Effect of irradiation and storage on biogenic amine contents in ripened Egyptian smoked cooked sausage

Mohamed A. Rabie · Abbas O. Toliba

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Abstract The effects of γ -irradiation upon the biogenic amine inventory in Egyptian smoked cooked sausages were investigated for the first time during storage for up to 90 days at 4 °C. Typical contents of biogenic amines in non-irradiated sausages ranged between 125.50 and 596.18 mg/kgDW; irradiation with 4 and 6 kGy decreased said total contents to 105.20–94.82 and 104.98–26.44 mg/kgDW respectively, by the end of storage. Putrescine and cadaverine were the major amines in non-irradiated samples—where it accounted for 33% and 29% respectively, of the total by 90 days; however, tyramine dominated in irradiated samples with 2, 4 and 6 kGy, where it accounted for 44, 52 and 42%. On the other hand, the histamine content in non-irradiated sausage by 90 days of storage (i.e. 109.12 mg/kgDW) clearly exceeded the maximum allowable of 50 mg/kg, unlike happened in their irradiated counterparts. Therefore, the dramatic reduction observed in the histamine levels suggests use of this preservation technique for that traditional meat food.

Keywords Traditional foods · Food safety

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M. A. Rabie · A. O. Toliba
Department of Food Science, Faculty of Agriculture,
Zagazig University,
Zagazig, Egypt

M. A. Rabie (✉)
Instituto de Tecnologia Química e Biológica,
Universidade Nova de Lisboa,
Apartado 127,
2781-901 Oeiras, Portugal
e-mail: marabie@zu.edu.eg

Introduction

Microbial fermentation is one of the oldest methods of food preservation, and is common practice in meat processing; the consequent decrease in pH prevents growth of such food-borne pathogens as *Clostridium* spp. However, the high protein levels in the original meat matrix, and the proteases made available during said fermentation—namely upon lysis of the starter culture brought about by an excessively low a_w , provide the precursors for eventual decarboxylase-type reactions. These reactions eventually lead to formation of biogenic amines, BAs (Brink-ten et al. 1990); in chemical terms, these compounds are organic bases with aliphatic, aromatic or heterocyclic structures, which can be found (although unwanted) in several foods.

One of the most important parameters that influence BA formation in sausages is thus the nature of the starter culture, and also the degree to which it can withstand ecological competition with unwanted contaminating microorganisms (Maijala et al. 1995). Formation of BAs in meat has indeed been attributed to the action of microorganisms belonging to the *Pseudomonas*, *Enterococcus* and *Lactobacillus* genera, besides members of the *Enterobacteriaceae* family (Halász et al. 1994; Silla-Santos 1996). Most meat starter cultures commercially available contain mixtures of Lactic Acid Bacteria (LAB) and Coagulase-Negative Cocci (CNC)—mainly *Staphylococcus* and *Kocuria* spp., besides yeasts and moulds to a lesser extent (Hammes et al. 1990).

The most frequent BAs in processed meat products are putrescine, cadaverine, histamine and tyramine (Demeyer et al. 2000); in particular, spermidine and spermine contents change only slightly during processing or storage afterwards (Hernández-Joyer et al. 1997). From a toxicological point of view, consumption of excessive amounts of BAs

can pose a health risk, especially in unhealthy or compromised individuals. Typical symptoms are nausea, hot flushes, cold sweat, palpitations, headaches, red rash, and high or low blood pressure. Unfortunately, BAs are also known to be precursors of carcinogens, e.g. *N*-nitrosamines (Shalaby 1996).

From a toxicological point of view, consumption of excessive amounts of BAs can pose a health risk, especially in unhealthy or immuno-compromised individuals. Typical symptoms are nausea, hot flushes, cold sweat, palpitations, headaches, red rash, and high or low blood pressure. Unfortunately, BAs are also known to be precursors of carcinogens, e.g. *N*-nitrosamines (Shalaby 1996). Determination and control of BAs in foods therefore holds a great interest—not only owing to their intrinsic toxicity, but also because they can be used as indicators of food spoilage (Silla-Santos 1996; Awan et al. 2008); this is especially the case of histamine, putrescine and cadaverine, with regard to fresh fish, meat and vegetables (Visessanguan et al. 2004).

There is no official regulation being enforced pertaining to the limits of BAs specifically in fermented sausages—although some authors have suggested 100 mg/kg of histamine as the limit before a sufficiently high risk arises for healthy individuals (Brink-ten et al. 1990); the allowable maximum level of tyramine in general foods is 100–800 mg/kg, and 1080 mg/kg of tyramine has actually been reported to be toxic (Shalaby 1996). The European Union (EU) had established regulations for histamine levels, such that they should be below 100 mg/kg in raw fish and below 200 mg/kg in salted fish for species belonging to the Scombridae and Clupeid families (Montel et al. 1999). While, the Food and Drug Administration (FDA) established a level of 50 mg/kg histamine as an indication of potential health risk in fish (FDA 1995). The Nutritional codex of the Slovak Republic recommended the maximal tolerable limit for both the histamine (200 mg/kg in fish and fish products) and tyramine (200 mg /kg in cheese) (Halász et al. 1999).

Because of the severely detrimental effects of thermal processing, the food industry has resorted to a number of alternative preservation techniques to extend the shelf life of foods (WHO 1994); one of them is irradiation, which is effective when used alone or in combination with other techniques. Gamma-irradiation has indeed a positive impact upon reduction of viable numbers of microorganisms and viruses; furthermore, it appears to reduce nitrosamine formation from nitrite in meat products, as well as overall allergenicity in protein-rich foods (Byun et al. 2000; Ahn et al. 2002; Byun et al. 2002). Significant progress has recently been made in using γ -irradiation to reduce the levels of BAs in food (Kim et al. 2004; Kim et al. 2005; Min et al. 2007a, b). Safer apparatus and more extensive knowledge on the effects upon the food matrix, and

eventually upon human health, of γ -irradiation has led to a significant growth of the US market of irradiated fresh and frozen meat over the latest decade.

The first regulations on irradiation of meat were published by USDA in 1985 regarding inactivation of *Trichinella spiralis* in pork carcasses, and irradiation of red ground meat was authorized in 1997. Promoted by FAO in the *Codex Alimentarius* in 2003, irradiation has meanwhile been adopted in more than 50 countries—especially the USA, Egypt, China and several countries of Latin America. However, irradiation is still far from being a general practice: in Europe, for the moment only herbs and spices are allowed to be irradiated. On the other hand, FDA in the USA has issued approvals for irradiation: of general foods, at doses not exceeding 1 kGy to control insects and other arthropods; of fresh foods, at doses not exceeding 1 kGy to inhibit maturation (e.g. ripening and sprouting); of poultry, at doses not exceeding 3 kGy to control food-borne pathogens; of fresh iceberg lettuce and spinach, at doses not exceeding 4.0 kGy to extend shelf-life; of red meats (e.g. beef, lamb and pork), at doses not exceeding 4.5 kGy in the case of fresh meat 7.0 kGy in the case of frozen meat; of fresh and frozen mollusc and shellfish (e.g. oysters, mussels and clams), at doses not exceeding 5.5 kGy for control of *Vibriobacterium* and other foodborne microorganisms; of dry and dehydrated enzymes, at doses not exceeding 10 kGy to control microorganisms; and of dry or dehydrated flavour substances (e.g. spices and vegetable seasonings), at doses not exceeding 30 kGy to control insects and microorganisms. Hence, no legal support still exists for irradiation of fermented foods; however, a body of knowledge has started to build up in the specific field of fermented sausages, e.g. Byun et al. (2004) who aimed at using irradiation to control *N*-nitrosamines, and recently Kwon et al. (2010) who aimed at maximizing shelf life while retaining acceptable quality attributes.

According to previous studies (Kim et al. 2003; Kim et al. 2004), γ -irradiation was effective in reducing the content of BAs in both aqueous solutions and fermented soybean paste; the mechanism claimed therefore was a delay in the initial growth of contamination microorganisms. In particular, Min et al. (2007a, b) concluded that irradiation was effective in reducing the major BAs in ground beef and pork, following inoculation with three different microorganisms. Gamma irradiation reduced the BAs formation significantly ($P<0.05$) during the storage of Blue cheese up to 90 days, especially at doses above 4 kGy and specifically of histamine after 60 day of storage, together with an acceptable level of sensory decay (Rabie et al. 2011).

Very scarce data are indeed available pertaining to the use of γ -irradiation for reduction of the major BAs in sausages—so this manuscript intends to contribute to fill

such a gap, aiming at an eventual legal acceptance based on scientific grounds. Note that this type of food is associated with a long cultural heritage in Mediterranean countries, and is rather important from a social-economic point of view in such countries as Egypt. Therefore, the main goal of this study was to assess the effect of various doses of γ -irradiation upon BA formation in Egyptian smoked cooked sausages throughout storage.

Materials and methods

Sample materials

Packaged Egyptian smoked cooked sausages were purchased from a well-respected sausage factory in Cairo (Egypt) from three independent batches; and three sausages were selected, also at random,—and previous experience indicated that it was representative of regular quality smoked cooked sausages in the Egyptian market; they had been manufactured from frozen ($-50\text{ }^{\circ}\text{C}$) beef meat and beef back fat. After irradiation, the sausages were stored for 0, 30, 60 and 90 days, at $4\text{ }^{\circ}\text{C}$.

Performance of γ -irradiation

The packaged Egyptian smoked cooked sausages were irradiated using a ^{60}Co irradiation source, in a Russian Gamma Chamber at the Nuclear Research Center (Atomic Energy Authority, Abu Zabel, Egypt). The source strength was ca. 100 kCi, with a dose rate of 2 kGy/h, and was applied at $12\pm0.5\text{ }^{\circ}\text{C}$. The irradiation doses tested with were 0, 2, 4 and 6 kGy.

Determination of dry weight

Dry weight measurements were made on 3 g-samples of the Egyptian smoked cooked sausages, after drying in an WS50

oven (MLW, Germany) at $105\text{ }^{\circ}\text{C}$ until constant weight—according to Hungarian standards (MSZ EN ISO 1666:2000).

Extraction of biogenic amines

The BAs in the Egyptian smoked cooked sausage samples were extracted according to the method by Simon-Sarkadi and Holzapfel (1994). Ten ml of 10%(v/v) trichloroacetic acid (TCA) was added to 3 g-samples, and the mixture was shaken for 1 h using a Laboshake (Gerhardt Ls 500i, Germany). The extract was then filtered through Whatman No.1 filter paper. To remove fat, the samples were kept at $-20\text{ }^{\circ}\text{C}$ for 1 day, and then centrifuged in a T24 centrifuge (MLW) at $7000\times g$ for 15 min. The supernatants were finally collected, and filtered through a $0.25\text{-}\mu\text{m}$ membrane filter (NALGENE, USA).

Quantitation of biogenic amines

Analysis of five biogenic amines, viz. histamine, tyramine, cadaverine, putrescine, spermine and spermidine were performed in duplicate using an AAA 400 amino acid analyser (Ingos, Czech Republic), equipped with an Ostion LG ANB ion-exchange column ($6\times3.7\text{ cm}$): separation was by a stepwise gradient elution using Na^+/K^+ citric buffers at pH 6.0 (A), 5.7 (B), 5.45 (C). Colorimetric detection was at 570 and 440 nm, after post-column derivatization with ninhydrin (Csomos and Simon-Sarkadi 2002). The operating parameters were: column temperature of $60\text{ }^{\circ}\text{C}$; eluant and reagent flow rates of 0.30 and 0.20 mL min^{-1} , respectively; reactor temperature of $121\text{ }^{\circ}\text{C}$; sample volume of 100 μL ; and total run time of 92 min. Calibration curves were prepared in advance: identification and quantification of each biogenic amine was by comparing retention time and peak area, respectively, between actual samples and standards. A typical chromatogram of BAs standards are shown in Fig. 1.

Fig. 1 Typical chromatogram of biogenic amine standards, encompassing (by order of elution): histamine (His), tyramine (Tyr), putrescine (Put), cadaverine (Cad), spermidine (Spd) and spermine (Spm)

Statistical treatment

Differences in amine contents during storage in non-irradiated and irradiated smoke cooked sausage and between the different irradiation doses were tested by t-test to detect significant variation between the means of biogenic amines during storage. Statistical comparison was based on three samples for each treatment for each specific storage time using a two-way ANOVA, at a significance level of 0.05 (SPSS, Chicago IL, USA).

Results and discussion

The levels of total biogenic amines increased as storage time elapsed in control sausage ($P < 0.05$), total contents of BAs in non-irradiated samples ranged in 126.03–596.46 mg/kgDW as shown in Fig. 2a; six different BAs (viz. histamine, tyramine, putrescine, cadaverine, spermidine and spermine) could be found—meat and meat products are known to contain all such BAs (Koehler and Eitenmiller 1978; Nakamura et al. 1993; Santos-Buelga et

al. 1986; Shalaby 1993, 1996). Kim et al. (2005) reported 6 different biogenic amines in pepperoni sausage (viz. putrescine, cadaverine, β -phenylethylamine, spermidine, spermine and tyramine). Putrescine, cadaverine, tyramine and histamine were also detected in French sausages (Montel et al. 1999). Tryptamine and phenylethylamine were not considered here because they appear only at trace levels, and were thus of a marginal relevance when compared with the aforementioned six BAs.

The non-irradiated Egyptian smoked cooked sausages underwent a significant increase ($P < 0.05$) in their contents of histamine (21.64–108.88 mg/kgDW), tyramine (23.55–42.93 mg/kgDW), putrescine (20.28–196.56 mg/kgDW) and cadaverine (16.15–174.82 mg/kgDW) throughout storage (Fig. 2a); conversely, said increase was dramatically lower in irradiated sausages. The aforementioned levels may pose a public health risk: in fact, the toxic limits for histamine lie in 40–100 mg/kgDW, and contents above 50 mg/kgDW may cause poisoning, whereas, tyramine contents over 100 mg/kgDW may cause migraine (Maijala et al. 1993). On the other hand, putrescine and cadaverine were the major BAs found in non-irradiated Egyptian

Fig. 2 Changes in concentration of selected biogenic amines in **a** non-irradiated and **b** irradiated Egyptian smoked cooked sausages storage time. * Values are presented as average $\pm$ standard deviation, $n=3$; data pertaining to each biogenic amine followed by different superscripts are statistically different from each other, at the 5% significance level

smoked cooked sausages—where it represented by itself ($P<0.05$), 33% and 29% from the total BAs by 90 days (Fig. 2a); occurrence of cadaverine in meat products has been related to lysine decarboxylase activity. Hernández-Joyer et al. (1997) found that tyramine and cadaverine were the main BAs formed during sausage production. Furthermore, such BAs as cadaverine and putrescine and histamine have been proposed as chemical indicators of the hygienic conditions prevailing in the initial raw material, and during handling and processing—as their accumulation can be associated with the activity of contaminant bacteria (Bover-Cid et al. 2003; Halász et al. 1994; Hernández-Joyer et al. 1997).

The variation in the total BAs contents of Egyptian smoked cooked sausages irradiated with distinct doses are

shown in Fig. 2b. Irradiation with 2 kGy was increased the total BAs 46%, while, irradiation with 4 and 6 kGy decreased the total BA contents by 1.1-fold mg/kg 4.0 fold-mg/kg respectively, during storage. Tyramine was the dominant BA in all irradiated Egyptian smoked cooked sausages—where it represented 44% for 2 kGy, 52% for 4 kGy and 42% for 6 kGy by 90 days of storage (Fig. 3a, b & c). The changes during storage in BAs levels in Egyptian smoked cooked sausages irradiated at 2 kGy are depicted in Fig. 3a. Putrescine and cadaverine levels increased ($P<0.05$), 1.7-fold mg/kg and 1.6-fold mg/kg, respectively; these results are not in agreement with Kim et al. (2004) who reported that 2.5 kGy-irradiation induced radiolysis of putrescine in an aqueous model system, with full destruction at 5 kGy. Result was reported by Min et al. (2007a, b):

Fig. 3 Changes in concentration of selected biogenic amines in Egyptian smoked cooked sausages during storage following irradiation at **a** 2 kGy, **b** 4 kGy and **c** 6 kGy. *Values are presented as average $\pm$ standard deviation, $n=3$; data pertaining to each biogenic amine followed by different superscripts are statistically different from each other, at the 5% significance level

the amounts of putrescine were significantly reduced by irradiation—from 5.6 to 0.9 in breast, and from 11.2 and 4.6 in thigh. On the other hand, Kim et al. (2003) did not observe a significant difference in BA content between controls and γ -irradiated Korean fermented soybean pastes, immediately after irradiation. Spermidine and spermine also decreased during storage in Egyptian smoked cooked sausages irradiated at 2 kGy ($P<0.05$); they accounted for 8% and 11%, respectively, of the total BA content by 90 days. Min et al. (2007a, b) found as well that spermidine decreased significantly in chicken breast, following irradiation up to 2 kGy. Smoking, cooking and irradiation at low dose (2 kGy) of the Egyptian sausage may be increased the level of these biogenic amine mentioned above.

As apparent from inspection of Fig. 3b, all BAs detected in Egyptian smoked cooked sausages underwent reduction in their levels during storage after having been irradiated at 4 kGy ($P<0.05$). Cadaverine underwent the greatest reduction (2.3-fold), seconded by putrescine (2.1-fold) and histamine (1.3-fold) ($P<0.05$); the realisation pertaining to cadaverine is particularly relevant, as the safety of the product might be compromised owing to its initial high levels. Spermidine and spermine decreased their levels during storage in irradiated Egyptian smoked cooked sausages, after irradiation at 4 kGy ($P<0.05$); they accounted for 8%, respectively, of the total BA inventory by 90 days.

As illustrated in Fig. 3c, irradiation of Egyptian smoked cooked sausages at 6 kGy ($P<0.05$), had the greatest effect upon BAs (as expected). Cadaverine, histamine, tyramine, and putrescine were significantly reduced, by 7-, 4.5-, 2- and 1.5-fold, respectively, by 90 days ($P<0.05$). Note that Brink-ten et al. (1990) claimed that 10–80 mg/kg of tyramine is toxic in foods. Kim et al. (2005) reported that tyramine in vacuum- and CO_2/N_2 -packaged pepperoni underwent a significant reduction by 30 days upon γ -irradiation.

Tabor and Tabor (1984) and Pegg (1986) claimed that spermidine and spermine are ubiquitous components of meat. These two BAs in Egyptian smoked cooked sausages previously irradiated at 6 kGy ($P<0.05$), showed significantly lower levels by 90 days (11 and 9%, respectively) than in non-irradiated ones which increased by (55–67%, respectively). Note that when proteins are irradiated, their tertiary structure can undergo changes (Stevenson 1992). Because beef and poultry meat contain substantial amounts of water, the radicals produced there from via irradiation will likely disturb the protein aqueous structure, and hence their physicochemical features—via fragmentation, cross-linking, denaturation or oxidation. This may be the reason why irradiation can increase the contents of certain BAs. However, Kim et al. (2005) stated that direct breakdown of BAs in meat products is likely less extensive than indirect reduction, which is achieved through depletion of the initial

microbial load effected by irradiation. On the other hand, the spermidine level was reduced in irradiated pepperoni sausage under air and vacuum conditions, by 30 days of storage, whereas, spermine in pepperoni irradiated at 5 kGy and 10 kGy, and maintained under an atmosphere of CO_2/N_2 , exhibited significantly higher levels than their air-packaged counterparts by the same storage time (Kim et al. 2005).

Other compounds with relevance for public health are affected by irradiation of fermented sausages; this is the case of N-nitrosodimethylamine and N-nitrosopyrrolidine (Byun et al. 2004), which may be associated with carcinogenesis. Likewise, oxidation is also promoted by irradiation, which may lead to off-flavours (Kwon et al. 2010)—especially well above 2 kGy; in addition, faster formation of volatiles may occur, most of which will be lost as storage time elapses in unpackaged feedstock (as usually done). However, these compounds stood out of the scope of this research effort—so a further check on health and organoleptic issues is recommended prior to release of processed traditional fermented sausages to the market.

Conclusions

The biogenic amine contents of non-irradiated smoked cooked sausage increased significantly ($P<0.05$) during storage general, γ -irradiation reduced them significantly ($P<0.05$), especially notorious following 6 kGy irradiation. Therefore, γ -irradiation appears to be effective in preventing formation of BAs, thus allowing the shelf life of Egyptian smoked cooked sausages to be significantly extended.

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