Analysis of White Particles in Xuanwei Ham

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Abstract: Xuanwei Ham is a famous ham in China with some white particle spots often appeared in ham, mostly in ham muscle. However, examined by microbiological methods and observed by light microscope, there are neither microbes nor parasites in the white particle spots. Proteins in particles would decompose significantly, and two main proteins with molecular weights of respectively 51000 and 13200 were detected by gradient SDS-PAGE in particles. The content of tyrosine in white particles was eight times higher than that in normal ham muscle, which indicated that the particles might be decomposed protein, tyrosine and some other component. All the contents of inorganic elements analyzed are less than those in ham muscle, and the schondroitin sulfate occurred in white particle implie that pathological disease might occur in muscle at the particle position during live stage of pig. Inoculating yeast on ham during salting can prevent the formation of particles effectively.

Key words: Xuanwei Ham; white particles; tyrosine; component

宣威火腿白点的分析

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摘 要：宣威火腿是中国三大著名火腿之一，常常有白点在火腿中出现，影响产品外观。通过微生物学方法和

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Jiaotou fermented CSB had higher sensory scores in interior structure and cohesiveness, but had lower sensory scores in odor and stickiness. The general sensory scores of two groups were not significantly different. The TPA results indicated that Jiaotou fermented CSB was significantly superior to Furong™ CSB in hardness, gumminess, chewiness, cohesiveness and adhesiveness ($p<0.05$) respectively, but only significantly better to Dangju™ CBS in cohesiveness ($p<0.05$). In general, Jiaotou fermented CSB had better eating quality indicated by parameters of TPA, while its odor preference varied.

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显微镜观察，发现这些白点既不是微生物，也不是寄生虫。白点中的蛋白质降解程度很高，经梯度SDS-PAGE凝胶分析，发现白点含有两种主要蛋白质，其分子量为51000和13200。在白点中的酪氨酸含量是正常火腿肌肉的8倍。表明白点可能是蛋白质降解产物、酪氨酸结晶和一些其它成分。所有无机成分的含量经分析都少于火腿肌肉。在白点中还有硫酸软骨素出现，表明在猪的活体阶段在白点部位的蛋白质发生了某种病理性变化。在火腿腌制阶段火腿上接种酵母能有效的减少火腿白点的形成。

关键词：宣威火腿；白点；酪氨酸；成分

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Xuanwei Ham is one of three famous hams in China, and is produced in Xuanwei district of Yunnan province in China with more than a thousand years history. There are more than 10000 tons per year. White particles often appear in some ham in the lean meat portion occasionally in fat. Their shapes are round, spindle, rod, or irregular. The diameter is in 0.5mm to 5mm, the color is milky or chalky, and the character is fragile (Fig. 1). The particles usually cause unfavorable consumer concerns and are thought to be the ham quality problem, and even thought harmful to health.

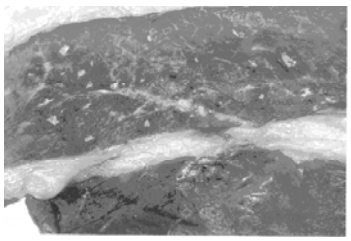


Fig.1 White particle in ham muscle

The formations of white film developed on the cut surface or the surface of vacuum-packed dry cured ham slices were found by Toldrá F, et al[1]. wheresa, They demonstrated that the film was composed of protein materials. Bañón S, et al[2] thought the presence of white precipitates was an alteration in dry-cured ham which was closely related to the changes in its proteins. The precipitates might appear in crystalline form, and the rupture of tissue membranes during freezing might favor the nucleation and the growth of tyrosine crystals. Denaturized proteins are especially sensitive to the attack of proteolysis enzymes released following the rupture of cellular structures by ice crystals. The release of tyrosine during the curing process has an enzymatic origin[3-4], and depends on the cathepsin and calpain activity. Tyrosine is less soluble in water than other amino acids found in meat, and has a special tendency to form precipitates when the moisture content of the ham falls.

White particles in Xuanwei Hams someimes seem to be different from the above reports. The development mechanism and the composition of white particles in Xuanwei Hams, however, had not yet been reported.

1 Materials and Methods

1.1 Procedure of ham process

Usually, the pigs breed called Wujin bred in Xuanwei district are slaughtered on Spring Festival in February annually, and then the legs are cut off from the carcass and cooled for about 10 hours. The meat temperature drops to 5～6℃, with the leg weight about 8～12kg and trimmed neatly. Brush salt on ham for the first time, and squeeze the blood liquid from vessel thoroughly by hand. The salt used is 2kg/100kg ham. After 2 days, put salt on ham for the second time and squeeze the liquid further, with the quantity of salt as 4～5 kg/100kg ham. After 2 days, salt is put on ham for the third time, with the quantity of salt as 1～2kg salt/100kg ham and this step is only supplementary to some ham if the ham have not had enough salt on it afer being judged objectively. Then pile up the hams and drip the liquid by gravity naturally. After hanging the hams in rows regularly half month, ventilate the storage room at sun days and shut up windows at rain days. The ham will mature primarily at Duanwu Festival usually in June and mature thoroughly at Medium Autumn Festival usually in September.

1.2 Sampling white particles

Xuanwei Hams cured about one year were obtained from Xuanwei Food Company and Xuanwei Ham Cannery (Yunnan, China). Surfaces of ham were cleaned by cotton swabs dipped with alcohol (75%) before cutting. White particles on the cut surface were picked out aseptically with a pincette free from ham muscle and placed in aseptic beakers for study. Ham muscle without white particles from the same ham was also taken and stored at 4℃ as control.

1.3 Microbiological study

10 g of white substance was homogenized with phosphate buffer solution (PBS, pH7.0), after serial dilution with PBS. The gradient solution was spread on nutrient agar (Difco) plate or potato dextrose agar (Difco) plate, and incubated at 35℃ or 25℃ for a period of time to cultivate bacteria or fungi

under aerobic and anaerobic condition, respectively.

Particles and ham muscle were observed by a light microscope (×40～400) for parasites detection and structure inspection.

1.4 Chemical assays

Conventional method[5] was employed to measure moisture and ash, total nitrogen and protein were measured following the Kjeldahl method[6], lipid was analyzed by Soxhlet method[5].

Elements of Fe, Ca, Zn, S, Mn, Na, Cu, P, K and Mg were determined by ICP-AES[7]. Cl-was determined according to the publication of Zhongshan University[8].

Chondroitin was determined according to Dische Z, et al[9].

For analysis of amino acids, sample hydrolysates were prepared by putting 6 mg (dry weight) of white particles and ham muscle(dry weight) in an ampoule containing 2 ml 6N HCl spearately. The sealed ampoules were kept at 100℃ for 18h. After cooling, the hydrolysates were filtered with Whatman No 1 filter paper. The quantities of amino acids in the filtrates were analyzed with an amino acid analyzer (Hitachi 835-50, Tokyo, Japan), programmed from 34 to 53℃ within 68 min, using a lithium high performance column. All analyses were carried out in duplicate.

1.5 Electrophoresis analysis

White grain of ham muscle were mixed with a portion of solution containing 0.1mol/L KI and 0.1mol/L sodium phosphate buffer (pH7.0) respectively. Each mixture was homogenized and then centrifuged (1800r/min). The supernatants were prepared for electrophoresis. A portion of supernatant was mixed with the same volume of 0.125 Tris-HCl (pH6.8) containing 4% (W/V) sodium dodecyl sulfate (SDS), 10% glycerol, and 0.01% bromophenol blue. The mixture was boiled for 2min. A portion of boiled mixture was loaded on a 5%～20% linear polyacrylamide gradient gel, according to Walker[10] method, under a constant voltage of 100V. Bovine albumin (molecular weight 66000), egg albumin (45000), glyceraldehydes-3-phosphate dehydrogenase (36000), trypsinogen (24000), trypsin inhibitor (20100), and α-lactalbumin (14200) were used respectively as standard markers and were purchased from Sigma Chemical Co.Ltd. The gel was fixed, stained, and destained as described by Neuhoff, et al[11].

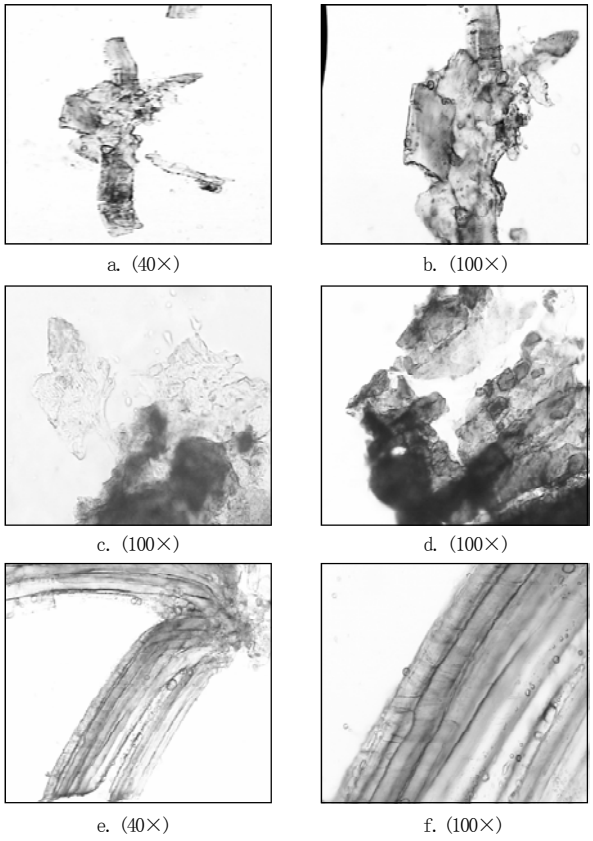
1.6 Inoculate yeast on ham

Yeast strain was cultured in potato-dextrose culture containing 10% NaCl, and then mixed with salt for inoculation on 600(10.5Ton) hams at salting time. The inoculation dose of yeast culture(>2×10^{10}/ml) which was cultured at shake flask (175r/min, 25℃)7days was 0.1% of ham, while the control group consisting of 600 hams was processed according to traditional method, and then the other procedure was processed according to traditional procedure.

2 Results and Analysis

2.1 Microscopic observation and isolation of microbes

We have not found any bacteria or fungi from the culture plates spreading with the homogenates of white particles after incubation.



a. (40×) b. (100×) c. (100×) d. (100×) e. (40×) f. (100×)

Fig.2 Microscopic observation

By taking different shapes of white particles from ham muscle on a glass plate covered with cover plate and observing by light microscope, we found that the particle consisted of broken fragment of myofibril and with irregular substance of fragile character. The particles were neither parasites nor the parasite's eggs. Contrast to normal ham muscle kept integrated (Fig.2 e, f), the myofibril in particle broke into small pieces intensely (Fig.2 a, b, c, d). That demonstrated that the muscle at the particle might undergo severe decomposing process.

2.2 Chemical composition of the white particles

The contents of amino acids in white particles and

Table 2 Comparison of ash and some inorganic elements between white deposit and ham muscle (dry weight, g/g)

	Ash (%)	Ca	Fe	Zn	Mg	S	P	Na	Cl^-
White deposit	8.9	29.57	26.01	8.43	151	948	1180	14400	21900
Ham muscle	15.9	71.02	32.17	45.16	265	2600	3410	37400	53700

Note: K, Mn, Cu could not be detected.

normal ham muscle of Xuanwei Ham were shown in table 1. From table 1, we could find that tyrosine accounts for 17.32% of the total weight of the particle, while the tyrosine in particle is 8 times higher than that in ham muscle. Because tyrosine solubility is low in water, we might think that the crystal of tyrosine forms the particle in that part. This result was somewhat different from the Banón S, et al[2], where they reported that tyrosine accounted for 70.54% of the precipitate, but in our report, tyrosine accounted for only 17.32% of the particle.

Table 1 Free amino acid composition in the white deposit

	Deposit	Ham muscle
Asparticacid	2.11	3.67
Threonine	1.18	2.09
Serine	0.97	1.64
Glutamic acid	3.64	2.07
Glycine	1.17	1.77
Alanine	1.46	2.18
Valine	1.67	2.46
Isoleucine	1.34	2.12
Leucine	1.53	3.71
Tyrosine	17.32	1.86
Phenylalanine	3.23	2.48
Lysine	1.40	2.18
Arginine	1.11	3.20
Histidine	0.68	1.77
Methionine	1.13	0.99
Proline	traces	1.07
Tryptophane	traces	0.68
Cystine	ND*	0.67
NH_3	0.29	0.22
Total	40.23	41.92

Note: *Not detected.

From table 2, we could see that all the inorganic elements determined in particle are lower than those in normal ham muscle. That might be caused due to the reason that the crystal of tyrosine decrease the inorganic components such as NaCl quantities in white particle.

Table 3 Comparison of some component between ham muscle and white deposit (fresh weight%)

	Amino acids	Protein	Fat	Chondroitinsulfate	Water
White deposit	12.9	24.2	9.11	2.7	8.6
Ham muscle	9.9	30.0	10.66	0	29.6

From table 3, we could find that the chondroitin sulfate appeared in white particle mainly existed in cartilage and did not appear in muscle. This might imply that the pathological disease might occur in live muscle. Water content in white particle is severely decreased further. This might imply that the white particle is crystal of some substance, which is insoluble in water.

2.3 Protein in white particles

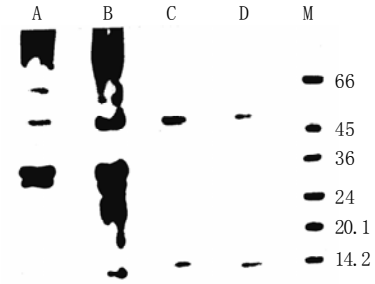


Fig.3 Proteins in ham muscle and white particle

From the appearance in a gradient SDS-PAGE gel of white grain, there were ham muscle homogenates (Fig. 3), varieties of proteins present in ham muscle (lane A and B), while only two main proteins (molecular weight 51000 and 13200) were observed in the white particles (lane C and D). These two proteins were also detected in normal ham muscle. This result was different from Toldrá, et al[1] in that the main proteins were 37000, 41000, 56000, and 65000 in molecular weight respectively in white film developed on cut surface of vacuum- packed dry-cured ham slices. That less protein variety was detected in white particles of Xuanwei Ham demonstrated that the protein decomposed intensely in white particleposition.

This result might be consistent with García-Garrido's J A, view that the formation of white spots was due to tyrosine particles once marked in hams of defective texture[12], where an increased proteolytic activity led to increasing level of free amino acids such as tyrosine. Intense massage in Xuanwei Ham process might increase the destruction of myofibrils, and the destructive myofibril was more sensitive to proteinases in meat like cathpsin and calpain.

2.4 Inoculating yeast on ham could prevent the appearance of white particle

In our other experiments, we found that yeast is benefi-

cial to ham quality. No white particle occurred in any of hams inoculated with 0.1% yeast culture that had been identified as *Hansenula xuanweiensis* Jiang sp. nov[13] at salting time with particles appearing about only 5% of the ham in control group. But the reason why inoculating yeast could prevent the formation of particle in ham muscle was yet unknown. We speculated that the released tyrosine might be absorbed by vast yeast in multiplication and growth with the result of decreasing tyrosine thoroughly and finding no tyrosine to crystal. The tyrosine in inoculation ham keeps unchanged in comparison with the non- inoculated ham muscle.

Table 4 Comparison of amino acids contents between inoculated and traditional ham (% dry weight)

Amino acids	Inoculated	Traditional	Increase
Total quantity	59.46	54.71	8
Cystine	0.80	0.89	−10.11
Tryptophane	0.74	0.64	15.6
Proline	2.12	1.89	12.2
Arginine	3.20	3.41	−6.16
Histidine	2.14	1.92	11.5
Lysine	5.26	4.70	11.9
Phenylalanine	3.11	2.76	12.7
Tyrosine	1.49	1.51	−1.32
Leucine	4.86	4.51	7.8
Isoleucine	3.03	2.76	9.8
Methionine	1.18	1.30	−9.23
Valine	4.16	3.11	33.76
Alanine	3.90	3.21	21.50
Glycine	2.66	2.54	4.70
Glutamate	11.14	9.98	11.6
Serine	2.05	1.99	3.0
Threonine	2.60	2.40	8.30
Aspartate	5.02	5.19	−3.28

3 Conclusion

In conclusion, the particles in Xuanwei Ham were not microbes, parasites, but the particles of tyrosine, developed from the denatured protein in ham muscle as metaprotein and some other components. The metaprotein might be formed during the pig's live stage, and the intense massage procedure intensified the destructed protein in ham muscle. All the metaprotein was easily decomposed by proteinase, so, the crystallization of tyrosine formed at the metaprotein position. The particle should not do any harm to health. Inoculating yeast on ham at salting time could prevent the formation of particle in the ham. Inoculation yeast on ham at salting time could prevent the formation of white particle effectively.

But the white particles have some special characteristics such as component type and kinds of protein which were different from other reports. Different pig breed and process might cause this phenomenon.

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