Proteolysis of porcine muscle cured with *Monascus purpureus* as a starter culture

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Introduction

Two important actions of meat curing process were diffusing the curing agents into meat organism and ripening the meat product. Proteolysis is a major change during curing, due to the peptides and amino acids are formed by proteolysis and contribute to desirable flavor. Proteolytic action originate from use of starter culture and non microbial source (endogenous meat enzyme). Starter cultures are widely used to shorten the ripening period, ensure color development, enhance the flavor, replace or reduce the chemical preservant usage (Baccus, 1986).

Monascus purpureus was originally grow on rice, wheat and bran. The fungi producing red pigment used as food colorant, acid protease and amylase for foods fermentation. Hung-tsao meat is a famous Chinese food, which are made by monascus koji and pork. Some researches about *Monascus* applied on meat were studied. Yasuda et al. (1993, 1995) investigated the chemical and physical change of tofuyo, and found that soy bean protein was digested by monascus protease . Yasuda, et. al. (1991) showed the acid protease from *Genus Monascus* was active on human hemoglobin, milk casein and bovine serum. The optimum pH and temperature of the enzyme were 3.2 and 50 ℃, respectively. Tsai (1977) also reported that the optimum pH of monascus protease was 3.0-6.0. Although the fungi have been used for cure meat for hundreds years in Orient countries, no research has been devoted to the proteolysis on meat protein. The purpose of this study was to determine the proteolytic activity on porcine meat and test the change of nitrogen compounds during curing time.

Materials and Methods

preparation of samples

Aseptic l*ongissimus dorsi* muscle was ground through a plate having 10 mm hole, then added 1.3 % salt, 0.2 % polyphosphate, 4 % sugar and inoculated with 10 % of *Monascus* culture broth. Starter culture of *Monascus purpureus* was prepared from yeast extract broth contained 10 % glucose and 0.8 % yeast extract then incubated at 35 ℃ for 6-7 days. The control was added the same curing agents as above and 150 ppm of sodium nitrite except *Monascus* culture broth, then was stored at 25 or 4 ℃ for curing. Samples were collected at the 0, 2nd, 4th, 6th, 10th, and 14th day. Tyrosine concentration was used as an indicator for proteolysis of porcine meat in this experiment. All collected samples were stored at -18 ℃ for total nitrogen (TN), total soluble nitrogen (TSN), non protein nitrogen (NPN), and free amino acid nitrogen (FAAN)of meat protein during curing.

Analytical methods.

Proteolysis on porcine protein was presented by free tyrosine concentration/per gram of cured meat during curing. Hull's method (1947) was used to assay the tyrosine concentration. Determination of total nitrogen and the NPN were performed by Demasi's method (1990). Changes of myofibrillar and sacoplasmic protein were done by SDS-Polyacrylamide gel electrophoresis modified by Uytterhaegen *et al.* (1994).

Results and Discussion

The tyrosine concentration of cured meat was showed in Table 1. The tyrosine concentration of the control and *Monascus* treatment increased with curing time increasing. At the 6 day, The tyrosine concentration of the control and *Monascus* treatment at 25 ℃ were significantly higher ($P<0.05$) than those at 4 ℃. All samples produced off-flavor when curing at 25 ℃ for 4 day. After the 10th day during curing time, the rate of the tyrosine concentration of *Monascus* treatment significantly faster than the control when curing at 4 ℃. The TSN, NPN and FAAN of the control and the sample with *M. purpureus* increased slightly with curing time increasing. The Concentration of TSN and NPN of the control were higher than the sample with *M. purpureus* when cured at 25 ℃, but lower at 2-4 ℃. The FAAN of the sample with *M. purpureus* was higher than the control ($p<0.01$) at 25 and 2-4 ℃ curing period. A stronger intensity band below 29 Kd and a lighter intensity band of 97.4 Kd of sarcoplasmic fraction were existed on SDS-PAGE electrophoregram when pork cured at 25 ℃ for 4 day. No significant difference between the control and the sample with *M. pursuers* was observed in this research

Table 1. The tyrosine concentration (*umol* %)of pork with or without *M. purpureus* were cured at 2-4 or 25 ℃ during curing time

Curing time	25 ℃		2-4 ℃	
(Day)	Control	*Monascus* cured	Control	*Monascus* cured
0	80.5[a]	107.2[a]	80.5[a]	107.2[a]
2	147.2[a]	168.8[a]	127.1[a]	189.0[b]
4	175.6[a]	195.9[a]	151.0[a]	190.5[a]
6	251.0[a]	243.6[a]	197.0[b]	207.6[b]
10			198.2[a]	231.7[b]
14	—	—	205.3[a]	287.9[b]

[a-b] Values within the same row with different superscripts are significantly different ($P<0.05$)
—: Non detection

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