REVIEW



Biogenic amines in meat and meat products and its public health significance: a review

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Abstract Biogenic amines are anti-nutritional nitrogenous bases formed by the action of microbial decarboxylases on free amino acids. They are found widely in varying concentrations in meat and meat products. Public health significance lies in their toxic effects associated with high levels in meat and meat products. Owing to their consistent presence with microbial spoilage they are utilized as quality indicator in terms of spoilage/ freshness of meat and meat products. The reason for the formation of these amines is multi-factorial however the poor quality meat is the most important one, contributing substrate for microbial decarboxylases. Their presence can be analytically determined in the food stuffs by employing various techniques. The key to control biogenic amines is the good manufacturing practices. Many new technologies have also been emerged to reduce the levels of these amines to permissible limits.

Keywords Biogenic amines \cdot Meat and meat products \cdot Public health \cdot Freshness indicator

Introduction

Biogenic amines are organic nitrogenous bases of low molecular weight that possess biological activity. On the basis of

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² Department of Animal Genetics and Breeding, College of Veterinary Science, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana 125004, India source, these are grouped into two types, endogenous and exogenous biogenic amines. Endogenic amines are neurotransmitters produced by different tissues (de novo biosynthesis) and are transmitted locally or via the blood system. These are further divided into three classes i.e., catecholamines (dopamine, norepinephrine, epinephrine), indolamines (serotonin, 5-hydroxytryptamine, melatonin) and histamines. They have many physiological functions like growth regulation, neural transmission, mediators of inflammation etc. (Onal 2007). Exogenic amines are natural anti-nutritional factors detected in both raw and processed foods (Santos 1996). They are formed by the removal of the α -carboxyl group from amino acids and they are usually named after corresponding precursor amino acid e.g., histidine is decarboxylated to produce histamine tryptophan to tryptamine, tyrosine to tyramine and lysine to cadaverine. However, putrescine can be produced from three amino acids: glutamine, arginine and agmatine (Stadnik and Dolatowski 2010). Arginine, thus formed is easily converted to agmatine or as a result of bacterial activity can be degraded to ornithine from which putrescine is formed by decarboxylation (Fig. 1).

Biogenic amines have been reported to present in different food stuffs where they are associated with poisoning. For example, two well known biogenic amine poisonings, 'scombroid poisoning' from fish of *Scombridae family* and 'cheese reaction' from cheese consumption are associated with histamine and tyramine, respectively (Stadnik and Dolatowski 2010). The first reported case of biogenic amines poisoning occurred in 1967 in the Netherland and involved Gouda cheese (Stratton et al. 1991). Biogenic amines have also been reported in widely varying concentrations, in meat and meat products (Kim et al. 2005; Min et al. 2007a, b; Ntzimani et al. 2008). Meat being rich in protein content is more important product with respect to amine decarboxylation. Meat consumption is increasing especially in developing countries,

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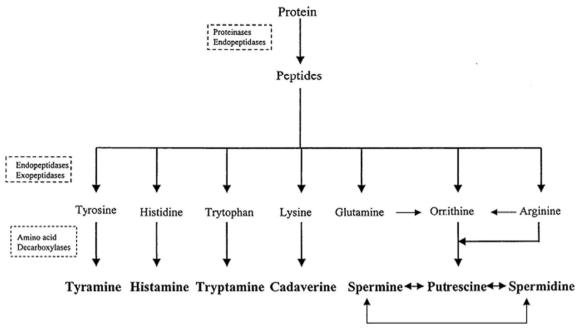


Fig. 1 Formation of biogenic amines (adopted from Ruiz-Capillas and Jiménez-Colmenero 2004)

where the modest average annual per capita consumption of 10 kg in the 1960s has increased to 26 kg global meat production and there will be an increase from the current annual production of 267 million tons in 2006 to nearly 320 million tons by 2016 (FAO 2007). This increased consumption has also increased the threat of meat borne poisoning associated with biogenic amines, thereby requires a special consideration. Besides poisoning, biogenic amines also determine the freshness/quality of the product as they are consistently found in association with spoilage, so referred as freshness or quality indicators.

As biogenic amines are associated with the safety and quality of meat and meat products it is necessary here to review the different aspects viz., their presence, the level of presence, factors influencing their formation, their importance, legal limits, analytical methods and measures to control their formation.

Biogenic amines in meat and meat products

The most prevalent biogenic amines in meat and meat products are tyramine, cadaverine, putrescine, and also histamine (Ruiz-Capillas and Jiménez-Colmenero 2004; Stadnik and Dolatowski 2010). The concentrations of these amines tend to vary depending on the different types of products (Table 1).

Amongst meat products, fermented meat products constitute considerably higher amount of biogenic amines. The main reason for higher amount is nonprotein nitrogen fraction (free amino acids), the main precursors of biogenic amines which increases during fermentation. The amino acid concentration is further related with the activity of endogenous meat enzymes which is in turn favoured by the denaturation of proteins as a consequence of acidity increase, dehydration and action of sodium chloride (Suzzi and Gardini 2003). Additionally, the microorganisms responsible for the fermentation process may contribute to biogenic amines accumulation (Latorre-Moratalla et al. 2010). However, the concentration of biogenic amines may vary in fermented products with comparable microbial flora, indicating that the complex interaction of factors is involved in their production. Lange et al. (2002) reported tryptamine level of 17 mg \cdot kg⁻¹ in salami detected with high performance liquid chromatography. Kim et al. (2005) reported putrescine, tyramine and spermine in fermented pepperoni sausages as 2.6, 0.9 and 9.6 mg·kg⁻¹ respectively. Papavergou et al. (2012) examined biogenic amines in Greek retail market fermented meat products and found tyramine, putrescine, histamine and cadaverine at high concentrations ranging from: 0 to 510, 0 to 505, 0 to 515 and 0 to 690 mg \cdot kg⁻¹, respectively.

Formation of biogenic amines

Biogenic amines are formed as a consequence of microbial activity (decarboxylases) (Fig. 1). Bacterial decarboxylases are not very specific, but their activity varies according to the bacterial species and strain (Bardocz 1995). Thus, biogenic amines accumulation in foods require the availability of precursors i.e., free amino acids (FAA), the presence of microorganisms with amino acid decarboxylases, and favourable conditions for their growth. Hence, all the factors bearing on

Table 1 Levels of biogenic amines in meat and meat products	t and meat pro	oducts							
Biogenic amines (mg/kg)									
Meat and meat product	Histamine	Tyramine	Tyramine Cadaverine Putrescine	Putrescine	Tryptamine	Tryptamine Phenylalanine Spermidine	Spermidine	Spermine	References
Pork raw	4.7	Ι	13.3	7.8	Ι	I	7.0	67.1	Halasz et al. 1994
Beef raw	ND-1.1	ND	ND	ND-1.75	I	I	1.9-4.2	28.7-44.6	Hernandez-Jover et al. 1996a
Dry sausages	<1-200	3-320	<1-790	<1.850	<10-91	>1-48	<1-14	19-48	Eerola et al. 1997
Adult bovine meat samples	I	10.71	18.54	2.08	20.42	I	2.20	27.15	Vinci and Antonelli 2002
Salami	<ld< td=""><td>I</td><td>I</td><td>1</td><td>17</td><td>1</td><td>Ι</td><td>I</td><td>Lange et al. 2002</td></ld<>	I	I	1	17	1	Ι	I	Lange et al. 2002
Onion sausage	<ld< td=""><td>Ι</td><td>Ι</td><td>Ι</td><td>32</td><td>I</td><td>Ι</td><td>Ι</td><td>Lange et al. 2002</td></ld<>	Ι	Ι	Ι	32	I	Ι	Ι	Lange et al. 2002
Ham	I	I	I	I	7.5	1	I	Ι	Lange et al. 2002
Pepperoni sausage (fermented)	Ι	0.9	Ι	2.6	Ι	Ι	Ι	9.6	Kim et al. 2005
Pepperoni sausage (fermented) after 20kGy	I	0.2	I	Complete destruction	I	1	I	4.2	Kim et al. 2005
Beef	Ι	24.7	Ι	4.7	I	I	Ι	28.4	Min et al. 2007b
Beef after irradiation at 2Gy	I	9.3	I	2	I	1	Ι	22.4	Min et al. 2007b
Pork	I	1.3	I	2.3	I	Ι	Ι	31.3	Min et al. 2007b
Pork after irradiation at 2Gy	Ι	0.8	Ι	0.3	Ι	I	Ι	25.9	Min et al. 2007b
Smoked turkey fillets (aerobic packaged)	32.9	25.0	Ι	I	4.1	I	Ι	I	Ntzimani et al. 2008
Smoked turkey fillets (skin-packaged)	11.9	4.3	I	I	2.8	I	I	I	Ntzimani et al. 2008
ND not detected, <ld below="" detection<="" limit="" of="" td="" the=""><td>ection</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></ld>	ection								

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production of the substrate (FAAs), the enzyme, and also their level of activity, affect the type and amount of biogenic amines present in each case (Ruiz-Capillas and Jiménez-Colmenero 2004).

Two mechanisms of action, for amino acid decarboxylation, have been identified; a pyridoxal phosphate dependent reaction and a non-pyridoxal phosphate dependent reaction (Eitenmiller and De Souza 1984).

The presence of decarboxylases is closely tied to microbiological aspects which thereby influence the final level of biogenic amines. The microbiological aspects are further related to the bacterial species, strain (decarboxylase+ve) and their growth. Additionally, availability of substrate (free amino acids, FAA) is associated with the raw material (meat composition, pH, handling conditions, etc.) as the substrate source and reaction medium. Ruiz-Capillas and Jiménez-Colmenero (2004) reviewed that these factors are related to each other and are further influenced by the technological processes associated with types of meat derivative (steak, roast, ham, ground, restructured, comminuted, fresh, cooked, smoked, fermented, etc.) and storage conditions (time/temperature, packaging, temperature abuses, etc.). Therefore, the activity of substrate and enzyme is also related with the combined action of these factors which ultimately determines the final concentrations of biogenic amines (Fig. 2) and thus reviewing of these factors would further help to take steps to control the levels of biogenic amines.

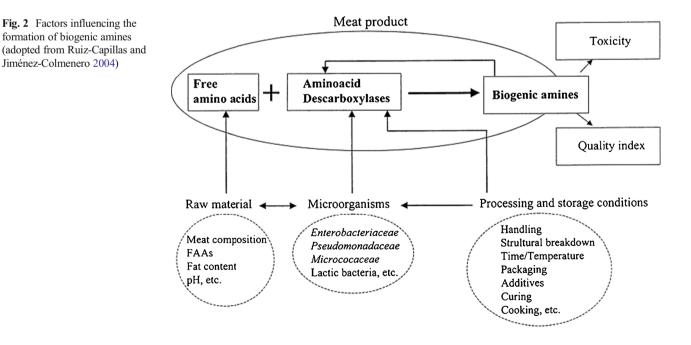
Raw materials

The factors like meat composition (presence of FAAs, their concentrations, fat content), pH, and source of raw material

affect the formation of biogenic amines. Proteolysis is a crucial factor, because it is directly related to availability of free amino acids that provide a substrate for BA formation. During storage and treatment, the concentrations of precursors increase as a result of proteolytic events, due essentially to many of the microorganisms present (Komprda et al. 2008). It has also been documented that fat content influences the formation of biogenic amines (Hernandez-Jover et al. 1997). This phenomenon has been most intensively studied in cheese, where it has been observed that the concentration of biogenic amines decreases along with fat content.

Various studies have shown that the type of meat source can influence the formation of biogenic amines. Some authors (Wortberg and Woller 1982; Vidal-Carou et al. 1990) have reported that histamine and tyramine formation tends to be found less in packed meats made only with pork (e.g., cooked ham and cured ham) than in other meat derivatives containing mixtures of beef and pork (salami, salchichon, chorizo or Bologna sausages), where there was a greater tendency to form tyramine.

The level of biogenic amines is greatly influenced by net pH balance as pH affects the production by two mechanisms (European Food Safety Authority Panel 2011). One is affecting the growth by acidity which inhibits the growth of microorganisms (Maijala et al. 1993). The other affects the production and activity of the enzyme because in low pH environment, bacteria are more stimulated to produce decarboxylase as a part of their defence mechanisms against the acidity (Bover-Cid et al. 2006). As the pH decreases, decaboxylase activity increases and thereby increases the production of biogenic amines. Halasz et al. (1994) documented that histidin decarboxylase activity increases in acid media, with an



optimum pH range of 4 to 5.5. The final pH of the meat, which can vary depending on a variety of factors, could, therefore, have a considerable influence on the production of biogenic amines. High pH, for instance in DFD (dark, firm and dry) meat, favours microbial proliferation which promotes FAAs, but limits decarboxylase activity. The opposite is true of PSE (pale, soft, exudative) meat. Therefore, the meat type also (PSE and DFD) affects factors directly involved in the activity of amino descarboxylases enzymes and the final presence of biogenic amines in these meat (Ruiz-Capillas and Jiménez-Colmenero 2004).

Microbial contaminant

Amino acid decarboxylase+ve microorganisms play an important role in formation of biogenic amines (Fig. 3). This ability has been associated with some groups of microorganisms. For example, putrescine and cadaverine production is frequently found in enterobacteria, and tyramine production is reported in the majority of enterococci. However, within microbial groups, this ability to produce biogenic amines is a strain-specific characteristic and is more widely distributed among certain genera or species which suggest that horizontal gene transfer may account for their dissemination between strains (Lucas et al. 2005). In addition, the enzymes of pathways involved in biogenic amine production can be encoded by unstable plasmids and only strains harbouring BA-related plasmids are able to produce BA (Lucas et al. 2005). The synthesis of BAs in bacteria may be associated with the supply of energy and to help protect from acid stress (Foster 2004). Slemr (1981) also reported that there was no formation of biogenic amines in sterile meat, and that when concentrations increased in meat, they did so along with microorganisms.

Although species of many genera such as *Bacillus*, *Citrobacter*, *Clostridium*, *Klebsiella*, *Escherichia*, *Proteus*, *Pseudomonas*, *Salmonella*, *Shigella*, *Photobacterium* and the lactic bacteria *Lactobacillus*, *Pediococcus* and *Streptococcus* are capable of decarboxylating one or more amino acid (Brink et al. 1990) but, decarboxylase activity in meat products is attributed chiefly to *Enterobacteriaceae*, *Pseudomonadaceae*, *Micrococcaceae* and lactic bacteria. Numerous researchers have sought to establish a relationship between the formation of biogenic amines in meat and meat products and the activity of various types of microorganisms (Table 2).

Starter culture

The choice of suitable starter culture with aminooxidase activity is fundamental in preventing the formation of high levels of biogenic amines in fermented meat products (Suzzi and Gardini 2003; Karovičová and Kohajdová 2005). Lactic acid bacteria are the most widely used microorganisms used in meat industry as starter culture together with micococci and/or coagulase-negative staphylococci because of their acidification property as well as proteolytic and lipolytic activities which are responsible for the color formation and aroma development (Suzzi and Gardini 2003; Latorre-Moratalla et al. 2010). Moreover, starter LAB able to compete with nonstarter bacteria during the later phase of ripening and throughout storage can further avoid excessive biogenic amines production.

The inability of the culture to form biogenic amines but also its ability to grow well at the temperature intended for processing of the product and competitiveness in suppressing the growth of wild amine producing microflora should be taken into consideration in the selection of starter cultures

Fig. 3 Biogenic amine biosynthesis pathways in bacteria predicting the membrane antiporter protein delivering the amino acid substrate into the cell and removes (excretes) the decarboxylated product from the cytoplasm. (Adopted from Bover-Cid et al. 2000); Amino acid decarboxylase (aaD)

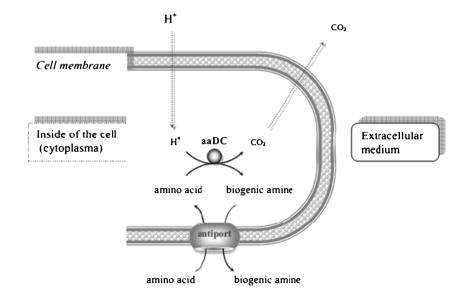


Table 2	Index of biogenic amines in meat and meat products

Products	Biogenic amines	Refernces
Fresh beef meat	Putrescine and cadaverine	Slemr 1981
Bologna sausages	BAI=Putrecine+cadaverine+Histamine+tyramine	Wortberg and woller (1982)
Raw and cooked ground beef	Tyramine, Putrecine	Sayem-El-Daher et al. 1984
Pork meat at 6-8 °C	BAI=Putrecine+cadaverine+Histamine+tyramine	Hernandez-Jover et al. 1996
Dry sausages	Tyramine, Histamine, Putrecine and cadaverine	Eerola et al. 1996
Broiler chicken cuts stored in modified atmosphere	Tyramine, putrescine and cadaverine	Rokka et al. 2004
Fresh beef meat fresh and packed in aerobic atomospher with biopolymers	Spoilage index: Ttyramine and cadaverine	Galgano et al. 2009
Turkey meat under modified atmospheric packaging	Freshness indicators: Cadaverine or Putrescine+cadaverine+tyramine	Fraqueza et al. 2012

(Suzzi and Gardini 2003). The formation by the strains of bacteriocin (for example curvacin A) can increase their competitiveness (Hammes and Hertel 1996). A rapid pH decrease caused by amine negative starter cultures can largely prevent biogenic amines accumulation in sausages (Bover-Cid et al. 2001a). However, the selection of lactic acid bacteria with application in meat fermentation has to take in consideration the various, specific requirements of the fermentation process (Roig-Sagues and Eerola 1997).

Starter cultures are not always able to control the decarboxylase-positive strains. The discrepancies observed in their efficiency in BA control during sausage fermentation and ripening could depend on the raw meat microbiological quality and the characteristics of natural microflora, in particular aminepositive nonstarter LAB (Maijala and Eerola 1993; Roig-Sagues and Eerola 1997). These microorganisms are often responsible for BA formation in fermented sausages (Maijala and Eerola 1993).

Masson et al. (1996) found that tyramine producing strains in dry sausage belonged to *Carnobacterium*, *Lactobacillus curvatus* and *L. plantarum*, whereas *Micrococcaceae* and *L. sake* did not produce tyramine. It has been suggested that the use of amino-negative starters comprised of *L. sakei* or *Pediococus pentosaceus* could prevent the formation of biogenic amines in dry sausage (Bover-Cid et al. 2001b), although any reduction would always depend on other factors influencing formation, especially the raw material (Fig. 3).

Mixed starter cultures (*L. sakei, S. carnosus* and *S. xylosus*) greatly reduced (about 90 %) the presence of putrescine, cadaverine and tyramine in Spanish sausages (Bover-Cid et al. 2000). Likewise, similar decreases in tyramine, cadaverine, and histamine concentration in sausages using amine negative mixed (staphylococci plus lactobacilli) starter cultures were observed by Maijala et al. (1995a). In French sausages, high concentration of histamine was found in industrial product added with starter cultures rather than in artisanal sausages (Montel et al. 1999). The use of starter cultures (*P. Pentosaceus* and *S. xylosus*; *L. sakei* and *S. xylosus*) did not reduce biogenic amine accumulation in Italian dry sausages Salsiccia and Soppressata (Parente et al. 2001). A slight reduction of tyramine, cadaverine and putrescine was observed in fermented sausages added with *M. carnosus* plus *L. plantarum* and *M. carnosus* plus *P. Pentosaceus* (Hernandez-Jover et al. 1997). Selected *L. sakei* strains were able to reduce biogenic amines (with the exception of tyramine), also in the presence of an amine-positive Lactobacillus strain (Roig-Sagues and Eerola 1997).

Similarly, the presence of a selected starter culture (L. sakei CTC494) reduced BA accumulation during ripening, but only if raw meat was characterised by a good quality with Enterobacteriaceae count not exceeding 10^3 cfu g⁻¹ (Bover-Cid et al. 2001c). The addition of the same strain of L. sakei, along with proteolytic S. carnosus and S. xylosus, decreased biogenic amines accumulation in the production of Fuet (Bover-Cid et al. 2000) and reduced the total biogenic amines content of 80-90 % with respect to the sausages without starter cultures added. A 50 % reduction in biogenic amines was observed also in sausages fermented by L. curvatus CTC371 in association with a proteolytic strain of S. xylosus, which increased the free amino acid availability (Bover-Cid et al. 2001b). In contrast, the use of single starter cultures of Pediococcus cerevisiae and L. plantarum did not decrease tyramine and total biogenic amines contents with respect to spontaneous fermentation (Buncic et al. 1993).

Meat processing

Amine content and profiles may vary depending on various extrinsic and intrinsic factors during the processing, such as pH, redox potential, temperature, additives, curing, the size of the sausage (Latorre-Moratalla et al. 2008). The pH is a key factor influencing the amino acid decarboxylase activity. Bacterial amino acid decarboxylases usually have acid pH optimum. Decrease of pH results in increasing decarboxylase activity of bacteria. In these conditions bacteria produce more decarboxylases as part of their protective mechanism. However, rapid and sharp reduction in pH is known to reduce the growth of the amine-positive microorganisms (Maijala et al. 1995b). The redox potential of the medium also influences biogenic amines production. Conditions resulting in a reduced redox potential stimulate histamine production, and histidine decarboxylase activity seems to be inactivated or destroyed in the presence of oxygen (Karovičová and Kohajdová 2005). Amine formation by bacteria is decisively influenced by temperature too. Temperature between 20 and 37 °C is optimal for the growth of the most bacteria containing decarboxylases, decreased temperature stops their growth (Karovičová and Kohajdová 2005).

Additives have also been found to have control on biogenic amines. Sausage containing potassium sorbate, and ascorbic acid showed a significant reduction in biogenic amine accumulation (Bozkurt and Erkmen 2004). Sodium nitrites (45 to 195 ppm) in sausage decreased biogenic amine production, (Kurt and Zorba 2009). This confirms the findings of Bozkurt and Erkmen (2004) that sodium nitrite and sodium nitrate inhibit biogenic amine production. The addition of 0 to 1 % glucono-delta-lactone into meat decreased histamine and putrescine production through a pH drop in meat (Maijala et al. 1993). The addition of sugar may also slightly reduce biogenic amine formation (Bover-Cid et al. 2001a). Mah and Hwang (2009) documented that glycine also inhibits the amine forming activity of microorganisms. According to Suzzi and Gardini (2003) biogenic amines accumulation decreases markedly with the increase of NaCl concentration, while proteolytic activity is higher for intermediate concentration of salt, pointing out that there is not necessarily a correlation between these two variables. Karovičová and Kohajdová (2005) reported that presence of sodium chloride activates tyrosine decarboxylase activity and inhibits histidine decarboxylase activity.

A relationship was also found between biogenic amines content and the size of dry fermented sausages. The diameter of the sausage affects the environment in which microorganisms grow; for example, salt concentration is usually lower and water activity is higher in sausages with a larger diameter. A larger diameter may be one of the reasons for a higher production of certain amines, such as tyramine and putrescine. Generally, biogenic amines level in the bigger diameter sausages were higher than in the thinner sausages and in the central part of the sausages than in the edge (Suzzi and Gardini 2003; Ruiz-Capillas and Jiménez-Colmenero 2004).

Hygiene and storage conditions

The way by which raw materials are handled affects the production of biogenic amines. The importance of using measures focused on the hygienic quality of both raw material and processing units to avoid the development of aminogenic contaminant bacteria and in turn, to reduce biogenic amines content, is well known. Smith et al. (1993), for example, detected only tyramine consistently over 120 days storage at 1 °C among all the amines (histamine, phenylethylamine, tryptamine, and tyramine) after the decontamination of beef carcasses (washing with chlorine and lactic acid), followed by vacuum packing of cuts. However, proper hygiene may not be enough to avoid some biogenic amines formation and other technological measures must be applied (Latorre-Moratalla et al. 2010).

The amine production is greatly affected by storage conditions (temperature and time). Generally the amine production rate increases with the temperature. Conversely, biogenic amine accumulation is minimised at low temperatures through inhibition of microbial growth and the reduction of enzyme activity. The optimum temperature for the formation of biogenic amine by mesophilic bacteria has been reported to be between 20 and 37 °C, while production of biogenic amine decreases below 5 °C or above 40 °C. Klebsiella pneumonia was reported to produce cadaverine more extensively at 20 °C than at 10 °C, whereas Enterobacter cloacae was able to produce putrescine at 20 °C but not at 10 °C (Halasz et al. 1994). Unsuitable storage temperatures (i.e., temperatures >5 °C), prolonged storage, or temperature abuses during storage have a two-fold effect: on proteolysis due to increased microbial growth promoting penetration in the muscle; on amino decarboxylase activity (Maijala and Nurmi 1995).

Significance of biogenic amines in meat and meat products

Biogenic amines in products are important for two main reasons. First reason is their toxicological effects due to intake of foods containing high concentrations of biogenic amines and due to their interaction with some medicaments (Bardocz 1995). Second is their use as biogenic quality index role as an indicators of quality and/or acceptability in some foods (Hernandez-Jover et al. 1997; Ruiz-Capillas and Moral 2001).

Toxicological effects

In normal circumstances, the human body is able to rapidly detoxify histamine and tyramine absorbed from foods by metabolizing them to physiologically less active degradation products by involving the process acetylation and oxidation mediated by the enzymes monoamine oxidase (MAO; EC 1.4.3.4), diamine oxidase (DAO; EC 1.4.3.6), and polyamine oxidase (PAO; EC 1.5.3.11) (Bardocz 1995). However, if these detoxifying mechanisms are upset, either because of high amine intake or because the individual is allergic or is deficient in aminooxidases due to consumption of or treatment with oxidase enzymes (e.g., monoamine oxidase inhibitor, MAOI), biogenic amines may build up in the body and could cause serious toxicological problems (Halasz et al. 1994). Hence, the toxicity of biogenic amines will depend on factors associated with the food itself (quantitative and qualitative) as well as factors associated with the consumer (individual susceptibility and state of health).

Conditions which enhance the severity of toxicological effects

The severity of the clinical symptoms caused by BAs depends on the amount and variety ingested, individual susceptibility, and the level of detoxification activity in the gut. Individuals with respiratory and coronary problems or those with hypertension or vitamin B₁₂ deficiency are sensitive to lower doses of biogenic amines (Bardocz 1995). People with gastrointestinal problems (gastritis, irritable bowel syndrome, Crohn's disease, stomach and colonic ulcers) are also at risk because the activity of oxidases in their intestines is usually lower than that in healthy individuals (Stadnik and Dolatowski 2010). Patients, who are taking medicines with inhibiting effect to MAO, DAO and/or PAO such as painkillers, psychopharmaceutics, and drugs used for the treatment of Alzheimer's and Parkinson's diseases might have a changed metabolism of biogenic amines, which can cause health problem (Latorre-Moratalla et al. 2008). Other compounds, like alcohol and acetaldehyde, also can augment the toxic potential of biogenic amines, since they promote the transportation of these through the intestinal wall (Ruiz-Capillas and Jiménez-Colmenero 2004; Stadnik and Dolatowski 2010). The toxic potential of these dietary amines is even more alarming if we consider that approximately 20 % of the European population regularly consumes MAOI drugs as antidepressants, which inhibit aminooxidase activity (Ruiz-Capillas and Jiménez-Colmenero 2004).

Polyamines, such as putrescine, cadaverine, spermidine and spermine, do not exert a direct toxic effect, however, inhibit histamine or tyramine detoxifying enzymes and thus act as enhancers of their toxicity. These amines in the intestinal tract compete for the detoxifying enzymes that tend to increase the level of histamine and tyramine in blood (Karovičová and Kohajdová 2005; Onal 2007).

Associated symptoms

Biogenic amines in diet can cause pharamacological and toxicological effects due to their psychoactive and vasoactive properties (Stadnik and Dolatowski 2010). The most conspicuous symptoms of consumption of high doses of biogenic amines are vomiting, respiratory difficulties, perspiration, palpitation, hypo- or hypertension and migraine (Kordiovska et al. 2006).

Psychoactive amines, such as histamine, can cause some neurotransmission disorders due to their action as false neurotransmitters. Histamine exerts its toxic effects by interacting with two types of receptors $(H_1 \text{ and } H_2)$ on cellular membranes. Vasoactive properties are exhibited either as vasoconstrictor or as vasodilator. Some aromatic amines (tyramine, tryptamine, and β -phenylethylamine) show a vasoconstrictor action while others (histamine and serotonin) present a vasodilatador effect in blood vessels, capillaries and arteries, causing headaches, hypotension, flushing, gastrointestinal distress and oedemas (Onal 2007). The vasoconstrictor amines, largely tyramine, have been proposed as the initiators of hypertensive crisis in certain patients and of dietary-induced migraine. The physiological effects of tyramine include: peripheral vasoconstriction, increased cardiac output, increased respiration, elevated blood glucose, and release of norepinephrine (Onal 2007).

Polyamines are getting great attention for their presence in meat and meat products which have no adverse health effects as such, but have been described as potential precursors of stable carcinogenic N-nitrosamines and to enhance the growth of chemically induced aberrant crypt foci in the intestine (Stadnik and Dolatowski 2010). Formation of N-nitroso compounds, constitutes an additional toxicological risk associated to biogenic amines, especially in meat products that contain nitrite and nitrate salts as curing agents (Karovičová and Kohajdová 2005; Onal 2007). The reaction between nitrosating agents and primary amines generates short-lived alkylating agents that react with other components in the food matrix to generate products (mainly alcohols) devoid of toxic activity in the relevant contents. Secondary amines are known to form carcinogenic N-nitrosamines by reaction with nitrosating compounds, while tertiary amines produce a range of labile N-nitroso products. However, as primary biogenic amines can convert to secondary amines, not only on heating but also during storage at room temperature, and further reaction with nitrite can occur. In fatty foods, such as bacon, at high temperature and in the presence of water, the carcinogen N-nitrosopyrrolidine and N-nitrosopiperidine can be formed from secondary amines such as putrescine or spermidine (Karovičová and Kohajdová 2005; Onal 2007).

Biogenic amine quality index

The concentrations of some biogenic amines (tyramine, putrescine, and cadaverine) normally increase during the processing and storage of meat and meat products, whereas others (spermidine and spermine) decrease or remain constant (Ruiz-Capillas and Jiménez-Colmenero 2004; Stadnik and Dolatowski 2010). Therefore, their amounts and ratios have been proposed as an index of the hygienic conditions of raw material and/or manufacturing practices since their amount increase during microbial fermentation or spoilage (Latorre-

Moratalla et al. 2008: Stadnik and Dolatowski 2010). Many attempts have been made to establish a relationship between meat quality and changes in the content (individual or combined) of biogenic amines in different meat derivatives, thus have been used as quality indexes and indicators of unwanted microbial activity in meat and cooked meat products (Table 2). It generally has proven more difficult to apply similar quality criteria to fermented products. This is at least partly because biogenic amine concentrations vary much more widely in fermented products than in fresh meat and cooked meat products (Table 1), because of the number of different factors involved in their formation. These factors include the type and degree of contamination of raw materials, which are promoted by structural breakdown, manufacturing practices, certain processing stages, and the use of starters. All these factors vary according to the nature of the product and, in some cases, can mask changes in the type and concentration of biogenic amines through the different phases of treatment and storage, delaying visible signs of spoilage and/or off-odour development.

Meat freshness should be evaluated by considering an amine index, which includes all the biogenic amines related to meat spoilage. As tyramine increases considerably during meat storage, this biogenic amine should also be included in a biogenic amine index (BAI). This is the case of the BAI of putrescine+cadaverine+histamine+tyramine, proposed by Wortberg and Woller (1982) and Hernandez-Jover et al. (1996a, b). Wortberg and Woller (1982) established 500 mg. kg^{-1} as the limit for Bologna sausage, minced beef and pork. Hernandez-Jover et al. (1996a, b) suggested the following limits: BAI $<5 \text{ mg} \cdot \text{kg}^{-1}$ for good quality fresh meat; between 5 and 20 mg kg^{-1} for acceptable meat, but with initial spoilage signs; between 20 and 50 mg \cdot kg⁻¹ for low meat quality; finally, $>50 \text{ mg} \cdot \text{kg}^{-1}$ for spoiled meat. Not all spoilage or starter microorganisms can decarboxylate free amino acids. Even within the same species, not all strains develop the same decarboxylating capacity, so that a low biogenic amine concentration does not always signal good microbiological quality. There is, therefore, no simple matter to establish a biogenic amine index that reliably predicts quality for products of this kind (Ruiz-Capillas and Jiménez-Colmenero 2004).

Legal limits

Determination of the exact toxicity threshold of biogenic amines in a given food product is extremely difficult, because their effect does not depend on their presence alone, but is also influenced by other compounds and by the specific efficiency of the detoxifying mechanisms in different individuals (Ruiz-Capillas and Jiménez-Colmenero 2004; Stadnik and Dolatowski 2010). However, legal limits have been established by European Union (EU) and United States food and drug administration (USFDA) and have also been suggested by many authors.

Histamine is the amine most studied with regard to its toxicological effects. An intake of 5–10 mg of histamine can be considered as defecting to some sensitive people, 10 mg is considered as tolerable limit, 100 mg induce a medium toxicity and 1000 mg is highly toxic (Karovičová and Kohajdová 2005). Other authors (Hernandez-Jover et al. 1997; Gardini et al. 2001) suggested following toxic limits of histamine: 8– 40 mg causes slight poisoning, 40–100 mg intermediate poisoning and over 100 mg can cause intensive poisoning. On the basis of data from food intoxication outbreaks a legal upper limit of 100 mg histamine.kg⁻¹ food and 2 mg.lt.⁻¹ of ethanol has been suggested.

The EU has established regulations according to which histamine level should be below 100 mg \cdot kg⁻¹ in raw fish, below 200 mg \cdot kg⁻¹ in salted fish for species belonging to the Scombridae and Clupeidae families and up to 400 mg \cdot kg⁻¹ in cured products (Karovičová and Kohajdová 2005). Less is known about the toxic doses of other amines.

FDA has established 50 mg \cdot kg⁻¹ guideline for histamine in seafood. Seafood products containing above this level of histamine must not be used for human consumption and are subjected to recalls (FDA 2002).

Determination of biogenic amines in meat and meat products

Determination of amines is oriented towards two reasons, a) their potential toxicity and b) the possibility of using them as food quality markers. Some of the major applications of biogenic amines analysis are: quality control of raw materials, intermediates and end products, monitoring fermentation processes, process control, research & development (Onal 2007).

For separation and quantitative determination of biogenic amines in foods several methods have been developed (Karovičová and Kohajdová 2005). The extraction of amines presents the critical step of the process and it affects negatively the analytical recoveries.

Different approaches have been made to quantify biogenic amines in biological matrices after extraction, such as thinlayer chromatography (Fadhlaoui-Zid et al. 2012), gas chromatography (Almeida et al. 2012), and most commonly high performance liquid chromatography (HPLC) (Zhai et al. 2012). These methods are well approved. Different conditions of HPLC for the determination of biogenic amines in meat and meat products have also been documented by my workers (Smela et al. 2003; Saccani et al. 2005).

The extent of spoilage of muscle food products was determined through measurement of volatile biogenic amines from several sources: chicken, turkey, beef, pork and fish and the amines were monitored by ion mobility spectrometry (Karpas et al. 2002). An amperometric biosensor using pea seedling amine oxidase (PSAO) as molecular recognition element for the determination of biogenic amines has been recently characterized in flow injection analysis (FIA) by Telsnig et al. (2012).

Control of biogenic amines

The inhibition of microbial growth and their decarboxylases activity can control the biogenic amines formation. The prevention of biogenic amine formation in food has, therefore, been achieved using temperature control, using high-quality raw material, good manufacturing practices, the use of nonamine forming (amine-negative), amine oxidizing starter cultures for fermentation or the use of enzymes to oxidize amines (Dapkevicius et al. 2000), the use of microbial modelling to assess favorable conditions to delay biogenic amine formation (Emborg and Dalgaard 2008), packaging techniques (Mohan et al. 2009), high hydrostatic pressure (Penas et al. 2010), irradiation (Kim et al. 2005; Min et al. 2007a; Min et al. 2007b), and food additives (Mah and Hwang 2009).

Use of specific starter culture

Starter cultures used in fermentation can also delay the formation of biogenic amines (Bover-Cid et al. 2001b; Latorre-Moratalla et al. 2008). Certain specific starters are either amine-negative (not able to decarboxylate amino acid into biogenic amines) or amine oxidizing (oxidize biogenic amines) into aldehyde, hydroden peroxide, and ammonia) bacteria (Bover-Cid et al. 2000; Suzzi and Gardini 2003) which require optimal growth conditions to dominate over biogenic amine producing (Xu et al. 2010) and other contaminant bacteria (Maijala et al. 1995a; Maijala et al. 1995b).

Bacteria described as biogenic amine oxidizers include *Micrococcus varians* (Leuschner and Hammes 1998b), *Natrinema gari* (Tapingkae et al. 2010), *Brevibacterium linen* (Leuschner and Hammes 1998a) and *Lactococcus sakei*, *Lactobacillus curvatus* (Dapkevicius et al. 2000).

Microbial modelling

With the focus on controlling growth and predicting risk factors, microbial modelling can be employed to study the growth and inactivation of microorganisms. Modelling microorganisms responsible for biogenic amine formation (Emborg and Dalgaard 2008) has been used to explore options for biogenic amine control.

Irradiation

Shelf life extension of food products treated with irradiation has been applied to many foods including pork and beef (Min et al. 2007b), sausage (Kim et al. 2005) and chicken (Min et al. 2007a). Ground pork and beef inoculated with Alcaligenes faecalis, Bacillus cereus and Enterobacter cloacae were treated with gamma irradiation doses of 2 kGy. The total amount of biogenic amines (histamine, tyramine, spermidine, betaphenylethylamine, tryptamine, cadaverine, and putrescine) formed during 24 h storage at 4 °C was reduced by the treatment (Min et al. 2007b). While irradiation delays the formation of some biogenic amines, there are reports of irradiation enhancing the formation of other biogenic amines, for instance, Min et al. (2007a) reported reduction in biogenic amines in raw chicken breast and thigh meat using irradiation at a dose of 2 kGy, but the increment of some of the biogenic amines level (histamine, spermidine, and spermine), perhaps because irradiation changes the structure and physiological properties of enzymes that form biogenic amines.

Packaging

Packaging (MAP, vacuum and active packaging) may also play an important role in delaying the production of biogenic amines due to inhibition of microorganisms or the enzyme producing biogenic amines. There are reports on the successful control of biogenic amines through packaging e.g., MAP of chicken meat viz. Patsias et al. (2006) studied precooked chicken meat under air and MAP (30 % CO₂/70 % N₂) at 4 °C for up to 23 days, when the biogenic amines levels were compared after 23 days of storage under MAP, putrescine and tyramine were reduced compared with packaging under air.

Ozone decontamination

Ozone treatment of meat has found to be useful to control the putrescine and cadaverine levels. Ozone decontamination resulted in lower levels of putrescine (32, 37 mg·kg⁻¹) and cadaverine (132, 30 mg·kg⁻¹) in poultry meat as compared to that in controlled chilled poultry (Mercogliano et al. 2014).

Conclusions

Biogenic amines are constantly present in meat and meat products and their determination is important for consumers with respect to both toxicological points of view as well as to determine freshness/quality of the product. Monitoring of raw materials and products at multiple points is essential to evaluate the relevance of various factors contributing to amine formation and accumulation in fermented foods. Many new methods have also been emerged to delay or reduce the production of these amines which can be employed to avoid the ill health effects related with biogenic amines.

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