Change of Peptides and Free -Amino Acids Contents during Nanjing Dry-Cured Duck Processing

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***Abstract*—** *In order to explore the relationship between the change of peptides and free-amino acid (FAA) and its unique flavour, Dry-cured duck samples of different processing phases were used to study the change of free-amino acid by High Performance Liquid Chromatography (HPLC) in this paper, meanwhile the trichloroacetic acid precipitation method for modeling use to establish the quantitative predicated peptides. The changes of small peptides and free amino acids in the process were studied. The results showed that the level and amount of proteolysis increased with the processing time at traditional technology, meanwhile the amount of peptides were positively correlated with FAA contents (R^2=0.86).*

***Keywords*— dry-cured duck, Free-amino acids, proteolysis, Peptides.**

I. INTRODUCTION

Special flavor of cured meat product depended on special processing technology of different countries and regions, Monica Flores [1] and other scientists studied the flavor and sensory descriptions of correlation of Serrano ham of amino acids, peptides etc by HPLC and capillary electrophoresis and sensory analysis method. the flavor material with proportional combination could produce special dry pickled flavor characteristics. Ya-jun zhang etc[2] studied the relationship between protein degradation products and ham quality in jinhua ham. The analysis showed that the essential amino acid composition of proteins in jinhua ham made were more consistent with recommendation model and produced a large number of low molecular weight proteins and peptides after processing. The Nanjing dry-cured duck was a kind of traditional Chinese meat product, which was popular with consumers. However, the study on the mechanism of protein degradation in the processing of protein has not been reported.

Based on the different stages of processing in Nanjing dry-cured duck as the research object, the change of small peptides and free amino acid were determined during product process by HPLC, which provided theoretical basis for the modern control technology for Nanjing dry-cured duck.

II. MATERIAL AND METHOD

2.1 Experimental Materials

The thigh muscle sample during seven control critical points of manufacturing process (raw material, dry salting, wet salted, fold, dried 5 d, 10 d dry, dry 15 d) were removed as test materials, three animals were selected from every process control point respectively, transferred to lab and stored at -80^{o}C for analysis. Samples provided by the Nanjing Rurun Group Jin Furun Food Co., LTD.

2.2 Reagents and Instruments

2.2.1 Reagents

acetonitrile, trifluoroacetic acid, pure chromatographic; 2 mercaptoethanol, sodium dodecyl sulfate, Tris, disodium hydrogen phosphate, sodium dihydrogen phosphate etc, analytical pure;acrylamide (Shanghai colorful), methylene double acrylamide (Promega), TEMED (Promega), ammonium persulfate (Shanghai colorful), the molecular weight protein Marker (Promega).

2.2.2 Experimental Instruments

High speed dispersion (ultra-turrax T25), high-speed centrifuge (Beckman allegra 64R), 10kDa ultrafiltration membrane, high performance liquid chromatograph (Agilent1100), electrophoresis (602S stabilized current meter)

2.3 Test Methods

2.3.1 Study on small peptide of protein in duck：

3g duck thigh muscles were accurately weighed, added 60ml0.2mol/L phosphate buffer solution (pH6.5), homogenized with high-speed disperser (6000rpm) 3min, then high-speed centrifugation (10,000×g, 4^{o}C) 20min. the supernatant 1ml took out,

added 2.5ml acetonitrile, centrifuge (15,000×g, 4°C, 20min), 2.5ml supernatant took out, vacuum drying to remove water and organic solvents, with 40μl of eluent A (0.05% Fluoroacetic acid). 20μl of sample took into the C18 column (inner diameter 4.5mm, length 250mm) for HPLC determination. Eluent A (0.05% trifluoroacetic acid), eluent B (acetonitrile: water: trifluoroacetic acid = 60: 40: 0.04) [3,4,5].Elution process: first with eluent A plus 1% eluent B washed 5min respectively, and then gradient elution, eluent B from 1% gradually was increased to 100%, elution 25min, eluent speed control with 0.9ml/min, detection wavelength of 214 nm.

2.3.2 Determination of Free amino acid (FAA)

With reference to Ventanas[4] and Cordoba[6] and other methods, some changes have been made. The specific method was as follows: after the natural thawing of the sample, the visible fat and fascia were removed , minced, about 5g (accurate to 0.001g) weighed ,deionized water 20ml added, in the ice bath with ULTRA TURRAX (IKAT18basic, German) (22,000 rpm, each 10s, interval 10s), then 10% of the sulfosalicylic acid 20ml mixed evenly, at 4℃or 17h, the medium speed filter paper, the filtrate adjusted first with 4mol/LKOH to the pH Value 6.0, and then with deionized water to 50ml, 10kDa ultrafiltration membrane ultrafiltration to remove macromolecules. The ultrafiltrate was derivatized with AccQ Fluor Reagent Kit (P/N WAT052880) and the free amino acid content in the sample was determined by HPLC.The main technical parameters of HPLC: AccQ·Tag column (Nava-PakC18, 3.9×150mm, F0.4μm), column temperature 37°C; injection volume 10μl; Waters515 double pump gradient elution, eluent A AccQ Tag Liquid A was diluted 11 times with ultrapure water. The eluent B was 60%. The elution rate was 1.0 ml·min^{-1}, the elution time was 50 min, and the Waters 2487 UV detector was used.

2.4 Statistical Analysis

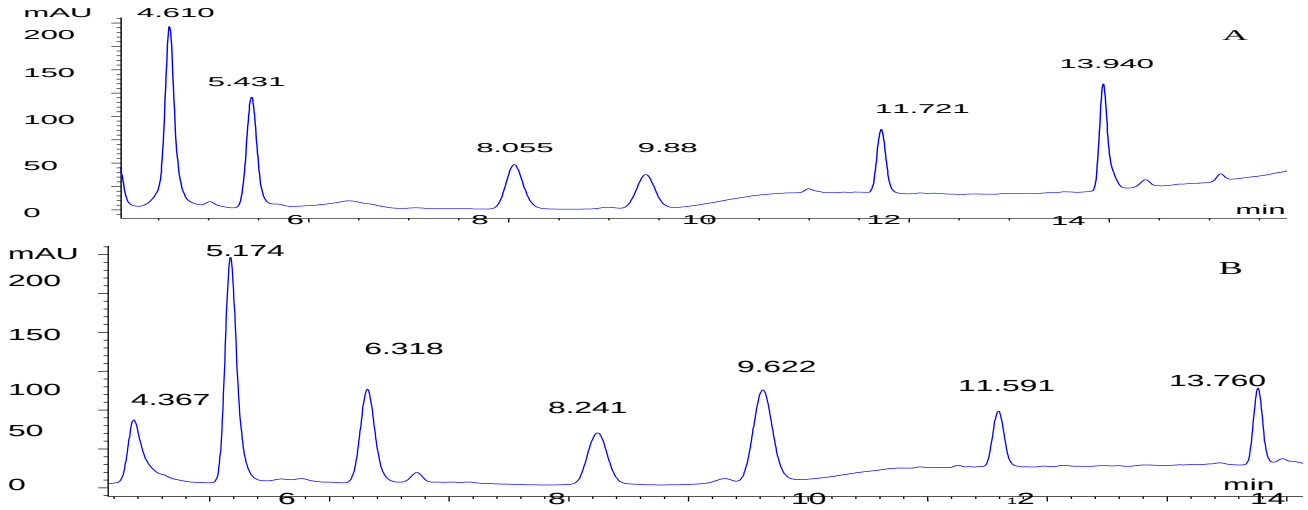
Using SASO.2 ANOVA analysis of variance, and with Duncan's multiple comparison, the difference was significant($p \leq 0.05$).

III. RESULTS AND DISCUSSION

3.1 Changes of Protein Peptides in Duck Muscle

It can be seen from Figure 1, in the processing stage of the duck, from the raw material to dry 5d retention time was short, the content of the polarity material in the dry picking stage first declined, and then increased and stabilized. In the dry stage of 10d, a new peptide peak appeared at 7.002, 12.632 and 17.429min, and the peptide peak were decreased at 6.330 and 9.664min. This may be related to the fact that "high levels of small peptides imparted flavor to the product and low levels of peptides made the product sweet" [7,8].

The main peak area of various major peptides in thigh meat was shown in Table 1. RP-HPLC analysis of small peptides showed that a total of ix significant peptides elution peaks were obtained in the ducks and the peak area of each elution peak was calculated by integral method. The number of elution peaks were consistent with that of Eugenio Rodriguez-Nune[9], and the first six peaks were concentrated in the first 14 min, indicating that the polarity of the polypeptide in the duck was mostly strong. ($P < 0.05$), which was probably due to the fact that the peptides were dissolved in the pickled liquid during the pickling stage ($P < 0.05$), and the total area of the peak increased significantly ($P < 0.05$), followed by dehydration mature process, endogenous enzyme decomposition of the protein as a short peptide



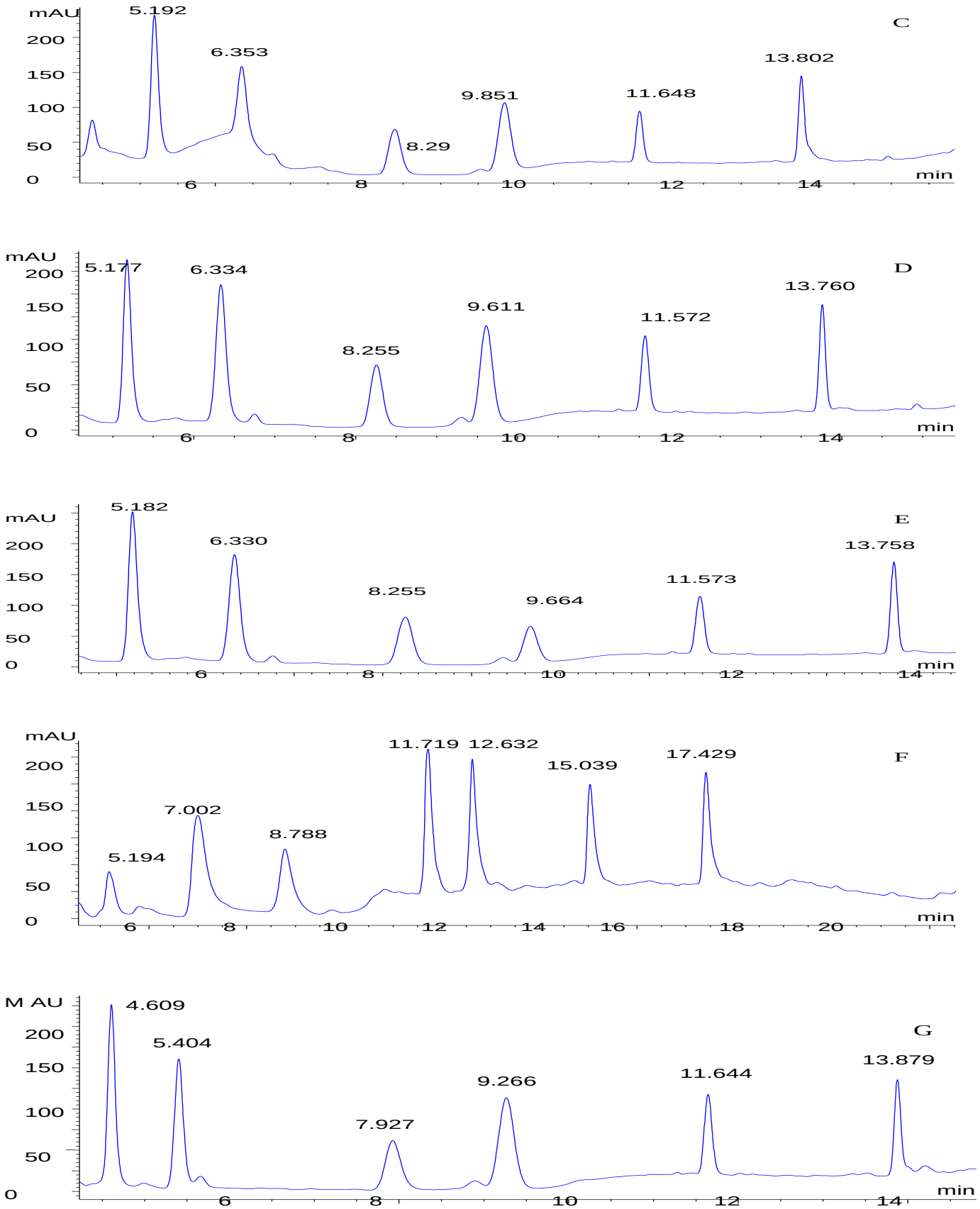


FIGURE 1: HPLC PATTERNS OF PEPTIDES IN LEG EXTRACT WITH DIFFERENT PROCESSING PERIOD

A-material ; B-cured ; C-salted ; D-overlaped ; E-drying 5d ; F-drying 10d ; G-drying 15d

TABLE 1
THE RESULT OF PEPTIDES OF HPLC IN LEG DURING PROCESSING PERIOD OF DRY-CURED DUCK [UNITS: AREA（ mAU*s）

Retain time(min)	raw material	cured	salted	overlaped	drying 5d	drying 10d	drying 15d
4.90	1284.80 ±187.71^{c}	1828.93±370 .84^{a}	406.50±15 9.50^{d}	1202.33±11 3.82^{c}	1659.17±1 85.73^{ab}	453.30 ±23.21^{d}	1332.47 ±145.04^{bc}
6.02	825.40±68.4 1^{e}	957.60±134. 86de	1436.17±9. 41^{ab}	117.77 306.39^{bcd}	1364.20±3 39.16^{abc}	1649.47± 69.36^{a}	1071.30±34. 65^{cde}
8.29	531.50±37.4 3^{c}	694.131±41. 22^{b}	739.33 ±1.55^{b}	669.37 ±72.39^{b}	878.90 ±35.21^{a}	900.83 ±24.83^{a}	783.93± 86.94^{a}
9.88	435.40±27.1 5^{c}	1152.13±295 .37^{a}	1099.53±2 3.99^{ab}	956.50 ±354.55^{ab}	758.40 ±187.79^{bc}	1217.70± 22.65^{a}	1268.57±20 4.34^{a}
11.77	403.77±67.0 4^{d}	443.90±68.6 8^{cd}	477.70±10. 16^{cd}	513.37 ±90.50^{c}	670.46 ±34.48^{b}	1056.73± 18.77^{b}	636.23± 13.10^{a}
13.99	676.13±33.0 2^{bc}	516.77± 13.70^{d}	813.30± 26.46^{a}	560.40 ±117.73^{cd}	772.27 ±31.58^{ab}	818.37± 19.37^{a}	742.13±128. 84^{ab}
total	4156.98±398 .54^{b}	5593.44±101 8.60^{a}	4972.56±1 79.12^{ab}	5077.02±10 54.88^{ab}	6103.38± 372.39^{a}	6102.42± 113.05^{a}	5934.64± 164.75^{a}

Note: Area are expressed as mean ± S.D. of triplicates; means within the same line with common superscripts are not significantly different (P>0.05).

3.2 Changes of Free Amino Acids in Duck Muscle

The determination of the major free amino acids in thigh meat was shown in table 2. In the processing of duck, in addition to arginine, threonine, cysteine little change, the other kinds of free amino acid concentration with the processing have increased to varying degrees. Compared with the pre-pickled, most of the free amino acids in the duck were increased by 1-2 times, among which the glutamic acid increased most, more than 2 times, followed by aspartic acid, serine acid and isoleucine. At the end of the processing, the higher content of free amino acids was glutamic acid, arginine, histidine and threonine. From the point of view the changes in content, aspartic acid, proline, tyrosine, valine, lysine, isoleucine and leucine were consistent, at the beginning of pickled stage were little changed, and later each stage of continuous rise($P < 0.05$). From the total amount of change, the free amino acid from the beginning of the raw materials to dry 5d continuous increase, and then a slight decline, which might be the amount of decomposition was greater than the amount produced by hydrolysis, decomposition into flavor small molecular components such as aldehydes, Alcohol compounds [10].

TABLE 2
MAIN FAA CONTENT IN LEG DURING PROCESSING PERIOD OF DRY-CURED DUCK (unit：mg/100g)

Items	material	cured	salted	drying 5d	drying 10d	drying 15d
Asp	12.25±0.81bc	12.07±1.31bc	10.16±2.96c	16.37±1.15ab	17.61±1.42ab	19.92±6.73a
Ser	13.77±1.37b	24.84±2.03a	21.55±2.68a	24.59±3.74a	27.29±1.56a	22.70±7.25a
Glu	19.91±0.59b	38.79±12.22a	36.73±3.16a	46.19±1.55a	50.21±5.90a	43.6397±15.34a
Gly	8.13±1.14c	15.02±2.09ab	11.66±1.00bc	16.47±0.67a	15.94±1.85ab	15.72±4.84ab
His	18.72±2.00c	32.43±3.79b	39.67±10.17ab	16.47±0.67a	42.16±1.39ab	35.02±8.26ab
Arg	77.57±24.24c	203.93±64.31a	189.35±43.07ab	230.64±27.22a	151.72±18.72abc	119.55±34.51bc
Thr	20.96±2.97b	47.24±16.86a	48.55±6.08a	53.35±4.74a	57.28±9.24a	38.86±14.67ab
Ala	14.58±1.85b	30.73±11.62a	29.64±6.04a	35.01±2.85a	34.95±4.16a	27.85±9.62a
Pro	10.74±0.73c	11.66±4.89c	13.54±1.12bc	16.88±0.97abc	20.14±1.99ab	21.52±6.73a
Cys	5.60±1.38a	1.36±0.73b	0.96±0.24b	0.12±0.50b	0.20±0.41b	8.81±5.00a
Tyr	12.32±1.66b	11.05±3.22b	10.99±2.84b	13.77±1.72ab	13.65±1.81ab	18.24±5.37a
Val	10.46±0.78c	11.21±2.94bc	10.74±2.45c	16.75±2.03ab	16.79±1.94ab	18.24±5.99a
Met	10.09±0.34b	6.46±1.74c	6.65±1.51c	9.00±1.25bc	8.40±1.04bc	13.07±2.61a
Lys	14.76±1.05b	19.93±5.16ab	19.15±3.63ab	28.99±2.90a	29.87±4.67a	29.87±11.98a
Ile	10.06±0.55bc	7.61±1.94bc	7.08±1.61c	11.54±1.47b	11.41±1.44b	15.61±4.40a
Leu	11.80±0.81b	15.83±4.09ab	16.31±3.13ab	22.78±3.45a	21.64±2.36a	21.86±7.03a
Phe	10.73±0.59b	8.37±2.47b	8.75±1.72b	12.55±2.44b	11.83±1.48b	17.18±4.63a
total	282.51±38.62b	468.56±134.02a	481.38±86.62a	602.09±54.92a	545.90±66.01a	487.72±154.41a

Note : FAA are expressed as mean ± S.D. of triplicates; means within the same line with common superscripts are not significantly different (P>0.05).

3.3 The Correlation between Small Peptides and Amino Acids

Using the correlation analysis of SAS8.2, the correlation equation y=1.0879x + 384.43 (R^2 = 0.86) which was obtained for the small peptide and amino acid.

IV. DISCUSSION

The processing time of the ducks was shorter (about 21 days) and the salt content of the products was higher (about 10%), which could be neglected the role of microorganisms in the formation of small peptides and free amino acids. In contrast muscle endogenous protease had strong ability to decompose muscle protein, among which cathepsin B, H, L, D and other decomposition was strong. In the process of processing, the muscle protein was first acted upon by cathepsin/calcein, decomposed into short peptides, and then decomposed by aminopeptidase to produce free amino acids. Capillary electrophoresis showed that a large number of peptides were produced by peptidase during the dry maturation of ham, especially in I and III dangks, some of which were associated with special tastes [11]. The peptide peptides obtained by RP-HPLC showed that the increase in peak area could be noted in the accumulation of free amino acids during maturation.

FAA in the cured products in the taste characteristics has been reported. Ventanas [12] studied the degradation of muscle protein in Iberian ham processing and found that muscle protein degraded in different degrees during processing, and the content of peptide, nonprotein nitrogen and free amino acid increased gradually. Zhang Yajun et al [2] studied Jinhua ham in the aspartic acid, glutamic acid on the role of flavor, phenylalanine, methionine, isoleucine, valine, histidine and other effects on bitter taste, serine, Glycine, arginine, proline and other effects on the sweet. In the processing of flakes and ducks, the FAA with flavor had a specific proportion combination and high NaCl content, which was the key factor to produce flavor characteristics.

V. CONCLUSION

During the different stages of processing in nanjing dry-cured duck, the content of small peptides showed a significant increase ($P < 0.05$) at the beginning stage, then decreased in the dry pickled stage and finally increased and stabilized. By using HPLC analysis of the free amino acids, in addition to arginine, threonine, cysteine little be changed, the other kind of free amino acids concentration with the processing time had different degrees of increase, which was a flavor of glutamic amino acid could be risen highest. From the total amount of change, the free amino acid from the beginning of the raw materials to dry 5d could be continuously increased, and then could be slightly declined.

ACKNOWLEDGMENT

This work is supported by Fund Project: Institutional collaboration research (NSFPT1415).

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