Report of the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN) on the application of ionizing radiation for sanitizing fresh meat, meat preparations and meat products

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Abstract

In the EU, food irradiation is regulated by two Directives. Directive 1999/2/EC regulates general and technical aspects for carrying out the process, labeling of irradiated foods and the conditions for authorizing food irradiation and Directive 1999/3/EC provides a positive Community list of foods and food ingredients authorized for treatment with ionizing radiation. So far, this list contains a single food category: dried aromatic herbs, spices and vegetable seasonings, and the authorized maximum absorbed dose is 10 kGy. In Spain, the specific regulation for the treatment of food with ionizing radiation, Royal Decree 348/2001, does not authorize the processing of foodstuffs of animal origin and only allows the treatment of dried aromatic herbs, spices and vegetable seasonings. Nevertheless, taking into account different documents published by the FDA (Food and Drug Administration) and the EFSA (European Food Safety Authority), it can be concluded that meat irradiation might be an effective tool to reduce the content of pathogenic and spoilage microorganisms not considered by Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs. In regard to potential toxicological considerations, foods irradiated in the range established by the legislation seem to be safe and nutritionally suitable. However an increase in quantity and variety of irradiated foodstuffs in the European Union is bound to predictably increase their daily intake in all population ranges and therefore it would be necessary to review the current studies to determine the actual risk due to a larger and ampler intake of radiolytic compounds.

Key words

Ionizing irradiation, meat and meat derivates.

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Introduction

Food irradiation is a high energy non-heat physical treatment that uses ionising radiations. During the treatment, the food is briefly exposed to a source of radiant energy, which may be one of three types: Gamma rays, X-rays or accelerated electrons (EU, 1999a). The process is conducted in an authorised protected installation.

Food irradiation is used for a number of purposes. The most usual objectives authorised by current legislation (EU, 1999a) include the reduction of the incidence of food toxi-infections due to the destruction of pathogenic micro-organisms, preventing microbial alteration with the destruction of the contaminating biota, the reduction in the loss of food products by slowing down or preventing ripening, germination or ageing processes in the foods and the elimination or reduction of plagues harmful to plants and of pesticides.

In the European Union, food irradiation is regulated through two directives. Directive 1999/2/EC (EU, 1999a) regulates the general and technical aspects for carrying out the process, labelling irradiated food and the conditions required to authorise food irradiation; and Directive 1999/3/EC (EU, 1999b) establishes a positive Community list of foods and food ingredients authorised for treatment with ionising radiation. To date, this list contains only one single food category: dried aromatic herbs, spices and vegetable seasonings and the maximum authorised absorbed dose is 10 kGy.

These directives established a deadline for Member States to maintain their previous authorisations provided that the foods and food ingredients concerned were supported by a favourable report from Scientific Institutions or from the Scientific Committee on Food (SCF) of the European Commission, and the absorbed doses did not exceed the established limits. In addition, the European Commission proposes a draft Regulation in relation to ionising radiations, but to date has not initiated any proceedings.

In accordance with article 4, section 6, of Directive 1999/2/EC seven Member States have maintained their previous authorisations: Belgium, France, Italy, the Netherlands, Poland, the United Kingdom and the Czech Republic. Some of them authorise for the treatment of products of animal origin with ionising radiation, as summarised in Table 1.

Table 1. Products of animal origin, the treatment of which using ionising radiation is authorised in Member States

	Belgium	France	Netherlands	United Kingdom	Czech Republic
Chicken meat	Yes	Yes	–	–	Yes
Farmed Poultry	Yes	Yes	–	–	Yes
Poultry	Yes	–	–	Yes	Yes
Mechanically recovered poultry meat	Yes	Yes	–	–	Yes
Offal of poultry	Yes	Yes	–	–	Yes
Frozen frog legs	Yes	Yes	Yes	–	Yes
Dehydrated blood, plasma and coagulates	Yes	Yes	–	–	Yes
Fish and shellfish	Yes	–	–	Yes	Yes
Frozen shrimps	Yes	Yes	–	–	Yes
Prawns	–	–	Yes	–	–
Egg white	Yes	Yes	Yes	–	Yes
Casein and caseinates	Yes	Yes	–	–	Yes

Source: List of Member States' authorisations of food and food ingredients which may be treated with ionising radiation (EU, 2009).

Nevertheless, in accordance with the latest report from the Commission on foods and food ingredients treated with ionising radiation, in 2011, only Belgium, France and the Netherlands made use of their authorisations for irradiating food products of animal origin (EU, 2012).

In Spain, the specific regulation governing the treatment of food with ionising radiation, Royal Decree 348/2001 (BOE, 2001), only permits the treatment of aromatic herbs, spices and vegetable seasonings, excluding all other food products.

In 2004, the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN) prepared a report relating to the application of ionising radiation to food. However, the report was published prior to the new European legislation on food hygiene, completed in 2005. Therefore, the Scientific Committee of the AESAN is asked to draw up a report on the application of ionising radiation for the hygienisation of fresh meat, meat preparations and ready-to-eat meat products, in which the following should be assessed:

1. The efficiency of the application of ionising radiation for the hygienisation of fresh meat, meat preparations and ready-to-eat meat products, with consideration for the existence of control measures for microbial contamination already established under European Union legislation.
2. Possible risks arising from the intake of products treated with ionising radiation for the health of consumers.

Definition of products referred to in the terms of reference of this report

1. Definition of meat, fresh meat, minced meat and mechanically separated meat

In accordance with Regulation (EC) No 853/2004 (EU, 2004b), "meat" is defined as the edible parts, including blood and offal, of the following animals:

- Domestic ungulates: domestic bovine (including *Bubalus* and *Bison* species), porcine, ovine and caprine animals, and domestic solipeds.

- Poultry: farmed birds, including birds that are not considered as domestic but which are farmed as domestic animals, with the exception of ratites.
- Lagomorphs: rabbits, hares and rodents.
- Wild game: wild ungulates, lagomorphs and birds, as well as other land mammals that are hunted for human consumption, including mammals living in enclosed territory under conditions of freedom similar to those of wild game.

"Fresh meat" means meat that has not undergone any preserving process other than chilling, freezing or quick-freezing, including meat that is vacuum-wrapped or wrapped in a controlled atmosphere.

"Minced meat" means boned meat that has been minced into fragments and contains less than 1 % salt. "Mechanically separated meat" (MSM) means the product obtained by removing meat from flesh-bearing bones after boning or from poultry carcasses, using mechanical means resulting in the loss or modification of the muscle fibre structure.

2. Definition of meat preparation

In accordance with Regulation (EC) No 853/2004 (EU, 2004b), "meat preparations" are defined as fresh meat, including meat that has been reduced to fragments, which has had foodstuffs, seasonings or additives added to it or which has undergone processes insufficient to modify the internal muscle fibre structure of the meat and thus to eliminate the characteristics of fresh meat.

3. Definition of meat product

"Meat products" are processed products resulting from the processing of meat or from the further processing of such processed products, such that the cut surface shows that the product no longer has the characteristics of fresh meat (EU, 2004b).

4. Definition of ready-to-eat food

In accordance with legislation (EU, 2005), "ready-to-eat food (RTE)" is defined as food intended by the producer or manufacturer for direct human consumption without the need for cooking or other processing effective to eliminate or reduce micro-organisms of concern to an acceptable level. In the scope of this report, it refers to meat products, the consumption of which, in general terms, does not require subsequent processing or cooking.

European food hygiene regulation

Regulations (EC) No 852/2004 and 853/2004 (EU, 2004a, 2004b) lay down the applicable bases on the hygiene of foodstuffs for food business operators. Specifically, Regulation (EC) No 852/2004 indicates the general obligations of the industry with respect to hygiene and Regulation (EC) No 853/2004 applies these to food products of animal origin. Both regulations base the exercise of hygiene on the analysis of hazards and the control of critical points and indicate the obligations which food operators must comply with as regards microbiological criteria, in order to guarantee the hygiene of the processes and food safety.

Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs (EU, 2005) and its subsequent amendments (Regulation (EC) No 1441/2007, Regulation (EU) No 365/2010 and Regulation (EU) No 209/2013) establish microbiological criteria for certain micro-organisms, and the applicable regulations that must be met by food operators on applying the general and specific hygiene measures considered in Regulation (EC) No 852/2004. In these regulations, the only pathogens listed in the food safety criteria for meats, meat preparations and meat products are *Salmonella* and *Listeria monocytogenes*, in the case of ready-to-eat products.

Micro-organisms from fresh meat and derived products. Pathways of contamination and recontamination

Micro-organisms that alter meat reach the meat as a result of infection of the living animal (endogenous contamination), or by *post-mortem* invasion (exogenous contamination).

The notable success obtained in the control of pathogens that caused some classic zoonoses following the application in the European Union (EU) of Regulation (EC) No 2160/2003 on the control of salmonellosis and other zoonotic agents (EU, 2003), and more specifically, following the application of the sanitation programmes is of note. This, combined with veterinary inspections of slaughterhouses, prevent the release for consumption of diseased animal meat and carcasses with injuries characteristic of different animal diseases. Nevertheless, the micro-organisms that do not produce symptoms or injuries and that go unobserved in the *ante-* and *post-mortem* health inspections of the animals in the slaughterhouse are becoming more and more of concern.

The possibilities of contamination and microbial growth increase during the processing of the carcass, in the cutting up and in the preparation of meat derivatives, as the surface in contact with the environment is greater. Environmental and handling conditions (equipment, tools, operators, and many other factors), and the characteristics of the meat finally determine the quantity and quality of the micro-organisms present in fresh meat. In view of the huge variety of sources of contamination, the types of micro-organism usually found in meats are many and varied. These micro-organisms include pathogens such as *Clostridium botulinum*, *Clostridium perfringens*, *Bacillus cereus*, *Staphylococcus aureus*, *Salmonella* spp., *Campylobacter* spp., verotoxigenic *Escherichia coli*, *Yersinia enterocolitica*, *Listeria monocytogenes*, hepatitis E virus and *Toxoplasma gondii*.

The types and quantity of micro-organisms present in the meat-based products also depends on the sanitary conditions of the environment from which the meat originates, on the properties and microbiological quality of certain added ingredients, on the care taken by the operators processing and handling the product and on the subsequent conditions for storage, handling and distribution, for example, heat treatment or slicing (Cheftel and Culioli, 1997).

The latest report from the European Food Safety Authority on zoonoses, zoonotic agents and food-borne outbreaks (EFSA, 2013) reveals aspects that require consideration in respect to meat microbiology. In 2011, the pathogen with the highest number of confirmed cases of food-borne toxi-infections produced in humans was *Campylobacter*, with 220 209 cases. In the case of this pathogen, the increase in the number of cases has been constant over the last four years and its presence in poultry meat from all the countries in the European Union continues to be high, with an average of 31.3 % positive samples.

Campylobacter is followed by *Salmonella* with 95 548 cases and a reduction of 5.4 % with respect to 2010, and of 37.9 % with respect to 2007, demonstrating the efficiency of the control programmes on farms, in slaughterhouses and of the products. Minced meat and meat preparations are the product categories in which non-compliance of microbiological criteria was highest.

The number of cases of listeriosis amounted to 1 476, a figure slightly lower than that of 2010, with a percentage of deaths of 12.7 %. The highest number of non-compliances with microbiological criteria occurred in fermented sausages. With respect to verotoxigenic strains of *E. coli*, 9 485 cases were recorded, with a 2.6 % increase with respect to 2010. From the cases in which the serogroup was identified, O157 was the most frequent group. Since 2008, the number of cases has been increasing. In products of animal origin, the most frequent isolations occur in bovine meat. In 2011, 7 017 cases of yersiniosis were confirmed, an increase of 3.5 % with respect to 2010. The most frequent isolations occurred in pork meat and meat products.

As indicated above, the regulation on microbiological criteria (Regulation (EC) No 2073/2005) does not include the obligation to detect in meat, meat preparations and meat products major pathogens including *Campylobacter*, verotoxigenic strains of *E. coli* or *Yersinia enterocolitica*. The presence of these pathogens in raw meat implies that, as a result of cross-contamination, they may reach many other food products, surfaces, industrial equipment and food handlers. Consequently, raw meat, meat preparations and meat products must be handled with extreme care. Heat treatment and other processing techniques may destroy the pathogenic agents present in the meat or the ingredients added, thus guaranteeing the final safety of the marketed product. Nevertheless, it should be remembered that some meat derivatives are sliced after the heat treatment or curing/maturing process, and an additional contamination could be produced from the equipment and surfaces with pathogens such as *Listeria monocytogenes*. The marked tendency of this bacterium to form biofilms should also be considered. *Listeria* attaches itself to surfaces through the synthesis of extracellular polysaccharides. Many studies have shown the resistance of these biofilms to the use of disinfectants. Regulation (EC) No 2073/2005 establishes for ready-to-eat food maximum levels of *Listeria monocytogenes* of 100 CFU/g during the shelf-life of the product. However, in other countries, the criteria established for this bacteria is zero tolerance; that is, the absence of *Listeria monocytogenes* in 25 grams.

Efficiency of the application of ionising radiations in the hygienisation of fresh meat, meat preparations and meat products

Ionising radiation destroys the micro-organisms by causing injury to critical cellular elements, in the majority of cases, genetic material. This damage prevents multiplication and various cellular functions. The damage in the genetic material takes place as the result of a direct collision of the radiant energy or as a result of the ionisation of an adjacent molecule, usually water, that interacts with the genetic material. In addition, radiation produces other effects due to the direct or indirect interaction with various cellular components, such as membranes, enzymes and cytoplasmic elements. These interactions may have a lethal action in themselves, although in the majority of cases they are not lethal provided that there is no damage to the genetic material. These interactions may have a decisive role in the survival of bacteria with sub-lethal alterations, as a cell that has not received lethal

genetic damage can be destroyed by other forms that complicate or prevent the survival of the cell. One important aspect is that the damage is random and is not linked to a specific genetic "target" or cellular component. This circumstance is a significant factor in the explanation of the bacterial mechanisms of radioresistance (AESAN, 2004).

There is a broad variation in the sensitivity of the different organisms to radiation. The radioresistance of the micro-organisms can be ordered from higher to lower as indicated below: virus > bacterial spore > Gram-positive bacteria > Gram-negative bacteria > moulds and yeasts > parasites (AESAN, 2010) (EFSA, 2011b).

Several aspects are of concern to the different scientific and research organisations as regards the use of irradiation on food products and its effect on micro-organisms, the main ones being the possibility of mutation, the transformation into pathogen micro-organisms or the reversal of virulence in attenuated micro-organisms, the stimulation of toxin production, the reduction of natural food microbiota and that this technology be used to substitute correct hygiene practices or to mask altered products, as it would also destroy indicator micro-organisms (AESAN, 2004) (EFSA, 2011b). The involvement of pathogenic micro-organisms in food-borne outbreaks resulting from the elimination of the natural microbiota of the irradiated food is a theory that appears to have been rejected in several studies of irradiated chicken meat and beef, in which it was demonstrated that the growth of *Salmonella* spp. and *Escherichia coli* O157:H7 was the same as in the non-irradiated matrices (Szcazwiska et al., 1991) (Dickson and Olson, 2001).

Mutation in bacteria and other organisms is a well-known process and, although it may occur spontaneously, ionising radiation is one of the processes recognised as mutagenic, similar to other physical and chemical processes (AESAN, 2004) (EFSA, 2011b). However, while it does not appear that irradiation induces pathogenicity in non-pathogenic bacteria, it does appear to reduce the virulence in other pathogens (AESAN, 2004). In addition, other authors have already demonstrated that irradiation does not appear to increase antibiotic resistance, nor do those resistant to antibiotics appear to be more resistant to radiation (EFSA, 2011b). In addition, Levanduski and Jaczynski (2008) demonstrated that, similar to that occurring with other inactivation techniques, *E. coli* has the capacity to develop resistance to radiation with accelerated electrons if the same populations of the bacteria in the food are subjected to continuous radiation, although the mechanism through which the radioresistance is developed remains unknown.

It should be noted that the application of ionising radiation in the doses authorised for the treatment of food does not render inactive the bacterial toxins and mycotoxins preformed in the food (EFSA, 2011b).

The existing systems for food safety management are based on an integrated approach from the place of primary production until the placing on the market or export of the product. In particular, the principles of Good Hygiene Practices (GHP) and the HACCP system must be applied throughout the food chain, in accordance with Regulation (EC) No 852/2004 (EU, 2004a). Furthermore, food products must comply with the food safety and hygiene conditions established in other Regulations, including No 853/2004 and No 2073/2005 (EU, 2004b, 2005). Moreover, Royal Decree 348/2001 indicates, in its article 4, that products to be subjected to ionising radiation must be in suitable sanitary conditions of

health. This means that treatment with ionising radiations does not remove the obligation to comply with applicable food hygiene regulations (BOE, 2001).

Moreover, Directive 1999/2/EC, in its considerations, states that: "(13) whereas foodstuffs may only be treated by the action of ionising radiation if there is a food hygiene need, or a demonstrable technological or other advantage, or benefit to the consumer and if they are wholesome and in a proper condition, since ionising radiation should not be used as a substitute for hygiene or health practices or good manufacturing or agricultural practice".

Irradiation may be an effective tool for reducing or eliminating micro-organisms (altering and pathogenic) from food, particularly in meat, meat preparations and meat products for consumption raw or in sliced meat products. The dose values of pathogens such as *Campylobacter* spp., *Yersinia* spp., verotoxigenic *Escherichia coli* or the plant forms of *Bacillus cereus*, required to reduce a population of micro-organisms by 90 % (D_{10}) are between 0.14-0.30 kGy, depending on the product type. *Salmonella* spp., *Listeria monocytogenes, Staphylococcus aureus* and the plant forms of *Clostridium perfringens* have D_{10} values of between 0.40-0.80 kGy. Spores are more resistant than plant forms with D_{10} values of 3.4 kGy for the spores of *Clostridium botulinum* type A and B (EFSA, 2011b).

A hygienisation treatment has just been authorised only for bovine carcasses. Regulation (EU) No 101/2013 concerning the use of lactic acid to reduce microbiological surface contamination on bovine carcasses (EU, 2013) authorises food business operators to use lactic acid to reduce microbiological surface contamination on bovine carcasses or half carcasses or quarters at the level of the slaughterhouse.

Undoubtedly, this regulation will open the way for the authorisation of new meat hygienisation systems which, in no event, should be considered as a replacement for good hygiene practices at the time of slaughter or the operating procedures, nor as an alternative for complying with the requirements established in the Hygiene package regulations.

Potential risks from the intake of irradiated food for the health of consumers

1. Toxicological considerations of irradiated food

The World Health Organization (WHO, 1999), based on the *in vivo* toxicity studies described in the scientific bibliography and used for the risk assessment of irradiated foods, established that foods irradiated even with doses higher than 10 kGy are considered safe and nutritionally suitable. The revised studies, principally conducted on rodents and monkeys, are numerous, although the majority are not representative (mainly due to the use of a deficient methodology, statistical analysis, and lack of precision in the exact conditions of irradiation). Some of the published works on animals used for research assess the toxicological properties of the radiolytic products and indicate that some compounds of 2-alchilcyclobutanone may cause *in vitro* injury to DNA. However, as there are no *in vivo* studies, the genotoxic risk to man is not considered. As regards other radiolytic products, no relevant toxicity studies have been published.

Subsequently, the *Agence Française de Sécurité Sanitaire des Aliments* (AFSSA, 2007), in an assessment of other more recent scientific papers, did not submit any further new information for the safety assessment. Negative results have been described in *in vitro* genotoxicity studies of gamma-irradiated foods (1.5-30 kGy) that include the Ames test, a chromosome aberration study of mammal

cells, and of the micronucleus (Hyun-Ja et al., 2001) (Sung-Kee et al., 2001) (Kim et al., 2003) (Hong-Sun et al., 2004) (Yook et al., 2004) (Yu et al., 2004) (Il-Jun et al., 2005) (Kang et al., 2005) (Yook et al., 2005) (Nazzaro et al., 2007) and *in vivo* genotoxicity studies in which the animals in question (rodents, rat and mouse) receive irradiated food in their diet (Yook et al., 2005).

Nor has any new information been obtained with respect to long-term toxicity studies. Hagiwara et al. (2005), in a chronic toxicity study (90 days) of rats treated in their diet with the sweetener, irradiated thaumatin (5.0 kGy), did not observe any adverse effects that could be attributed to its intake (2 889 mg/kg b.w./day). A number of studies of the carcinogenicity and reproduction of several generations of rats, mice, dogs, and monkeys fed for two years with irradiated food at doses of 27.9 and 55.8 kGy did not reveal any effects related to the treatment. Only in the studies of reproduction of several generations of rats was a small fall or rise in body weight observed. This appears to be related to nutrition and the lack of palatability of the diet.

The only adverse effect described in recent publications is the occurrence of leucoencephalomyelopathy (LEM) in cats fed on irradiated feed (36.3-47.3 kGy and ≥50 kGy) (Palmer and Cavanagh, 1995) (Hendricks et al., 2001) (Cassidy et al., 2007) (Child et al., 2009) (Caulfield et al., 2009) (Duncan et al., 2009), although in felines (cheetah, lion) a clinical-pathological of spontaneous occurrence syndrome with no defined aetiology has been described (Palmer et al., 2001) (Maratea et al., 2006) (Cassidy et al., 2007).

In the studies described by Caulfield et al. (2009), some cats fed with a diet irradiated with Gamma rays (25.7-53.6 kGy) for 224 days had injuries typically associated with LEM. These authors found that diets irradiated with doses of 25.7-38.1 kGy and with doses of 38.1-53.6 kGy revealed concentrations of peroxides in the order of 10 to 60 times higher than those observed in non-irradiated diets, together with reductions in the concentration of vitamin A of 43-48 %. Nevertheless, it has not been clearly demonstrated that a vitamin A deficiency or an increase in peroxides, or a combination of the same, is the cause of the neurological effects associated with LEM. Moreover, it is not possible to make a correlation between LEM disease in cats and its possible appearance in man, where the physiopathology of this alteration has not been well-established. It should also be noted that the neurological effects observed in cats were observed at irradiation doses that exceed the authorised range of doses for foods for human consumption (1-10 kGy).

Very limited information is available regarding studies on humans. Controlled studies have been conducted on young people in the United States Navy (Bierman et al., 1958), fed on irradiated foodstuffs (25-40 kGy) for 15 days, and no adverse effects were observed. Similarly, Plough et al. (1957) conducted studies on healthy human volunteers fed for 15 days with canned irradiated pork (30 kGy) and no adverse effects were observed. A later study (Shao and Feng, 1988) on young people fed for 90 days with a variety of irradiated foods, including meat (irradiated at a dose of 8 kGy) did not describe any adverse effect. No further studies of clinical trials on humans have been described.

Considering the data available in the bibliography and that the quantity of irradiated foodstuffs in the European Union is very limited, the EFSA (2011a) concludes that there is no immediate cause for concern about irradiated foods, although the possible significance for human health of the effect of LEM registered in cats must be clarified.

2. Induced radioactivity and toxicological aspects of radiolytic products

There is no data that indicates the existence of induced radioactivity in irradiated foodstuffs. In fact, some studies have shown that the radioactivity induced in minced meat treated with X-rays at 7.5 MeV is insignificant and may even be lower than that naturally present in some foods (Grègoire et al., 2003).

The irradiation of food products is a controlled process but one which produces detectable changes in the food, especially of a chemical nature. Although many of these changes are minor and do not differ excessively from those produced by other treatments, mainly heat treatments, they must be considered, especially if the food also undergoes any other type of process during its production. In general, the quantity of chemical reactions induced by the radiation of foods depends on many factors, of which the most important are the dose absorbed, the type of installation, the presence or absence of oxygen and the temperature. The composition of the food and its physical state (frozen, fresh, solid, liquid, etc.) also have an influence.

Radiolytic products are stable chemical products resulting from different reactions between free radicals and excited ions, which produce in a primary manner, highly reactive intermediate products. The result of the chemical reactions is dependent on the food type (EFSA, 2011a). The effect on the molecules is greater, the larger the size of the molecules, where the nucleic acids are the most affected molecules. In addition, the irradiation of water molecules produces free radicals with marked oxidising or reducing properties that are highly reactive. In fact, the secondary effects of irradiation of foods are considered to be greater the higher the aqueous content of the food (AESAN, 2004).

Although numerous publications have been written about irradiated foods, the number of relevant publications on the assessment of the safety is limited. The majority of the studies look at the toxicological properties of the radiolytic products, principally the 2-alchilcyclobutanone compounds (2-ACB). From the four main fatty acids, palmitic, stearic, oleic and linolenic, the corresponding cyclobutanones formed are 2-dodecylcyclobutanone (2-dDCB), 2-tetradecylcyclobutanone (2-tDCB), 2-tetradecenylcyclobutanone (2-tDeCB) and 2-tetradeca-5',8'-dienylcyclobutanone (2-tDdeCB). Some publications on studies of genotoxicity and studies of chronic toxicity of the 2-ACBs demonstrate a cytotoxic and genotoxic capacity, at least *in vitro*, depending on the degree of unsaturation and the length of the fatty acid chain (Hartwig et al., 2007). It has not been possible to define the risk to humans given the absence of *in vivo* studies. Certain studies of chronic toxicity with 2-ACBs in rats show a significant incidence of bowel tumours (Raul et al., 2002). There is no convincing evidence that the alchilcyclobutanones are genotoxic or mutagenic when consumed in a normal diet (O'Bryan et al., 2008). Sommers et al. (2006) admit that further studies of the 2-ACB are required due to their potential toxicity in humans, but they also state that they should be studied in the context of the overall human diet and remembering that the irradiation of food might reduce the number of diseases transmitted by the food, admissions to hospital and deaths.

Until recently, the 2-ACB had not been observed in non-irradiated food. However, Variyar et al. (2008) published their detection in cashew nuts and nutmeg which had not been irradiated. To the contrary, Chen et al. (2012) were unable to detect or identify these markers in samples of non-irradiated nutmeg *(Myristica fragrans)*, from five different sources, when compared to sources subject to irradiations of up to 5 kGy, in which they were detected and identified.

During the irradiation, other defined radiolytic products appear, some furans, hydrocarbons and cholesterol oxides, which may even appear with conventional heat treatments. Furans are considered as possible carcinogens for man (EFSA, 2004). The hydrocarbons formed after irradiation of the respective triglycerides (palmitic, stearic, oleic and linolenic acid) are considered to be potentially genotoxic due to their molecular structure. The cholesterol oxides are associated with several toxic effects such as cytotoxicity, mutagenesis or carcinogenicity, and have also been directly correlated to the development of arteriosclerosis and coronary heart disease in humans (Guardiola et al., 1996) (Nam et al., 2001) (Meyner et al., 2005) (O'Bryan et al., 2008).

3. Alterations due to irradiation in the quality of the foodstuff

In general, in meat products, irradiation accelerates lipid oxidation as the ionising radiation generates hydroxyl radicals, a powerful indicator of this type of oxidation. Hydroxyl radicals, the most reactive species of oxygen, are generated by ionising radiation from water molecules. Usually, meat has a water content equal to or more than 75 %, and therefore irradiation may generate hydroxyl radicals in these products. The oxidative changes induced by irradiation in meat are dose-dependent. Various substances are used to prevent or minimise the oxidation of the lipids by irradiation (antioxidants), including phenolic antioxidants. Certain compounds of polyphosphates, including sodium tripolyphosphate, are excellent metal chelates and inhibitors of lipid oxidation. Vacuum packaging or packing in a controlled atmosphere, and the addition of organic acids such as citric and ascorbic acid to fresh meat, are efficient measures for reducing the problems of colour alteration in irradiated meat; ascorbic acid, thanks to its higher antioxidant effect in aerobic packaging, is more efficient than citric acid for reducing colour intensity in irradiated meat (Nam and Ahn, 2002) (Ahn et al., 2013).

Proteins

The chemical reactions produced due to the irradiation of proteins depend on the structure of the same, on their state (native or denaturation), on their physical state, on the amino acid composition, on the presence of other substances in the food and on the irradiation treatment itself (EFSA, 2011a). The treatment of hazelnuts with doses of 10 kGy produces protein denaturation and aggregation, modifying the structure (Dogan et al., 2007), and at much lower doses the protein profile of black truffles is modified (Nazzaro et al., 2007). It must be remembered that any modification to the protein profile of a food may modify its allergy potential.

Irradiation may also produce radiolytic products with a low molecular weight derived from the peptides, including ammonia, ketonic acids, products similar to the amides and diamino acids (EFSA, 2011a).

The main changes induced by the irradiation of proteins affect the amino acids, the most sensitive of which are the aromatic and sulphur-containing ones. Thus, irradiation of phenylalanine produces the appearance of three isomers of tyrosine (para-, meta- and ortho-) and the irradiation of amino acids such as cysteine, phenylalanine and glycine results in α, α'-diamino acids (Hein et al., 2000) (EFSA, 2011a).

Lipids

Irradiation of lipids leads to several chemical reactions, the intensity of which depends on a number of factors, such as their concentration, their physical state, unsaturation profile, the presence of antioxidants in the food, environmental conditions, the irradiation treatment and the type and conditions of storage (EFSA, 2011a).

In general terms, irradiation accelerates the lipid oxidation process, which is highly significant in foods with a high content of fats and very unsaturated fatty acids, in which numerous free radicals are formed due to this oxidation (O'Bryan et al., 2008). Although this effect can be reduced in the presence of antioxidants in the food, these cannot always prevent the organoleptic alteration produced by volatile sulphured substances, with a very low odour threshold (EFSA, 2011a), and others. In general, the use of low temperatures, the presence of oxygen, antioxidants and suitable packaging materials minimise lipid oxidation (Stefanova et al., 2010).

In addition, lipid irradiation may generate 2-ACB and certain hydrocarbons. In fact, the detection of these compounds is used in chemical methods of reference for the detection of irradiation (standard European methods EN1785 and EN1784, respectively).

Triglycerides and fatty acids generate hydrocarbons as radiolytic products. Polyunsaturated acids are more susceptible than the monounsaturates and the saturates. Moreover, irradiation produces a significant reduction in polyunsaturated fatty acids in the food (Ahn et al., 2013). Irradiation may also induce *cis-trans* isomerisation, such that *trans* fatty acids may appear in irradiated food, even at absorbed doses of less than 8 kGy (Brito et al., 2002).

Lipid irradiation may also favour the appearance of cholesterol oxides, a group of sterols with a similar structure to that of cholesterol but which contains hydroxyl, ketone or epoxide groups in the sterol nucleus or to the cholesterol side chain (Nam et al., 2001) (EFSA, 2011a). Although the quantities found in irradiated meat by the different authors do not appear to be higher than those found in meat processed with other treatments, it is important to indicate that the cholesterol oxides are compounds with known atherogenic, cytotoxic, mutagenic and carcinogenic effects (Guardiola et al., 1996).

Carbohydrates

Irradiation produces modifications in the mono- and polysaccharides at the doses authorised in the legislation, resulting in aldehydes, formic acid and hydrogen peroxide (Raffie et al., 1981) (Fan, 2003).

Furans may form in ready-to-eat food (RTE) containing glucose, fructose or sucrose, irradiated at the authorised doses. Moreover, the quantity of furans detected in the food appears to be higher than that produced in other treatments and tends to increase at a more acid pH (Fan, 2005). Fan and Sommers (2006) found that the production of furans tends to be greater when irradiation is applied to aqueous solutions of the ingredients present in RTE foods, such as turkey or beef sausages, than when applied to the food as such, in the form it reaches the consumer. In addition, treatment with ionising radiation seems to compensate the production of furans due to the preliminary heat treatment to which this type of RTE product is subjected, with the exception of foods that are richer in carbohydrates and ascorbic acid, such as juices (Fan, 2005).

Vitamins

Irradiation of foods leads to vitamin losses similar to those of other treatments, mainly heat.

Sensitivity to radiation depends on the type of vitamin, water-soluble vitamins being the most sensitive. Of these, thiamine is the most sensitive and very high losses have been observed in meats (Stewart, 2009), especially pork. Folic acid also undergoes high losses in meats irradiated at doses of up to 3 kGy (Galán et al., 2010) (Galán et al., 2013). On the other hand, riboflavin, vitamin B6, vitamin B12 and niacin appear to be more radioresistant (Fox et al., 1989).

Liposoluble vitamins have variable sensitivities to irradiation, with vitamin E being the most sensitive, especially when the food is irradiated in the presence of oxygen (EFSA, 2011a). Vitamin K is the most resistant.

In general terms, the vitamins appear to be more sensitive when irradiated in solution than when they are irradiated in the food (EFSA, 2011a).

Other components and ingredients in the food

Inorganic salts

Inorganic anions hardly react with the primary radicals, except for nitrates which, in the presence of solvated electrons, are transformed into nitrites. Gamma irradiation appears to enhance the capacity of ascorbic acid to reduce the nitrites. Thus, the formation of N-nitrosamines appears to be lower in meat products treated at doses of more than 5 kGy (Ahn et al., 2004).

Effects on anti-nutrients

The effects of irradiation on certain components considered as anti-nutrients have been observed, principally, in studies conducted at irradiation doses higher than the authorised and recommended doses. However, some authors have observed relevant reductions in the levels of phytates and tannins in pulses irradiated at doses of 5 kGy (El-Niely, 2007). It should be noted that other studies conclude that the effect of radiation on phytates and tannins in food such as millet is very low if the food is not subjected to a subsequent heat treatment (ElShazali et al., 2011).

Effects on additives

Some additives may be affected by irradiation and may contribute to the potential appearance of harmful radiolytic products in the food. Benzene was detected in samples of irradiated RTE turkey ham in one study (Zhu et al., 2005). The benzene came from the decarboxylation of potassium benzoate, an additive present in the turkey ham.

4. Packaging materials

The majority of the food products are irradiated once packed in order to prevent recontamination and to maintain the quality of the food. Irradiation of packaged food is a globally recognised technology used to preserve food and is an alternative to heat sterilisation techniques. Therefore, the characteristics of the packaging materials are of great importance from the food safety viewpoint. If the packaging is suitable, irradiation should not compromise its functional properties, nor should it facilitate the

migration of unsuitable or hazardous components from the packaging material to the food (ICGFI, 1999) (AESAN, 2010). Section 179.45 of the Code of Federal Regulations Title 21 of the USA (FDA, 2006) defines the classes of packaging materials for use during irradiation treatment, its requirements and specifications. In this document the maximum irradiation dose authorised for the treatment of packaging materials is 10 kGy (FDA, 2006).

The irradiation of pre-packed foods leads to chemical and physical changes in the plastic packaging materials (Buchalla et al., 1993a) (FDA, 2008). The principal chemical changes in the polymeric material are caused by two reactions that compete together: polymerisation and degradation. The former prevails in inert atmospheres and in vacuums, while the second dominates in the presence of oxygen (FDA, 2008). The interaction of the ionising radiation with the packaging materials results in free radicals and ions that affect the polymer and the low molecular weight compounds present in the plastic material (AESAN, 2010), and also leads to the appearance of new compounds, in addition to increasing the quantity of those already in existence. If new compounds appear, these have a low molecular weight and unique chemical markers have not yet been identified in the irradiated packaging material (IFST, 2006), as these depend on the material. The appearance of degradation products is highly dependent on the presence of oxygen and on the irradiation dose applied. It is in these conditions that the behaviour of the materials, especially new materials, should be studied (FDA, 2008).

Once the degradation products have joined the packaging material, they may migrate to the packaged foods and cause their chemical contamination. Few studies have been made of the effects of ionising radiation on packaging materials. In the few available studies, it is noted that in effect the materials degrade to a greater or lesser extent, increasing the concentration of migrant substances. However the nature and quantity of the migration very much depends on the material and on the intensity of radiation applied (Buchalla et al., 2002) (Ito et al., 2005) (Jeon et al., 2006) (Park et al., 2006) (Félix et al., 2008) (Oliveira et al., 2012). Radiations of less than 10 kGy do not result in substantial changes, but these increase with higher levels of radiation. In multi-layer materials the problem is worse, as there are many more components originating in the adhesive, inks and the substrate itself (material used in each layer) susceptible to degradation. Some of those identified include 1,3-di-tert-butylbenzene, 2,6-di-tert-butyl-1,4-benzoquinone, 4-tert-butylphenol and the butyric and valeric acids, these last ones being responsible for bad odours. In polyamides, however, no degradation was observed due to the effects of the irradiation until reaching doses of 12 kGy.

In general terms, it has been observed that irradiation dose not modify the permeability and deterioration of the mechanical properties, and in certain polymers it can be controlled with suitable stabilisers. Volatile products have been observed after the irradiation of low density polyethylenes and polypropylenes, the main ones being hydrocarbons (C_3-C_{13}), alcohols (C_2-C_3), aldehydes (C_2-C_5), ketones (C_4-C_8) and carboxylic acids (C_2-C_5) (Buchalla et al., 1993a) (Tyapkova et al., 2009) (Lee, 2010). The changes induced by the irradiation depend on the chemical structure of the polymer, the composition (presence of additives, principally) and the preliminary processing of the plastic, together with the irradiation conditions. Several studies have demonstrated that the global migration towards the food is greater as a consequence of the irradiation of the materials, especially in fatty media. A transfer

of colour and odour has also been observed on using certain plastic compounds. Additives, especially antioxidants, are destroyed during irradiation and a specific increase in migration may be observed for this reason. Organic tin heat stabilisers used in PVC degrade to stannic chloride (IV) ($SnCl_4$) and migration of these compounds to the food increases after gamma irradiation (Buchalla et al., 1993b).

Further studies of the effects of the migration of packaging materials on irradiated meat products are required. Some countries with specific regulations, such as the United States, have authorised the irradiation of a multitude of materials in contact with foods (FDA, 2008). The principal problem is that in recent years new food contact materials have appeared, including oxygen barrier materials, which are complex multilayer materials, the majority of which are constructed combining different materials with adhesives, and for which it is difficult to assess the safety. Therefore, from the existing data, the need to study the behaviour of each packaging material selected for the irradiation process is inferred.

5. Organoleptic characteristics of the irradiated products

Although this is not a specific aspect of food safety, the organoleptic alterations due to the irradiation of food products must be considered, as in spite of the potential benefits from the irradiation of meat, meat products and meat preparations, these qualitative aspects limit their use in the meat industry.

Studies conducted on pork, turkey and beef show that the irradiation results in the appearance of volatile substances responsible for the characteristic odour due to the increase in the quantity of hydrocarbons and sulphured substances, in particular methyl mercaptan and hydrogen sulphide (Stewart, 2010); in addition, it accelerates lipid oxidation and modifies the colour of fresh meat and meat products (Lee and Ahn, 2005) (Stewart, 2010) (Yang et al., 2011) (Ahn et al., 2013). This characteristic odour has even been described as a "wet dog smell", "sweet smell", "metallic smell" or "burnt smell" and depends on the doses applied. The components of the meat principally involved in the production of these unpleasant odours appear to be the lipids (Stewart, 2010), although some authors have established that the smell from the substances produced by the radiolytic degradation of the sulphured amino acids is stronger and more astringent, and that the contribution of the volatile substances from the lipids to the odour is small in comparison (Lee and Ahn, 2003).

Some of these effects can be counteracted with the use of suitable packaging (Nam et al., 2001) (Nam and Ahn, 2003), with the irradiation of frozen products (Nam et al., 2002) and with the use of antioxidants, combinations of antioxidants and seasoning, such as rosemary, onion or garlic (Lee and Ahn, 2005) (Nam et al., 2006) (Ahn et al., 2013). However, these measures are not always able to prevent the organoleptic alteration produced by certain sulphured volatile substances with a very low odour threshold (EFSA, 2011a).

In other products, such as freshly cut vegetables, the increase of phenolic compounds due to irradiation produces colour reactions and blotching on the leaves (Fan, 2005).

According to the EFSA (2011b), the fear of irradiation making an altered food saleable by reducing the perception that it has been altered is unfounded. Irradiation cannot improve the odour, flavour or visual appearance of the altered food. If an unaltered food receives a non-sterilising dose, this food will display the characteristics of an altered product if it is in fact altered. The SCF (2003) indicated that "concern about the abusive use of irradiation to sanitise altered food contaminated at unacceptable

levels is unfounded, as irradiation does not restore the appearance and organoleptic characteristics of the altered food".

6. Nutritional considerations

The Food and Drug Administration (FDA, 2012) has revised the possible nutritional losses of irradiated meat. Based on the knowledge that the macronutrients in the diet (proteins, fats and carbohydrates) and minerals (calcium, iron) are not significantly altered by irradiation at permitted doses, and although certain vitamins may undergo reductions (such as thiamine), the FDA (Food and Drug Administration) concludes that there is no adverse effect on the nutritional aspect due to the use of ionising radiation in non-refrigerated meat, at a maximum ionising radiation dose of 4.5 kGy.

In accordance with the conclusions of the Joint FAO/WHO/IAEA Study Group on irradiation at high doses (WHO, 1999) in relation to the nutritional effects of high doses (>10 kGy) on micro and macro nutrients, irradiated food is nutritionally equivalent or superior to heat-sterilised food.

7. Microbiological considerations

The FDA (2012) has examined the effects of the alterations induced by the radiation on the microbiological profile of meat, and on the growth of micro-organisms including *Clostridium botulinum* to determine the microbiological safety of irradiated meat and its subproducts, concluding that the irradiation of frozen or refrigerated meat and its subproducts at a maximum dose of 4.5 kGy does not pose a public health risk from common pathogens, including *Clostridium botulinum*.

The EFSA (2011b) confirmed the absence of microbiological risks for the consumer resulting from the use of irradiation and its consequences on the microbial biota. To do so, in the report, they considered the selective effect on microbial biota of the food, the production of mutations, the effects on the production of toxins, and the possibility of developing radioresistance, among other points.

Conclusions of the Scientific Committee

With respect to Term of Reference 1

Meat and meat products must be prepared in accordance with Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs and its subsequent amendments. In addition, Directive 1999/2/EC, in the recitals states that: "foodstuffs may only be treated by the action of ionising radiation if there is a food hygiene need, or a demonstrable technological or other advantage, or benefit to the consumer and if they are wholesome and in a proper condition, since ionising radiation should not be used as a substitute for hygiene or health practices or good manufacturing or agricultural practice".

Undoubtedly, irradiation may be an effective tool for reducing or eliminating micro-organisms (altering and pathogenic) from food. On the other hand, and in line with the first term of reference of this report and the foods considered within, compliance with Regulation (EC) No 2073/2005 would guarantee the safety of said food with respect to the pathogenic micro-organisms considered therein. However, irradiation would permit the control of pathogenic micro-organisms not considered in Regulation (EC) No 2073/2005, such as *Campylobacter* spp., Shiga toxin-producing *Escherichia coli*, *Yersinia enterocolitica*, etc., contributing to the marketing of safer foods. In addition it would

provide an additional measure of control for those micro-organisms considered in Regulation (EC) No 2073/2005.

With respect to Term of Reference 2

In accordance with existing data and studies, it can be concluded that doses of ionising radiation between 1-10 kGy are safe for their use in meat (uncooked, refrigerated and non-refrigerated) and its derivatives (EFSA, 2011).

There is no data indicating the existence of induced radioactivity in irradiated foodstuffs. In fact, some studies have shown that the radioactivity induced in minced meat treated with X-rays at 7.5 MeV is insignificant and may even be lower than that naturally present in some foods (Grègoire et al., 2003).

With respect to the toxicological considerations of the irradiated food, the WHO (1999), based on the *in vivo* toxicity studies described in the scientific literature and used for the risk assessment of irradiated foods, established that those foods irradiated even with doses of more than 10 kGy are considered safe and nutritionally suitable. In addition, the EFSA (2011a) concludes that there is no immediate cause for concern about irradiated foods, although the possible significance for human health of the effect of LEM registered in cats must be clarified.

The quantities of cholesterol oxides found in irradiated meat by the different authors do not appear to be higher than those found in meat processed using other treatments. Nor is there convincing evidence that the alchilcyclobutanones are genotoxic or mutagenic when consumed in a normal diet.

Nevertheless, if the EU were to extend the irradiation authorisation to all these products, including RTE products, the foreseeable increase in the daily intake throughout the population would require a review of existing studies, as it is not possible, based on the available data, to infer the risk to which the population might be exposed as a result of a greater and broader consumption of radiolytic substances.

The irradiation of frozen or refrigerated meat and its byproducts at a maximum dose of 4.5 kGy does not appear to pose a public health risk due to the modification of common pathogens, including *Clostridium botulinum*. Nor are there microbiological risks for the consumer due to the use of irradiation and its consequences in the microbial biota.

There does not appear to be an adverse effect from the ionising radiation on the nutritional characteristics of non-refrigerated meat, at maximum doses of 4.5 kGy. Nor do there appear to be nutritional changes in comparison to heat-sterilised foods at doses higher than 10 kGy.

Studies are recommended on the effect that irradiation may have on the packaging material selected, in order to guarantee that the irradiation process does not alter either the chemical or the organoleptic properties of the packed product.

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