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# Application of the yeast comet assay in testing of food additives for genotoxicity

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ABSTRACT

Different kinds of additives are widely applied in food industry. The rationale for their use is preservation, coloring or sweetening of diverse foods. Though it has been proven that some additives possess cytotoxic effect they are still used in practice. As a justification of their use is being their low concentration of application. We have used the method of Comet assay to detect minimal concentrations at which a group of selected food additives could damage DNA. Five substances, commonly added in foods and one in pharmaceutical drugs showed DNA damaging effectively at concentrations lower than that used in practice. Additionally, we have compared the sensitivity of higher eukaryotic cells with yeast to genotoxic effect of these compounds. The higher sensitivity of yeast cells revealed by the comet assay was undoubtedly demonstrated.



## 1. Introduction

Different additives have been used in food industry for centuries. Initially, their application was meant only for food preservation by pickling (with vinegar), salting, or by adding sulfur dioxide (as in some wines). Later on, certain additives and supplements have been applied to improve the taste and the appearance of food. With time some of them have been found to possess toxicity and were prohibited from use. For example, the use of the food preservative furylfuramide (AF-2) has been banned since 1974 when it has been proven to be carcinogenic for experimental animals (IARC, 1983) and for humans (Sugimura & Nagao, 1981). Similarly, many azo compounds including Butter yellow demonstrated genotoxic and carcinogenic activity in laboratory animals and therefore were forbidden (Combes & Haveland-Smith, 1982). It has to be noted, however, that regardless the harmful effect of food additives they still are used. Saccharin and its sodium salt, for instance, are extensively used sweeteners primarily because of their value to diabetes patients, although sodium saccharin has manifested carcinogenic effect in experimental animals (IARC, 1999).

Remarkably, some additives and pharmaceuticals can possess minor toxicity but in low concentrations may induce certain genotoxicity (Mpountoukas, Vantarakis, Sivridis, & Lialiaris, 2008). Genotoxins might change DNA by direct cleavage or by modifications of various chemical groups in DNA. Most DNA damages are potentially dangerous for the cell and the organism as a whole. They can lead to cell death or can potentiate carcinogenic processes (Bertram, 2000). The most deleterious effect is executed by modifications in DNA which are then transferred undetected in progeny and thus, can appear later in offspring's life. Therefore, monitoring the genotoxic potential of such substances is of high priority nowadays, especially of those used in food industry.

A large number of methods have been developed to detect genotoxicity (Jenkins & Parry, 2000; Kirsch-Volders et al., 2000; Madle et al., 1994; Maron & Ames, 1983; Parry & Shamsher, 1990; Zimmermann, Kern, & Rasenberger, 1975), however none of them detects *per se* damages in the structure of DNA. Furthermore, many of these techniques are difficult to perform, expensive and require a large number of cells.

Single Cell Gel Electrophoresis (SCGE) also known as Comet Assay has gained popularity as a test for detecting genotoxins (Moller, Möller, Godschalk & Jones, 2010; Ostling & Johanson, 1984).

*Abbreviations:* [1]4-AAP, 4-aminoantipyrine; AF-2, furylfuramide; CA, standard Comet Assay; DMSO, dimethyl sulfoxide; $LD_{50}$, median lethal dose; SCGE, Single Cell Gel Electrophoresis; YCA, Yeast Comet Assay.

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**Table 1**
Detailed description of the tested compounds with their practical applications.

| Compound | Description | Foods, beverages and drugs | Concentrations used in practice [mmol/L] |
|---|---|---|---|
| *Preservative* | | | |
| Sodium nitrite[a] | Sodium nitrate is an inorganic compound. It is used in manufacturing of diazo dyes, nitroso compounds and other organic compounds. The sodium nitrite can interact with secondary amines in stomach, forming carcinogenic N-nitrosamines. | Meat, poultry and game products in whole pieces/cuts, non-heat-treated<br>Cooked fish and fish products<br>Salmon substitutes, caviar and other | 0.1–2.5 |
| *Additive* | | | |
| Caffeine[b] | Caffeine is a plant alkaloid, structurally related DNA purine bases. It is probably the most frequently ingested pharmacologically active substance in the world. At doses relevant to general human consumption, caffeine exerts most of its pharmacological effects by acting as an antagonist of adenosine receptors | Coffee<br>Tea<br>Coca-Cola, energy drinks, soda products<br>Milk chocolate<br>Caffedrine capsules and NoDoz tablets<br>Prolamine | 0.1–14.4 |
| *Coloring agents* | | | |
| Indigo carmine[c] (FD & C [g] blue No 2) | Indigo carmine is an organic compound with a distinctive blue color. Nearly all indigo produced today is synthetic. | Jams, jellies, marmalades<br>Fruit preparations, including pulp, purees, fruit toppings and coconut milk<br>Cocoa and chocolate products<br>Chewing gum<br>Fine bakery wares<br>Fresh fish<br>Soups and broths<br>Gastrointestinal endoscopy | 0.1–4.3 |
| Erythrosine[d] (FD & C [g] red No 3) | Erythrosine is a synthetic dye and used as a food coloring agent. It is a host of other applications such as printing inks, a dental plaque disclosing agent. | Candied fruit<br>Edible ices, including sherbet and sorbet<br>Fermented milks, heat-treated after fermentation<br>Jams, jellies, marmalades<br>Fruit preparations, including pulp and fruit Toppings<br>Sugar-based confectionery including hard and soft candy, nougats, etc.<br>Chewing gum | 0.05–0.5 |
| *Additive* | | | |
| Fast Green[e] (FD & C [g] green No 3) | Fast Green is a synthetic triarylmethane food dye, which is widely used as food colorant. | Canned or bottled (pasteurized) fruit<br>Jams, jellies, marmalades<br>Vegetables and seaweeds in vinegar, oil, brine, or soybean sauce<br>Pre-cooked pastas and noodles<br>Food supplements<br>Aromatized alcoholic beverages (e.g., beer, wine and spirituous cooler-type beverages, low alcoholic refreshers)<br>Egg-based desserts and fruit preparations, including pulp, purees, fruit toppings and coconut milk | 0.1–0.75 |
| *Pharmaceutical metabolite* | | | |
| 4-aminoantipyrine[f] (4-AAP) | 4-AAP is presumed that in the cell 4-AAP undergoes acetylation and that this modified form can cause DNA damage. | 4-AAP is a metabolite of different drugs (aminopyrine, metamizole – a pyrazolone non-steroidal anti-inflammatory drug) with significant analgesic, antipyretic and anti-inflammatory activity. | 0.5–8.4 |

[a] Codex alimentarius commission (2010); OECD SIDS (2005).
[b] IARC (1983); Smith, Smith, Miners, McNeli and Proudfoot (2000); Smith, Gupta, and Gupta (2007); OECD SIDS (2012); Nehlig (2002).
[c] Codex alimentarius commission (2011); Barrows, Lipman, and Bailey (2003); Kobylewski and Jacobson (2010).
[d] Codex alimentarius commission (2011); Abdel Aziz, Shouman, Attia, and Saad (1997); EFSA (2011); Kobylewski and Jacobson (2010).
[e] Codex alimentarius commission (2011); Mittal, Kaur, and Mittal (2009); Kobylewski and Jacobson (2010).
[f] Żukowski and Kotfis (2009); Agundez, Carrillo, Martinez, and Benitez (1995); Agundez, Martinez, & Benitez (1995); Tenga, Liua, Li, and Zhanga (2011); Chen and Chen (2009).
[g] FD & C – Federal Food, Drug and Cosmetic Act.