

**Review****Research progress of biogenic amines in fermented sausages: A review**

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Abstract

Biogenic amines (BAs) widely exist in fermented sausages, and high concentrations of BAs are harmful to human health. Therefore, rapid detection of BAs in fermented sausages, and effective control of BAs require urgent attention. The present review aims to expound the toxicity of BAs, analyse their formation mechanism and the influencing factors, and identify some effective control measures, so as to provide a basis for further studies on BAs in fermented sausages.

Keywords

fermented sausage,
biogenic amine,
information mechanism,
inhibition measures

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Introduction

Fermented sausage is a type of meat products made from livestock and poultry meat. After mincing, chopping, emulsifying, and other operations, the meat is filled into natural or artificial casing, and then fermented by long-term natural or artificial control (Gonzalez-Tenorio *et al.*, 2013). Typical sausage products include salami, Sichuan sausage, and Guangzhou sausage (Komprda *et al.*, 2001). During fermentation, the macromolecular protein in raw meat is degraded into micromolecular polypeptides and amino acids, which are more conducive for the formation of sausage flavour, and human digestion and absorption (Maijala *et al.*, 1995); fatty acids are also gradually decomposed and oxidised to form carbonyl compounds such as aldehydes and ketones. Besides, carbonyl compounds are further dehydrated, hydrolysed, and cyclised to form lactone compounds, thus giving meat products a good flavour (Wang, 2017). However, certain microorganisms during fermentation may cause decarboxylation of various free amino acids to form corresponding biogenic amines (BAs) (Papavergou *et al.*, 2012). In the present review, the formation pathway of BAs, and the factors affecting their formation in fermented

sausages were analysed, and the measures to inhibit their formation were identified.

Biogenic amines and the toxicity

BAs are low molecular weight organic compounds with biological activity and nitrogen content. Based on the chemical structure, BAs can be divided into heterocyclic (*e.g.*, histamine and tryptamine), aliphatic (*e.g.*, putrescine, cadaverine, spermidine, and spermidine), and aromatic (*e.g.*, tryptamine and β -phenylethylamine) compounds. Based on the number of amines, BAs can be divided into monoamine (*e.g.*, tyramine and phenylethylamine), diamine (*e.g.*, histamine, putrescine, and cadaverine), and polyamine (*e.g.*, spermidine and spermidine) (Önal, 2007).

BAs widely exist in common food products; the content of BAs in meat and fish products is often used as a quality index to monitor their freshness, while BAs in alcoholic beverages are gaining much research interest recently (Hernández-Jover *et al.*, 1997; Sirocchi *et al.*, 2013). Although the content of BAs is not high in alcoholic beverages, ethanol is naturally an inhibitor of amine oxidase, and ethanol with a volume fraction of more than 12% can inhibit more than 91% of amine oxidase, thus resulting in

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increased toxicity of BAs (García-Ruiz *et al.*, 2011). Therefore, the limits of BAs in alcoholic beverages are more stringent than those in other food products (Milheiro *et al.*, 2019). In addition, BAs are also detected in soy sauce pickles (Zou *et al.*, 2012), douche (Park *et al.*, 2019), and cheese (Liu *et al.*, 2018); and the content of BAs varies with food types and processing methods.

Low concentration of BAs (< 5 mg/kg) play an important role in the natural metabolism and physiological function of human body (Suzzi and Torriani, 2015). They participate in the synthesis of proteins, hormones, and nucleic acids, support the growth and proliferation of normal cells, and affect the normal maintenance of blood pressure and body temperature. They also affect membrane stability, stress and senescence response, and act as neurotransmitters (Naila *et al.*, 2010; Ramani *et al.*, 2014). However, high concentrations of BAs (> 100 mg/kg) can cause diseases such as hypertension, headache, vomiting, and respiratory disorders; among them, histamine and tyramine are the most toxic, which can lead to the symptoms of "mackerel poisoning" and "cheese reaction", respectively (McCabe-Sellers *et al.*, 2006; Dai, 2008; Hungerford, 2010). Histamine poisoning is caused by the intake of high concentration of histamine, which makes the normal metabolic mechanism unable to detoxify it. Symptoms of histamine poisoning are similar to allergies, and are characterised by neurological and gastrointestinal reactions such as headache, nausea, vomiting, diarrhoea, pruritus, flushing, and urticaria, as well as rhinorrhoea and hypotension. Tyramine is more toxic, and exerts its toxicity faster, and its toxicity will be increased by the presence of other BAs such as histamine (Del Rio *et al.*, 2017).

Excessive intake of tyramine in the diet can lead to migraine, neurological disorders, respiratory disorders, high blood pressure, gastrointestinal diseases, and allergic reactions. In severe cases, it can lead to stroke, heart disease, and even shock, thus directly endangering life (Alves *et al.*, 2017). Although the toxicity of cadaverine and putrescine is weak, they can inhibit the activities of histamine- and tyramine-related metabolic enzymes (Wójcik *et al.*, 2020). In addition, putrescine (Shalaby, 1996; Eerola *et al.*, 1997; Hernández-Jover *et al.*, 1997), cadaverine (Maijala and Eerola, 1993), spermine (Anli *et al.*, 2004), and spermidine can also be combined with nitrite to form carcinogenic nitrosamine.

Formation mechanism of BAs in fermented sausages

The precursors of BAs are generally amino acids. The formation of BAs in food products generally requires three preconditions. First, the presence of free amino acids in the foods. Second, the presence of decarboxylase-producing microorganisms as well as their survival and reproduction. Third, the presence of decarboxylase activity (Santos, 1996).

BAs are mainly produced by decarboxylation of amino acids or transamination of aldehydes and ketones. They are substances in which the H atom of NH₃ group has been replaced by alkyl or aromatic groups. The decarboxylation of amino acids is to remove the α-carboxyl group of amino acids to produce corresponding BAs and CO₂ under the action of microorganisms producing decarboxylase (Naila *et al.*, 2010). As shown in Figure 1, cadaverine is the product of decarboxylation of lysine under the action of tyrosine decarboxylase.

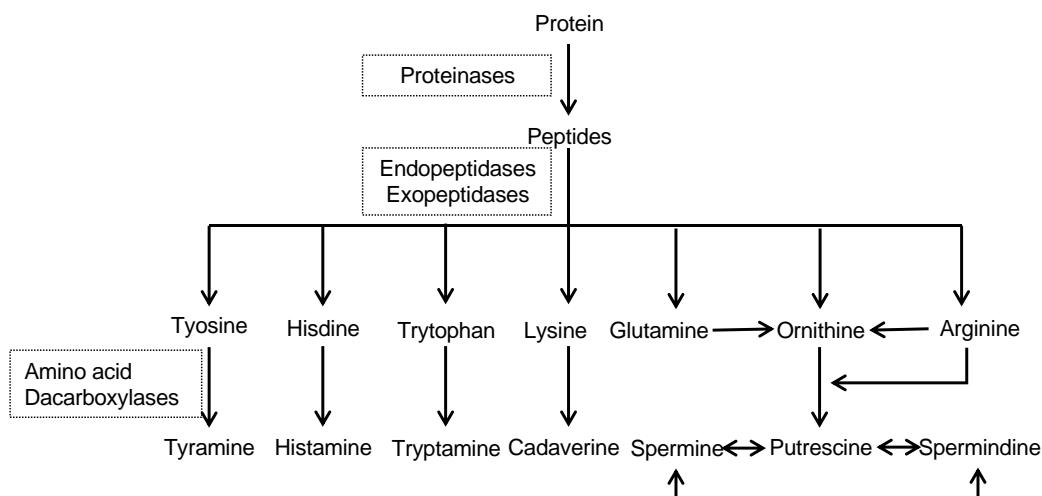


Figure 1. The formation mechanism of biogenic amines.

However, the formation of putrescine, spermine, and spermidine is more complicated, and involves multiple steps. Firstly, arginine is transformed into ornithine under the action of arginase. Certain bacteria can also convert glutamic acid into ornithine. Afterwards, ornithine is decomposed into putrescine by ornithine decarboxylase. Finally, putrescine forms spermidine under the catalysis of spermidine synthetase, and spermidine forms spermine under the action of spermine synthetase (Arena and Manca, 2001).

The processing conditions of fermented sausages such as high protein content, long maturation period, long protease hydrolysis period, and a drop in pH are favourable for the accumulation of BAs (Suzzi, 2003). The main BAs in fermented sausages are tyramine, histamine, cadaverine, and putrescine (Dabadé *et al.*, 2021). Latorre-Moratalla *et al.* (2017) found that tyramine (found in 95% of the samples) and histamine (found in 66% of the samples) were the most common and abundant BAs in fermented sausages.

Main factors affecting the formation of BAs in fermented sausages

The formation of BAs is mainly related to specific microorganisms. Due to the differences in climates and environments in different countries and regions, there are great differences in BAs in fermented sausages (Hu *et al.*, 2018). The accumulation of BAs in fermented sausages is mainly due to the metabolism of amino acids by contaminating microorganisms from the environment (Bodmer *et al.*, 1999), especially in traditional fermented sausages, where BAs content is relatively high. Bacterial amino acid decarboxylase plays a fundamental role in the formation of BAs. Therefore, the microorganisms that produce them are extremely important elements. Slemr and Beyermann (1985) reported that the production of BAs increased with the increase in microorganisms in sterile meat without BAs. Dabadé *et al.* (2021) found that the positive correlation between the number of microorganisms and production of BAs in fermented meat products was stronger than that in other foods. At present, amino acid decarboxylase genes are found in *Lactobacillus*, *Pediococcus*, *Lactococcus*, *Streptococcus*, *Enterococcus*, *Clostridium*, *Klebsiella*, *Escherichia*, *Proteus*, *Pseudomonas*, *Sardinia*, and *Streptococcus* (Li, 2007; Landete *et al.*, 2007; Feng *et al.*, 2013; Li *et al.*, 2018). It was

reported that most of the microorganisms producing BAs in fermented food are Gram-negative bacteria; while in fermented sausages, Gram-negative bacteria are often inhibited. Hence, Gram-positive bacteria, especially lactic acid bacteria, are the main strains producing BAs in fermented sausages (Marcobal *et al.*, 2012). Komprda *et al.* (2010) isolated *Lactobacillus plantarum*, *Lactobacillus brevis*, and *Enterococcus faecalis* from fermented sausages, which are tyramine- and histamine-producing bacteria. Trevino *et al.* (1997) also found that tyramine and putrescine were the main components in fermented beef sausages, and histamine and cadaverine were detected in the later stage of fermentation. Danilovic *et al.* (2011) showed that *Lactobacillus* isolated from fermented sausages had the activity of histamine decarboxylase, and *Lactococcus* and *Leuconostoc* could produce tyramine. Meng *et al.* (2010) analysed the ability of 63 strains of lactic acid bacteria to produce BAs, and only three strains produced histamine, while the others all produced tyramine.

The environmental conditions during the processing and storage of fermented sausages not only affect the metabolism and growth of microorganisms, but also affect the formation of BAs such as temperature and pH (Visciano *et al.*, 2020). Due to the vigorous growth and metabolism of microorganisms at higher temperature, the accumulation of BAs in fermented sausages will also increase at higher temperature. Therefore, temperature is the most important factor affecting the formation of BAs. Wang *et al.* (2015) studied the Chinese fermented sausages that were stored at -18, 0, 4, and 25°C for 20 days, and the contents of histamine in the fermented sausages at different storage temperatures were determined. Their results showed that the contents of histamine were higher than the initial amount ($p < 0.05$), except at -18°C. Even histamine at 0 and 4°C had a slight increase. Hence, it was suggested that fermented sausages should be stored at -18°C after ripening. Wang *et al.* (2020) found that the tyramine content of fermented fish at 25°C was 2.5 times higher than that at 15°C.

pH also affects the production of BAs in fermented sausages (Gardini *et al.*, 2001). Generally, the growth of amine-producing microorganisms is inhibited at low-acid environment. Wang *et al.* (2019) found that inoculating *Lactobacillus plantarum* and *Staphylococcus aureus* in fermented sausages can effectively reduce tyramine formation during

fermentation. However, Linares *et al.* (2009) found that the coding gene of decarboxylase could be induced at low pH; the increase of tyramine production by *Enterococcus durans* at low pH was due to the significant induction of gene expression of decarboxylase (TDCA) and transporter (tyrp), but these were not expressed at neutral pH. The TDC and aguA1 genes of *Lactobacillus brevis* which were involved in the production of tyramine and putrescine, respectively, were transcriptionally induced at low pH (Arena *et al.*, 2011). In addition, the production of BAs is related to the protective effect of bacteria on acidic environment. The production of BAs by lactic acid bacteria is the stress mechanism of the body. Under the condition of relatively low acid and nutrient deficiency, lactic acid bacteria metabolise basic BAs in order to maintain their own growth and regulate the stress (González de Llano *et al.*, 1998). Overall, some researchers have shown that rapid acidification could reduce the growth of decarboxylated microorganisms in fermented sausages that increase the BAs (Gardini *et al.*, 2001). Therefore, the optimal pH of fermented sausages should be established (Kumar *et al.*, 2017).

The content of free amino acids in raw materials, degree of contamination, processing methods, presence of additives, and other factors will also affect the formation of BAs in fermented sausages (Jaworska *et al.*, 2020; Xu *et al.*, 2020a). Dabadé *et al.* (2021) found that except tryptophan, the concentration of BAs was significantly positively correlated with the content of corresponding free amino acids. Liang *et al.* (2014) found that the content of free tyrosine increased to about 50 and 30 mg/kg after 10 h storage at 4 and 25°C, respectively. These tyrosines are likely to be utilised by amine-producing microorganisms in the subsequent fermentation process, thus resulting in the accumulation of tyramine in the product.

Any processing of raw materials will involve the process of structural damage such as grinding, chopping, and slicing which are conducive for microbial contamination and the formation of BAs. Xu *et al.* (2020b) found that the degree of contamination of raw meats had a significant impact on the content of BAs in fermented beef sausages. The total content of BAs in beef sausages made from slightly contaminated beef was 118.82 mg/kg, and that of beef sausages made from heavily contaminated beef was 182.32 mg/kg. Roseiro *et al.* (2010) pointed out that in traditional Portuguese dry

fermented sausages, the total concentration of BAs observed in the processing and storage stages increased continuously, while tyramine, putrescine, and cadaverine reached alarming levels. If decarboxylase negative starter was not added, the final product should not be prolonged.

The addition of fermented sausage ingredients also affected the accumulation of the total content of BAs in beef sausages to a certain extent (Yu *et al.*, 2021). Sun *et al.* (2018a) suggested that compound spices (cinnamon, clove, and star anise mixed at a ratio of 1:1:1) could effectively inhibit the accumulation of BAs in Harbin dry sausages ($p < 0.05$), and the inhibition effect increased with increasing spices amount ($p < 0.05$). Bover-Cid *et al.* (2001) found that sugar limited the formation of BAs in fermented sausages; sugar acidified during fermentation, which affected the formation of BAs. Jia *et al.* (2020) used liquid chromatography-mass spectrometry (LC-MS) to determine the inhibitory effect of star anise, Fructus Tsaokuo, cinnamon, clove, cassia seed, fennel, laurel leaf, and nutmeg on the accumulation of BAs in dry fermented mutton sausages. Among these, cinnamon and fennel extracts had the highest inhibitory activity. Increasing the salt content of fermented meat products can also effectively reduce the content of BAs in products (Liu *et al.*, 2020). Roseiro *et al.* (2006) found that the higher salt concentration (6% vs. 3%) of traditional dry fermented pork sausages resulted in the significant ($p < 0.001$) decrease in the level of BAs. BAs are heat-resistant, so heat treatment has little effect on BAs (Ruiz-Capillas and Herrero, 2019).

Research progress on detection methods of BAs

There are many methods available for the detection of BAs in fermented foods, such as high performance liquid chromatography (HPLC), ion exchange chromatography gas chromatography (ICE), thin layer chromatography, capillary electrophoresis (Steiner *et al.*, 2009; Li *et al.*, 2012), matrix assisted laser desorption / ionisation mass spectrometry (Su *et al.*, 2015), and electronic nose (Wojnowski *et al.*, 2019). However, due to the advantages of high column efficiency, accurate quantitative analysis, and high sensitivity, HPLC has become the most commonly used analytical tool for BAs. Jastrzebska *et al.* (2018) used liquid chromatography tandem mass spectrometry to analyse BAs in alcohol samples. 5-Bis-(trifluoromethyl) phenyl isothiocyanate (BPI) is a

simple and rapid method for derivatisation of histamine, tyrosine, tryptamine, and 2-phenylethylamine. The results showed that the intra-day precision of this method was between 1.26 and 4.99%, while the inter-day precision ranged from 2.89 to 6.12%. The methods for the detection of BAs have been constantly improved (De Mey *et al.*, 2012; Liu *et al.*, 2018), especially the sample pre-treatment method, but the price of measurement is always very expensive, and requires professional analytical skills which include extraction, pre-treatment, and analysis.

In recent years, there were many new technologies for the detection of BAs in foods such as nanofiber membrane test paper technology, competitive fluorescent molecularly imprinted polymer (MIP) detection method, hyperspectral imaging technology, biosensor, and surface plasmon resonance (SPR). Yurova *et al.* (2018) electrospun cellulose acetate (CA) doped with 2 mg/mL fluorescent amine reactive chromotropic dye py-1 into a uniform anion pad, and dropped the cationic BAs extracted from the sample onto the nanofiber pad. Py-1 reacted with different concentrations of BAs in foods through the changes of colour, reflection, and fluorescence. The fluorescence was excited by ultraviolet lamp, and the digital camera acted as a detector for digital image processing. When compared with the polymer film embedded with the same dye, the sensitivity of the nanofiber pad test paper was six times higher. Mattsson *et al.* (2018) synthesised MIPs by using histamine as template, and applied them to batch binding assay similar to competitive fluorescence immunoassay to prepare competitive fluorescence MIP for quantitative BAs. The biggest challenge of this method is that complex sample matrix may block the polymer binding sites. Taking fish extract as an example, the liquid-liquid extraction method of BAs was optimised so that the polymer can combine with the analyte to the maximum extent. The MIP is most sensitive to histamine, but it can also bind to tyramine, which is similar to histamine in structure.

The detection of BAs is gradually developing in the direction of convenience, low cost, large-scale use, and rapid on-site detection. Choi *et al.* (2021) developed a colloidal gold nanoparticle colorimetric sensor with carbon disulphide (CS_2) as additive for rapid on-site detection of BAs. Colloidal gold was synthesised by reduction reaction of gold ions with citric acid as stabiliser, and its alkalinity was detected by particle aggregation effect. The combination

possibility of different types of basic gold and colloidal gold was determined, and the mixed reactivity of basic gold and colloidal gold was further confirmed. The absorption spectrum was used to estimate the chromaticity difference and diffuse reflectance spectrum of samples. The morphology of colloidal gold nanoparticles and the aggregation behaviour of CS_2 in colloidal gold nanoparticle solution were observed by transmission electron microscope. Raman spectroscopy was further used to characterise the peaks of CS_2 , Cad, and $\text{CS}_2\text{-Cad}$ molecules. The sensing analysis was carried out systematically in the presence and absence of CS_2 . In the presence of CS_2 , the direct torque control (DTC) formed by BAs was confirmed by high absorption spectrum analysis.

Inhibition methods of BAs

First of all, we should control the source of meat processing, control the microbial status of raw meats, reduce the initial microorganisms of raw meats, and try to choose fresh or low-temperature raw meats with short storage time. In the processing of meat products, control the production process such as reducing water activity, appropriate pH, low temperature, and new sterilisation methods to reduce the growth of amine-producing microorganisms and spoilage bacteria, so as to achieve the purpose of inhibiting BAs. In the present review, the inhibition of BAs by starter, plant natural products, and other methods was described.

Starter to inhibit BAs in fermented sausages

At present, the formation of BAs in fermented sausages is mainly inhibited by the screening and development of starters, including three types of starters: the first one is to reduce the level of amino acids in precursors, the second one is to use amine oxidase to decompose BAs, and the third one is other types of starters (Ruiz-Capillas and Jimenez-Colmenero, 2004).

Free amino acids are precursors of BAs, and their contents are mainly affected by raw meat quality and fermentation process. Due to the action of cathepsin and microorganisms, macromolecular proteins will be degraded during the storage of raw meat, and the content of peptides and free amino acids in the raw meat will increase (Chen, 2017). The sources of free amino acids are the free amino acids in the raw meat produced by hydrolysis of cathepsin and exogenous protease (Peng *et al.*, 2020). During

fermentation, cathepsin in meat and exogenous protease produced by microorganisms can hydrolyse protein and produce a large number of free amino acids, thus increasing the risk of accumulation of BAs, especially the role of microorganisms such as lactic acid bacteria which are common in fermentation, which not only secrete protease to degrade protein in meat but also hydrolyse protein by reducing pH (Özogul and Hamed, 2017; Wang, 2017). The decrease in pH caused by lactic acid bacteria can also increase the activity of cathepsin in meat, and then accelerate the degradation of protein and the formation of free amino acids (Alfaia *et al.*, 2018). However, the selection of starter strains with weak protease synthesis ability can reduce the content of free amino acids and control the formation of BAs, but the weakening of protein degradation ability will affect the formation of flavour substances in fermented sausages and the digestion and absorption of human body (Van Ba *et al.*, 2016). Therefore, on the premise of choosing starter which can reduce free amino acids, the formation of flavour compounds in fermented sausage should also be considered.

Amine oxidase is a kind of active protein. Its main function is to oxidise and reduce BAs to corresponding aldehydes, ammonia, and hydrogen peroxide (Cooper, 1997). Therefore, amine oxidase can degrade BAs (Li and Lu, 2020). Amine oxidase includes monoamine oxidase (MAO), diamine oxidase (DAO), and polyamine oxidase (PAO); therefore, microorganisms with amine oxidase can be screened and selected to degrade BAs in fermented sausages. In meat starter cultures, some lactic acid bacteria and coagulase negative *Staphylococcus* can also produce MAO to degrade tyramine (Li and Lu, 2020). Sun (2016) isolated three tyramine-degrading starter strains from Sichuan sausages, namely *Enterococcus faecium* R2, *Enterococcus faecalis* R6, and *Staphylococcus squirrel* P11, which indicated that the tyramine content in fermented meat products could be reduced by screening suitable lactic acid bacteria and coagulase negative *Staphylococcus*. Guarcello *et al.* (2016) found that many starters for cheese production have the ability to degrade BAs, and the strains contain genes related to amine oxidase. Zhang *et al.* (2015) found that *Lactobacillus rhamnosus*, *Bacillus subtilis*, *Staphylococcus saprophyticus*, *Staphylococcus xylosus*, *Pediococcus pentosaceus*, and *Lactobacillus plantarum* all contained amine oxidase, which was inoculated into smoked horse sausage as starter, and the effect of

these strains on the content of BAs during sausage ripening was studied. Their results showed that these six strains could degrade and ferment in varying degrees, and the degradation degree of BAs in sausages was more than 40%.

Screening strains with degradation ability of BAs, screening strains without tyrosine decarboxylase, screening BAs negative bacteria, and using compound starter to inhibit accumulation of BAs are also the development direction of starter (Dias *et al.*, 2020; Roselino *et al.*, 2020; Serio *et al.*, 2020). Zhao *et al.* (2020) isolated two strains of bacteria with strong degradation ability of BAs from Chinese soybean paste, namely *Pediococcus acidilactici* M28 and *Staphylococcus carnosus* M43. *Pediococcus acidilactici* M28 could degrade eight kinds of BAs, and *Staphylococcus carnosus* M43 could degrade histamine and tyramine, the most common toxic substances in fermented food. Liang *et al.* (2020) used *Lactobacillus sake*, *Pediococcus pentosaceus*, and *Staphylococcus xylosus* as compound starter (1:2:2) to produce fermented beef jerky, which significantly inhibited the accumulation of putrescine, cadaverine, and histamine in dried meat. Van Ba *et al.* (2016) found that the compound starter of *Staphylococcus aureus* and *Lactobacillus sake* could reduce the putrescine and tyramine content in fermented sausages. Saelao *et al.* (2018) suggested that the use of *Lactococcus lactis*, which does not produce tyramine, can reduce the tyramine content in Thai fermented shrimps. Kim *et al.* (2019) also proved that the use of fermentation agents without producing BAs is an effective way to reduce the content of BAs in foods. Sun *et al.* (2019b) inoculated *Lactobacillus plantarum* and *Staphylococcus xylosus* in Harbin air-dried sausages, and found that tyramine content in the product during fermentation decreased by 23%, and the number of Enterobacteriaceae in the starter was lower than that in the control group (natural fermentation group).

Plant-derived natural products to inhibit BAs in fermented sausages

Plants are abundant sources of chemical substances of numerous bioactive compounds including extracts and essential oils from roots, stems, leaves, flowers, and buds. They are recognised as safe, and often added to foods as antimicrobial agents, strengthening and improving food quality.

At present, the plant-derived natural products used to inhibit or reduce the production of BAs in

meat products mainly include phenolic compounds (*e.g.*, phenolic acids, gingerol, gallic acid, eugenol, carvacrol, flavonoids, quercetin, and flagellin), terpenes, alkaloids (*e.g.*, caffeine and berberine), and other antibacterial compounds (*e.g.*, vitamin C, vitamin E, allicin, and organic acids) (Houicher *et al.*, 2021). These compounds had a certain antibacterial effect which leads to the inactivation of microorganisms by acting on biomembrane. There may be three ways: (1) by inhibiting the activity of amine-producing microorganisms; (2) by inhibiting the reproduction of harmful microorganisms; and (3) by inhibiting the activity of protease-producing bacteria, thus the content of free amino acids in the precursor was reduced.

Yavuzer *et al.* (2021) used safflower extract and balsam pear extract to inhibit the accumulation of putrescine, cadaverine, histamine, tyramine, and other BAs produced by fish spoilage bacteria. The antibacterial properties of balsam pear may be related to a variety of bioactive components of phenolic compounds, especially gallic acid, saponins, peptides, alkaloids, and vitamins. The antibacterial activity of safflower extract may be related to its phenolic compounds such as lignans and flavonoids. Lu *et al.* (2015) added plant extracts (*e.g.*, tea polyphenols, cinnamon, ginger, and fennel essential oil) to smoked horse sausages, and designed three groups of fermentation agents, plant extracts, and mixed extracts of plant extracts to compare the results. The results showed that both plant extracts and starter cultures inhibited the accumulation of BAs (*i.e.*, tramines, cadaverine, histamine, and tyrosine) and the growth of pathogenic bacteria. The inhibitory effect of plant extracts was stronger than that of fermentation agents. Zhang *et al.* (2017) extracted rose polyphenols from rose, and added them to traditional fermented dry sausages. It was found that a certain amount of rose polyphenols could prevent the increase of pH value, lipid oxidation, and inhibit the formation of BAs. Rose polyphenols can promote the growth rate of lactic acid bacteria, and reduce the total number and diversity of bacteria. Sun *et al.* (2018b) studied the inhibitory effect of spice extracts (*e.g.*, cinnamon, clove, and fennel) on the accumulation of BAs in Harbin dry sausages. Their results showed that spice extracts could significantly inhibit the accumulation of BAs, especially cinnamon extract. The addition of spice extract inhibited the growth of pathogenic bacteria, reduced lipid

oxidation, inhibited the formation of TVB nitrogen, and improved the sensory quality of sausages.

Other methods to inhibit BAs in fermented sausages

The inhibition of amine-producing microorganisms by controlling their exogenous growth environment can also inhibit the accumulation of BAs. For example, different packaging methods have been used to inhibit the growth of amine-producing microorganisms.

Sun *et al.* (2019a) used the combination of compound starter and vacuum packaging to inhibit the accumulation of BAs in Harbin dry sausages. They found that the combination of compound starter and vacuum packaging could effectively inhibit the accumulation of BAs, and the combination effect of the two methods was better.

Yew *et al.* (2014) stored Indian mackerel in vacuum at 30% CO₂ + 65% N₂ + 5% O₂, 60% CO₂ + 35% N₂ + 5% O₂, 80% CO₂ + 15% N₂ + 5% O₂, and 100% CO₂ for 12 days at 5 ± 1°C. It was found that histamine decreased in the packaging containing different concentrations of CO₂, but spermine was not significantly affected. High concentration of CO₂ had a significant inhibitory effect on tyramine, while putrescine and spermidine increased at low concentration of CO₂ (30%).

Zhao *et al.* (2021) analysed the effects of aerobic packaging, NaCl solution package, and vacuum packaging on reducing the content of BAs and nitrite in pickles during storage. Their results showed that vacuum packaging could reduce the number of lactic acid bacteria and yeast to 6.98 and 1.21, respectively. The accumulation of histamine, tyramine, and putrescine in pickles was more effectively inhibited by vacuum packaging, and the production of cadaverine was inhibited by NaCl solution package and vacuum packaging. At the end of storage, the nitrite content in vacuum packaging was 56.04 and 36.31% less than that in aerobic packaging and NaCl solution package, respectively.

Conclusion

The sources of BAs in fermented sausage are complex, and there are many influencing factors. Therefore, it could be easy to produce a large amount of BAs in products. At present, researchers should not only continue to research and develop amine-reducing starter strains, but also investigate their relationship with amine-producing microorganisms,

to ensure the flavour and texture of fermented sausage while reducing amine. In addition, it is urgent to monitor the content of BAs in foods, and establish the permissible limits. In China, there are no laws or regulations on the limits of BAs in foods, except for fish (Zhang, 2015). It is thus necessary to improve, perfect, and standardise the BA limits.

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