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Changes in taste compounds of duck during processing

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Abstract

Nanjing cooked duck is one of the few low temperature cooked meat products in traditional Chinese featured meat products. It is famous for its delicate processing, savoury, and tender flavour. Taste compounds of Nanjing cooked duck during processing, namely free amino acids, peptides and nucleotides, were analyzed. During boiling, both free amino acids and flavour nucleotides decreased significantly. Most peptides decreased during processing and the changes of total peptides were consistent with the nucleotides. Results showed that the special processes before boiling, especially brining, produced an increased of effect on the taste compounds, which could be the reason for the savoury taste of Nanjing cooked duck.

Keywords: Cooked duck; Taste compounds; Free amino acids; Peptides; Nucleotides

1. Introduction

Flavour is a very important component of the eating quality of meat and much research has been aimed at determining those factors, during the production and processing of meat, which influence flavour quality (Mottram, 1998), non-volatile substances that may stimulate the taste buds (taste compounds). Taste sensations are defined as sweet, salty, bitter and sour (acid). Moreover, a further basic sensation called 'umami' has been described as the taste of monosodium glutamate (MSG) and 5′-nucleotides, such as 5′-inosinate (IMP) and 5′-guanylate (GMP) (Mateo, Domínguez, Aguirrezábal, & Zumalacárregui, 1996). Amino acids, peptides, inorganic salts and nucleotides are the main taste compounds in meat products (Ruiz et al., 1999). It is well known that amino acids and peptides contribute to the taste of a wide variety of foods. Furthermore, some amino acids and peptides have been shown to intensify, modify or mask the flavours of certain foods (Maga, 1994). There have been many studies on the taste compounds of some meat products, such as several famous dry-cured hams (Careri et al., 1993; Martín, Antequera, Ventanas, Benítez-Donoso, & Córdoba, 2001; Rodríguez-Nuñez, Aristoy, & Toldrá, 1995; Ruiz et al., 1999; Sentandeu et al., 2003), fermented sausage (Mateo et al., 1996), Frankfurters and dry sausages (Maga, 1998). However, to our knowledge, no study has so far been reported regarding duck meat products.

Cooked duck products are popular foods in China for their flavour and nutritional value. About thirty million ducks are consumed annually, just in Nanjing city. Nanjing cooked duck, one of the few low temperature meat products in traditional Chinese featured meat products, is famous for its delicate processing, savoury, and tender flavour. No study, however, has been done to examine the effects of different processes on the changes of taste compounds in Nanjing cooked duck. Our work sought, on the one hand, to determine the quantities of the major part of the taste compounds, namely free amino acid, peptides and nucleotides, in traditional Chinese Nanjing cooked duck, and on the other hand, to study the changes of taste compounds of duck meat during processing to provide theoretical bases for the quality improvement of Chinese duck meat products.

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2. Materials and methods

2.1. Processing and sampling of Nanjing cooked duck

Thirty-six lean-type Cherry Valley ducks were taken on the day after slaughter, each of which was about 1.5 kg. They were processed by dry curing, brining, roasting and boiling. Each duck was dry-cured using 100 g stir-fried salt with *Illicium verum* Hook. f. for 2 h. As for brining, the brine for Chinese traditional feature meat products was used, which contained not only excessive salt, but also some spice components, such as *Allium fistulosum* L., *Illicium verum* Hook. f., and *Zingiber officinale* Roscoe. The brining process was 4 h. Roasting process was 1 h at 90 °C for drying the carcass and decreasing the subcutaneous lipid. A low boiling temperature from 85 °C to 90 °C was used for the tender taste, and the boiling time was 40 min. Six control ducks were treated without any spices in the dry-curing and brining, but the other procedures were the same as for the test ducks. At the end of each processing stage, 72 duck breasts were trimmed of subcutaneous fat, vacuum-packaged and stored at −20 °C.

2.2. Chemical analysis

Moisture was determined, following the ISO recommended method 1442 (ISO, 1996a).

Fat was determined by following the ISO recommended method 1443 (ISO, 1973).

Salt was determined by following the ISO recommended method 1841-1 (ISO, 1996a, 1996b).

Free amino acids (FAAs) were analyzed with an 835-50 amino acid auto-analyzer (Hitachi Co., Japan).

Peptides were analyzed, following the procedure described by Martín, Antequera, and Córdoba (1998). A 10 g sample was deproteinized with 0.6 N perchloric acid by homogenizing. The homogenates were centrifuged at 15,300*g* for 10 min and filtered through Whatman No. 54 paper. After the pH of the filtrates was adjusted to 6 with 30% potassium hydroxide, they were filtered again. Twenty microlitres of filtrate were used for the HPLC analysis, using a Supelcosil LC-18 containing octadecyldimethylsilyl, 25 cm × 4.6 mm (5 μm particle size) form Supelco (Bellafonte, PA, USA) and a UV detector (214 nm). The eluents used were (A) water (HPLC grade) and (B) acetonitrile containing 0.1% of trifluoroacetic acid. The flow rate was 1 ml/min and the following gradient was employed: initial of 3.2% B; 0.5 min linear change to 4.5% B; 5 min linear change to 8.5% B; 10 min linear change to 11.5% B; 22 min linear change to 99% B and kept for 12 min, then equilibrated at 3.2% for 10 min.

For nucleotides, 10 g of each frozen meat sample were minced and extracted with 30 ml of 5% $HClO_4$ in an ice bath. The extracts were homogenized (3 × 10 s at 20,000 rpm with cooling on ice) with a Ultra-Turrax (Ika, German) macerator, centrifuged at 10,000*g* for 20 min at 4° and the supernatant filtered through Whatman No. 54 paper. The pH of the filtrate was then adjusted to 6.5 with potassium hydroxide and each was filtered again. Twenty microlitres of filtrate were used for the HPLC analysis, using a ZORBAX SB-C18 25 cm × 4.6mm (5 μm particle size, Agilent, USA) and UV detector (254 nm). A constant temperature was used. The eluents used were (A) 0.72% (v/v) triethylamine containing 0.35% (v/v) of phosphate buffer at pH 6.5 and (B) methanol (HPLC grade). The flow rate was 1 ml/min and the following gradient was performed: initial of 0% B; 15 min linear change to 5% B and kept 20 min, then equilibrated at 2.5% B for 10 min. Each nucleotide was quantified by the calibration of the authentic compounds (Sigma).

2.3. Statistical analysis

All the taste compound data were expressed on the base of salt and lipid-free dry matter (DM). The effects of process on the taste compounds of duck meat were evaluated by factorial analysis of variance. Means were compared using Duncan's multiple-range test at the significance level of 0.05. All analyses were carried out using the SPSS11.5 software.

3. Results and discussion

3.1. Free amino acids

Table 1 shows the contents of free amino acids in Nanjing cooked duck throughout the processing. A varied degree of increase in free amino acids of duck was observed from raw duck to the stage before boiling. During the dry-curing stage, free amino acids showed irregular variations and the total free amino acids showed no significant changes. After brining and roasting, most free amino acids increased from 20% to 100% of that in raw duck. There was an abrupt decrease during boiling in all free amino acids, during which most free amino acids decreased by 50%. Whether free amino acid content increases or decreases depends on the balance between free amino acid formation and degradation. The increase in free amino acids of duck meat before boiling is mainly due to the activity of aminopeptidases, while the decrease of free amino acids after boiling is principally related to the formation of volatile compounds from the amino acids (Buscailhon, Monin, Cornet, & Bousset, 1994). Free amino acids constitute a potential source of volatile compounds as follows: (i) through Strecker degradation of valine (Val), isoleucine (Ile) and leucine (Leu), giving 2-methyl-propanal, 2-methyl-butanal and 3-methylbutanal, (ii) generation of dimethyl disulfide compounds from sulfur-containing amino acids, such as methionine, cysteine and cystine, and (iii) the generation of a several pyrazines from Maillard reactions (Toldrá, Aristoy, & Flores, 2000).

The most abundant free amino acids detected in the final product were glutamic acid (Glu), and alanine (Ala), which were generally up to 100 mg 100 g^{-1} of DM, as was consis-

Table 1
Free amino acid concentrations in breast meat at different processing stages of Nanjing cooked duck[A]

	Raw duck	Dry-cured duck	Brined duck	Roasted duck	Nanjing cooked duck	Control duck
Asp	75.7 ± 1.30^{a}	98.0 ± 3.93^{b}	86.7 ± 4.07^{c}	103 ± 3.10^{b}	37.4 ± 0.23^{d}	22.3 ± 0.05^{e}
Thr	151 ± 2.61^{a}	196 ± 7.86^{b}	173 ± 8.13^{c}	205 ± 6.20^{b}	74.6 ± 0.47^{d}	44.7 ± 0.11^{e}
Ser	76.9 ± 3.80^{a}	97.9 ± 5.03^{b}	93.7 ± 1.26^{b}	109 ± 7.17^{c}	57.6 ± 1.63^{d}	42.8 ± 0.13^{e}
Glu	156 ± 9.09^{a}	178 ± 9.47^{a}	171 ± 2.88^{a}	211 ± 15.42^{b}	109 ± 3.61^{c}	78.3 ± 0.30^{d}
Gly	76.1 ± 3.38^{a}	104 ± 3.98^{b}	87.8 ± 1.82^{c}	104 ± 5.76^{b}	53.3 ± 1.29^{d}	39.9 ± 0.19^{e}
Ala	158 ± 8.56^{a}	172 ± 8.57^{a}	162 ± 1.97^{a}	203 ± 13.31^{b}	119 ± 3.38^{c}	93.9 ± 0.62^{d}
Cys	15.4 ± 1.02^{a}	9.82 ± 1.79^{b}	18.5 ± 0.32^{a}	17.0 ± 1.97^{a}	6.99 ± 1.54^{bc}	6.70 ± 0.14^{bc}
Val	47.3 ± 2.17^{a}	50.1 ± 2.21^{ab}	63.1 ± 0.69^{c}	66.4 ± 4.15^{c}	42.5 ± 0.78^{ad}	27.3 ± 0.22^{e}
Met	21.9 ± 0.92^{a}	18.8 ± 0.78^{b}	31.7 ± 0.45^{c}	30.4 ± 1.90^{c}	21.1 ± 0.20^{ab}	15.8 ± 0.56^{d}
Ile	23.0 ± 1.46^{a}	18.0 ± 0.75^{bc}	29.2 ± 0.26^{d}	35.2 ± 2.14^{e}	22.3 ± 0.39^{a}	16.0 ± 0.16^{c}
Leu	38.2 ± 1.94^{a}	29.6 ± 1.64^{b}	55.1 ± 0.63^{c}	64.1 ± 4.85^{d}	29.9 ± 0.96^{b}	20.0 ± 0.23^{e}
Tyr	37.9 ± 2.08^{a}	34.4 ± 2.15^{a}	53.5 ± 1.17^{b}	59.6 ± 4.62^{b}	25.0 ± 1.99^{c}	19.1 ± 0.43^{c}
Phe	28.4 ± 2.02^{a}	21.2 ± 1.56^{b}	39.3 ± 0.48^{c}	42.5 ± 3.19^{c}	18.7 ± 0.52^{b}	13.7 ± 0.49^{d}
Lys	28.9 ± 1.72^{a}	27.9 ± 1.35^{a}	44.8 ± 0.80^{b}	59.7 ± 4.06^{c}	32.9 ± 0.88^{a}	16.6 ± 0.07^{d}
His	12.0 ± 1.59^{a}	10.7 ± 0.75^{a}	18.3 ± 0.27^{b}	23.1 ± 1.89^{c}	11.7 ± 0.43^{a}	7.11 ± 0.19^{d}
Arg	63.5 ± 2.88^{a}	61.7 ± 3.38^{a}	82.5 ± 1.93^{b}	105 ± 8.04^{c}	60.2 ± 2.03^{a}	40.4 ± 0.67^{d}
Total	1011 ± 44.7^{a}	1128 ± 52.0^{ab}	1211 ± 25.0^{b}	1438 ± 86.4^{c}	723 ± 19.1^{d}	505 ± 0.45^{e}

[A] Contents of free amino acids were in mg 100 g^{-1} on the basis of duck meat dry matter and expressed as means ± standard error ($n = 4$). Means with different superscripts in the same row indicate significant difference ($P < 0.05$).

tent with the report for Iberian ham and Parma ham (Martín et al., 2001), while cysteine (Cys), phenylalanine (Phe) and histidine (His) were the least abundant, usually less than 20 mg 100 g^{-1} DM. Compared with the control duck, the free amino acids content of Nanjing cooked duck was significant higher ($P < 0.05$) except Cys ($P > 0.05$), which could be explained by the high level of free amino acids accumulated in the stages before boiling. Furthermore, the desirable amino acids which possess umami (Asp and Glu) and sweet (Ala, Gly and Ser) tastes in Nanjing cooked duck were significantly greater ($P < 0.05$) than in the control, which could be one of reasons contributing to the delicious taste of Nanjing cooked duck.

3.2. Peptides

A total of 14 peaks, corresponding to peptides, was detected using reverse-phase HPLC (Table 2). The method used to analyse the peptides of the perchloric acid- soluble fractions did not allow the determination of their amino acid composition (Martín et al., 2001). Contrary to the trend observed in the total free amino acids content, a decreasing tendency in the chromatography area of the total peptides was observed throughout the processing, which indicated that less proteolytic breakdown took place leading to the formation of peptides in duck meat. During the dry-curing, there were decreases in some peptides and total peptides, while most peptides and total peptides in brined duck were more abundant ($P < 0.05$) than in dry-cured duck and total peptides returned to the initial level. Thus, most peptides did not change ($P > 0.05$) during the roasting, but the total peptides decreased significantly ($P < 0.05$). During boiling, most peptides and total peptides decreased ($P < 0.05$) and most peptides were found in significantly smaller quantities ($P < 0.05$) in the final product than in raw duck. Comparing Nanjing cooked

Table 2
Peptide concentrations in breast meat at different processing stages of Nanjing cooked duck[A]

	Raw duck	Dry-cured duck	Brined duck	Roasted duck	Nanjing cooked duck	Control duck
Peak1	2.08 ± 0.07^{a}	3.77 ± 0.15^{b}	3.28 ± 0.21^{c}	3.41 ± 0.10^{bc}	4.68 ± 0.05^{d}	5.44 ± 0.08^{e}
Peak2	87.6 ± 5.36^{a}	63.3 ± 8.74^{b}	90.3 ± 4.72^{a}	74.1 ± 2.99^{b}	44.3 ± 0.91^{c}	36.4 ± 1.61^{c}
Peak3	79.7 ± 2.68^{a}	63.6 ± 1.77^{b}	75.9 ± 0.88^{a}	70.9 ± 10.49^{c}	62.3 ± 0.63^{b}	47.1 ± 1.83^{d}
Peak4	0.58 ± 0.06^{ab}	0.70 ± 0.05^{ac}	0.89 ± 0.02^{d}	0.79 ± 0.06^{cd}	0.34 ± 0.00^{ef}	0.23 ± 0.01^{f}
Peak5	1.36 ± 0.16^{ab}	1.46 ± 0.03^{a}	2.19 ± 0.03^{c}	2.21 ± 0.05^{c}	1.57 ± 0.08^{a}	1.14 ± 0.09^{b}
Peak6	5.32 ± 0.30^{a}	5.27 ± 0.19^{a}	6.78 ± 0.08^{b}	6.42 ± 0.12^{b}	3.37 ± 0.07^{cd}	3.08 ± 0.10^{d}
Peak7	2.58 ± 0.60^{ab}	3.40 ± 0.42^{bc}	4.31 ± 0.39^{c}	3.42 ± 0.09^{bc}	2.91 ± 0.13^{ab}	1.50 ± 0.07^{d}
Peak8	15.3 ± 2.06^{a}	11.8 ± 0.31^{b}	15.3 ± 0.07^{a}	14.9 ± 0.29^{a}	10.5 ± 0.03^{b}	7.58 ± 0.06^{c}
Peak9	8.76 ± 0.30^{ab}	7.21 ± 0.30^{c}	9.83 ± 0.28^{ad}	8.93 ± 0.14^{ab}	14.2 ± 0.34^{e}	11.0 ± 0.09^{d}
Peak10	9.36 ± 0.25^{a}	2.56 ± 0.24^{b}	7.80 ± 0.16^{c}	3.27 ± 0.38^{d}	2.33 ± 0.07^{b}	1.61 ± 0.12^{e}
Peak11	25.5 ± 1.64^{a}	6.21 ± 1.13^{b}	7.02 ± 0.51^{b}	5.19 ± 0.29^{b}	14.0 ± 0.44^{c}	11.7 ± 0.74^{cd}
Peak12	30.7 ± 1.69^{a}	45.5 ± 0.50^{b}	49.1 ± 0.68^{c}	46.7 ± 0.71^{bc}	23.9 ± 0.90^{d}	16.1 ± 0.17^{e}
Peak13	3.57 ± 0.23^{a}	3.62 ± 0.04^{a}	5.10 ± 0.08^{b}	5.13 ± 0.25^{b}	2.81 ± 0.05^{c}	2.39 ± 0.05^{d}
Peak14	0.03 ± 0.00^{a}	0.18 ± 0.02^{b}	0.09 ± 0.00^{c}	0.08 ± 0.01^{c}	0.45 ± 0.00^{d}	0.49 ± 0.01^{d}
Total	273 ± 4.12^{a}	219 ± 13.90^{b}	278 ± 8.12^{a}	245 ± 6.54^{c}	187 ± 3.86^{d}	146 ± 5.12^{e}

[A] Contents of peptides were in area 100 g^{-1} on the basis of dry matter and expressed as means ± standard error multiplying 10^{4}($n = 6$). Means with different superscripts in the same row indicate significant difference ($P < 0.05$).

duck to the control, nine peptides and total peptides in the former were significantly more abundant ($P < 0.05$) than in the latter. Considering that the increase in the area of the total peaks took place only during brining, the process of brining appears to be the specialty for Nanjing cooked duck.

Small peptides, as well as FAAs, can directly contribute to flavour or indirectly contribute as precursors of other flavour compounds (Sentandeu et al., 2003). As free amino acids are the ultimate products of proteolysis, the small peptides present would represent a dynamic state in which both formation and breakdown occurred. The increase of free amino acids could explain the decrease of peptide during the process before boiling, and boiling accelerated peptide transformation to other flavour compounds, consequently, the content of peptides decreased continually. The importance of peptides in the sensory perception of food has been recognized for some time. In some foods, particularly cheeses, the occurrence of sweet, biter, and sour tastes, as well as brothy and nutty flavours has been correlated with the presence of certain peptides derived from the proteolysis of milk protein (Sentandeu et al., 2003). Dipeptides, such as Gly–Leu, Pro–Glu or Val–Glu, play an important role in flavour enhancement probably by virtue of their buffering capacity, while others, e.g., glutamic acid oligomers, are effective as bitterness-masking agents (Gill, López-Fandiño, Jorba, & Vulfson, 1996). It has also been demonstrated that peptides contribute to the improvement in flavour of meats during refrigerated storage (Nishimura, Rhue, Okitani, & Kato, 1988). It is important to continue to investigate the generation of peptides as they contribute to the specific flavour of Nanjing cooked duck.

3.3. Nucleotides

As shown in Table 3, the contents of 5′-inosinic acid (5′-IMP) and 5′-guanosine monophosphate (5′-GMP) decreased continuously during the stages before boiling while they increased significantly ($P < 0.05$) during boiling, which is opposite to the changes of inosine. The conversion reaction among nucleotides, during processing, accounted for certain regular changes of nucleotides. For example, IMP was transformed into inosine, probably by endogenous enzymes, which could explain the opposite changes between IMP and inosine during processing. During boiling, the degradation of adenosine diphosphate (ADP) produced the highest level of flavour nucleotides, 5′-IMP and 5′-GMP, and simultaneously, the content of 5′-adenosine monophosphate (5′-AMP) increased abruptly ($P < 0.05$).

5′-IMP was the most abundant nucleotide in the final product with average quantities of 241 mg 100 g^{-1}in DM of duck breast and inosine was the second major compound (166 mg 100 g^{-1} of DM), while 5′-GMP and ADP were found at lower concentrations. As we know, among the adenosine triphosphate (ATP) derivatives, IMP is predominant in meat extract 24 h after slaughter. This compound is gradually transformed into inosine and hypoxanthine in the meat flesh (Watanabe, Tsuneishi, & Takimoto, 1989). Nucleotides decrease to undetectable levels during the long curing process in sausages (Mateo et al., 1996) and in Parma hams (Kohata, Numata, Kawaguchi, Nakamura, & Arakawa, 1992). As for our study, the duration of the full process was less than one day and the concentrations of flavour nucleotides, especially 5′-IMP found in Nanjing cooked duck, were well above their umami taste threshold 140 ppm. Furthermore, the contents of flavour nucleotides in Nanjing cooked duck were as high as in some mushrooms, such as king oyster mushrooms, shiitake, ear mushrooms and black poplar mushrooms (Yang, Lin, & Mau, 2001). Inosine produced a bitter taste and hypoxanthine (Hx) did not account for any taste response (Mateo et al., 1996). All these facts could imply that nucleotides would be very relevant to the taste of Nanjing cooked duck. All the nucleotides were found in significantly larger quantities ($P < 0.05$) in the final products than in the control. It seems that the processes of dry-curing, brining and roasting produced a decelerating effect on the reduction of flavour nucleotides.

From the results of this work, it can be concluded that the processes before the boiling, especially brining, produced an increase effect on the taste compounds, which could be the reason for the savoury taste of Nanjing cooked duck. To determine the relationship of the taste of duck meat products to their taste compounds, further sensory evaluation is needed.

Table 3
Nucleotide concentrations in breast meat at different processing stages of Nanjing cooked duck[A]

Nucleotides[B]	Raw duck	Dry-cured duck	Brined duck	Roasted duck	Nanjing cooked duck	Control duck
5′-IMP	470 ± 3.87^{a}	251 ± 1.66^{b}	197 ± 10.60^{c}	164 ± 2.80^{d}	241 ± 14.9^{b}	182 ± 0.99^{cd}
5′-GMP	18.9 ± 0.88^{a}	6.98 ± 0.95^{b}	6.04 ± 0.68^{bc}	3.67 ± 0.26^{c}	10.4 ± 1.64^{d}	4.99 ± 0.16^{bc}
5′-ADP	73.4 ± 1.35^{a}	33.3 ± 0.42^{b}	37.7 ± 0.66^{c}	39.5 ± 0.40^{c}	13.1 ± 1.11^{d}	11.0 ± 0.34^{d}
5′-AMP	5.34 ± 0.33^{a}	2.50 ± 0.25^{bc}	3.41 ± 0.20^{ab}	0.27 ± 0.05^{c}	24.6 ± 2.10^{d}	17.0 ± 0.19^{e}
Inosine	223 ± 1.34^{a}	249 ± 2.04^{b}	297 ± 2.14^{c}	319 ± 1.68^{d}	166 ± 8.97^{e}	105 ± 0.54^{f}
Hx	40.6 ± 0.51^{a}	25.1 ± 0.37^{b}	44.5 ± 1.10^{c}	49.2 ± 0.72^{d}	32.0 ± 1.51^{e}	26.0 ± 0.32^{b}
Flavor nucleotides[C]	489 ± 3.62^{a}	258 ± 2.15^{b}	203 ± 10.65^{c}	167 ± 2.91^{d}	251 ± 15.5^{b}	187 ± 1.06^{c}

[A] Contents of nucleotide were in mg 100 g^{-1} on the basis of dry matter and expressed as mean ± standard error ($n = 6$). Means with different superscripts in the same row indicate significant difference ($P < 0.05$).
[B] 5′-IMP, 5′-inosinic acid; 5′-GMP, 5′-guanosine monophosphate; 5′-ADP, 5′-adenosine diphosphate; Hx, Hypoxanthine.
[C] Flavour nucleotides: 5′-IMP+5′-GMP.

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