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Toxicological potential of 2-alkylcyclobutanones – specific radiolytic products in irradiated fat-containing food – in bacteria and human cell lines

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Abstract

Food irradiation has been considered as a safe processing technology to improve food safety and preservation, eliminating efficiently bacterial pathogens, parasites and insects. This study aims to characterize the toxicological potential of 2-alkylcyclobutanones (2-ACBs), radiolytic derivatives of triglycerides, formed uniquely upon irradiation of fat-containing food. In irradiated food they are generated proportionally to fat content and absorbed radiation dose.

The cyto- and genotoxic potentials of various highly pure synthetic 2-ACBs were studied in bacteria and human cell lines. While pronounced cytotoxicity was evident in bacteria, no mutagenic activity has been revealed by the Ames test in *Salmonella* strains TA 97, TA 98 and TA 100. In mammalian cells genotoxicity was demonstrated mainly by the induction of DNA base lesions recognized by the Fpg protein as determined by both the Comet Assay and the Alkaline Unwinding procedure. Formation of DNA strand breaks was observed by the Alkaline Unwinding procedure but not by the Comet Assay. The extent of cytotoxicity and genotoxicity were dependent on chain length and degree of unsaturation of the fatty acid chain. Further studies will have to clarify mechanisms of action and potential relevance for human exposure situation.

Keywords: Food irradiation; 2-Alkylcyclobutanones; Cytotoxicity; Genotoxicity; Mutagenicity; Oxidative DNA lesions

1. Introduction

Food irradiation is gaining interest in light of the increasing incidence of foodborne diseases in the last decades (ICGFI, 1999; WHO, 2002), as it efficiently reduces the population of pathogens such as *Salmonella*, *Listeria*,

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Campylobacter, *Escherichia coli* 0157:H7 and others, but also parasites and insects (Lutter, 1999; Molins, 2001; Steele, 2001). The process has been approved by FDA and WHO, and endorsed also by many countries and organizations (Diehl, 1995; Thayer et al., 1996; IAEA, 2004). The positive list of irradiated products varies between countries but is often limited to spices, herbs, seasonings, some fresh or dried fruits and vegetables, seafood, meat and meat products, poultry and egg products. Irradiation can be performed either with γ-rays produced from a radioactive nuclide such as ^{60}Co or with an electron beam or X-rays generated from machine sources (Codex Alimentarius, 2003; Mermelstein, 1999). The Codex General Standard for Irradiated Foods (Codex Alimentarius, 2003) recommends that the maximum absorbed dose delivered to a food subjected to radiation processing should not exceed 10 kGy. This dose permits the pasteurization of food mostly without altering its organoleptic characteristics (Diehl, 1995; Molins, 2001). Doses above 10 kGy may be applied when necessary to achieve a legitimate technological purpose, e.g. for sterilization purposes such as for hospital meals or foods which need safe long-term storage without refrigeration such as for astronauts, soldiers, mountain climbers or campers (WHO, 1999).

The consideration of food irradiation as a toxicologically safe process by organisations such as WHO, the American Medical Association, the American Dietetic Association, the Institute of Food Technologists and the Institute of Food Science and Technology was mainly based on feeding studies with laboratory animals. Thus, in a very large experiment, the so-called Raltech study, highly irradiated chicken meat was fed to various laboratory animals. The mean radiation dose applied to the chicken meat was a sterilising dose of 58 kGy, and the meat was administered as 35% on a dry weight basis in the animal diet. No evidence of genetic toxicity in the Salmonella/mammalian microsome mutagenicity assay (Ames test) applying five strains of *Salmonella typhimurium,* namely TA 1535, TA 1537, TA 1538, TA 100 and TA 98 was observed when testing the meat extracts. The sex-linked recessive lethal mutation test with *Drosophila melanogaster* also produced no evidence of mutations caused by irradiated chicken meat extracts. No teratogenic effects in mice, hamsters, rats and rabbits were observed. Furthermore, no treatment-related abnormalities or changes were observed in dogs or mice during multigeneration studies. Thus, it was concluded that both the nutritional, genetic and toxicological studies provided no definitive evidence of toxicological effects in mammals due to ingestion of chicken meat sterilized by ionizing radiation (Thayer et al., 1987).

Nevertheless, some indications for potentially toxicologically relevant alterations during food irradiation came from experiments investigating isolated compounds generated uniquely in the course of this process. Thus more than 30 years ago the occurrence of 2-alkylcyclobutanones (2-ACBs) in highly irradiated synthetic triglycerides has been reported (Letellier and Nawar, 1972). Almost more than 20 years later, among the different products that are formed in food upon irradiation, these compounds have also been detected in irradiated fat-containing food such as chicken, pork, lamb, beef and mechanically recovered meat, and also in irradiated liquid whole egg (Stevenson and Hamilton, 1990; Stevenson, 1996). Recently, they have also been identified in irradiated cheese, fish (sardine, trout, salmon), ground beef patties, mangoes, papayas (Ndiaye et al., 1999a; Stewart et al., 2000; Gadgil et al., 2002) and in rice irradiated at a very low dose (0.1 kGy) as well as in pre-cooked meals containing low amounts of irradiated ingredients (Ndiaye et al., 1999b).

The 2-ACBs are formed as a result of the radiation-induced cleavage of triglycerides. They have the same number of carbons (n) as their fatty acid precursors, with an alkyl chain of ($n-4$) carbons in ring position 2 (Stevenson, 1996; Stewart et al., 2000). They have been found exclusively in irradiated fat-containing food, and have until now never been detected in non-irradiated foods treated by other food processes such as freezing, heating, microwave heating, UV irradiation, high pressure processing, or simple preservation treatments (Crone et al., 1992, 1993; Ndiaye et al., 1999a). Thus, under the current analytical limits of detection, these compounds are considered to be unique markers for food irradiation.

With respect to their bioavailability, 2-ACBs were recently detected in the adipose tissue and faeces of rats fed with pure synthetic 2-ACB in the drinking fluid (Horvatovich et al., 2002). Previous experiments using Comet Assay and measuring DNA strand breaks indicated a slight genotoxic potential both in rat and in human colon cells of 2-dodecylcyclobutanone in an *in vitro* study (Delincée and Pool-Zobel, 1998), and also in an *in vivo* experiment with rat colon cells (Delincée et al., 1999). In addition, a tumour-promoting effect in rats exposed to a colon carcinogen has been observed for some 2-ACBs (Raul et al., 2002). Furthermore, a recent study demonstrated the induction of DNA damage detected by Comet Assay as well as an increased frequency of translocations determined by FISH predominantly in LT97 human colon adenoma cells as well as in primary human colon cells after treatment with 2-dodecylcyclobutanone (Knoll et al., 2006). Within this context, an extensive study has been conducted to determine the potential cyto- and genotoxicity of several 2-ACBs varying in chain length in different cell lines, including different bacterial and mammalian test systems. Especially the development of new synthesis methods (Miesch et al., 1999, 2002) enabled the production of sufficient amounts of highly pure 2-ACBs including 2-decyl-(2-DCB), 2-dodecyl-(2-dDCB), 2-tetradecyl-(2-tDCB) and 2-(tetradec-5′-enyl)–cyclobutanone(2-tDeCB), the radiation-induced derivatives of myristic, palmitic, stearic and oleic acids, respectively, to perform these toxicological studies. In addition, also γ-stearolactone, a potential oxidation product of 2-tetradecylcyclobutanone, was included.