STUDY ON THE MUTAGENECITY OF INDUSTRIAL AND TRADITIONAL BREADS PRODUCED IN IRAN BY AMES ASSAY

[1]Haddad, Khodaparast,M. H. and [*]Moazzami, Ataollah,

[1]Food science department of Ferdowsi University of Iran; *Food scince department of Upsala University of Sweedn . E-*mail: haddad1945@yahoo.com*

ABSTRACT

In this study the Dichloromethane (DCM) extracts of three samples comprising traditional and industrial breads (Sangak, Taftoon and Lebanese) produced by direct flame in Mashhad-Iran were studied by Salmonella-microsomal assay (Ames test) to determine the mutagenic effect of compounds generated during baking process. The study indicated that the compounds extracted from breads had mutagenic effect on TA98 and TA100 strains of *Salmonella typhimorium*. The mutagenecity onTA98 was more than TA100 and the mutagenic effect of Lebanese extract was more conspicuous than Sangak and Taftoon.

INTRODUCTION

Benzopyrene is the first chemical compound of polycyclic aromatic hydrocarbons, which its relation with cancer was discovered in 1775. The heating of carbonates in the absence of oxygen forms heterocyclic aromatic compounds. However, high amounts of these substances are presented in smoked products and also barbecued beeves (6).

Most of these compounds have carcinogenic properties and in the presence of metabolic activators, have mutagenic effects on TA98 and TA100 strains of *Salmonella typhimorium*. Formation of these compounds in food products has a direct relation with temperature. For instance, increasing the temperature of starch from 370 to 650°C will change the concentration of Benzopyrene from 0.7ppb to 0.17ppb. High temperature causes formation of identical substances of amino acids and fatty acids (6, 8).

In ordinary conditions of bread baking, the temperature of bread surface reaches to 400°C. In new processes of bread production, increase of temperature causes the possibility for the formation of this kind of components. Temperature raise, increases reactions of Maillard and pyrolysis of amino acids as well. The components, formed by this way, are heterocyclic amines and also have mutagenic effects onTA98 and TA100 (2, 4, 9).

In the following research, a comparison between several amounts of formed compound during different industrial and traditional heating processes on bread production have been examined.

MATERIALS AND METHODS

1. **Samples**: The experiment is done on three different samples each divided into three, for examining the effect of mutagen agents and their mutagenic effects were studied after a week.

2. Extraction: For the extraction of hydro cyclic substances, instructions of Stavric (1993) were carried out (7). In this way, first, sample was dried until reaching constant weight according to standard methods, and then crushed. Samples were weighed and acidified by solution of 0.2 N

HCl and extracted by Dichloromethane within 48 hours. In this step, Dichloro-methane was separated from samples and after the removal of whole solvent, to perform Ames test, four concentrations, according to the dry contents of primary samples, of final extract were dissolved in a solution of Dimethyl sulphoxide.

3. Mutagenecity test: This test is based on increasing the numbers of *Salmonella*'s colonies in minimal glucose culture due to the reversible mutation, which its mutagen agents are studied. Mutation contributes the ability of growth in minimal histidineless media to those strains, which need histidine in natural conditions. To activate these mutagen agents, activator enzymes (S9) were used. In the first step, the phenotype of the strains was confirmed according to the Ames instructions and numbers of spontaneous returns of TA98 and TA100 in the presence of metabolic activators was defined 25 and 90 respectively. According to Ames assay, (S9) metabolic activators were extracted from mouse liver that had been treated by enzyme inducer. During mutagenecity test, 0.5 ml of S9 was added to a sterile tube; next 0.1 ml of a one-branched culture media with the density of $2 .10^9$ cfu / ml and 0.1 ml of extract solution were added. After 20 minutes incubation at 37°C and addition of 2 ml agar from the surface of minimal glucose culture, the collection was cultivated. These plates were incubated at 37°C for 48 hours and after observing the grassy-green colonies, their numbers were counted as a measure for the presence of mutagen agents in culture media.

RESULTS AND DISCUSSIONS

In biological Ames assay, the increase in concentration of mutagens in plate will increase the number of bacterial colonies and if this increasing occurs in linear order with high gradient, the mutagenecity of studied samples will be confirmed. In the performed experiments, all samples had mutagenic effects on both TA98 and TA100 strains. While effects on TA98 were more (Figure-1).

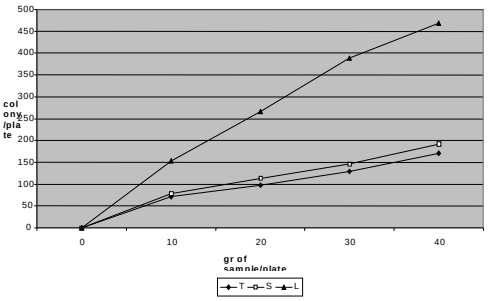

Figure-1: Mutagenic effects of bread extracts on TA98

TA98 is sensible to the mentioned mutagen agents, which will be "Framed shift" after their mutagenic action. Substances resulted by Maillard reaction and pyrolysis of amino acids; also heterocyclic and polycyclic aromatic compounds are some of the examples of this group of components, which are formed at high temperatures in food products. With the increase of temperature, there will be an increase in these compounds (1). In this research, mutagenecity levels in Lebanese samples for both TA98 and TA100 strains were more than of Sangak and Taftoon. The difference can be the cause of the fact that during the baking process, Lebanese bread was exposed to direct flame at 350°C to 400°C whereas Sangak and Taftoon were baked at 250°C to 270°C.

During thermal processes and the increase of temperature, reactions between naturally occurring carbohydrates and amino compounds form some materials, which can make "Base Substitution Mutation"in TA100 strain. Formation of

these kinds of substances depends on the temperature of the baking process. In Lebanese baking in comparison with other samples, high amounts of these compounds are the result of high temperature (Figure 2).

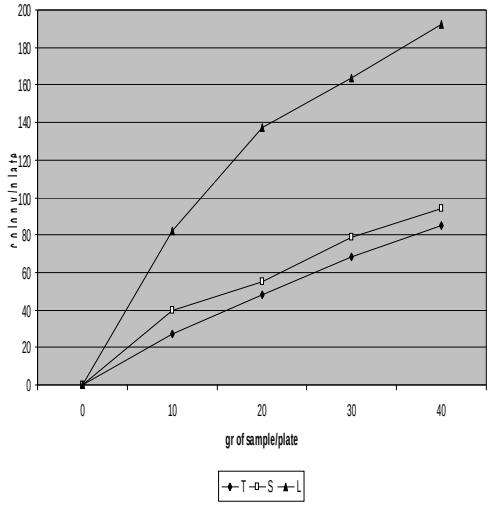

Figure2: Mutagenic effects of bread extracts on TA100 **L**: Lebanese, **S**: Sangak, **T**: Taftoon

CONCLUSION: The use of high temperature and also direct flame in Lebanese production will increase the amount of mutagen agents in this kind of bread comparing to the other samples. During the use of direct flame, the temperature of bread surface will rise immediately at contact point of fire and this can form some polycyclic aromatic compounds in bread surface. Experiments have shown that a 200°C increasing in temperature will cause an increase of 100% in mutagenecity of Lebanese comparing to Sangak and Taftoon. Concentrating on the relation between mutagen agents and outbreak of cancer and also carcinogenic characteristics of aromatic compounds, which are produced in these samples due to high temperature, more attention on choosing the proper temperature is necessary. As a result of the experiments, it is strongly recommended not to use direct heat in bread baking process.

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