

# Measurement of trimethylamine concentration and evaluation of pig meat natural quality by a spectrophotometric method

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**Abstract** Pig meat off-flavor is attributed to trimethylamine (TMA) concentration, and it is considered as the precursor of the fishy off-flavor problems. In this study, TMA concentrations in pig meat were determined, and the interactions with breed and gender effects were discussed. In addition, the TMA threshold for meat off-flavor and pig meat natural quality was measured in relation to meat storage and movement, and the influential factors including the pig breed and storage time were discussed. The results indicated positive effects on the precursor of the fishy off-flavor and the TMA threshold. Native breeds were found to have lower TMA concentrations than European breeds ( $P < 0.01$ ), and females and castrated males had significantly lower TMA concentration than males ( $P < 0.01$ ). The threshold concentration of TMA when meat was classed as off-flavored was  $25 \mu\text{g} \cdot \text{g}^{-1}$ , and this occurred after 35–38 h of storage. The natural qualities, such as appearance, flavor, color and overall acceptable scores declined significantly after 4 days in storage ( $P < 0.01$ ). It is concluded that pig meat off-flavor, breed and gender were essential factors affecting flavor for meat breeding programs, and storage time is important for pig meat natural quality.

**Keywords** pig meat, trimethylamine (TMA), influential factor

## 1 Introduction

Trimethylamine (TMA) is a tertiary amine gas at normal temperature and has a characteristic smell of rotting fish [1,2]. Its accumulation in meat affects the quality. Off-flavors in pork can sometimes be described as having a sour, fishy, metallic or other non-typical flavors including

boar taint [3,4]. Pig meat has different aromas associated with breed, gender, age, diet and carcass handling [5,6], but the basic factor is TMA concentration which determines the flavor of meat and is an important determinant of the natural quality of meat. TMA concentration is also associated with some genetic factors [7]. Loss of function mutations in flavin containing monooxygenase 3 are known to be associated with a fishy off-flavor in chicken eggs [8,9] and bovine milk [10,11] and Trimethylaminuria (TMAU) or fish-odor syndrome in human [12,13].

There are several factors which can influence TMA concentration, including animal age, gender, genotype, breed and carcass handling treatment. To minimize experimental error, we controlled diet, animal age and carcass handling.

The common view about pork flavor is that castrated males and females are more fragrant in smell and delicious in taste compared to normal males, and the native breeds are preferred over European breeds [14]. The consumption of meat and meat products mostly depends on color, appearance, flavor and taste [15–17]. Pig meat natural quality such as, color, appearance, flavor and acceptability and TMA concentration depend mostly on storage time.

In this study, we measured trimethylamine concentration and some natural qualities in pig meat, to evaluate the influence of flavor and acceptability of meat storage at room temperature, and provide scientific suggestions for flavor as a meat breeding priority.

## 2 Materials and methods

### 2.1 Experimental animals and sample collections

Eighty-five leg meat samples were collected from four different pig breeds/lineages including Xiang Mini pig (XP), Jinhua pig (JP), and hybrid line of Duroc, Landrace

and Yorkshire (YLD). All pigs were 1 year old having received the same diet, and collected from Beijing and Hangzhou, China. The experimental pigs included 30 females, 40 castrated males and 15 normal males.

All experimental animals received humane care. Pig slaughter procedures were according to the Humane Slaughter Programs of China approved by the World Society for Protection of Animals. The meat was obtained within 2 h after slaughter; the separable fat and connective tissue were removed, packed in low density polyethylene bags and transferred to the freezer at  $-80^{\circ}\text{C}$  until processed. Details of pigs used in this study are shown in Table 1.

**Table 1** Sample details for pigs used in this study

Breed	Gender			Total
	Female	Castrated male	Male	
JP	10	10	5	25
XP	10	10	5	25
YLD	10	20	5	35
Total	30	40	15	85

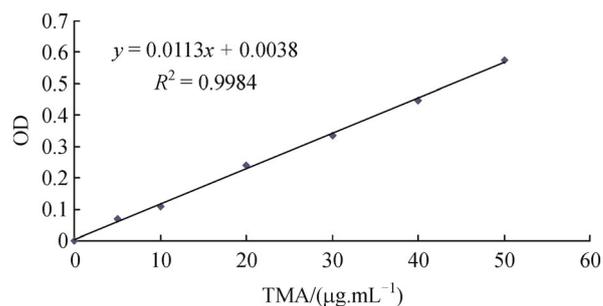
Note: XP, Xiang Mini Pig; JP, Jinhua Pig; YLD, Hybrid line of Duroc, Landrace, and Yorkshire.

## 2.2 TMA measurement

The procedure used to measure TMA concentration was a modification of a method used for chicken meat by Ward et al. [18]. Meat samples (100 mg) were collected in 2 mL tubes, to which 1 mL 10% trichloroacetic acid ( $4^{\circ}\text{C}$ ) was added and the contents quickly homogenized. The liquid phase was transferred into a new 2 mL tube and 1 mL 10% trichloroacetic acid added and mixed with vibration, before centrifugation at  $3000\times g$  for 3 min. 0.2 mL 10% formaldehyde solution, 2 mL anhydrous toluene, 0.6 mL 1:1 potassium carbonate solution was then added to the supernatant, which was shaken up and down for 10 min before the liquid of the toluene phase was transferred to a plastic tube and 0.2 g anhydrous sodium sulfate added and thoroughly mixed. After mixing, 1 mL of liquid was transferred to a new plastic tube and 1 mL of 0.02% picric acid added, forming a yellow TMA-N-picric acid complex. For each sample 200  $\mu\text{L}$  liquid was transferred to a 96-well EIA/RIA plate (Corning Incorporated, USA) and its absorbance measured photometrically at 410 nm (wavelength of maximum absorbance) in a spectrophotometer (TECAN infinite M200, Switzerland). A 10% trichloroacetic acid solution was used as the blank.

A standard curve with 14 TMA-N concentrations ranging from 0 to  $50\ \mu\text{g}\cdot\text{mL}^{-1}$  was used to estimate the TMA concentration of each meat sample ( $R^2 = 0.9984$ ).

The method was modified from Kretzschmar et al. [19]. Dry TMAH ( $\text{C}_3\text{H}_9\text{N}\cdot\text{HCl}$ ) (0.682 g) was added to 1 mL of HCl and diluted with double distilled water up to 100 mL to give a stock solution of  $50\ \mu\text{g}\cdot\text{mL}^{-1}$  TMA-N. This stock solution, was further diluted with double distilled water to give solutions of 0, 5, 10, 20, 30, 40 and  $50\ \mu\text{g}\cdot\text{mL}^{-1}$  of TMA-N, and the standard curve for estimation of TMA concentration was established (Fig. 1).



**Fig. 1** Standard curve for estimation of trimethylamine (TMA) concentration

## 2.3 Sample details for TMA off-flavor threshold

Eighteen leg meat samples from three different breeds were used, 3 females and 3 castrated males from each of Xiang Mini, Jinhua and hybrid line of Yorkshire, Landrace and Duroc pigs. Each sample was divided into three equal parts which were respectively evaluated for 3 days storage (0–72 h) at room temperature ( $13\text{--}16^{\circ}\text{C}$ ), and TMA concentrations measured at different times.

## 2.4 Evaluation of meat appearance, flavor scores and overall acceptability

The standard meat appearance, flavor and acceptability scores of meat was evaluated using an 8-point scale, where 8 is excellent, and 1 is extremely poor [20], every 24 h. The experienced test panel consisted of 30 scientists and post graduate students of the College of Animal Science and Technology, China Agricultural University, Beijing, China, which judged the samples. The panelists were trained according to the guidelines of the American Meat Science Association, 1995. The panelists evaluated the samples for flavor scores ranging from 1 to 8. Average scores were taken for each assessment time.

## 2.5 Measurement of meat color

Meat color was measured every 24 h, using star series equipment [Opto-star (SFK technology A/S, Denmark)] [21]. Surface color was measured at different locations. Results for individual meat samples for color were averages of four readings across each sample surface.

## 2.6 Statistical analysis

TMA concentrations, meat appearance, flavor and color scores and overall acceptability in different breeds, days and genders were analyzed by the General Linear Model (GLM) procedure of the SAS 9.1 program (Statistical Analysis System, 1999). The linear model used to fit the quantitative traits included: the contemporary group effect considered as follows:  $y_{ijkl} = u + b_i + g_j + e_{ijkl}$ , where  $y_{ijkl}$  is TMA concentration for each sample,  $u$  is population mean;  $b_i$  is the effect of breed  $i$  ( $i = 1, 2, 3, 4$ );  $g_j$  is effect of gender  $j$  ( $j = 1, 2, 3$ );  $e_{ijkl}$  is the random residual for each sample.

## 3 Results

### 3.1 Meat TMA concentrations in different breeds and genders

The least square means (LSM) of meat TMA value in different pigs are shown in Fig. 2. The results indicate that there were significant differences between samples ( $P < 0.01$ ).

The breed effect showed that TMA concentration was significantly different between the different breeds and genders. YLD ( $14.51 \pm 0.085 \mu\text{g} \cdot \text{g}^{-1}$ ) had significantly higher TMA concentrations than XP ( $11.07 \pm 0.095 \mu\text{g} \cdot \text{g}^{-1}$ ) and JP ( $12.19 \pm 0.095 \mu\text{g} \cdot \text{g}^{-1}$ ) ( $P < 0.01$ ). The gender effect showed that normal males ( $15.63 \pm 0.120 \mu\text{g} \cdot \text{g}^{-1}$ ) had higher TMA value than castrated males ( $11.69 \pm 0.076 \mu\text{g} \cdot \text{g}^{-1}$ ) and females ( $10.44 \pm 0.086 \mu\text{g} \cdot \text{g}^{-1}$ ) ( $P < 0.01$ ).

### 3.2 Threshold concentration of TMA in pig meat for it to be considered off-flavored

The threshold concentration for TMA off-flavor in pig meat was determined by three steps. First, one part of a meat sample was evaluated for smell every 12 h, and the

test panel used the 48-h point. Secondly, the second part of the meat sample was evaluated every 2 h for a period from 36 to 48 h, and the test panel used the 38-h point. Finally, the last part of the meat sample was evaluated every 0.5 h over the period from 36 to 38 h, and the test panel used at 37 h (Fig. 3). The threshold TMA concentration was found to be  $25 \mu\text{g} \cdot \text{g}^{-1}$  at 37 h when the meat smelled off (Jinhua pig).

Analysis of pig meat TMA threshold associated with breeds and storage period is shown in Fig. 4. The analysis indicated that the period to reach the TMA threshold is different for different breeds.

### 3.3 Association analysis of appearance, flavor scores, color scores, and overall acceptability in different breeds and storage periods

Association analysis showed that appearance, flavor scores, color scores and overall acceptability in pig meat had significant associations with different breeds and storage periods ( $P < 0.01$ ) (Table 2).

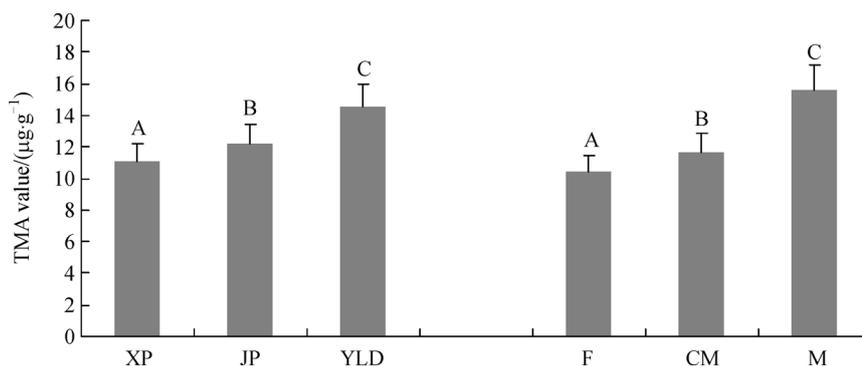
The appearance after 0 and 1 day storage was better than after 2 and 3 days for each breed (Table 2).

The flavor score was higher after 0 and 1 day than after 2 and 3 days. The overall acceptability after 0 and 1 day was greater than after 2 and 3 days (Table 2).

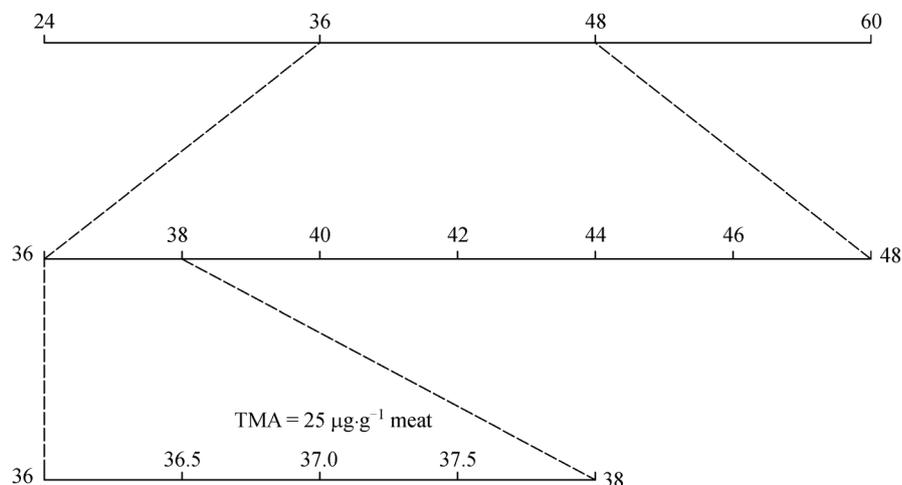
The color scores had significant associations with different breeds and days ( $P < 0.01$ ) (Table 2).

## 4 Discussion

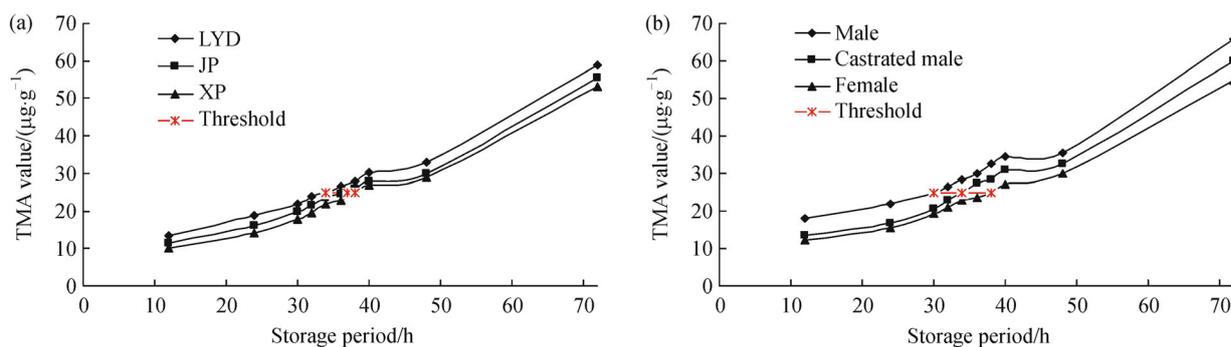
Trimethylamine is a common product produced during pig meat spoilage, and the quality of meat depends mainly on TMA concentration. Ward et al. [18] showed that the TMA should be not more than  $4 \mu\text{g} \cdot \text{g}^{-1}$  in egg yolk. However, there is variability in the ability of individuals to detect TMA [22]. In this study, the TMA value of different breeds and genders had a wide range from 8.3 to  $18.5 \mu\text{g} \cdot \text{g}^{-1}$  meat. One reason is that the TMA measurement methods



**Fig. 2** Pig meat TMA concentrations for breeds and genders. The data are presented as the least square means  $\pm$  SEM (standard error of the mean). A, B and C indicated significant difference at  $P < 0.01$  level. XP, Xiang Mini Pig; JP, Jinhua Pig; YLD, Hybrid line of Duroc, Landrace, and Yorkshire; F, females; M, males; CM, castrated males.



**Fig. 3** Identification of trimethylamine (TMA) concentration threshold for meat of Jinhua pig to smell off by trained test panel



**Fig. 4** Breed (a) and gender (b) effects on time taken to reach the trimethylamine (TMA) off-flavor threshold

have some errors, and another reason is that there can be some amines present that artificially amplify the measured TMA concentration. Although  $4 \mu\text{g}\cdot\text{g}^{-1}$  is an appropriate benchmark, it is possible that eggs with yolk TMA concentrations above that would be acceptable to some consumers [18].

Xiang Mini pigs and Jinhua pigs are typical native breeds used for meat production in China. They have lower bodyweight and lower genetic selection, but have significantly lower TMA concentrations than European pigs (hybrids of Pietrain and Landrace and hybrids of Yorkshire, Landrace and Duroc) which have higher genetic selection for meat productivity.

Regarding gender, the current data suggested that normal males have higher TMA values than castrated males and females.

Traditionally in China, people prefer meat from castrated pigs because it is considered more delicious. Therefore, further study is needed to establish whether the more delicious meat has less TMA. In 1979, Pearson and Butler found that the TMA oxidation defect is present in both male and female chickens and there is no difference between the genders in regards to their sensitivity to

rapeseed meal. Further study is on this process is needed for pigs.

In humans, Cashman et al. [23] found that in unaffected individuals, TMA composes 0 to 9% of total urinary trimethylamines (i.e., TMA + TMAO), whereas TMA comprises greater than 40% of the total urinary trimethylamines in individuals with severe trimethylaminuria. Lundén et al. [11] found that TMA concentrations between 1 and  $37 \mu\text{g}\cdot\text{g}^{-1}$  in milk resulted in a fishy taint. They also mentioned that this fishy off-flavor was influenced by environmental factors such as the presence of TMA precursors or FMO inhibitors in the feed.

The concentration of TMA for the different breeds increased with meat storage time. In this study, meat TMA threshold for off-flavor used was  $25 \mu\text{g}\cdot\text{g}^{-1}$ , which was detected between 35 and 38 h storage depending on the breed.

The appearance, flavor scores and overall acceptability of meat declined significantly ( $P < 0.01$ ) during the 4 days of storage. The steep decline in scores was attributed to the liberation of fatty acids [24], oxidation of fat [25] and increased TMA concentrations. An abrupt reduction in overall acceptability during the storage period was mainly

**Table 2** Associations of appearance, flavor scores, color scores, and overall acceptability with different breeds and days

Treats	Breeds	Storage period/days (hours)			
		0 (0)	1 (24)	2 (48)	3 (72)
Appearance	XP	8.00±0.06 <sup>A</sup>	7.33±0.06 <sup>B</sup>	5.45±0.06 <sup>C</sup>	2.00±0.06 <sup>D</sup>
	JP	8.00±0.05 <sup>A</sup>	7.15±0.05 <sup>B</sup>	4.95±0.05 <sup>C</sup>	2.00±0.05 <sup>D</sup>
	YLD	8.00±0.04 <sup>A</sup>	7.08±0.04 <sup>B</sup>	4.78±0.04 <sup>C</sup>	2.05±0.04 <sup>D</sup>
Flavor scores	XP	8.00±0.05 <sup>A</sup>	6.98±0.05 <sup>B</sup>	5.48±0.05 <sup>C</sup>	2.48±0.05 <sup>D</sup>
	JP	8.00±0.06 <sup>A</sup>	6.73±0.06 <sup>B</sup>	5.35±0.06 <sup>C</sup>	2.40±0.06 <sup>D</sup>
	YLD	7.93±0.06 <sup>A</sup>	6.38±0.06 <sup>B</sup>	4.95±0.06 <sup>C</sup>	2.05±0.06 <sup>D</sup>
Color scores	XP	65.28±0.34 <sup>A</sup>	59.36±0.34 <sup>B</sup>	54.73±0.34 <sup>C</sup>	51.37±0.34 <sup>D</sup>
	JP	44.95±0.36 <sup>A</sup>	42.03±0.36 <sup>B</sup>	40.23±0.36 <sup>C</sup>	37.60±0.36 <sup>D</sup>
	YLD	49.78±0.26 <sup>A</sup>	48.33±0.26 <sup>B</sup>	46.42±0.26 <sup>C</sup>	44.57±0.26 <sup>D</sup>
Overall acceptability	XP	8.00±0.05 <sup>A</sup>	7.35±0.05 <sup>B</sup>	5.15±0.05 <sup>C</sup>	0.98±0.05 <sup>D</sup>
	JP	8.00±0.05 <sup>A</sup>	7.15±0.05 <sup>B</sup>	4.75±0.05 <sup>C</sup>	0.92±0.05 <sup>D</sup>
	YLD	8.00±0.06 <sup>A</sup>	7.02±0.06 <sup>B</sup>	4.65±0.06 <sup>C</sup>	0.90±0.06 <sup>D</sup>

Note: XP, Xiang Mini pig; JP, Jinhua pig; YLD, hybrid of Yorkshire, Landrace and Duroc. The data are presented as the least squares means±SEM (standard errors of the mean). A, B, C and D indicated significant differences at  $P < 0.01$  significance level.

attributed to a decline in flavor of the pig meat. This study showed that the natural quality of meat was acceptable after 1 day and 2 days storage, and 10 h into the third day.

The color scores of meat declined significantly ( $P < 0.01$ ) during 4 days of storage. This decline was due to lipid oxidation and oxidized compounds subsequently reacting with amino acids during non-enzymatic browning of the product [26].

In this study we used only 100 mg meat for measurement of TMA concentration, for ease of sample collection and processing and to contain costs. In previous studies larger samples (20 g or more) [27] were used, which were difficult to collect and process.

## 5 Conclusions

Breed and gender have influenced the TMA concentration of pig meat. The native breeds had lower concentrations than European breeds. Females had the lowest TMA concentrations, and castrated males had lower concentrations than normal males. The threshold concentration of TMA for pig meat to be considered to have an off-flavor was  $25 \mu\text{g} \cdot \text{g}^{-1}$ . The results provide valuable information of TMA concentration and off-flavor, which is related to natural meat quality and encourage further research on TMA concentration and off-flavor in relation to meat quality.

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All applicable institutional and national guidelines for the care and use of animals were followed.

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