an important issue in food safety[5].

Various short term screening methods have been developed to detect mutagenic/ carcinogenic substances. They have played an important roles not only in screening suspected chemicals but also in studying the mechanisms of mutagenesis and carcinogenesis, and have provided useful information for assessing the genetic effects of chemicals on humans[6]. Yeast has several attributes, which makes it attractive as test organisms for the rapid screening of chemicals in food. It has relatively short life cycle and, therefore, responds rapidly to chemical toxicity. It is easily handled and inexpensively maintained. Its rate of multiplication is such that a large number of homogeneous organisms are available for use in toxicity test procedures[7].

A current approach for assessing cytotoxicity is to monitor respiratory activity, a sensitive, non-specific subcellular target site[8]. Microbial dehydrogenase activity can be used to evaluate microbial viability because in microbial respiration, the dehydrogenases participate in the transport of electrons from substrate to final electron acceptors in an electron transport system (ETS). Tetrazolium salts act as artificial electron acceptors along ETS, becoming reduced to form insoluble formazans; therefore microbial activity can be assessed using 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyltetrazolium chloride (INT) reduction to the photometrically measured end product, INT-formazan[9].

The purpose of the present study was to develop a simple, rapid and practical toxicity test, based on monitoring changes in respiratory activity of Saccharomyces cerevisae. Further, using this test, potential genotoxicity of a number of packaged food products was evaluated.

EXPERIMENTAL

Materials and methods

Samples

The toxicity of the following food products was investigated: canned fruits, packaged fruit juices, packaged soup and fresh raw vegetables and fruits.

Fresh vegetables and fruits were taken peeled and crushed in a grinder and their extracts were taken to assess their cytotoxic potential.

Baker's yeast assay

Baker's yeast assay[10] was carried out by preparing a 1% (v/v) suspension of yeast in sterilized saline solution (0.85% NaCl) as the suspending fluid. The yeast suspension was stirred for 15 minutes to break up yeast floc using magnetic stirrer. Food sample (0.2 mL) was added to 0.8 mL of yeast suspension and incubated for 30 minutes at 30^{o}C with shaking. To this, 0.1 mL of INT solution (0.2%) and 0.1 mL of 10% solution of yeast extract were added and the mixture was incubated in the dark at 30^{o}C for 1 hr with shaking. The reaction was stopped with 0.1 mL of 37% formaldehyde.

The proportion of respiring cells was determined as follows: one or two loopfuls of the yeast suspension was spread on a glass slide, air dried, counterstained with 0.025% malachite-green and blotted after 1 minute; 500 cells were examined with bright field microscopy (100X) and the number of respiring cells (green cells with red INT-formazan cryatals) and non-respiring cells (green cells) were determined and counted.

The proportion of respiring cells was calculated following exposure to each food sample concentration. Results were expressed as percent inhibition compared with negative controls. EC50 values and EC 20 values (concentration exhibiting 50% and 20% reduction in the percentage of respiring cells) were calculated using regression analysis. All data presented in this study represent means of duplicate runs.

Statistical analysis

EC 50 and EC 20 values were calculated utilizing software XLSTAT 2012.1.01-Dose effective analysis.

RESULTS AND DISCUSSION

Rapid lifestyle transformation, particularly among those living in urban areas, has resulted into a dramatic increase in the demand for packaged and canned food. These foods contain chemicals either in the form of packing material, canned coatings, food additives, etc. Wide varieties of packaging materials are used now days in food industry to increase the shelf life of food. These packaging materials include plastics, tinplate, and tin-free steel etc.[11] Under normal conditions of use small amounts of the plasticizers have been reported to migrate into the food. Phthalate plasticisers have reported to possess carcinogenic effect as revealed in some toxicological studies[12]. In present study a rapid and reproducible assay based on respiratory activity inhibition of *Sacchromyces cervisae* was used to evaluate the

toxicity screening and assessment of different packaged and canned food and also fresh raw vegetables and fruits.

The results of respirometric test for four packaged food and fresh fruits and vegetables are summarized in Table 1. Dose related response curve of packaged food samples and fresh fruits and vegetables is depicted in Figure 1 and Figure 2, respectively.

Table: 1: Respiratory activity responses and regression coefficients of packaged and fresh foods

Baker's yeast assay			
Food samples	**EC20*2**	**EC50***	**R2**
Company -1 Orange fruit Juices	0.0093	0.9043	0.309
Company-2 Orange fruit juice	0.2913	4.3631	0.184
Canned Fruits	0.0096	1.7797	0.246
Packaged soups	0.1081	8.4151	0.81
Spinach	0.55999	7.4868	0.211
Cabbage	0.294	9.6673	0.122
Pomegranate	0.7593	10.8316	0.195
*EC values in Dose (µL)			

Among all the tested food samples minimum values of EC 20 and EC 50 were obtained for Company-1 orange fruit juice. (Inhibition of 20% and 50% respiring cells at the concentration as low as 0.0093 and 0.090 respectively), so it was found to be most cytotoxic.

Among the fruit juices tested from two different companies i.e. Company-1 Orange Juice and Company-2 Orange juices, it was found that there is significant difference in their EC20 and EC50 values. Fruit juice from Company-1 having higher cytotoxic potential (Inhibition of 20% and 50% respiring cells at the concentration as low as 0.0093 and 0.090, respectively for company-1 while EC 20 and EC 50 value for Company-2 was 0.2913 and 4.3631 much higher than that of Company-1).

From Table 1, it can be visualized that canned food also possess significant cytotoxic potential though its EC 20 and EC 50 values are less than that of fruit juices. Cytoxicity of canned food can also be due to migration, in the food, of epoxy based solution coatings, which are used for laquer coating on food cans on food storage vessel[13-14]. This epoxy resin

phenol and its hydrolysis products when examined for genotoxicity, they were found to be genotoxic.

As seen from the dose response curve showing percent of respiration inhibition with increasing concentration of packaged food and canned food (Fig. 1), maximum cytotoxicity was seen in case of Tropicana fruit juice. (EC 20 and EC 50 values 0.0093 and 0.090, respectively). Further Packaged soups and canned food samples were found to be toxic to eukaryotic yeast cells. Least cytotoxicity was shown by packaged soups (with EC 50 values as high as 8.4151).

Cytotoxicity in case of preserved food samples might be due to preservatives and additives added in them. There are many chemicals that get on or into foods during production, processing, or packaging other than preservatives and can result into cytotoxicity for e.g. residues of pesticides, herbicides, and fungicides used on fruits and vegetables; residues of detergents used in washing foods; and residues of detergents and sanitizers used on utensils and equipments which are likely to carry over into foods.

Further, when raw vegetable and fruits were analyzed (Fig. 2), it was observed that compared to packaged or canned foods, raw products had much lesser toxicity. Spinach showed maximum cytotoxicity (with EC 20 and EC 50 values at 0.599 and 7.4868) followed by cabbage (with EC 20 and EC 50 values at 0.294 and 9.6673, respectively). Compared to fresh vegetables fresh fruits were found to posses lesser cytotoxicity. Pomegranate was found to have EC 20 and EC 50 values as high as 0.7593 and 10.8316.

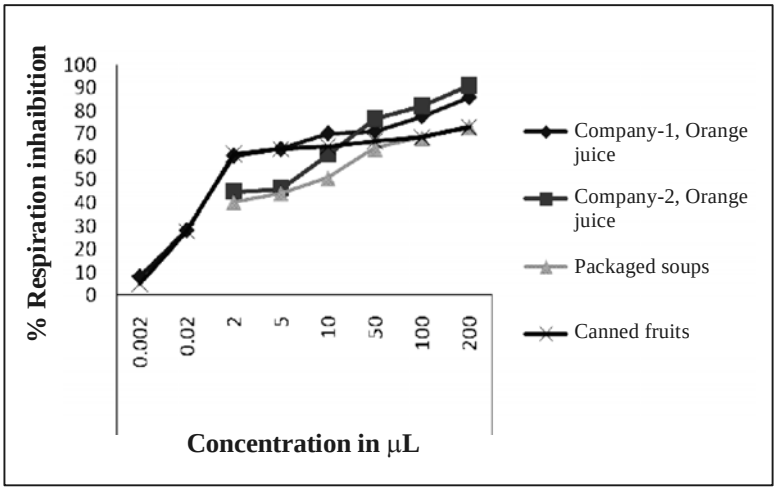

Fig. 1: Dose response curve of packaged food products

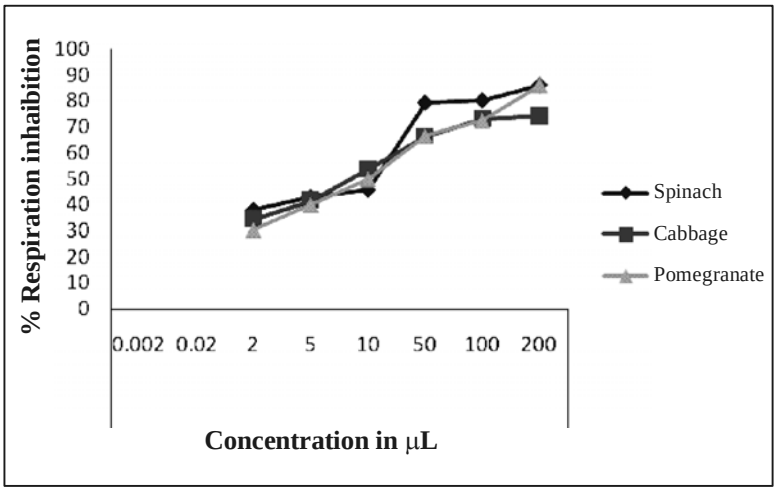

Fig. 2: Dose response curve of fresh fruits and vegetables

It is therefore evident that the raw fruits and vegetables are not having genotoxic potential. However, the steps of packing or addition of food additives or preservatives are making these food products cytotoxic.

Thus, there is an urgent need to pre-screen and analyzed packaged food products before they can be marketed for human consumption. Further, Baker's yeast assay has proved to be simple, rapid and inexpensive assay to test cytotoxicity. Baker's yeast, readily available in dry pellets from commercial sources, is inexpensive and stable at room temperature for relatively long periods. No tedious and time consuming culture techniques are necessary since the commercial preparations may be used simply by suspending them in saline or deionized water. The cells can be easily observed using a low cost compound microscope. The assay can also be adapted to field conditions. Baker's Yeast assay give result in 24 hours so it can be effectively used for pre-screening of large number of variety of food products, before and after their marketing.

For the food samples tested a dose related increase in the number of dead cells can be clearly seen by Fig. 1 and Fig. 2.

ACKNOWLEDGEMENT

The authors are thankful for the financial support provided by UGC Major Project entitled "Genotoxicity evaluation of wastewaters discharged from hospitals in Jaipur city" (File No. 40-113/ 2011).

REFERENCES

1. A. N. Gomurgen, Cytological Effect of the Potassium Metabisulphite and Potassium Nitrate Food Preservative on Root Tips of Allium Cepa, L. Cytologia, **70**, 119-128 (2005).

2. L. Growther, R. Parimala, G. Karthiga, J. Vimalin Hena, K. Kalimuthu and K. B. Sangeetha, Food Additives and Their Mutagenicity, The Internet J. Nutrition and Wellness, **7** (2009).

3. T. Sifa, Genotoxicity of Five Food Preservatives Tested on Root Tips of Allium Cepa, L. Mutat. Res. Genet. Toxicol. Environ. Mutagen., **62**, 4-14 (2007).

4. S. Suarez, R. A. Sueiro and J. Garrido, Genotoxicity of the Coating Lacquer on Food Cans, Bisphenol A Diglycidyl Ether (Badge), its Hydrolysis Products and a Chlorohydrin of Badge, Mutation Research, **470(2)**, 221-228 (2000).

5. S. H. Shoeibi, N. Rahimifard, B Pirouz, R Yalfani, S. R Pakzad, S, Samiee and M. Hamedani Mutagenicity of Four Natural Flavors: Clove, Cinnamon, Thyme and Zataria Multiflora Boiss, J. Medic. Plants, **8**, 89-96 (2009).

6. N. Mathur, P. Bhatnagar, P. Bakre, Assessing Mutagenicity of Textile Dyes from Pali (Rajasthan) using Ames Bioassay, Applied Ecology and Enviorn. Res., **4**, 111-118 (2005).

7. G. Bitton, Bacterial and Biochemical Tests for Assessing Chemical Toxicity in the Aquatic Environment, A Review, CRC Crit. Rev. Environ. Control, **13(1)**, 51-67 (1983).

8. M. E. Haubenstricker, S. E. Holodnick, K. H. Mancy, M. J. Brabec, Rapid Toxicity Testing Based on Mitochondrial Respiratory Activity, Bull. Environ. Contam Toxicol., **44**, 675-680 (1990).

9. R. J. Dutton, G. Bitton and B. Koopman, Rapid Test for Toxicity in Wastewater Systems, Toxic Assess, **2** (1986) pp. 147-158.

10. G. Bitton, B. Koopman and H. D. Wang, Baker's Yeast Assay Procedure for Testing Heavy Metal Toxicity, Bull Environ. Contam. Toxicol., **32**, 80-84 (1984).

11. K. Marsh and B. Bugusu, Food Packaging-Roles, Materials, and Environmental Issues, J. Food Sci., **72**, 39-55 (2007).

12. S. I. Shibko and H. Blumenthal, Toxicology of Phthalic Acid Esters used in Food-Packaging Material (1973) pp. 131-137.

13. P. A. Tice and J. D. McGuiness, Migration from Food Plastics, Part I, Establishment and Aims of the Pira Project, Food Addit. Contam., **4**, 267-276 (1987).

14. P. A. Tice, Pira Project on Migration of Monomers and Overall Migration, Food Addit. Contam., **5**, 373-380 (1988).

Revised : 07.08.2012 *Accepted : 09.08.2012*