Identification of Radiation Effects on Carcinogenic Food Estimated by Ames Test

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Received: 5/8/2015 Accepted: 20/10/2015

ABSTRACT

A major concern in studies related to carcinogenesis is the exposure to the exogenous carcinogens that may occur in food in both natural and polluted human environments.

The purpose of the present study is to examine some food products by Ames test to find out if food products carcinogenic then expose food to gamma radiation to find out the effect of radiation on it as a treatment.

In this study, the food samples were examined by Ames test (Salmonella typhimurium mutagenicity test) to find out that a food product could be carcinogenic or highly mutated. Testing of chemicals for mutagenicity is based on the knowledge that a substance which is mutagenic in the bacterium is more likely than not to be a carcinogen in laboratory animals, and thus, by extension, present a risk of cancer to humans. After that food products that showed mutagenicity exposed to gamma radiation at different doses to examine the effect of gamma radiation on food products.

This study represent γ radiation effect on carcinogenic food by using Ames test in the following steps: Detect food by Ames test using Salmonella typhimurium strains in which the colony count/plate for each food sample will show if food is slightly mutated or highly mutated or carcinogenic. If food is highly mutated or carcinogenic with high number of colonies/plate, then the carcinogenic food or highly mutated food exposed to different doses of radiation. The applied doses in this study were 0, 2.5, 5, and 10 (K Gy). Detect the radiation effect on food samples by Ames test after irradiation.

The study shows that mutated and carcinogenic food products estimated by Ames test could be treated by irradiation.

Keywords: Ames test, Carcinogenic, Salmonella typhimurium, γ irradiation.

1. INTRODUCTION

Our environment is full of potential carcinogens (cancer-causing agents) such as UV light, industrial pollutants, pesticides, food additives, and natural products such as tobacco. It is estimated that 90% of all carcinogens also are mutagens, and these carcinogens can cause cancers because they are mutagens (chemicals that cause mutation). They can change the nucleic acid sequence of DNA (¹). Many chemicals in our environment can cause genetic mutations and are potentially responsible for millions of cancer-related deaths.

The main purpose of nutrition is to provide energy for current and future consumption. Otherwise, food is necessary for body growth, maintenance, and tissue repair (²).
Food additives are substances added to preserve flavor or improve the taste and appearance of food. The continuous consumption of these food additives could be hazardous to human health. Nowadays, scientists are looking for food materials which can potentially prevent cancer occurrence. 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 and carcinogenic activity. There is a high correlation between carcinogenicity and mutagenicity. The importance of food contaminants in the link between diet and cancer has been widely studied and formal risk assessments are routinely completed by several governmental and international agencies (3).

The effect of sodium azide on the anaerobic carbohydrate metabolism of living yeast cells has been studied by many investigators. Sodium azide induces the production of a larger amount of carbon dioxide from a given quantity of glucose. Sodium azide is a rapidly acting, potentially deadly chemical that exists as an odorless white solid when it is mixed with water or an acid, sodium azide changes rapidly to a toxic gas with a pungent (sharp) odor. It also changes into a toxic gas when it comes in contact with solid metals (for example, when it is poured into a drain pipe containing lead or copper). The odor of the gas may not be sharp enough, however, to give people sufficient warning of the danger. The objective of the present study aims to demonstrate the efficacy of irradiation treatment for change the number of colonies of S. typhimurium with carcinogenic material (sodium Azide) of different types of food (4, 5).

Because carcinogenesis is a multistep process that can be affected by chemicals in a variety of ways, for simplicity, it is assumed that the carcinogenic processes can be categorized into two major modes of action. The first is a direct DNA-reactive mode of action whereby the compound or its active metabolite(s) reacts covalently with DNA in target cells to form chemical specific addition products (adducts) which leads to procarcinogenic mutations, followed by neoplastic transformation and neoplasm induction. The second mode of action is epigenetic in nature, in which the compound produces effects in target cells that either indirectly lead to neoplastic transformation or facilitate the development of neoplasms from cytogenetically transformed cells. Health Canada has identified the need for a standardized department wide approach for the risk assessment of carcinogens in foods (6). (e.g., pesticides, food chemical contaminants, veterinary therapeutics).

Ames test is a basic toxological test that can be used to determine if a substance is potentially genotoxic (7). It is a very easy and cheap test that can be set up in most labs. The Ames test is based on the assumption that mutagenicity is associated with carcinogenicity, and that mutagenic activity in bacteria is predictive of mutagenic activity in humans. The test was first validated in a study of 300 chemicals. Most of which were known carcinogens (8). It was subsequently validated in studies by the Imperial Chemical Industries (9), the National Cancer Research Institute in Tokyo (10) and the International Agency for Research on Cancer (11). The Food and Drug Administration (FDA) now uses the Ames test to screen many chemicals. Ames test measures the mutagenic strength of various chemicals in bacterial cells. It is a widely used method for testing potential mutagens because it does not require actual use of animals and it is inexpensive to perform. Mutagenic chemicals cause the mutant strains to revert back to the wild type (11).

Gamma irradiation technology has positive effects in preventing decay by sterilizing the microorganisms and by improving the safety and shelf-stability of food products without compromising the nutritional or sensory quality (12, 13). The process of food irradiation involves exposing the foods to ionizing radiation so that a prescribed quantity is absorbed radiation sources used for food irradiation are gamma-rays from the nuclides Co-60. Because irradiation does not heat the treated material, the food keeps its freshness (fish, fruits, vegetables) and its physical state (frozen or dried commodities) (14).

The study aims to detect the radiation effect on carcinogenic and mutated food products estimated by Ames test.
2. MATERIALS AND METHOD

For each test, the following factors must be considered: the amount of sample available for testing, the number of tester strains to be used, the exogenous activation requirements, the appropriate spontaneous and positive controls, the selection of the dose range of the sample to be tested, and the number of replicate plates needed for an appropriate analysis. In order to create reproducible results, manufacturer’s instructions and all procedures must be followed in as precise a manner as possible. Average calculated for the results of food product samples by Ames test and irradiation.

2.1. Sample Preparation

The Salmonella mutagenicity assay (Ames test) was performed according to the methods of Ames et al. 1975 (8) and Maron and Ames, 1983 (15). Fourteen food samples were used to be investigated by Ames test. Food samples include: dry food (Potato chips with three flavors (cheese, kabab, tomato), biscuits, noodles, powder juice, powder milk, infant milk), food with fats (beef luncheon, chicken luncheon, tomato paste, tuna, hard cheese) and produced food (cake improver, galley, chicken stock, Pepsi, chocolate, bonbons, juice). The food materials used in this study are commonly used and sold in the Egyptian market as prepared food forms.

For each sample, there are several preprocess steps before starting Ames test. The dry foods and non-powdered foods are smash and for beef luncheon, chicken luncheon smashed by machine called stomacher and weigh a mass of 100 g for each sample. Then, they were dissolved in distilled water as much as four times the weight of the sample. This water is to be sterilized and pipes, tips, top agar used in the sterilizer under the temperature of 121°C for 20 mints. Then the resulting liquid is filtered using a filter paper in sterile tubes. Finally, the resulting solution is filtered again through a syringe filter in order to be free from bacteria. We reiterate the previous steps three times to calculate the average of the final product.

2.2. Ames Test

The Salmonella mutagenicity assay (Ames test) was performed according to the methods of Ames et al. 1975 (8), and Maron and Ames, 1983 (15). Reverse mutation assays, the Salmonella, histidine reversion assay developed by Bruce Ames and coworkers, the revertant colonies are clearly visible in a uniform background lawn of auxotrophic bacteria. Each tester strain reverts spontaneously at a frequency that is characteristic of the strain. The number of revertants that arise spontaneously during the 48-h incubation is dependent on the final number of auxotrophs on the plate and that number is a function of the histidine concentration. We emphasize that the number of spontaneous revertants per plate is completely independent of the initial number of bacterial cells plated, within the limits, roughly, of $10^5$ to $10^8$ cells (16). The auxotrophs (background bacteria) are not counted but their number is assumed to be constant because the histidine concentration is constant. Acceptable ranges of spontaneous reversion may be somewhat different in different laboratories, but they should be relatively consistent within a laboratory. The test detects specific types of genetic events (point mutations) that occur at specific locations on the chromosome. Within our laboratory, each strain is maintained as a frozen permanent culture. They are opened only to subculture the strains for additional frozen permanent cultures or to prepare master plates. Prior to its use in a mutagenicity assay, several different concentrations of S9 fraction should be tested for metabolic activation potency against three known indirect-acting mutagens (17).

First, the materials and tools were sterilized. The sterilized 10ml broth inoculated with 100 culture of Salmonella typhimurium (TA100, TA98), incubated at 37°C for 16 hrs. Top agar was sterilized along with the histidine (1µg / 10ml) and sterilized agar media was poured to the sterilized petri plates with minimal glucose agar and allowed to be solidified. Bacterial culture, test chemical and S9 mix (rat liver extract) are incubated for one hour.
The top agar are incubated and water bath temperature of 45°C were used to keep the top agar in its liquid state without freezes and minimal glucose agar plates placed incubated inside the nursery to dry for 30min.

Fresh bacterial culture should be used for test and incubation time for the overnight fresh bacterial culture in nutrient broth oxioid no 2 should not be more than 16 hours for first time only they used it directly from the refrigerator and test the media every time in use to ensure that it can be used with incubate it for 24 hours in 37°C in single test tube.

Figure 1 represents the Ames test procedure in which the left hand side of the figure shows the control negative plate in which the colonies number/plate was few and the right hand side of the figure reveals the positive control plate in which the colonies number/plate was high with a carcinogenic material and in the middle the sample plate is represented.

The Protocol colony counter from the synopsis used in the present studies was obtained from first medical international and also used by Robert Mandavi Research Institute (RMI). The protocol counter is used to count plates in order to obtain a single-colony count per plate.

2.3. Interpretation of Mutagenisis

Mutagenesis by Ames test is evaluated by mutagen index \(^{(18)}\). Mutagenic index (MI) is the number of reverent colonies in test plate/ number of reverent colonies in control plates. In the present study, the number of reverent colonies in control plates was 106 colonies.

2.4. Irradiation

The food samples which investigated with the Ames test were irradiated using cobalt-60 Indian Gamma unit, at dose rate of 10KGY/38min at the National Center for Radiation Research and technology (NCRRT), Atomic Energy Authority, Cairo, Egypt. The applied doses in this study were 0,
2.5 kGy, 5kGy, and 10K Gy. After an irradiation, the samples were transferred and Ames test was performed again.

3. RESULTS AND DISCUSSION

According to the Ames theory which presented in 1982, in case the number of colonies on positive control medium (contained carcinogen) is two times more than the test sample. Salmonella typhimurium TA100 and TA98 are used for Ames test. The mutant strain, in need of histidin, directly receive from professor Ames (19).

**Fig. (2):** Ames test colonies counting for several samples the horizontal line represents food samples (Potato chips with cheese flavor, Luncheon meet, Luncheon chicks, Biscuits, Cake improver, powder milk, Noodles, Gully, Chicken stock, Tomato paste Tuna, Juice, Pepsi, Chocolate, Infant milk, Bonbons, Potato chips with kabab flavor, Potato chips with tomato flavor and Hard cheese) and the dash line represents the control positive for this test.

Figure 2 shows the colonies counting of Ames test for all the selected food samples. The selected powder milk, Noodles, Gully, Chicken stock, Tomato paste, Tuna, Juice, Pepsi, Chocolate, Infant milk, bonbons, Potato chips with kabab flavor, Potato chips with tomato flavor and Hard cheese. The control positive value (104 colonies per plate). These results show that, the number of colonies per plate excesses the control positive in potato chips and beef luncheon samples. These results proved that, there are four food samples have carcinogenic.

The carcinogenic food samples were irradiated using cobalt-60 source, at dose rate 10K Gy/38min; the applied doses (0, 2.5kGy, 5kGy, and 10 K Gy) were in agreement with Thayer, Josephson and Brynjolfsson in 1996 (20).

Figure 3 represents the variations in the carcinogenicity represented by the number of colonies in each potato chips with cheese flavor plate samples. The result showed that, the number of colonies represents the chips sample without being exposed to radiation shows more density than the number of colonies represents the chips sample exposed to radiation dose. It can be noticed that the colonies counting decreases with the increase of the irradiation doses.
Figure 4 shows the colony characteristics of Salmonella typhimurium on the plates of luncheon meet samples without and with different radiation doses. By comparing these plates, significant difference in the colonies density between these plates could be noticed. The results indicated that high colonies number of reverters was found on plate without irradiation and decreased after irradiation.

The relationship between the mutagenic interpretation (MI) and the radiation doses were drawn in the flowing figure. The average mutagenic interpretations are plotted on the y-axis, and radiation doses on the x-axis.

Figure 5 represents the mutagenic index variation with different radiation doses 0, 2.5, 5 and 10 K G y for the food samples which show a high degree of mutation when examined by Ames test (potato chips with cheese flavor, potato chips with kabab flavor, Potato chips with tomato flavor, beef
Table 1 shows the mutagen index for food samples that represent a high mutation degree (Potato chips with cheese flavor, Potato chips with kabab flavor, Potato chips with tomato flavor, Beef luncheon, chicken luncheon, infant milk, hard cheese, tomato paste and bonbons) variation with different gamma radiation doses.

Table 1 shows that the mutagen index of carcinogenic and mutated food products decreases with increasing the radiation dose and this is in agreement with Ahn H.J. et al at 2002 (21).

4. CONCLUSION

- The present study investigated the effect of $\gamma$ radiation on the mutated and carcinogenic food affected by carcinogenic material which estimated by Ames test.
- Ames test was used to detect the mutated and carcinogenic food samples in several food samples. Each sample has a larger colony count than control negative value with double value or more were irradiated with different doses (2.5, 5 and 10 K Gy).
- Ames test was repeated after irradiation for each carcinogenic and mutated food sample to show the effect of $\gamma$ irradiation as a safety tool for food.

The experiment shows that gamma radiation can be used as a treatment for carcinogenic and mutated food products which identified by Ames test (Salmonella typhimurium reverence test) using bacterial strains with specific characters.

5. REFERENCES


(8) Ames, B.N., McCann and J. and Yamasaki, Methods for detecting carcinogen and mutagens with the salmonella/ mammalian – microsome mutagenicity test at Mutation. res 31(1975), pp.347-364


(13) Kamat, A., Pingulkar, A., Bhushan, K., Gholap, B. and Thomas, A., Potential application of low dose gamma irradiation to improve the microbiological safety of fresh coriander leaves at Food Control, 14 (2003), pp.529–537.


