

Effect of Cooking Methods on the Chemical Compounds Associated with Umami Taste in Duck Breast Meat

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Abstract: Dry-curing, wet-curing, stewing and marinating are sequential Chinese traditional meat-processing methods dating back thousands of years ago. However, little information is available about the contribution of each processing stage to the umami taste in duck breast meat. Therefore, effects of those meat-processing methods on umami taste related chemical compounds were determined in this study. Dry-curing significantly increased free amino acids, oligopeptides and nucleotides and nucleotides degradation products ($P < 0.05$), however, in the following wet-curing, stewing and marinating stages, the percentages of inosine and hypoxanthine were gradually declined. Electronic tongue and principle component analysis results revealed the difference between dry-cured/wet-cured duck breast meat and marinated/stewed duck breast meat was due to the increase of free amino acids, oligopeptides and inosine monophosphate. Therefore, dry-curing is an important stage in meat processing, not only it produces a pool of taste related compounds, it also stimulates the enzyme activities in the muscle. Meanwhile, in the stewing stage, the umami taste profile of duck breast meat was gradually established. We can conclude that dry-curing and stewing are the two crucial stages to form the umami taste profile in duck breast meat.

Key words: Duck breast meat, free amino acids, oligopeptides, nucleotides, inosine monophosphate.

Nomenclature

ADP:	adenosine diphosphate
AMP:	adenosine monophosphate
IMP:	inosine monophosphate
IN:	inosine
Hx:	hypoxanthine

1. Introduction

Dry-curing, wet-curing, stewing and marinating are sequential Chinese traditional meat-processing methods dating back thousands of years ago. Those traditional recipes have handed down from one generation to another and formed a unique taste in meat [1]. During dry-curing, meat is first rubbed with salt and nitrite and then placed at 4 °C for 24 h.

Afterward, the meat is soaked in a mixture brine containing salt, and spices (e.g.: aniseed, red yeast rice, pepper, Sichuan peppercorn and ginger) at room temperature at least 2 h, this stage is called as wet-curing stage. When wet-curing stage is completed, the brine with meat is boiled and held no less than 1h named as stewing stage. Finally, meat is ripened by marinating under brines at the minimum period of 2 h at room temperature to finalize flavor [2].

Recently, several studies have been focused on non-volatile compounds of the meat products, including dry-cured hams [3, 4] and fermented sausages [5, 6]. All these researches indicated that a number of complex chemical and biochemical reactions occur during processing meat products: muscle proteins break down into small peptides and free amino acids. Although meat taste is a

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combination of all 5 taste dimensions (sour, sweet, salty, bitter and umami) [7], the intensity of umami taste plays a crucial role in the taste and palatability of meat products. The umami taste, which is described as savory, brothy or beefy, comes mainly from the free amino acids, oligopeptides as well as from certain 5'-ribonucleotides such as inosine-5'-monophosphate (IMP) [8]. In addition, those umami tastes related chemical compounds are also influenced by curing time [9, 10], curing agent usage as well as temperature [11, 12], and they are precursors for the formation of volatile odor-active compounds through Strecker degradation and Maillard reactions [13, 14].

In spite of the increasing demand for duck meat products day by day [2], little information is available about the contribution of each Chinese traditional processing stage to the final duck breast meat products. Therefore, the objectives of this research were to: (1) determine the umami taste related chemical compounds in duck breast meat during processing; (2) analysis the taste profiles in different processing stages by electronic tongues; (3) elucidate the mechanisms of umami taste formation in the final duck breast meat products.

2. Materials and Methods

2.1 Sample Preparation

The duck breast meat was obtained from a local processor. The meat was first dry-curing using salt (2%, w/w), sodium nitrite (0.01%, w/w) and sodium erythorbate (0.1%, w/w) at 4°C for 24 h. When the dry-curing stage was completed, a mixture brine containing salt (0.6%, w/w), aniseed (0.13%, w/w), red yeast rice (0.1%, w/w), pepper (0.24%, w/w), Sichuan peppercorn (0.07%, w/w) and ginger (0.17%, w/w) was prepared. Then, the dry-curing meat was marinated under the brine for 2 h at room temperature (wet-curing stage). At the end of wet-curing stage, the brine with meat was heated to 100 °C with an induction cooktop (Duxtop) at the highest setting (1, 800 watts) and holding for 1h (stewing stage). After

the stewing stage was done, the container was immediately cooled down with running water, and the meat was continuously to marinate for another 2 h at room temperature (marinating stage). The entire processing procedure was replicated three times at different time.

2.2 Proximate Composition Analysis

Compositional analysis was performed using AOAC [15]. Moisture content was determined by weight loss after 12 h at 105 °C in a drying oven. Fat content was determined using the Soxhlet solvent extraction system. Free amino acids were determined by the ninhydrin colorimetry method. Oligopeptides were determined by spectrophotometric assay based on Lowry's method [16].

2.3 Analysis of Nucleotides, Inosine and Hypoxanthine

Nucleotides and nucleotides degradation products were analyzed using the HPLC method of Liu et al. [2] and Hernández-Cázares et al. [17] with some modifications. Ten grams of minced meat were weighed and homogenized with 40 ml of 3.5% (v/v) perchloric acid using a Polytron homogenizer (Type PT 10/35, Brinkman Instruments Inc., Westbury, NY, USA) for 15 s at high speed. The precipitated proteins were removed by centrifugation at 10,000 rpm for 20 min at 4 °C. The supernatant was filtered through Whatman NO. 54 and neutralized with sodium hydroxide to pH 6.5. The neutralized extracts were centrifuged and filtered again as above, and the final solution was filtered with a syringe filter (Waters, Milford, MA, USA) before HPLC analysis.

A waters e2695 HPLC system equipped with a photodiode array detector and a LiChrospher100 RP-18 column (4.6 × 250 mm, 5 µm, Merck, Germany) was used for HPLC analysis. An aliquot of extract (10 µL) was injected using an auto-sampler. The following mobile phases were used to analyze different nucleotides and nucleotides degradation products: 0.04 M potassium dihydrogen

orthophosphate and 0.06 M dipotassium dihydrogen orthophosphate buffer at pH 6.8/methanol (90:10, v:v) was used to analyze ADP and IMP; 0.05 M KH_2PO_4 /methanol (99:1, v:v) was used to analyze AMP, hypoxanthine, and inosine. All the samples were detected at 254 nm with the flow rate 1.0 mL/min.

2.4 Electronic Tongues Analysis

The electronic tongue system (α -ASTREE II Liquid and Taste analyzer, Alpha MOS, Toulouse, France) consisted of a 16-position auto-sampler, an array of sensors, a reference electrode (Ag/AgCl), and a chemometric software package. The sensor array used in this study contained seven sensors designated by Alpha MOS as sensor set (ZZ, JE, BB, CA, GA, HA, JB). These sensors are based on the ChemFET technology (Chemical modified Field Effect Transistor) and coated with proprietary membranes that impart the non-specificity, low selectivity qualities to the sensors as well as cross-sensitivity to different components in solution.

Prior to analysis of samples, the electronic tongue sensors were conditioned (0.01 mol/L hydrochloric acid), calibrated (0.01 mol/L hydrochloric acid) and tested (diagnostic, 0.01 mol/L each of hydrochloric acid, sodium chloride, and monosodium glutamate) for proper functioning and stability. Following successful tests, each meat sample was analyzed seven times for a period of 120 s. To avoid carryover effects, the sensors were rinsed in deionized water after each measurement. The raw data thus obtained were multivariate in nature and expressed as voltage vs. time [18].

2.5 Statistical Analysis

Three replications of samples were used for each analysis. Data were analyzed by the GLM procedure of SAS (SAS 9.1 version) for different treatments. The fix effect of treatments and random effect of replications were included in the model. The differences in the mean values were compared by Duncan's multiple comparison method, and mean values and standard deviation were reported ($P < 0.05$). Discriminant function analysis (DFA) and principle component analysis (PCA) were performed on XLSTAT (2015).

3. Results and Discussion

3.1 Proximate Compositions

During the whole processing procedure, the moisture and fat contents were significantly decreased ($P < 0.05$), while a significant increase of sodium chloride content was observed at the dry-curing stage ($P < 0.05$), however, after the dry-curing stage, the concentration of sodium chloride was decreased and stayed in a constant level. These results is in agreement with the results of Liu et al. [2] who reported in Nanjing duck.

3.2 Oligopeptides and Free Amino Acids

The contents of oligopeptides and free amino acids in duck during the whole processing procedure were shown in Table 2. In the dry-curing stage, there was a significant increase ($P < 0.05$) for free amino acids at 16 h and 24 h for oligopeptides, respectively, however, in the following processing steps, the amount of oligopeptides and free amino acids were gradually

Table 1 Moisture, fat content and sodium chloride contents (%) of duck breast meat during different processing stages.

Stage	Moisture (g/100 g)	Fat (g/100 g)	Sodium chloride (g/100 g)
Raw	77.04 \pm 0.19 ^c	6.78 \pm 0.40 ^a	0.21 \pm 0.04 ^c
Dry-curing	76.39 \pm 0.48 ^c	6.09 \pm 0.10 ^{ab}	1.90 \pm 0.05 ^a
Wet-curing	71.81 \pm 0.46 ^b	5.93 \pm 0.08 ^{bc}	1.22 \pm 0.02 ^b
Stewing	64.63 \pm 0.18 ^a	6.40 \pm 0.28 ^{ab}	1.16 \pm 0.27 ^b
Marinating	67.42 \pm 0.11 ^a	4.83 \pm 0.55 ^d	1.07 \pm 0.13 ^b

^{a-c} Means with different letters within a column differ significantly ($P < 0.05$) (n = 3).

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Table 2 Oligopeptides and free amino acids contents of duck breast meat during different processing stages.

Stage	Oligopeptides [*]	Free amino acids [†]
Raw	33.14 ± 0.26 ^c	831.43 ± 43.79 ^b
Dry-curing		
8 h	34.67 ± 1.08 ^c	853.20 ± 34.34 ^b
16 h	40.21 ± 1.08 ^b	901.16 ± 18.54 ^{ab}
24 h	47.10 ± 0.67 ^a	956.68 ± 4.11 ^a
Wet-curing	23.07 ± 0.50 ^d	499.37 ± 15.80 ^c
Stewing	19.61 ± 0.29 ^e	329.37 ± 9.65 ^d
Marinating	19.75 ± 0.15 ^e	280.66 ± 6.70 ^e

^{a-c} Means with different letters within a column differ significantly ($P < 0.05$) ($n = 3$).

^{*} Contents of oligopeptides were in mg BSA eq./g dry matter.

[†] Contents of total free amino acids were in mg/100 g based on dry matter.

decreased ($P < 0.05$).

These results indicated that a massive proteolytic breakdown by enzymatic activities had been taken place in the duck muscles in the dry-curing stage, which led to producing low molecular peptides and free amino acids [19]. But in the following processing steps, the oligopeptides could be converted into other volatile compounds [10, 20].

The amount of salt, curing time and temperature are the key parameters to attribute taste formation. Salt can affect the muscle enzymes activities: Toldrá [21] and Zhao et al. [12] found that salt can inhibit cathepsin, alanyl and pyroglutamyl aminopeptidase's activity while enhance m-calpain activity; Sentandreu and Toldrá [22, 23] reported that dipeptidyl peptidases (DPP) can be inhibited by salt except DPP I, which was only moderately affected by high salt concentrations. The curing time is another factor: Flores et al. [9] reported that there was a significant increase in peptides and free amino acids during the 12 month drying in "Serrano" dry-cured ham than the 7 month drying. Similar results was found by Ruiz et al. [10] in dry cured Iberian ham. Temperature can also affect enzyme activities and lead to the different release rates of oligopeptide and free amino acids [11]. Zhao et al. [12] found that there was a significant interactive effect on cathepsin B and L activity with temperature and salt content ($P < 0.001$). In addition, the inhibiting effect of salt contents on cathepsin activity was strengthened by the increase in

temperature. Liu et al. [2] also reports that the free amino acid concentration increased significantly ($P < 0.05$) during the roasting stage in Nanjing cooked duck, which indicated that the reaching-temperature can stimulate protease and peptidase activities, and lead to the release of free amino acids.

3.3 Nucleotides and Nucleotides Degradation Products

Table 3 showed the changes of nucleotide and nucleotides degradation products during the whole processing procedures. In the dry-curing stage, the amounts of ADP, IMP and Hx were increased significantly ($P < 0.05$). A similar trend was found in inosine before 16 h, but a significant decrease was observed at 24 h ($P < 0.05$). Compared the amounts in the raw duck breast meat, those nucleotides and nucleotides degradation products increased about 21% to 115% after completing the dry-curing stage. However, in the subsequent wet-curing, stewing and marinating stage, the amount of IMP and inosine decreased significantly ($P < 0.05$). A slight increase was observed in ADP and AMP in the stewing stage, but the amount of those decreased in the following marinating stage.

During the dry-curing stage, the water is unavailable by increasing the extracellular osmotic pressure, which was attributed an increase of ADP, AMP, IMP, HX and inosine [24]. However, in the wet-curing stage, two opposite effects were applied in

Table 3 Nucleotides and nucleotides degradation products of duck breast meat during different processing stages¹.

Stage	Nucleotides and nucleotides degradation products ^{*,†}				
	ADP	AMP	IMP	Inosine	Hx
Raw	394.83 ± 1.80 ^c	6.45 ± 1.79 ^f	1425.01 ± 3.65 ^b	3697.98 ± 19.27 ^d	2617.12 ± 25.64 ^c
Dry-curing					
8 h	401.78 ± 10.67 ^c	35.92 ± 3.89 ^c	1079.30 ± 2.91 ^c	5371.10 ± 14.43 ^b	2437.84 ± 10.14 ^d
16 h	428.49 ± 5.29 ^b	44.41 ± 4.87 ^b	1184.57 ± 9.23 ^c	6442.49 ± 9.10 ^a	3356.06 ± 26.01 ^b
24 h	477.29 ± 18.33 ^a	13.88 ± 0.46 ^e	2240.52 ± 6.25 ^a	5244.42 ± 10.63 ^c	5053.64 ± 23.22 ^a
Wet-curing	41.08 ± 1.64 ^e	18.38 ± 0.20 ^d	891.59 ± 3.21 ^d	2194.50 ± 7.90 ^e	1542.91 ± 1.69 ^e
Stewing	94.24 ± 1.12 ^d	59.36 ± 1.67 ^a	792.91 ± 4.26 ^e	716.47 ± 5.14 ^f	432.26 ± 5.00 ^g
Marinating	49.63 ± 0.51 ^e	62.21 ± 0.98 ^a	560.22 ± 1.33 ^f	628.53 ± 2.01 ^f	594.71 ± 1.32 ^f

^{a-g} Means with different letters within a column differ significantly ($P < 0.05$) ($n = 3$).

^{*} Contents of Nucleotides were in $\mu\text{mol}/100\text{ g}$ based on dry matter.

[†]ADP: adenosine diphosphate; AMP, adenosine monophosphate; IMP: inosine monophosphate; Hx: hypoxanthine.

nucleotides degradation: a decrease of extracellular osmotic pressure provided by brine, which causes small molecules components leaking into the brine; the conversion of nucleotides by endogenous enzymes in muscle. As shown in Table 3, in the dry-curing stage, all the nucleotides and nucleotides degradation products increased significantly, which indicated a condensed effect caused by curing agents. However, in the subsequent wet-curing, stewing and marinating stages, the diluting effect was dominated factor in the changes of IMP and inosine. On the contrary, in the stewing stage, some ADP and AMP flowed back into muscle cells and conversion to Hx in marinating [25].

In the final product, IMP was the most prevalent nucleotide. The concentration of IMP was higher than the recognition taste threshold [7, 26] and could be acted as a flavor enhancer in muscle [27]. Liu et al. [2] reported that IMP was the most abundant nucleotide in Nanjing cooked duck and inosine was the second most abundant nucleotide, which was consistent with our results. The decrease of IMP after the dry-curing stage (Table 3) could imply a reduction in the intensity of meat flavor. However, other components, such as salt, glutamic and aspartic acids would replace this flavor potentiating effect and these amino acids were generated by proteolysis during the dry-curing process [28]. In fermented hams [29] and sausages [30], the concentration of nucleotides decreased to undetectable levels during the long curing time.

Similar reports of dry-curing ham were made by Hernández-Cázares et al. [17] who found that the AMP content disappeared completely after 7 months, and IMP was depleted after 200 days. However, in our study, the duration of the whole processing procedures was only 31 h, it is much shorter than those reported results.

3.4 Electronic Tongues

The results of discriminant function analysis (DFA) performed with data obtained from the electronic tongue were show in Fig. 1. The first two factors explained 86.22% and 12.71%, and electronic tongue showed an excellent ability to discriminate the duck breast meat in different processing stages. There was a clear separation among raw, dry-cured and wet-cured duck breast meat, while an overlap was observed in stewed and marinated ones, which also indicated there was little changes in the taste related chemical components at the last two stages.

The percentage changes of free amino acids, oligopeptides, nucleotides and nucleotides degradation products at each processing stage were listed in Table 4. Gradually increases were observed in free amino acids and oligopeptides after dry-cured 16 h. The changes in the nucleotides and nucleotides degradation products were more complex, but a high-level IMP content and a low-level inosine/hypoxanthine content were found in the final

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products. The principle components analysis for the percentages of oligopeptides, free amino acids and nucleotides and nucleotides degradation products of the seven duck breast meat products at different stages was showed in Fig. 2. According to this reduced-dimension plot, we can visualize the contributions of those variables to each different product. In the lower right quadrant of PC1, the major components contributing to the dry-cured 8 h and 16 h duck breast meat products were shown to be inosine,

while in the upper right quadrant of PC1, the dry-cured 24 h duck breast meat product was associated with hypoxanthine. In contrast, near the negative axis of PC1 and PC2 (lower left quadrant), the differentiation of the marinated and stewed duck breast meat products from others was attributed to the high percentage of IMP, free amino acids and oligopeptides. Kuchiba-manabe et al. [31] already reported that inosine and hypoxanthine have a bitter taste in food systems. Considering a relatively high

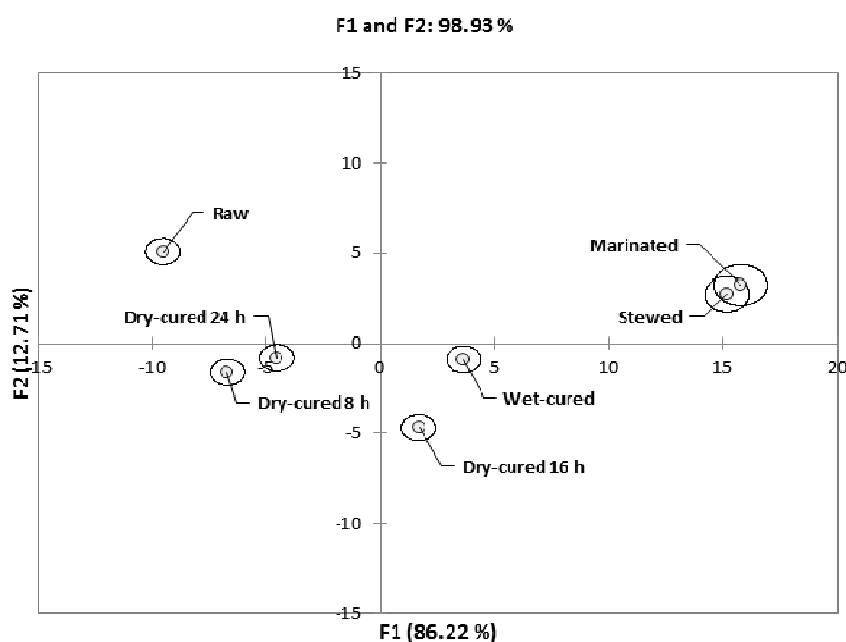


Fig. 1 Electronic tongue separation of duck breast meat in different processing stages using discriminant function analysis (n = 3).

Table 4 Changes of oligopeptides, free amino acids, nucleotides and nucleotides degradation products (%) in the duck breast meat during different processing stages*.

Stages	Nucleotides and nucleotides degradation products						
	Oligopeptides	Free amino acids	ADP	AMP	IMP	Inosine	Hx
Raw	1.13 ± 0.01 ^d	28.26 ± 1.49 ^b	5.73 ± 0.03 ^a	0.08 ± 0.02 ^d	18.99 ± 0.05 ^e	33.71 ± 0.18 ^d	12.11 ± 0.12 ^c
Dry-curing							
8 h	1.06 ± 0.03 ^e	26.11 ± 1.05 ^c	5.25 ± 0.14 ^b	0.38 ± 0.04 ^c	12.95 ± 0.03 ^f	44.09 ± 0.12 ^b	10.15 ± 0.04 ^d
16 h	1.06 ± 0.03 ^e	23.78 ± 0.49 ^d	4.83 ± 0.06 ^c	0.41 ± 0.04 ^c	12.26 ± 0.10 ^g	45.60 ± 0.06 ^a	12.05 ± 0.09 ^c
24 h	1.13 ± 0.02 ^d	22.86 ± 0.10 ^d	4.87 ± 0.19 ^c	0.12 ± 0.01 ^d	20.99 ± 0.06 ^c	33.61 ± 0.07 ^d	16.43 ± 0.08 ^a
Wet-curing	1.36 ± 0.03 ^c	29.47 ± 0.93 ^b	1.04 ± 0.04 ^f	0.38 ± 0.01 ^c	20.63 ± 0.07 ^d	34.73 ± 0.13 ^c	12.39 ± 0.01 ^b
Stewing	2.02 ± 0.03 ^b	33.89 ± 0.99 ^a	4.14 ± 0.05 ^d	2.12 ± 0.06 ^b	32.00 ± 0.17 ^a	19.77 ± 0.14 ^f	6.05 ± 0.07 ^f
Marinating	2.43 ± 0.02 ^a	34.54 ± 0.82 ^a	2.61 ± 0.03 ^e	2.66 ± 0.04 ^a	27.04 ± 0.06 ^b	20.75 ± 0.07 ^e	9.96 ± 0.02 ^e

^{a-g} Means with different letters within a column differ significantly ($P < 0.05$) (n = 3).

*The percentage is calculated by using each individually taste component divided by the sum amount of free amino acids, oligopeptides and nucleotides and nucleotides degradation products at the same processing stage.

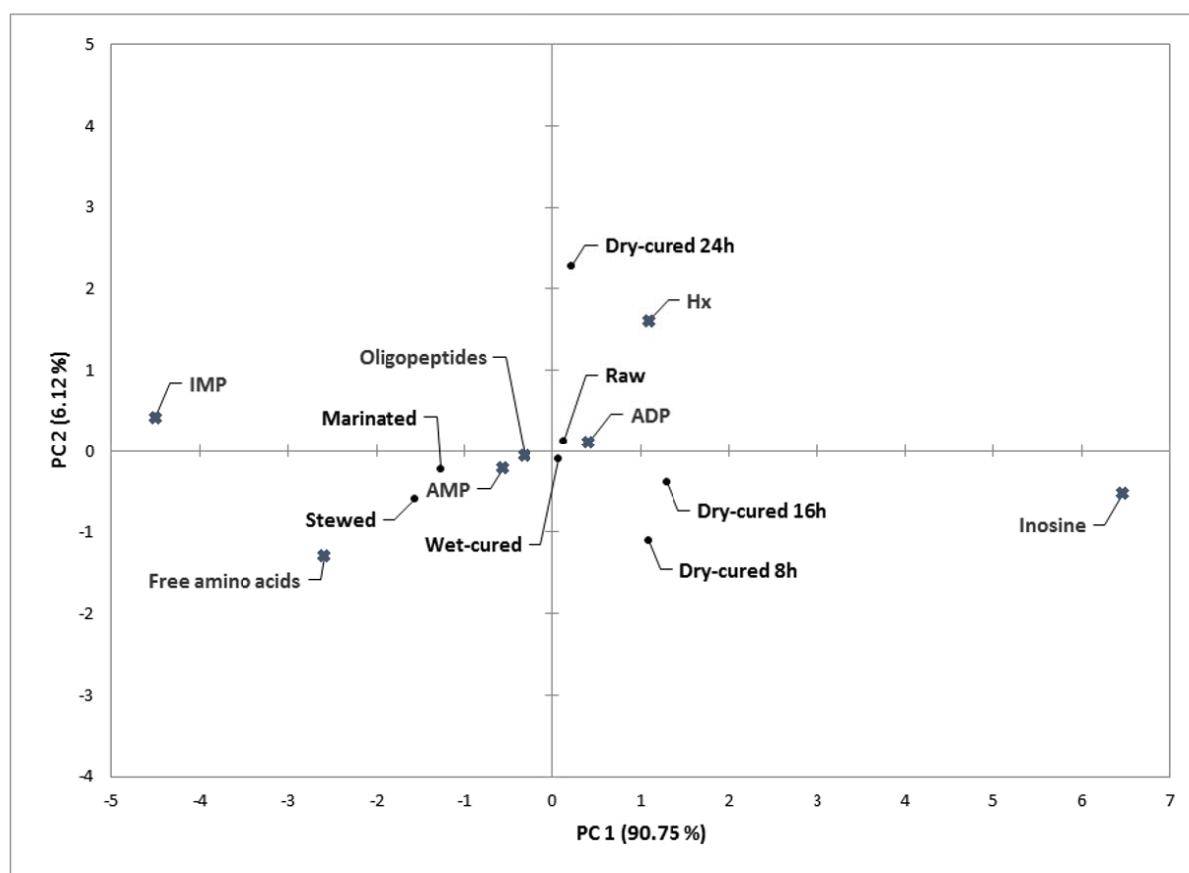


Fig. 2 Principal component (PC) analysis for oligopeptides, free amino acids, nucleotides and nucleotides degradation products (%) (*) of the seven duck breast meat products at different stages (■).

percentage of IMP, free amino acids, oligopeptides in the final product, we can speculate that umami related taste chemical compounds are the dominant taste profile in the final product.

4. Conclusions

Dry-curing is an important stage in meat processing, not only it produces a pool of umami taste related compounds (e.g.: free amino acids, oligopeptides, IMP), it also stimulates the enzyme activities in the muscle. In the stewing stage, the umami taste profile of duck breast meat was gradually established: the percentage of oligopeptides, free amino acids and IMP increased 0.66%, 4.42% and 11.37%, respectively, while the significant decreases were found in hypoxanthine (14.96%) and inosine (6.34%). Consequently, we can conclude that the dry-curing and stewing are the two crucial stages to form the

umami taste profile in duck breast meat.

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