Genotoxicity of Brown Parts of Some Ready Meals

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ABSTRACT

Recently, it was proved that some carcinogenic, mutagenic compounds such as heterocyclic aromatic amines (HCAs), polycyclic aromatic hydrocarbons (PAHs) and acrylamide (AA) formed during the cooking and roasting of foods, are known to induce tumors in rodent bioassays and may thus contribute to human cancer risk. A survey of some local meals were checked using the cytokinesis-block micronucleus assay on human lymphocytes *in vitro*, the mutagenicity values expressed as the ratio of binucleated (BN) to mononucleotide cells (MN). Different samples were collected from local markets including brown roasted poultry, brown or black parts of grilled fish, chips (potato, rice and maize), rusted brown layer of local bread and normal kids' candy. The results showed increased frequency of micronuclei formation in human lymphocytes *in vitro* from most of the tested samples. The BN/ MN ratios were: roasted brown parts in potato chips (44.8), roasted poultry parts(30.5), grilled fish (18.5), bread (14.3), whereas, lower values were recorded in potato chips with cheese flavour (12.6), candy kids' (12.5) and crisps corn-rice for kids (5.8).

The present work introduces alarm to re-evaluate the procedures used for preparation of ready meals and calls for the abandon of ingestion of brown parts of food during eating. Further studies are recommended to extrapolate this *in vitro* study on animal models *in vivo*.

Key words: ready meals, genotoxicity, heterocyclic aromatic amines, acrylamide.

INTRODUCTION

Cooking of food is a process unique to humans. It enhances the taste and the digestibility of food. However, it induces profound changes in all types of food. It has been well established that these changes may be of concern to human health (Sugimura & Sato, 1983, Felton & Knize, 1991, Felton *et al*., 1997). General cooking procedures such as broiling, frying, barbequing, heat processing and pyrolysis of protein rich foods like beef, chicken and fish induce the formation of potent mutagenic and carcinogenic compounds called heterocyclic amines (HCAs) (Ohgaki *et al*., 1991, Wakabayashi *et al*.,1992, Adamson *et al*. ,1994). These are potent mutagens and carcinogens in rodents, inducing tumors of several organs (Sugimura *et al*., 1983, Ohgaki *et al*., 1991)and in limited studies in monkeys (Phillips,1999). Both polycyclic aromatic hydrocarbons (PAHs) and also nitrated (nitro-PAHs) can be generated through thermal processing of foods such as drying, baking, grilling and smoking (Adamson, *et al*., 1994). However, these compounds also occur more widely as environmental pollutants through emission from a wide range of combustion sources including vehicle exhausts, furnaces, etc. and may also enter the human food chain through deposition on the surface of food crops (Durant *et al*., 1996).

Millard reactions or non-enzymatic browning involves amino acids with reducing sugars that undergo thermal treatment and often employed in cooking and industrial food processing. Millard reaction products (MRPs) are consumed regularly as part of the daily human diet, especially with bakery products, coffee, caramel or beer (Gerhardsson de Verdier *et al*., 1991, Faist *et al*., 2001, Murtaugh *et al.*, 2004). However, some reports regarding the possibility of presence toxicity of different MRPs (Gerhardsson de Verdier *et al*.,1991). In April (2002), scientists in Sweden-quite unexpectedly-discovered large amounts of acrylamide (AA)in foods rich in starch that have been heated at high temperatures., these included crisps, French fries, bread and crisp breads (Kim *et al*., 1991, FAO/WHO, 2002, European Commission, 2002).

The present work introduces fast simple technique to determine genotoxicity in brown parts of some common ready meals in Egypt.

MATERIALS AND METHODS

Materials

Samples of different fast food meals were collected from local markets in Cairo, Egypt. Samples included brown parts from Balady bread (common bread in Egypt), potato chips (two types, one as po-

tato free from additives and the other with cheese taste), and corn-rice crisp product. Roasted poultry was purchased from the local market. Whereas, grilled fish (Tilipia variety) was prepared at the laboratory and broiled. Also, sweet candy (caramel candy) samples were purchased locally. Only, dark brown parts were selected from bread, poultry, fish, potato chips whereas the processed food were tested directly for the genotoxicity test.

Methods:

Genotoxicity test

Preparation of blood samples

Heparinized blood was obtained from six healthy, non-smoker volunteers who had no recent diagnostic or occupational exposure to ionising radiation, laser, or chemicals and have no allergic responses. Buffy coats were separated and concentrated in plasma at a cell density of 2×10^5 cells /100ml. Aliquots of cells were distributed in 96 well tissue culture plates (Nunc, town and country). Every treatment was investigated in duplicate.

Cell Culture

Immediately, after addition of food samples, cells were transferred into 15-ml sterile plastic round bottom tubes containing only medium 199 (Sigma, Saint Loius MO, USA). Cells were incubated for 72 hours, adding cytochalasin B 48 hours before harvesting.

Harvesting of Cells

Forty eight hours after the addition of cytochalasin B, cells were collected and treated with 0.8% sodium citrate for 3-5 min and then fixed in methanol: acetic acid (5:1). Fixed cells were gently dropped onto clean microscope slides, air-dried and stained with 4% Giemsa (Sigma, Saint Loius MO, USA) using standard procedures (Fenech, 993).

Scoring Under the Microscope

Slides were scored at 1000X magnification using a Leica Biomed microscope (Leica Lasertechnik GmbH, Heidelberg, Germany). Identification of cytokinesis blocked binucleated cells and the frequencies of micronuclei in such cells were estimated according to the criteria stated (Fenech,1993). Binucleated cells were selected on the basis of having a well-preserved cytoplasm with two distinct nuclei of approximately equal size, which may be attached by a fine nucleoplasmic bridge or alternatively be overlapped. The micronuclei scored were therefore located within the cytoplasm and were not refractile nor linked to the main nuclei *via* nucleoplasmic bridge. From each culture, the ratio of binucleated (BN) to mononucleated cells (MN) was determined by counting the number of BN per 2000 MN. Additionally, 500 binucleated cells were scored for micronuclei.

RESULTS AND DISCUSSIONS

Heated food systems contain hundreds of chemical compounds, some being mutagenic and others being antimutagenic. Studies have indicated that food exposed to drying, frying, roasting, baking, and broiling conditions possess net mutagenic activity besides nonenzymic browning reactions which are involved in developing of mutagenicity. Caramelization and Millard reactions are responsible for producing melanoidins.Heat treatment of food is used extensively to increase the palatability of food. Baking, roasting, broiling, and frying of food cause the formation of flavour compounds and brown pigments.

The obtained results of tested samples are shown in Table (1) and Fig. (1). Potato chips, poultry and fish samples recorded high percentages of genotoxicity.

Table 1: Ratio* of binucleotide (BN) to mononucleotide cells (MN)as influenced by different roasted fast meal parts

Samples	Replicates				Average ± SD
Blank samples	2	2	1	3	2.0±0.707
Roasted bread spots	11	20	13	13	14.25±3.4
Potato chips					
additives free	41	54	39	45	44.75±5.76
chees tasty	10	14.5	15	11	12.63±2.2
Corn-rice crisps (Sandose)	5	10	4	4	5.75±2.5
Poultry (rosted)	20	34	42	26	30.5±8.29
Fish (grilled with wheat bran)	13	26	12	23	18.5±6.1
Sweet candy (caramel candy)	12	18	120	10	12.5±3.28

* The ratio of BN/ MN was determined by counting the number of BN per 2000 MN.

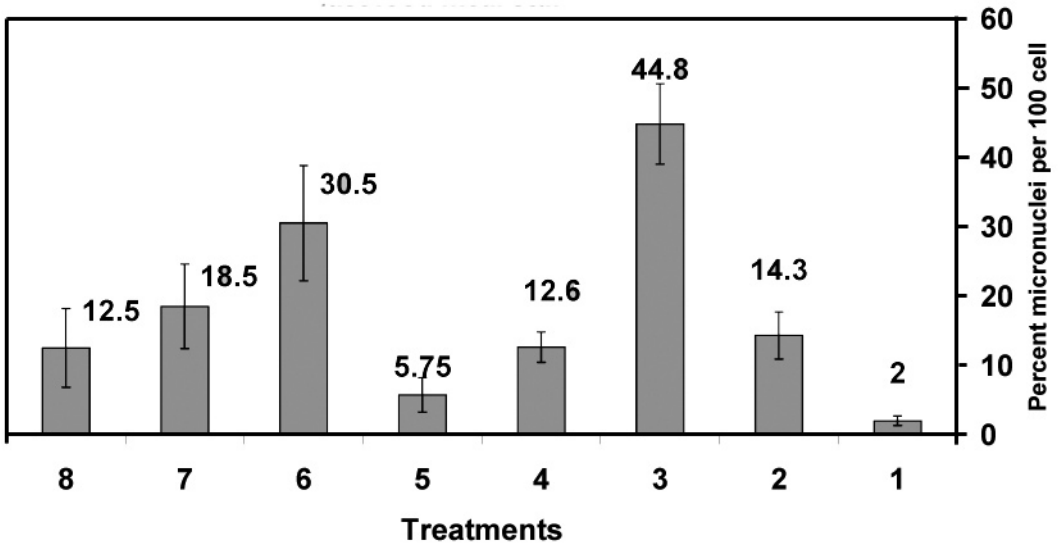

Fig. 1: Genotoxicity of roasted surface parts of different types of fast food mean stuffs

These values were significantly different from blank value. Samples of potato chips, poultry and fish samples recorded 44.8, 31.0 and 19.0, respectively. Whereas, significantly less values were found in bread, chips (with cheese taste) and sweet candy as 14.25, 12.6 and 12.5, respectively. In the same time, corn-rice crisp samples -recorded the significantly lowest values.

The brown surface samples especially meat and fish recorded high values of BN/ MN ratio as compared to blank samples. Our results are in harmony with those obtained by O'Brien & Morrissey (1989) and Jing & Kitts (2002) who reported that cooking at high temperature over 150°C either for roasting, frying, or broiling, initiate mutagenic compounds as HCPs and PAHs. Also, Lee & Shibamoto (2002) and Stadler, *et al* (2002) showed that HCA are presented with values ranged from 0.1 to 50 ppb, depending on the food and cooking conditions. Additionally, published reports suggest that risk of colorectal cancer or adenoma may be increased among individuals who consume meat with a heavily browned surface, but not among those who consume meat with a medium or lightly browned surface (European Commission, 2002, FAO/WHO, 2002, Jing & Kitts 2002).

Concerning, genotoxicity of potato chips which recorded high values (44.8), this may be attributed to the presence of HCA and acryl amide as showed in some publications. (Kim *et al*, 1991, FAO/WHO, 2002, European Commission, 2002). Further analytical studies revealed that processing of food rich in starch and protein is the main source of acrylamid (AA). The presence of some amino acids as asparagine, glutamine, methionine and cysteine plus glucose provided the essential ingredients for AA formation when heated above 120°C (Stadler *et al*, 2002).

Caramel candy results low level of genetoxicicty, which may be attributed to Millard reaction only at low temperature as showed by Skog *et al*. (1998).Although, Millard reaction products (MRPs) are consumed regularly as a part of the daily human diet. Some reports describe the different MRPs as mutagenic towards salmonella species (Stoner, *et al*., 1997). Although the products of these reactions impart important colours, flavours, and antioxidant properties to food, some of them also have toxic properties (Sugimura & Sato, 1983, Felton & Knize, 1991, Ohgaki *et al*., 1991, Wakabayashi *et al*., 1992, Adamson *et al*., 1994, Phillips, 1999).

As shown in Fig. (2), microscopic photos of cytokinesis-block micronucleus assay results on human lymphocytes *in vitro*. The extracts of the treated samples (the brown spots) showed positive results as different types of small micronuclei (Fig. 2). The reduction in genotoxicity in potato chips samples may be attributed to the presence of some spices which may play a role as antioxidants and partially preventing mutagenicity. Therefore, to reduce the genotoxicity effects, some advices can be followed as using rich sources of antioxidants such as some medicinal plants, spices, fruits and vegetables which have beneficial effects in preventing the development of many types of cancers (Sugimura & Sato, 1983, Felton & Knize, 1991,

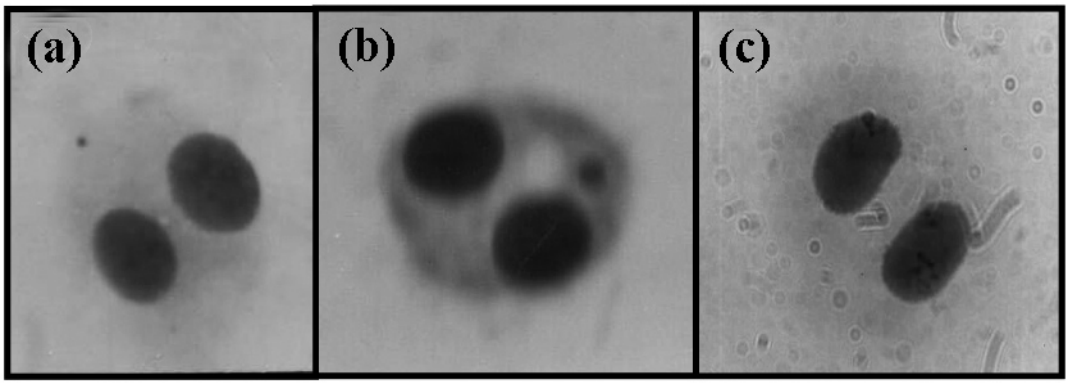

Fig. 2: Microscopic photos of micronuclei after exposure to genotoxic agent from food samples

(a), (b) cytochalsanin (b) blocked lymphocyte showing a small micronuclei indicating genetic instability due to exposure to a genotoxic agent, (c) cytochalsanin showing no small micronuclei from a control sample

Ohgaki *et al*.,1991, Wakabayashi *et al*.,1992, Adamson *et al*., 1994, Felton *et al*., 1997). Also, reducing cooked meat and fish in the daily diet can be advised to avoid HSAs (Yen & Hsieh, 1994, Oguri *et al*., 1998, Monti *et al*., 2001). In the same way by marinating of foods, removing the skin of cooked chicken or fish, and cutting away charred or burned areas in meat prior to consumption (Yen & Hsieh, 1994, Oguri *et al*., 1998, Monti *et al*., 2001).

Finally, common dry heat cooking methods used for preparing food could be the source of health hazards due to producing genotoxic or carcinogenic compounds as HCAs, PAHs ,AA or others. But alternative methods as indirect vapour, low thermal methods, using antioxidants sources with minimizing hazard sources can solve this problem.

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التأثيرات السمية على المستوى الجيني للأجزاء الغامقة في الوجبات الجاهزة

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ثبت حديثاً على حيوانات التجارب تواجد بعض المركبات المسببة للسرطان مثل المركبات الأمينية الحلقية، الهيدروكربونية الحلقية، الأكريلاميد والتي قد ينتج بعضها أثناء إعداد الطعام على درجات حرارة مرتفعة في تحمير الأغذية. وأوضحت هذه التجارب سمية عالية لهذه المركبات وكذا احتمال اشتراكها في إحداث سرطان للإنسان.

ويتناول هذا البحث استخدام تكنيك يسمى بالـ Cytokinesis-block micronucleus على كرات الدم البيضاء للإنسان *in vitro* حيث يؤدي وجود المركبات السامة إلى إنقسام الأنوية، والنتائج يعبر بها بنسبة الأنوية الشاذة إلى الطبيعية. وقد تم اختيار العينات التالية من الوجبات السريعة من السوق المحلي بالقاهرة حيث استخدمت الأجزاء البنية في كل من:

- الدواجن المحمرة، السمك المشوي بالرده، شرائح بطاطس (محمرة).
- شرائح بطاطس (بالأرز و بالذرة)، أجزاء من الخبز البلدي.
- ملبس الأطفال (كاندي).

وأثبتت النتائج على المستوى المعملي أن:

- أعلى النتائج سمية كانت في شرائح البطاطس المحمرة بنية اللون (٤٤.٨) ثم الدواجن (١٨.٥)، الخبز البلدي (١٤.٣).
- كانت أقل القيم في شرائح البطاطس المحمرة بطعم الجبن (١٢.٦)، ملبس (كاندي) للأطفال (١٢.٢٥) ثم شرائح البطاطس بالأرز والذرة (٥.٨).

ويدق هذا البحث ناقوس خطر ينبه إلى ضرورة اتباع الطرق الصحية السليمة لإعداد الوجبات السريعة لتلافي تكوين الأجزاء بنية اللون والمحترقة مع عدم الإسراف في تناول هذه النوعية من الوجبات السريعة بجانب ضرورة الإكثار من استخدام المواد الطبيعية المحتوية على مضادات الأكسدة. كما يحتاج هذا الأمر لمزيد من البحوث المستفيضة خاصة تحت الظروف المحلية على حيوانات التجارب مباشرة.