Food Chemistry 162 (2014) 206–214

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Genotoxicity of processed food items and ready-to-eat snacks in Finland

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ARTICLE INFO

Article history:
Received 31 August 2013
Received in revised form 6 March 2014
Accepted 13 April 2014
Available online 24 April 2014

Keywords:
Ames test
Food processing
Ready-to-eat food
Industrially processed food
Genotoxicity
Mutagenicity
Cytotoxicity

ABSTRACT

Processed foods are an insufficiently characterized source of chemical mutagens for consumers. Here, we evaluated the genotoxicity of selected food products in Finland. Mutagenicity was determined by the standard plate incorporation assay followed by methylcellulose overlay and treat-and-wash assays, using the *Salmonella* strains TA 100 and 98 with and without metabolic activation. Generally, the mutagenic activity of food samples was low, but exhibited lot-wise variation. Cold cuts of cold-smoked beef, grilled turkey, and smoked chicken (a single batch of each) were mutagenic in all three assays with the TA 100 strain with and without metabolic activation, indicating the mutagenic effect was not secondary to histidine release from the food products. However, none of the food extracts showing mutagenic potential induced DNA damage in vitro using the Comet Assay. Our findings imply that in Finland today, there are still products the production methods of which should be refined to reduce the potential risk of mutagenicity to consumers.

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1. Introduction

Food processing is the set of methods and techniques used to transform raw ingredients into food or to modify food properties for consumption by humans or animals. There has been a growing demand for produced foods globally due to changes in the lifestyle of the world population (Akintowa, Awodele, Emeka, & Osajare, 2007). The majority of these foods is processed and preserved using methods (physical and chemical) that have the potential to trigger formation of mutagenic, genotoxic and carcinogenic substances. It has been estimated that 1–2 g of potentially mutagenic substances are consumed by humans every day from food and beverages alone (Ames & Gold, 1990). Previous epidemiological studies have substantially furthered our understanding of the links between diet and genetic toxicity, and cancer (Akintowa et al., 2007; Francois et al., 2010; Sharif, Ghazali, Rajab, Haron, & Osman, 2008; Sinha et al., 2009). These studies have revealed that diet is a key contributor to human cancer with approximately 32% of cancers estimated as being attributable to dietary factors as a whole (Willet, 1998), although the contribution of dietary genotoxic substances is apparently far less prominent (World Cancer Research Fund/American Institute for Cancer Research, 2007).

According to a recent comprehensive analysis of the available literature data, the evidence is convincing for consumption of processed meat as a cause of increased risk of colorectal cancer; in the case of cancers of the oesophagus, stomach and lung, the causal relationship is suggestive (World Cancer Research Fund/American Institute for Cancer Research, 2007). High temperature cooking methods such as pan-frying and grilling/barbecuing produce compounds such as heterocyclic amines and polycyclic aromatic hydrocarbons (PAHs), which are well-known animal carcinogens (Ferguson, 2010). Highly carcinogenic *N*-nitroso compounds are also known to be formed during frying of nitrite-treated bacon and meat (Cross & Sinha, 2004), although humans are mainly exposed to *N*-nitroso compounds via endogenous synthesis in the stomach (Lutz, 1990).

Acrylamide, a compound that has gained considerable attention in recent years due to its high toxicity (Erkekoglu & Baydar, 2010), foremost its carcinogenicity (Hogervorst et al., 2010), and common occurrence in, for example, a variety of snacks, has been shown to form in carbohydrate-rich foods as a result of heat processing (Hogervorst et al., 2010). In the daily diet of the Swedes and the Dutch, product groups including potato crisps, French fries, coffee, bread, biscuits and breakfast cereals contribute more than 90% to the total intake of acrylamide (Konings et al., 2003; Svensson et al., 2003). This has also been reported for Finnish foodstuffs (Eerola, Hollebekkers, Hallikainen, & Peltonen, 2007). On the other hand, heterocyclic aromatic amines are often present in hamburgers (Knize et al., 1998), and commercially sold hamburgers have been reported to possess variable levels of mutagenic activity (Gabbani et al., 1998; Stavric, Matula, Klassen, & Downie, 1995). There are also reports of mutagenic activity in urine of subjects who have consumed foodstuffs processed at a high temperature (Gabbani et al., 1998; Peters et al., 2004).

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http://dx.doi.org/10.1016/j.foodchem.2014.04.055
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The mutagenic potential of commercially processed food products depends on a number of factors related to cooking conditions, such as the equipment used, ingredients, temperature and cooking time, as attested by considerable differences in the mutagenic activity among equivalent products from different manufacturers or restaurants (Knize, Dolbeare, Carroll, Moore, & Felton, 1994; Peters et al., 2004; Tikkanen, 1991; Tikkanen, Sauri, & Latva-Kala, 1993). For example, the Ames/*Salmonella* test shows a correlation between meat-processing temperature and the number of revertant colonies per gram of meat (Peters et al., 2004; Tikkanen, 1991). Also, industrial processing of food has a marked effect on the mutagenic activity of the final product, based on the variations recorded in equivalent products from various manufacturers (Tikkanen et al., 1993).

The presence of mutagenic compounds in commercially heat-processed foods such as meat, fish and poultry products was observed in Finland over twenty years ago (Tikkanen, 1991). Thereafter, a number of toxic compounds have been extracted at varying concentrations from such products in Finland (Eerola et al., 2007; Tikkanen et al., 1993). However, since the methods of preparing these products have probably undergone marked changes over the years, screening of the overall mutagenic potential of commercially produced foodstuffs is warranted to ensure that they do not represent a genotoxic hazard to consumers. To this end, the present study set out to use the Ames test together with complementary assays to investigate the mutagenicity of processed and preserved foodstuffs as well as some ready-to-eat snacks in Finland.

2. Materials and methods

2.1. Materials

All materials used in this study were of analytical grade. The NADP and glucose-6-phosphate used were obtained from Roche Biochem (Stockholm, Sweden). Aroclor-induced S9 from rat liver was purchased from Trinova Biochem (Giessen, Germany). *Salmonella enterica* sv. *typhimurium* strains TA 100 and TA 98 were obtained from Pasteur's Institute (Paris Cedex, France). Histidine, potassium chloride, magnesium sulphate, potassium phosphate dibasic anhydrous and sodium ammonium phosphate were purchased from Merck AG (Darmstadt, Germany). Magnesium chloride hexahydrate and citric acid monohydrate were acquired from VWR international (Leuven, Belgium). Biotin, tryptophan, methylcellulose (MC), dimethyl sulfoxide, benzo[*a*]pyrene, 2-aminoanthracene and sodium azide were obtained from Sigma–Aldrich (Steinheim, Germany).

2.2. Cell line

Human hepatocellular carcinoma-derived cell line (HepG2) was obtained from American Type Culture Collection through LGC standards (Boras, Sweden) and cultured in Eagle's Minimum Essential Medium (LGC standards, Boras, Sweden) containing 10% heat-inactivated fetal bovine serum (Sigma–Aldrich, Steinheim, Germany). The cells were maintained at 37 °C in a humidified atmosphere of 5% CO_2 in air atmosphere incubator (NuAire Inc., Plymouth, USA).

2.3. Sampling

Fourty-five samples of industrially processed and packaged food products and 15 samples of ready-to-eat snacks were evaluated for their mutagenic potential. The industrially processed and packaged food products were purchased from a popular supermarket in Helsinki (Prisma, Viikki), while the ready-to-eat food samples were acquired from a local representative (at SOKOS City Centre) of a global chain of hamburger restaurants also located in Helsinki, Finland. The sample types and manufacturers are listed in Supplementary Table 1. A total of three batches were collected at separate times. The cooking conditions such as time and temperature were not available. We carefully ensured that all products were extracted before the expiry date shown on the packages.

2.4. Extraction

Possible mutagenic compounds were extracted from the food samples using the method described by Peters et al. (2004) with slight modifications. Briefly, 20 g of food sample was homogenized with 80 ml of 1 mol/l NaOH at 24,000 rpm. The homogenate was mixed with 20 g of Extrelut refill material (VWR international, Helsinki, Finland) and then poured into an empty Extrelut 20 column. The organics were eluted from the Extrelut column with 40 ml of dichloromethane/toluene solution (95:5 v/v) into a cartridge. The organics were finally eluted with 5 ml of $MeOH–NH_4OH$ (9:1) solution and evaporated to dryness under a gentle stream of nitrogen in a fume hood. For determination of recovery, fresh meat was spiked with 50 µl of benzo[*a*]pyrene and 250 ng of 2-aminoanthracene in two different cases and extracted in the same way as above.

2.5. Cytotoxicity assays

The cytotoxic effect of the concentrations of food extracts used in this study was investigated by three independent assays measuring trypan blue exclusion, lactate dehydrogenase activity, and boar sperm motility.

2.5.1. Trypan blue test

HepG2 cells were grown in 24-well plates (VWR, Finland) until semi-confluent cells were obtained (48 h). This was followed by exposure of the cells to different concentrations of food extracts for 4, 24 or 48 h. After exposure, the cells were trypsinised using 0.25% (w/v) trypsin–0.53 mmol/l EDTA solution (LGC standards, Boras, Sweden). Trypsinised cells were transferred to 1.5 ml Eppendorf tubes and centrifuged for 5 min at 2,500 rpm. Pellets were then re-suspended in PBS, after which 10 µl of the cells were mixed with 5 µl (0.8 mmol/l) trypan blue dye before microscopic observation.

2.5.2. Lactate dehydrogenase (LDH) assay

The activity of lactate dehydrogenase (LDH) was determined in HepG2 cells exposed to the same concentrations of the food extracts used in genotoxicity assays. The LDH test measures plasma membrane integrity and it was performed according to the instructions provided in the Cytotoxicity Detection Kit^{PLUS} (LDH), version 6 (Roche Biochem, Stockholm, Sweden).

2.5.3. Boar sperm motility inhibition bioassay

Extracts of selected food samples were assessed for their mitochondrial toxicity using the boar sperm motility assay (Andersson et al., 2010). Briefly, 2 ml of boar semen in screw-capped exposure vials was exposed to 10 µl of food extracts for 30 min, 24 or 48 h at 20 °C. Vehicle (DMSO) exposure was prepared simultaneously with the test samples for each time point. After exposure, the vials were shaken gently to disperse the sperm cells, and 200 µl of the suspension was drawn into a warmed test tube and placed in a heating block (30 °C) for approximately 5 min to activate sperm motility. Sperm motility was assessed by dispensing warmed sperm suspension onto a microscopic slide using a pre-warmed capillary tube, and immediately observed with a 40-× inverted phase contrast objective.

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