



DETECTION AND CONTROL OF BIOGENIC AMINE CONTENT AND PHYSICAL/CHEMICAL PROPERTIES BASED ON HPLC METHOD IN FERMENTED FOOD

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ABSTRACT

In this paper, high performance liquid chromatography (HPLC) method for determination of the content of biogenic amines, methods and good stability, high precision, high recovery rate. Fermentation agent is the most important biogenic amines tyramine and histamine categories, because of its physiological toxicity, and cadaverine will strengthen due tyramine and histamine toxicity. Therefore, the reason why the event biogenic amines poisoning often occurs because we are biogenic amines thorough understanding is not enough, but also the lack of fast, efficient detection methods, the paper established HPLC detection of biogenic amines in food type and content of wine, soy sauce biogenic amines were measured and used to strengthen vaccination effectively reduce biogenic amines in fermented sausages content, which improve the quality of life and protect the public food safety has important practical significance.

1. Introduction

Food poisoning and certain toxicological properties of histamine and tyramine are closely linked, and therefore the study of biological amines can enhance and improve food quality and safety (Lu, 2014). China has a large number of hypertensive patients, they eat cheese, yogurt and other high tyramine containing foods, nausea, vomiting, and other symptoms touyunnaozhang This is because high blood pressure in patients with long-term use of reserpine, excellent drop Ning and other antihypertensive drugs, these drugs can inhibit the body monoamine oxidase activity, so food tyramine not decompose, accumulate in the body, more and more strongly stimulated the peripheral vascular system, causing indirect effects of sympathomimetic amines, vascular resistance increased, so more and more high blood pressure (Muthurs, 2014; Kim, 2014). In

severe cases, intracranial hemorrhage and coma and even death. Clinical studies can inhibit monoamine oxidase in vivo as well as anti-TB drug isoniazid, anti-tumor methyl thousand navel, styrene solution antidepressant and anti-inflammatory bacteria hang furazolidone tablets (Zarifi, 2014).

Use fermenting agent in the production of fermented sausages vital leaven quality is directly related to the quality of fermented sausage products, if you choose an inappropriate leaven production of fermented sausage, the product quality is likely worse than manual irrigation system, natural fermentation sausage (Yu, 2014). Current domestic production of fermented sausages imported multi-use commercial fermentation agent, but imports of commercial starter cultures may be due to the traditional Chinese sausage production technology and raw meat

sausage incompatibility can not become the dominant bacteria causing sensory quality reduction (Leroy, 2014). Therefore, based on Western-style fermented sausages, combined with China's traditional fermentation technology, the use of traditional meat products selected from the strain out of production for Chinese-style fermented sausage is worthy of further study (Thakur, 2015; Sunano, 2015).

Biogenic amines are a class of nitrogen-containing aliphatic, aromatic or heterocyclic compounds of low molecular weight, it is essential for the biological activity of cellular components, but biogenic amines have potential toxic effects, when accumulated to a certain extent the body will produce toxicity, so far in food research has become a hot issue of biogenic amines. In fermented sausages, biogenic amines is an important factor affecting the safety of dry fermented sausages, at home and abroad in recent years for the study of biogenic amines in fermented foods are deeply concerned. In this study, the content of biogenic amines index, from this laboratory strain deposited preferred high-quality security, for fermented sausage production strains, and strains were identified; In the preferred mixed culture of bacteria and strains of a single production of fermented sausage products and with the commercial production of fermented sausage products business fermenting agent quality compared to a suitable fermentation of a preferred agent in the production fermented sausage, fermented sausage industrial production basis.

2. Materials and methods

2.1. Biogenic amines and their physiological significance

Biogenic amines are a class of nitrogen-containing aliphatic (putrescine, cadaverine, spermine, spermidine), aromatic (tyramine, phenylethylamine) or heterocyclic (histamine, serotonin) low molecular weight compounds, mainly through amino acid decarboxylase or aldehyde and ketone amine and transamination

formation (Barbosa, 2014). Animals, plants and micro-organisms living cells have an important physiological role, the amount of biogenic amines contribute to the body's normal physiological function, the excess will cause adverse physiological reactions that can lead to high blood pressure, headache, his face flushing, rash. Sometimes mainly gastrointestinal disorders, including sudden vomiting and diarrhea, accompanied by abdominal pain and other symptoms, biogenic amines are often produced in the food rotting or fermenting process (Kang, 2015).

Metabolic pathways generated by biogenic amines was shown in Figure 1, the most common monoamines (histamine, tyramine and serotonin), diamine (putrescine and cadaverine), histidine, tyrosine, tryptophan, ornithine, and lysine, polyamines (spermine and spermidine) is formed by putrescine. biogenic amines not only generate hormones, nucleic acids, proteins and other substances precursors, but also generate nitroso carcinogens and substances precursors. Monoamine compound has obvious blood vessels and muscle relaxation or contraction, can inhibit epileptic seizures, mental activity and cerebral cortex plays an important role in the regulation of the heart have different degrees of positive inotropic and positive-frequency effects. Polyamines organism during growth, can promote the synthesis of DNA, RNA and protein, to accelerate growth and development of the organism, it is essential for the biological activity of cellular components, they have an important in regulating protein synthesis and function of nucleic acids role, biofilms may also be related with the stability. Due to the diversity of their roles in cell metabolism and growth, so they need to have a great amount of rapid growth in the organization. All cells can be synthesized by the use of external resources polyamines.

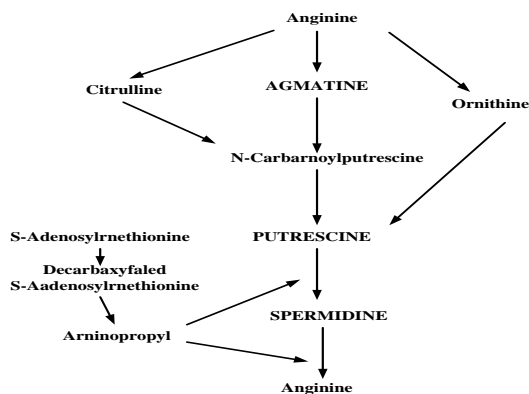


Figure 1. Metabolic pathways generated by biogenic amines

2.2. Preferred strains fermented sausages

In fermented sausage production process, the role of lactic acid bacteria play an important role, but not all are suitable for use as meat lactic acid fermentation agent. If the lack of meat in a competitive environment, some affect the sensory quality of meat, and some affect the safety of meat quality, in fermented foods and beverages, the use of leavening agents can

affect biogenic amines produced by different interaction between microflora can be directly or indirectly affected. Therefore, strains used as fermenting agents screening criteria should include an analysis of the situation to produce biogenic amines.

Weigh accurately tryptamine, phenylethylamine, putrescine, cadaverine, histamine, tyramine, spermidine, spermine each 50 mg, 0.4 mol than with perchlorate volume to 50 mL, made 1 mL reserve liquid reserve. Were taken over standard stock solution with 0.4mol than HClO₄ formulated into a final concentration of mixed standard solution 0.5, 1.0, 2.0, 5.0, 10, 20mL, the use of aluminum foil in the dark, refrigerator. The column was Asilent ZORBAXXDB-C18 (250 mm x 4.6 mm, 5 μm), the detection wavelength was 254 nm, injection volume 20 μL, column temperature 30°C, mobile phase A and water, using the gradient elution program displayed in the Table 1.

Table 1. Gradient elution program

Elution time/min	Mobile phase A/%	Mobile phase B/%
0.0	35.0	65.0
5.0	31.0	70.0
20.0	0.0	101.0
24.0	0.0	101.0
25.0	38.0	67.0
30.0	37.0	67.0

2.3. The main media

MRS medium: Peptone 10g, beef extract 10g, yeast extract 5g, hydrogen citrate diamine 2g, glucose 20g, Tween 801mL, sodium acetate, 5g, dipotassium hydrogen phosphate 2g, magnesium sulfate 0.5g, manganese sulfate 0.25g, distilled water 1000 mL, pH value 6.2-6.4, 121°C sterilization for 20min.

MS Medium: trypsin Chen 10g, beef extract 1 g, sodium chloride 75g, 1% phenol red reagent 2.5ml, D- mannitol 10g, distilled water 1000ml, PH value 7.2-7.4, 122°C sterilization for 20min.

Underlying medium: pancreatic peptone 0.5%, yeast extract 0.5%, beef extract 0.5%, 0.25% sodium chloride, glucose, 0.05%, 0.02% magnesium sulfate, 0.005% manganese sulfate, ferrous sulfate 0.04%, citric acid by 0.020 % vitamin B6 0.005%, 1.0% amino acids, 0.2% dipotassium hydrogen phosphate, calcium carbonate, 0.01%, agar 2.0%, pH 5.5.

Standards of biogenic amines: tryptamine, 2-phenylethylamine, putrescine, cadaverine, histamine, tyramine Spermine, Dan sulfonephthalein chlorine (Sigma Aldrich Company).

HPLC grade acetonitrile and acetone; ammonia, perchloric acid, sodium hydroxide, sodium bicarbonate, analytical grade; L tyrosine, histidine, lysine and biochemical reagents; homemade ultra pure water.

Acetonitrile: Shandong Yuwang Industrial Co., chromatography; Acetone: Nanjing Datang Chemical Co., chromatography; Ammonia: Nanjing Chemical Reagent Factory, AR; Perchlorate: Tianjin Xinyuan Chemical Plant, AR; Sodium hydroxide: Nanjing Chemical Reagent Factory, AR; Sodium bicarbonate: Nanjing Chemical Reagent Factory, AR; Wahaha purified water: purchased in the supermarket.

2.4. The main instruments and equipment

SW-CJ-I-type clean bench: Su net Group Suzhou Aetna; LDX a 50KBS vertical automatic electric pressure steam sterilizer: Shanghai Shen An Instrumentarija; HZO a high and low temperature oscillation incubator F160A: Shanghai Yuehua Instrument Co., Ltd.;

MDL9000 (B) -H-30-based desktop laboratory ultrapure water systems: Nanjing total Hing water equipment company; 2004MP analytical balance: STARIOUS; Desktop high-speed refrigerated centrifuge: Beckman Company; HPLC System: Waters Corporation USA.

3. Results and discussions

3.1. Qualitative analysis of the strains

In this experiment, B Lotus (double plate method) strains produce an amine preliminary analysis of the situation. As can be seen from Table 2, separated from the Dong acid meat production TD3 a 7 tyramine, ZJ3 a 4 tyramine and serotonin production. Only from qualitative analysis, staphylococcus AL> 3 can be used as an excellent safety fermenting agent. However, since some strains produce amines weak, reach BAP method detection limit, so the next step to make quantitative analysis.

Table 2. Strains were used and their activity of producing BA

Bacterial strain	Source	Tyramine	Histamine	Cadaverine
TD3-1	Dong Sour Meat	.	.	.
TD3-7	Dong Sour Meat	+	.	.
ZJ3-4	Dong Sour Meat	+	.	+
ZJ3-8	Dong Sour Meat	.	.	.
ALX3	Chinese mushroom	.	.	.

Figure 2 shows that the initial fermentation (0d), fermented group (A, B, C, D, E) of the total number of bacteria between 10⁷-10^{7.9}, while the total number of bacteria in the control group F group was 10. By the end of the fermentation (2d), the total number of bacterial fermentation group in 8.0lg cfu / g between a 9.0 lgcfu/g, while the control group, the total number of bacteria increased 7.6 lgcfu/g and at 7d reaches a maximum and then decreased slowly. to the production end (28d), the total number of bacteria in the test group 7.619cfu/g - 8.319cfu/g, the difference between the experimental groups was not significant

(P<0.05), while the control group, the total number of bacteria was 7.0 lgcfu/g. The results showed that in the control group throughout the production process the total number of aerobic mesophilic bacteria was significantly lower in the experimental group (P <0.05). Lactic acid bacteria and staphylococcus as the application leaven fermented sausages can be accelerated.

Sausage initial *E. coli* contamination from slaughter and segmentation as well as the use of appliances. Similar in all groups *Enterobacteriaceae* initial number of 4.0lg cfu/g a 5.0 lgcfu/g. But in the process of maturation of each group (A between B, C, D, E, F) change

significantly different ($P < 0.05$). At the end of fermentation (2d), F group compared with the control group, mixed fermentation agent B, group C decreased significantly, respectively 3.5 lgcfu/g and 3.7 lgcfu/g. may be due to lactic acid bacteria produce lactic acid fermentation agent

group classes to accelerate the rapid decline in the value of B, the demise of the *Enterobacteriaceae* group C, and the other may be lactic acid bacteria produce bacteriocins, thus inhibiting intestinal bacilli growth.

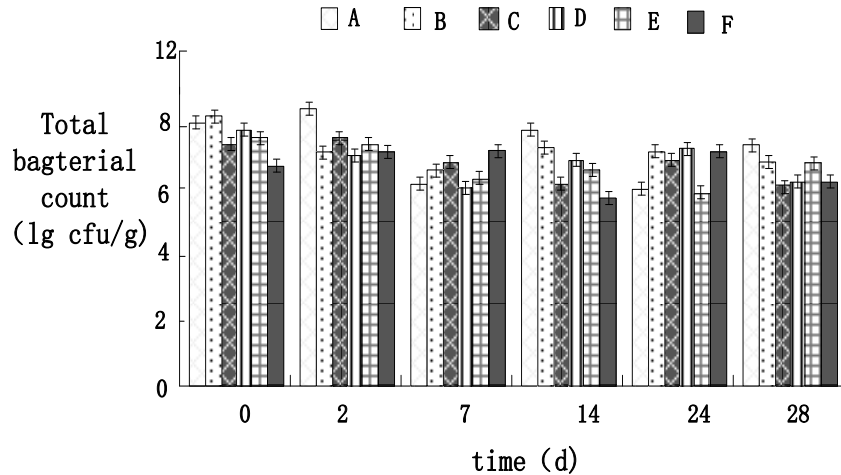


Figure 2. Different ferment fermented sausages during ripening total bacteria change

3.2. Different biogenic amines standard curve

In Figure 3, the standard curve biogenic amines can be seen, eight kinds of biogenic amines and their corresponding concentrations

peak area was linear correlation coefficients were greater than 0.99. By this method can accurately measure the content of biogenic amines in the sample.

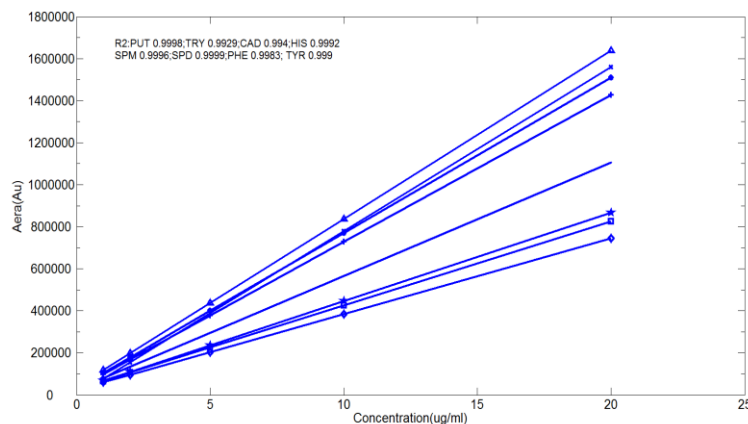


Figure 3. Standard curves of different levels of biogenic amines

Fermentation agent is the most important biogenic amines tyramine and histamine categories, because of its physiological toxicity, and cadaverine will strengthen due tyramine and histamine toxicity. Therefore, in this study, tyramine, histamine and cadaverine as standard

strains qualitative analysis was an amine. In this experiment, all isolates are not producing histamine, may be due to these strains do not carry histidine decarboxylase, histamine may be too little, not enough to make flat-panel color

change, it needs to do next quantitative analysis of biogenic amines.

This part of the study was determined by high performance liquid chromatography five strains tryptamine, a phenylethylamine, putrescine, cadaverine, histamine, tyramine, spermidine and spermine amount. ALX3 not produce an amine, was identified, respectively, sausage *Lactobacillus*, *Leuconostoc* bacteria and *Staphylococcus xylose*, these three bacteria are

common bacteria can be used as a leavening agent, and from fermented meat products more suitable for use as ferment fermented sausages.

Biogenic amine content of the fermentation process changes the situation shown in Figure 4. The results can be seen: raw meat itself with phenylethylamine, cadaverine, spermidine, spermine, and increased in the fermentation process, and raw meat substantially free of putrescine, histamine, tyramine.

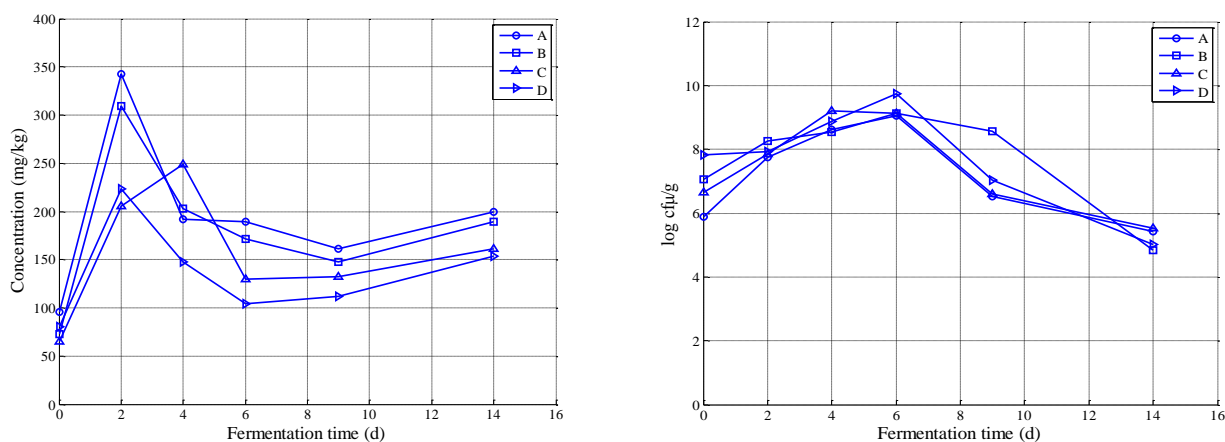


Figure 4. Biogenic amine content of the fermentation process changes the situation

Effect of putrescine and cadaverine and other secondary amines and other vascular amines (tyramine, histamine, tryptamine, phenylethylamine) is different, there is no adverse human health, but they are able to inhibit detoxification enzymes: diamine oxidase and light-methyl-converting enzyme activity and the potential impact of histamine content. Furthermore, the microbiological quality of their accumulation and products declined linked histamine toxicity in the presence of their increasing trend. From another point of view, it can also be considered biogenic amines are carcinogenic, as secondary amines can react with nitrite and are likely to form carcinogenic N-nitrosamines, because primary amine can be converted into a secondary amine, so not only heat, but also can be stored at room temperature the amine is further reacted with a nitrite.

3.3. Colony counts and pH change

Currently, sausage microbial fermentation agent commonly used are: bacteria (*Lactobacillus*, *Micrococcus*, *Staphylococcus*), yeasts and molds. The results (Figure 5 and Figure 6) can be seen, the number of lactic acid bacteria, total bacteria are showing a downward trend after the first rise. The first decline in six days a lot of lactic acid bacteria breeding trends and the corresponding pH value is, which is due to lactic bacteria break down carbohydrates produce a result of lactic acid. When the pH value of the product fell to its lowest value, along with the continuous loss of water, NaCl mass fraction relative increase, such an environment is no longer suitable for the rapid growth of lactic acid bacteria.

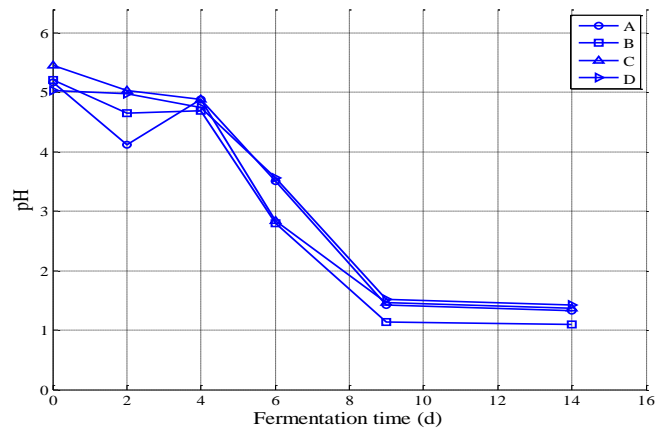


Figure 5. Different treatments in the fermentation process of change in the total number of bacteria

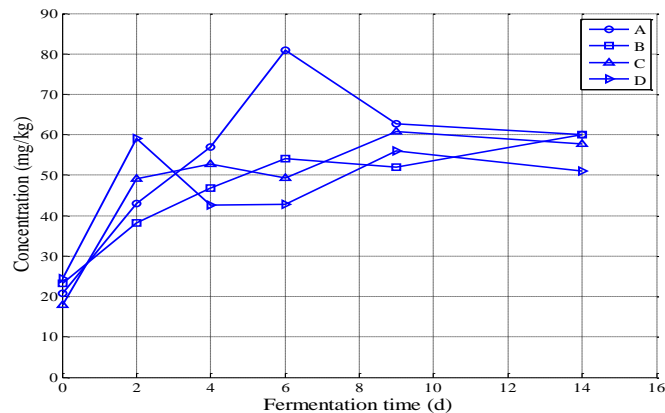


Figure 6. Sausage fermentation process pH changes

Therefore, the number of colonies of lactic acid bacteria is no longer a significant increase in pre-lactic acid fermentation bacteria quickly developed into advantages, the number of whole fermented sausage production process is much higher than bacteria, very close to the total number of bacteria, lactic acid is therefore the production of fermented sausages dominant bacteria and the final flavor of fermented sausages produced plays an important role. However, some species of lactic acid bacteria is to produce an amine, and as such, decarboxylase activity isolated from fermented sausages out of lactic acid bacteria have been studied extensively.

Between the same type of product, the

content of biogenic amines are very different, leading to many reasons for this change. The process and differences can lead to other conditions there of biological fermented sausage different amine content. To prevent or reduce the formation of biogenic amines in fermented sausages need to proceed in terms of raw meat, leavening agents and process conditions, etc., that use raw meat, do not produce amines ferment and leaven strains were grown in favor of process conditions to control and reduce the amount of amine.

3.4 Physical indicators

Color and flavor of the meat important sensory indicators for evaluating color quality of

fermented sausages important sensory index, is the first impression that people on food quality evaluation and consumer appetite and desire to buy has a great influence from Table 3 can be A, B, C, D, E group luminance values, red values were significantly higher than other groups protected, yellowness value lower than the other groups, and the difference is significant. this may

be because B, C, D, E group as a leavening agent added aureus, Staphylococcus nitrate reductase and catalase, the formation of sausage color plays an important role from the color analysis results, improved red Staphylococcus added value products and brightness values, improving the sausage color, improve the overall acceptability of the product.

Table 3. Color analysis of fermented sausages manufactured by different starter cultures

Process	L [*]	a [*]	b [*]
A	33.71±0.94b	12.17±0.44b	6.05±0.34b
B	39.86±3.94a	15.23±13.68a	4.77±0.49c
C	36.82±3.57ab	15.75±1.15a	4.97±0.30bc
D	37.88±1.94ab	13.97±3.19ab	4.71±0.69c
E	33.56±2.54b	13.03±0.30b	5.60±0.54bc
F	34.08±1.21b	11.55±0.94b	8.20±0.94a

Means within column with different letters were significantly different (P<0.05).data were shown as mean SD.

4. Conclusions

In this study, tyramine, histamine and cadaverine as standard strains qualitative analysis was an amine. The number of colonies of lactic acid bacteria is no longer a significant increase in pre-lactic acid fermentation bacteria quickly developed into advantages, the number of whole fermented sausage production process is much higher than bacteria, very close to the total number of bacteria, lactic acid is therefore the production of fermented sausages dominant bacteria, and the final flavor of fermented sausages produced plays an important role. However, some species of lactic acid bacteria is to produce an amine, and as such, decarboxylase activity isolated from fermented sausages out of lactic acid bacteria have been studied extensively.

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