Determination of Four Volatile N-nitrosamines in the Process of Chinese Preserved Meat

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Abstract. N-nitrosamines (NAs) are harmful substances which have been detected in meat products. In this study, four NAs namely nnitrosodimethylamine (NDMA), n-nituosomethylethylamine (NMEA), n-nitrosodiethylamine (NDEA) and n-nitrosopoperdine (NPIP) was determined in the process of Chinese preserved meat. GC-MS was used to analyze NAs. At each product stage the generation of NDMA is the largest, especially at the baking stage.

Introduce

N-nitrosamines(NAs) are widely present in meat products and considered to be carcinogens and mutagenics, which are formed by the reaction of secondary amines with nitrosating agents[1-2].The nitrosating agents including nitrates and nitrites ,are commonly used in the manufature of Chinese preserved meat. The concentration of NAs in food products depends on the method of cooking, cooking time and temperature, residual and concentration of na precursor and added nitrite concentration [3].The purpose of this paper was to establish mass spectrometry for determination of NAs in Chinese preserved meat.

Methods

Chinese preserved meat preparation. Including preparation the row meat, salting, baking, and ripening. First salting temperature 25 ℃ for 1 day , baking temparature 65℃ for 3 days and ripening tempareture 25℃ for 15 days.One hundred gram meat were randomly taken from each group at following stages: fresh meat, after alting, baking(1d), baking(3d),ripening(5d), ripening (10d), ripening(15d) . The minced samples were vacuum-package and stored at -20℃.The recipe of the Chinese perserved meat was depicted in table 1.

Table 1 the recipe of the chinese perserved meat

Material	pork	salt	White spirit	soy	Nitrite
concentration	100g	2g	3g	3g	0.015mg

Extraction and purification of volatile NAs .A steam-distillation method was adopted to extract and purify the NAs [4].One hundred gram samples were homogenized in 120ml of ultrapure water with 100g of sodium chloride.The homogenate was steamo distilled and approximate 200ml of the distillate was collected.After addition of 4ml 35%HCl, the distillate was extracted three times with 50ml of dichloromethane subsequently [5]. The extract was concentrated to 1ml with rotary evaporators and nitrogen blow the extract to 100μL.

Gas chromatography-mass spectrometry analysis. Samples were analyzed by American Varian's Varian 4000 GC-MS (gas chromatography mass spectrometry), the column model DB-5MS (30 m × 0.25 mm × 0.25 μm), the gas phase conditions: inlet temperature of 300 ℃, Helium

(purity99.999%) was used as the carrier gas with a flow rate of 1 mL/min, the injector temperature was set at 250 ℃,split ratio was 5:1, temperature program: Initial temperature 50 ℃, then 5 ℃ / min increased to 55 ℃, then at 15 ℃ / min was raised to 250℃, maintained 5 min; MS conditions: an ion trap mass separator, the ion source for EI, the ionization energy of 70 eV, the ion trap temperature of 220 ℃, transfer line temperature was 250 ℃, solvent delay for 2.5 min, the scanning range is 43 ~ 500 m / z, retrieval library for NIST 05.

Results and discussion

Fig.1 depicts the structure of four targets NAs .the sample of perserved meat were detected byGC-MS and got four kinds of NAs contamination. They are N-nitrosodimethylamine (NDMA), N-nituosomethylethylamine (NMEA), N-nitrosodiethylamine (NDEA) and N-nitrosopoperdine (NPIP) respective. The steam-distillation method can extract and purify them, so they are called volation NAs.
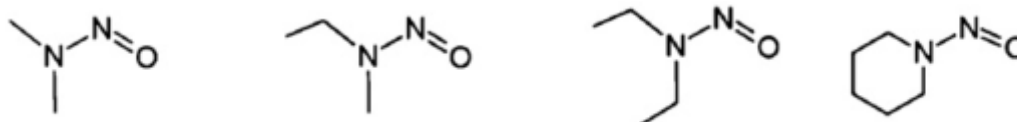

Fig.1 structure of the 4 nitrosanines analyzed in this study

Table 2 The average recovery ,standard deviation and relative standard deviation

Compound	Rec(%)	SD	RSD(%)
NDMA	77.69	0.031	1.96
NMEA	77.65	0.027	2.58
NDEA	83.90	0.092	2.32
NPIP	77.48	0.059	3.29

Experiments conducted to detect NAs contamination in the process of preserved meat. Quantitative date concerning toxic compounds (NDMA, NMEA, NDEA and NPIP) shows in table 2 and analysis of results for the following conclusions. From Fag.1, the experiment revealed the biggest NAs in preserved meat is NDEA and the smallest is NDMA at four stages. There have no NAs been detected raw meat and salting stage meat. NAs are formed in baking stage. The amounts NAs were found at the high meat processing temperature. It seems more probable that NAs were formed when meat were processed at higher temperature. On the first day of the baking, NAs have started to generation and the content of NDEA is biggest and NEMA is minimal. On the third day of the baking, the formation of the sum is the biggest. The generation of NDEA up to 9.492 μg/kg and in the proportion of total content is 74.13%.The maturation stage of NAs content decreased significantly. According to GB9677-1998 the restriction standard regulation of NAs in meat products, the NDMA cannot higher than3μg/kg and NDEA is5μg/kg. The most obvious reduction is NDEA in the process. NPIP is most abundant at 19 days and NPIP perhaps is the most difficult degradation NAs in the meat process.

Table 3 The level of four NAs in preserved meat

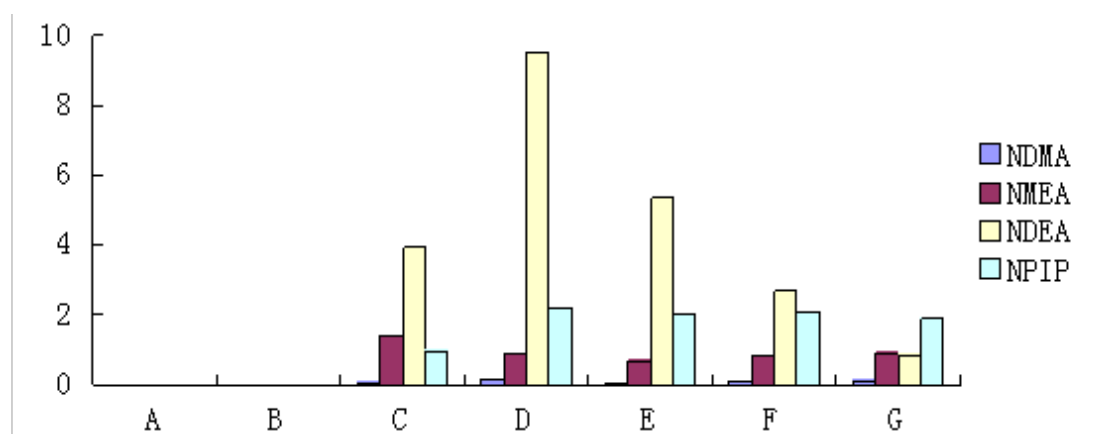| Time(days)	Concentration of nas (μg/kg)				Sum of four NAs (μg/kg)
	NDMA	NMEA	NDEA	NPIP	
0	0	0	0	0	0
1	0	0	0	0	0
2	0.098	1.434	3.933	1.002	6.467
4	0.200	0.939	9.492	2.171	12.804
9	0.087	0.745	5.363	2.001	8.195
14	0.124	0.868	2.690	2.062	5.744
19	0.151	0.960	0.914	1.910	3.936

Fig.3 The change of NAs in different processing stage

Conclusions

GC-MS was used to analyze NAs and 4 kinds of NAs were detected and accurately quantitative. The experiment revealed the biggest NAs is NDEA and the smallest is NDMA at preserved meat process. On the mature stage the four NAs decreased significantly and the least is NDMA.NDMA have a very low level in the process of production and the biggest contest is 0.151μg/kg. The contact of NDMA have an increased trend, the other three NAs reduced gradually.

Acknowledgements

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