Effects of Cooking Process on the Content of Nitrite in Sausage

Ya Li 1, Chun-Lu Gan 1, Mei Cheng, Wei-Wei Chen, Li-Rong Cao, Ting-Ting Zhao, Jiao-Jiao Zhang *

Guangdong Provincial Key Laboratory of Food, Nutrition and Health, Department of Nutrition, School of Public Health, Sun Yat-Sen University, Guangzhou 510080, China

* Author to whom correspondence should be addressed; E-Mail: zhangjj46@mail2.sysu.edu.cn; Tel.: +86-20-87332391; Fax: +86-20-87330446.

1 These authors equally contribute to this paper.

Article history: Received 10 March 2016, Received in revised form 25 April 2016, Accepted 28 April 2016, Published 2 May 2016.

Abstract: Nitrite is widely used in food industry, especially in cured meat products. The main function of nitrite includes the formation of characteristic color and flavor associated with cured meats as well as antimicrobial activity. However, the safety concern of nitrite has been increasing in recent years. This study is aimed to investigate effects of cooking process on the content of nitrite in sausage in order to remove nitrite from food. Effects of pretreatment before cooking on the content of nitrite in sausage were investigated and optimized, including temperature, time and material/solvent ratio. In addition, effects of different cooking methods on the content of nitrite in sausage were investigated, including boiling, steaming, and boiling with different vegetables (such as head of lettuce, carrot, broccoli and cucumber). Results showed that the optimal conditions for pretreatment were as follows, temperature of 80 °C, time of 60 min, and the solvent/material ratio of 20, which resulted in the 19.47% removal of nitrite in sausage. Furthermore, boiling with head of lettuce, carrot or broccoli could greatly remove nitrite from sausage. The pretreatment before cooking and different cooking methods developed in this work could be adopted to remove nitrite from food, which will reduce hazards of nitrite.

Keywords: sausage; nitrite; pretreatment; cooking process; removal; vegetable.
1. Introduction

The typical diet in most countries contains nitrate, nitrite and nitrosamine. Nitrite is widely distributed in nature environment and foods, such as soil, water, vegetable, meat, fish, wine, mushrooms, spices, processed smoked food and pickles. Therefore, the exposure of human to nitrite could be from drinking water, vegetables, cured meats and other foods.

Meat curing which includes the addition of salt, nitrite, and sometimes nitrate to fresh meat cuts, enables preservative effect by removing moisture and reducing the water activity of the meat. In cured meats, the main functions of nitrite include the formation of the characteristic reddish-pink color and flavor associated with cured meats as well as serving as an effective antimicrobial agent (Alahakoon et al., 2015). Firstly, nitrite could form nitric oxide during the curing process facilitated by reductants such as ascorbate. Nitric oxide reaction with myoglobin could form the nitrosylmyoglobin complex, which outline the basis for unique cured meat color (Parthasarathy and Bryan, 2012). Nitrosylmyoglobin is bright red in color and is an extremely unstable compound. During thermal processing, it could be converted to a stable, attractive reddish-pink compound nitrosohemochrome, due to the denaturation of the protein moiety of the myoglobin pigment. Secondly, the characteristic flavor of cured meat products is also attributed to the chemical reactions of nitrite and its associated reactions as described above. It was found that a residual nitrite level of 10-15 mg/kg is generally recommended as a reservoir primarily for the regeneration of cured meat color, and 50 mg/kg for developing the unique flavor differences between cured and uncured meat (Sindelar and Milkowski, 2012). Thirdly, nitrite could also retard the development of rancidity during storage and the subsequent warmed-over flavors developed upon heating of meat and meat products. Nitric oxide from nitrite could bind to and stabilize heme iron of meat pigments during the curing process (Bergamaschi and Pizza, 2011). Nitric oxide could also terminate lipid auto-oxidation, since it is a free radical. Lastly, nitrite in curing meat could produce antimicrobial effect, targeting bacteria at multiple sites by inhibiting metabolic enzymes, limiting oxygen uptake, and breaking the proton gradient (Ford and Lorkovic, 2002). Nitrite is well known as to suppress the outgrowth of C. botulinum spores in cured meat products and to completely control the botulism.

The health hazard of nitrite mainly takes part in acute toxicity and chronic toxicity. As for acute toxicity, intake of 0.3 to 0.5 g nitrite for general adults could cause poisoning, and consumption of 3 g could lead to death. The hemoglobin in normal human contain Fe$^{2+}$, and exercise the function of delivering O$_2$ and take away CO$_2$ in blood. When the excess nitrite is absorbed in human body, due to the strong oxidative power of nitrite, the myoglobin (Fe$^{2+}$) could be oxidized to methemoglobin (Fe$^{3+}$). Methemoglobin loses oxygen carrying capacity and results in tissue hypoxia, causing dyspnea, drop of blood pressure, skin cyanosis, dizziness, vomiting and diarrhea. In severe cases, it could cause death as

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a result of hypoxia. The chronic toxicity of nitrite is mainly associated with the formation of nitrosamines. Nitrosamines are chemical substances with strong toxic, mutagenic, neuro- and nephrotoxic, teratogenic and carcinogenic effects. The nitrosamines are easily formed by interaction of a secondary amino compound with nitrite under favorable conditions, such as near acidic pH and a product temperature of >130 °C (Cassens, 1995). The formation of N-nitrosamines in meat products depends on the cooking method, cooking temperature, and time; concentrations of residual or added nitrites; presence of N-nitrosamine precursors (Drabik-Markiewicz et al., 2011). Dimethylnitrosamine (DMNA) and diethylnitrosamine (DENA) are the most frequently occurring nitrosamines in our dietary foods and reveal the strongest toxic activity (Rywotycki, 2007). NDMA is a potent carcinogen, capable of inducing malignant tumors in various animal species in a variety of tissues, including liver, lung and gastric tissues (Anderson et al., 1996; Tricker and Preussmann, 1991). So far, numerous epidemiologic studies have found the potential risk of cancer about the dietary nitrate, nitrite, and nitrosamines intake (Alahakoon et al., 2015; Song et al., 2015).

In recent years, in spite of controlling the added amount of nitrite and using alternatives of nitrite, some researchers have been working on chemical or biological methods to reduce the nitrite in food. Several antioxidant plant extracts, such as ginger extract, aloe extract and tea polyphenols, as well as some microbes, such as the lactobacillus, have been found effective in reducing the nitrite residual in cured meat. However, few researches focused on the influence of pretreatment and cooking methods on the nitrite content of cured meat product (Li et al., 2012). Therefore, taking proper pretreatment before cooking might have the potential to reduce the nitrite content in cure meat. This paper is aimed to investigate the influence of pretreatment before cooking and different cooking methods (boiling, steaming, and boiling with different vegetables) on the nitrite content of sausage.

2. Materials and Methods

2.1. Chemicals

The sodium nitrite, zinc acetate, sodium tetraborate and potassium ferrocyanide were purchased from Tianjin Chemical Reagent Factory (Tianjin, China). The sulfanilic acid was purchased from Aladdin Industrial Corporation (Shanghai, China). The N-(1-naphthyl) ethylenediamine dihydrochloride was purchased from Kermel Chemical Reagent Co. Ltd (Tianjin, China). All chemicals used in the experiments were of analytical grade, and all the water used in this study was ultrapure water.

2.2. Sample Treatment
The cantonese sausage and the vegetables were bought from the supermarket in Guangzhou, China. The sausage was firstly ground in a grinder, and then 5.0 g of the ground meat was accurately weighed in a beaker according to the Chinese GB 5009.33-2010. A 12.5 mL of 50 g/L saturated borax solution was added to the beaker and stirred well. The sample was extracted with about 300 mL water of 70 °C into a 500 mL volumetric flask, and then the flask was heated in boiling water bath for 15 min. Afterwards, the volumetric flask was taken out and cooled to room temperature. A 5 mL of 106 g/L potassium ferrocyanide solution was added while shaking, and then 5 mL of 220 g/L zinc acetate solution was added to precipitate the proteins. Finally, water was added to the scale and placed for 30 min, and the upper fat supernatant was removed. The sample was filtered and the first 30 mL of the filtrate was abandoned. The remaining filtrate was collected for the subsequent experiment.

2.3. Determination of Residual Nitrite in Sausage

The AOAC official method for nitrite determination in meat is based on the Griess method which uses N-(1-naphthyl)ethylenediamine and sulphanilamide (Badea et al., 2004). The Chinese GB 5009.33-2010 for the detection of nitrite was established by a slight modification of the AOAC official method, and was selected as the standard protocol in this study. In brief, 2 mL of 4 g/L sulphanilamide was mixed to 40.0 mL of the filtrate and placed for 3 to 5 min. Then 1 mL of 2 g/L N-(1-naphthyl) ethylenediamine was added to the mixture to form a purple-red color and water was added to 50 mL. The absorbance was measured at 538 nm of wavelength after the mixture was placed for 15 min.

2.5. Determination of the Amount of Nitrite Dissolved from Sausage to Water

The amount of nitrite dissolved from sausage to water could reflect the elimination of nitrite in sausage by the treatment, and the procedure to measure the nitrite in water is much simplified. Thus, in each experiment, 20.0 g sausage was weighed accurately and added with corresponding amount of ultrapure water. After the treatment, the water is filtered to remove the floating fat and 40 mL of filtrate was absorbed and mixed with 2 mL of 4 g/L sulphanilamide solution. Then, 1 mL of 2 g/L N-(1-naphthyl) ethylenediamine solution was added to form the purple-red color and water was added to 50 mL. The absorbance was measured at 538 nm of wavelength after the mixture was placed for 15 min.

2.6. Removal of Nitrite in Sausage

The removal of nitrite (%) in sausage is obtained using the content of nitrite in water to divide its own content of nitrite.

All the experiments were performed in triplicate, and the results were expressed as mean ± SD.
(standard deviation).

3. Results and Discussion

3.1. Effects of Pretreatment before Cooking

3.1.1. Effects of Temperature

The influence of the temperature of the immersion water on the content of nitrite in sausage is shown in Fig. 1. Different temperatures were selected, that is 20, 40, 60, and 80 °C. The time of water immersion was set as 30 min, and the solvent/material ratio was set at 10 mL/g. The temperatures were maintained using the thermostat water bath. It could be seen from Fig. 1 that the content of nitrite in sausage has been decreasing with the increase of temperature from 20 to 80 °C, indicating that as the temperature increases, the amount of nitrite dissolved from sausage to water is increasing. Therefore, 80 °C was used in subsequent experiment.

![Figure 1](image_url)

**Figure 1.** Effect of the temperature of the immersion water on the content of nitrite in sausage.

3.1.2. Effect of Time

The influence of the time of the immersion on the content of nitrite in sausage is shown in Fig. 2. Different time (30, 60 and 120 min) was employed. The temperature of water was maintained at 80 °C using the thermostat water bath, and the solvent/material ratio was set as 10 mL/g. From Fig. 2, it could be seen that as the time extended from 30 to 60 min, the content of nitrite in water was increasing, which indicated that the amount of nitrite dissolved from sausage to water was increasing from 30 to 60 min. In addition, with the time extending from 60 to 120 min, the content of nitrite in sausage almost didn’t change. Therefore, 60 min was selected as the time of the immersion in the subsequent experiments.
Figure 2. Effect of the time of the immersion water on the content of nitrite in sausage.

3.1.3. Effect of Solvent/Material Ratio

The influence of the solvent/material ratio on the content of nitrite in sausage is shown in Fig. 3. The temperature was maintained at 80 °C using a thermostat water bath and the time was set as 60 min. From Fig. 3, it could be seen that as the solvent/material ratio increased from 5 to 20 mL/g, the content of nitrite in sausage was decreased, indicating that in the range of 5 to 20 mL/g, the amount of nitrite dissolved from sausage to water kept increasing. When the solvent/material ratio increased to 30 mL/g, the content of nitrite in sausage almost didn’t change. The reason could be that the higher ratio of solvent to material could accelerate mass transfer and facilitate the diffusion of nitrite into the water until the mass transfer process reached its maximum.

Figure 3. Effect of the solvent/material ratio on the content of nitrite in sausage.
3.1. Effects of Different Cooking Methods

The effects of different cooking methods on the content of nitrite in sausage are displayed in Fig. 4. The sausage was respectively steamed, boiled, and boiled with head of lettuce, carrot, broccoli, or cucumber, each for 30 min. Results showed boiling with head of lettuce, carrot or broccoli could greatly remove nitrite from sausage. This could be explained by, on the one hand, boiling water could help the nitrite dissolving from sausage to water; on the other hand, these vegetables were rich with antioxidants, which could react with the nitrite, thus decreasing the content of nitrite in sausage.

![Effect of the different cooking methods on the content of nitrite in sausage.](image)

**Figure 4.** Effect of the different cooking methods on the content of nitrite in sausage.

4. Conclusions

Sausage is a kind of globally popular and widely consumed meat product; however, the safety concern brought by the nitrite in sausage has also becoming an issue. In the present study, effects of pretreatment before cooking and different cooking methods on the nitrite in sausage were investigated. The results showed that the 3 factors (temperature of immersion water, time of immersion, and the solvent/material ratio) all had an influence on removal of nitrite from sausage. The optimal conditions were as follows, water immersion temperature of 80 °C, water immersion time of 60 min, and the solvent/material ratio of 20 mL/g, which resulted in the 19.47% removal of nitrite from sausage. Furthermore, boiling with head of lettuce, carrot or broccoli could greatly remove nitrite from sausage. This study could be instructive to daily life, since the pretreatment before cooking and different cooking methods developed in this work are easy to operate, and doesn’t have a significant impact on...
the flavor and taste of sausage in the subsequent cooking. Therefore, the methods developed in this work could be applied to daily life. Further study is still needed for exploring the more effective condition for the removal of nitrite from sausage.

Acknowledgements

This research was supported by the Laboratory Open Fund Project of Medical Students in Sun Yat-Sen University (No. 12 in 2015 Year).

References


