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Taste improvement of refrigerated meat treated with cold-adapted Protease

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Abstract

The cold-adapted protease produced by a deep-sea cold-adapted bacterium *Pseudoaltermonas* sp. SM9913 and the mesophilic protease produced by a mesophilic bacterium *Bacillus* sp. SM98011 were sprayed onto the surfaces of marine fish, pork and shrimp meat, respectively, and then stored at 0 °C for 6 days. The amounts of free amino acids in the hydrolysates of samples were determined. The results showed that the samples treated with cold-adapted protease released more free amino acids than those treated with mesophilic protease at 0 °C. The refrigerated meat samples treated with cold-adapted protease, released more taste amino acids and essential amino acids than those treated with mesophilic protease. Therefore, the cold-adapted protease had potential in improving the taste of refrigerated meat.

Keywords: Cold-adapted protease; Hydrolysate; Refrigerated meat; Free amino acid; Taste

1. Introduction

Among the various methods of meat and fish preservation, freezing is one of the most common techniques practised by man, due to the slowed rancidity during frozen storage. But after frozen storage, various meats showed increasing cooking loss (Jeremiah, 1980), decreasing protein extractability (Miller, Ackerman & Palumbo, 1980) and increasing drip loss (Sahagian & Goff, 1996). In order to maintain nutritional value and taste of the meat, frequently meat is stored at about 0 °C instead of at −20 °C after slaughtering; this is called refrigerated meat. Generally, refrigerated meat does not spoil at 0 °C for 6–7 days. Compared with frozen meat, refrigerated meat keeps its original colour and taste, and maintains nutritional value during the course of freezing and thawing.

It is known that important taste precursors are nucleotides, taste peptides, lipids, water-soluble proteins, sugar and especially free amino acids (Colombo, 1975). The main components of meat are insoluble proteins. When these proteins are partially hydrolysed during their refrigerated storage, free amino acids are released, and the taste increases remarkably. Common flavour enzymes are mesophilic proteases, which exhibit very low efficiency of proteolysis at 0 °C (Imm & Lee, 1999). Recently, work on psychrophilic microorganisms in polar regions, alps and glaciers, has been reported (Margesin, Palma, Knauseder, & Schinner, 1991). At low temperature, psychrophilic enzymes, produced by psychrophilic microorganisms, have higher catalytic efficiencies than the mesophilic enzymes produced by mesophilic counterparts, because of adaptation to low temperature. Many reports suggested that psychrophilic enzymes have high potential in many fields, such as the food industry, chemical industry and environmental protection (Margesin & Schinner, 1994). However, some reports here suggested that cold-adapted proteases could be used as of taste enzyme. In this paper, the effect of psychrophilic protease on improving the taste of refrigerated meat is studied and compared with that of mesophilic protease.

2. Materials and methods

2.1. Experimental strains

Psychrotrophic strain *Pseudoaltermonas* sp. SM9913 was isolated from sediment at 1855 m in the deep sea

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(Chen, Zhang, Wang, Gao, & Luan, 2001). Mesophilic strain *Bacillus* sp. SM98011 was preserved in our lab. Both of these can produce serine protease and their optimum catalytic pH is at 7.0.

2.2. Media

The LB medium (g/100 ml), which contained 1% peptone, 0.3% beef extract, was used to cultivate *Pseudoaltermonas* sp. SM9913. The medium for protease production (g/100 ml) contained 0.5% corn powder, 0.5% bean powder, 0.25% wheat bran, 0.1% $CaCl_2$, 0.4% Na_2HPO_4, and 0.03% KH_2PO_4. All above media were prepared with seawater. The medium for *Bacillus* sp. SM98011 growth and protease production was the same as that for *Pseudoaltermonas* sp. SM9913, except that the seawater was replaced with distilled water.

2.3. Proteases preparation

Pseudoaltermonas sp. SM9913 was cultivated at 12 °C, 200 rpm for 72 h. *Bacillus* sp. SM98011 was cultivated at 28 °C, 200 rpm for 36 h. The two cultures were centrifuged at 10,000 rpm for 20 min. The supernatants were collected and used as crude cold-adapted protease or mesophilic protease respectively.

2.4. Protease analysis

The activity of protease was determined by the digestion of casein. 1 ml diluted enzyme solution was mixed with 1 ml 2.0% casein in 50 mmol/l Tris–HCl (pH7.0) and incubated for 10 min at a given temperature. The reaction was stopped by the introduction of 2 ml of 0.4 mol/l trichloroacetic acid. Then the precipitate was removed by centrifugation (10,000 rpm). One millilitre of supernatant was neutralized with 5 ml 0.4 mol/l sodium carbonate and incubated with 1 ml of 1N Folin-Ciocalteu's reagent at 40 °C for 20 min. Then the absorbance at 660 nm was measured. One unit of activity was defined as the amount of enzyme that liberated 1 μg of tyrosine per ml of reaction mixture per min.

2.5. Samples preparation

The marine fish [*Ephippus orbis* (Bloch)], pork, and shrimp (*Penaeus chinensis*) meat were bought from the supermarket. The samples were stored at 0 °C before use. One hundred gram samples were put into plastic bags, separately, and 20 ml cold-adapted protease and mesophilic protease, which had been regulated to the same catalytic activity at optimum temperature, were sprayed onto the surface of the samples. The control was sprayed with the same amount of distilled water. Then, all treated samples were preserved at 0 °C. Six days later, the samples were immersed in 200 ml distilled water for 30min, and then, the solution was collected. 1 ml solution was mixed with 1 ml 4.0% sulfosalicylic acid, and precipitate was removed by centrifugation at 10,000 rpm for 15 min at 4 °C. The supernatant was used to analyse free amino acids.

2.6. Amino acid analysis

The free amino acids analysis was carried out in an automatic aminoanalysor, Hitachi 835.

3. Results and discussion

3.1. Comparison of characteristics of cold-adapted protease with mesophilic protease

Psychrophilic strain *Pseudoaltermonas* sp. SM9913 was isolated from the sediment in 1855-m, deep sea. Its optimum growth temperature was 12 °C and it could also grow normally at 0 °C. The optimum temperature of casein hydrolysis of the cold-adapted protease produced by *Pseudoaltermonas* sp. SM9913 was 35 °C (Chen et al., 2001). Mesophilic strain *Bacillus* sp. SM98011 was preserved in our laboratory. The optimum growth temperature was 30 °C. The optimum temperature of its mesophilic protease for casein hydrolysis was 50 °C (Fig. 1).

The protease activity at optimum catalytic temperature was defined as 100%. The relative activities of mesophilic protease and cold-adapted protease at 0 °C were 0.24 and 10.5% respectively (Fig. 2). The results indicated that the cold-adapted protease had a higher catalytic efficiency at lower temperature than the mesophilic protease.

3.2. The hydrolysis of refrigerated meat by Cold-adapted protease and mesophilic protease at 0 °C

The generation of free amino acids is very important for the taste of meat, because they directly contribute to

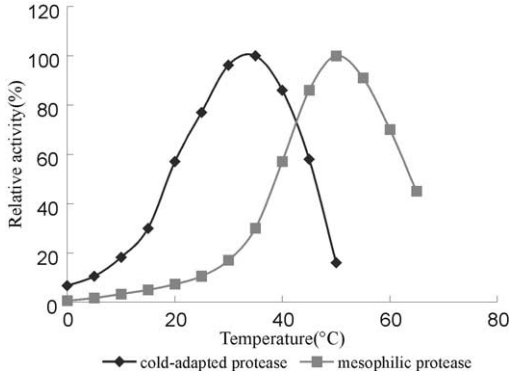

Fig. 1. Effects of temperature on the activity of cold-adapted protease from strain *Pseudoaltermonas* sp. SM 9913 and mesophilic protease from strain *Bacillus* sp. SM98011.

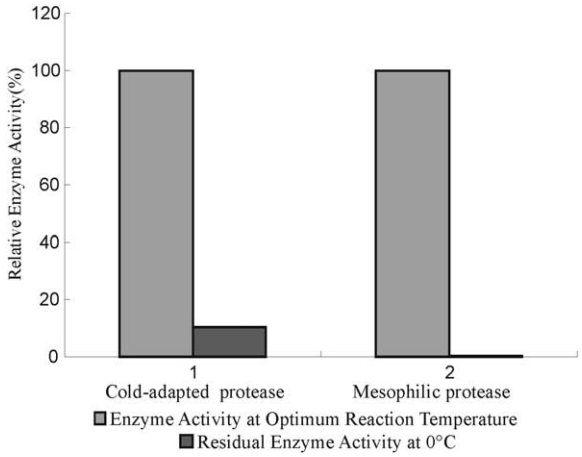

Fig. 2. Relative activities of cold-adapted protease and mesophilic protease at 0 °C.

the taste of meat (Mottram, 1994). The results in this paper show that the concentration of free amino acids in supernatant of the meat treated with cold-adapted protease increased more than the control (Table 1). The amounts of released free amino acids from marine fish, pork, and shrimp meat after the treatment with cold-adapted protease increased 1.18, 3.01, and 1.53 times respectively, than the control. As there were few free amino acids in cold-adapted protease solution, these free amino acids could be ignored. It was also observed that the marine fish, pork, and shrimp meat treated with cold-adapted protease released more free amino acids than those treated with mesophilic protease at 0 °C (Table 1). Before the treatment, both the cold-adapted protease and mesophilic protease solution had been regulated to the same catalytic activity at their optimum temperatures. After the cold-adapted protease treatment, the amount of released free amino acids from marine fish, pork and shrimp meat increased 23, 32 and 60% more than that with the mesophilic protease. This result indicated that the cold-adapted protease had higher proteolytic efficiency at low temperature than the mesophilic protease.

From Table 1, it was also found that few free amino acids were released in the control of pork, namely 0.097 g/100 g. Otherwise, relatively more free amino acids were released in the control of marine fish and shrimp meat, namely 0.299 g/100 g and 0.252 g/100 g, respectively. This was mainly due to the high activity of endogenous protease in aquatic products. This result indicated that the cold-adapted protease has greater potential for improving the taste of livestock and poultry meat.

3.3. The release of taste amino acids and essential amino acids in hydrolysate

Among the 20 amino acids composing proteins, various amino acids contribute to different tastes. Primary taste-active free amino acids responsible for umami and sweetness are Glu, Asp, Met, Ser, Thr, Gly, Ala and Pro, respectively (Fuke, 1994, chaptor 8). Glu is special for the delicious taste; the main component of monosodium glutamate is glutamine. Gly is sweet and, when it is heated, it generates formaldehyde. Ala and Cys will

Table 1
Comparison of the released amounts of free amino acids from refrigerated meat treated with cold-adapted protease and mesophilic protease at 0 °C for 6 days

Amino acid	Pork (%)			Marine fish (%)			Shrimp meat (%)		
	Control	Treated with cold-adapted protease	Treated with mesophilic protease	Control	Treated with cold-adapted protease	Treated with mesophilic protease	Control	Treated with cold-adapted protease	Treated with mesophilic protease
Asp	0.003	0.013	0.006	0.003	0.041	0.013	0.009	0.018	0.012
Thr	0.007	0.018	0.010	0.005	0.018	0.023	0.003	0.020	0.019
Ser	0.005	0.007	0.008	0.001	0.002	0.001	0.005	0.016	0.013
Glu	0.009	0.027	0.020	0.061	0.077	0.071	0.040	0.079	0.053
Gly	0.007	0.014	0.008	0.021	0.025	0.025	0.061	0.071	0.039
Ala	0.016	0.030	0.018	0.027	0.056	0.051	0.025	0.039	0.029
Cys	0.001	0.014	0.008	0.018	0.047	0.037	0.007	–	0.007
Val	0.007	0.028	0.017	0.024	0.045	0.040	0.011	0.034	0.023
Met	0.004	0.021	0.019	0.015	0.039	0.032	0.007	0.032	0.019
Ile	0.002	0.016	0.022	0.022	0.040	0.034	0.005	0.020	0.017
Leu	0.006	0.041	0.038	0.036	0.080	0.068	0.011	0.053	0.042
Tyr	0.002	0.014	0.023	0.002	0.024	0.012	0.006	0.040	0.027
Phe	0.007	0.045	0.043	0.022	0.045	0.048	0.009	0.059	0.032
Lys	0.006	0.027	0.022	0.028	0.066	0.042	0.019	0.045	0.032
His	0.004	0.013	0.011	0.002	0.015	0.016	0.007	0.014	0.009
Arg	0.003	0.031	0.014	0.001	–	0.004	0.022	0.083	0.020
NH_3	0.009	0.034	0.010	0.012	0.035	0.015	0.005	0.014	0.006
Summary	0.098	0.393	0.297	0.300	0.655	0.532	0.252	0.637	0.399

be degraded to produce the acetic aldehyde when heated. Heterocyclic compounds, especially those containing sulfur, are important taste compounds produced in the Maillard reaction, which provides savoury, meaty, roasted and boiled tastes. In addition when some amino acids combine with others, they will form special seafood or meat-like tastes (Mottram, 1998). After the refrigerated meat was hydrolysed by cold-adapted protease, the amount of released taste amino acids, which are responsible for umami and sweetness, increased markedly more than those from the control on treated with mesophilic protease (Table 2). After treatment with cold-adapted protease, the released Glu from pork increased 3.00 and 1.35 times more than that from control and that treated with mesophilic protease, respectively. After treatment with cold-adapted protease the released Asp from pork increased 4.30 and 2.17 times more than that from the control and that treated with mesophilic protease, and 13.0 and 3.15 times of Asp was released from marine fish than from the control and that treated with mesophilic protease, respectively. In addition, more free amino acids were released from the meat after the treatment with cold-adapted protease, when the meat treated with cold-adapted protease was cooked with the addition of glucose or sucrose; additional tastes are then produced through Marillard reactions (Mottram, 1998). Therefore, the refrigerated meat treated with cold-adapted protease had better taste than the control or that treated with mesophilic protease.

Essential amino acids are Thr, Val, Ile, leu, Met, Phe, Lys, His, Arg, which are not synthesized in the human bodies and animals, and need to be absorbed from the food. After cold-adapted protease hydrolysis, the concentration of essential amino acids in hydrolysate of meat markedly increased compared with both the control and that treated with mesophilic protease (Table 2). After proteolysis of the refrigerated pork with cold-adapted protease, the amount of essential amino acids increased 5.22 times than that in the control, and increased 1.23 times more than that treated with mesophilic protease. Therefore, the refrigerated meat treated with cold-adapted protease had better nutritional value than the control or that treated with mesophilic protease.

With improvement of living standards, the demand for meat is increasing, not only quantity but also freshness and taste. Scientists have previously adopted the frozen method to solve the problem of meat decay during storage. Many studies have proved that frozen storage was effective for avoiding rancidity, however, most meat shows a gradual deterioration in quality during frozen storage (Urbain, 1978). More recently, refrigeration at about 0 °C was proved to be a good method for short time meat storage (Lou et al., 2000). Compared with frozen meat, refrigerated meat maintains its the original colour and taste, and maintains nutritional value during freezing and thawing (Ngapo, Babare, Reynolds, & Mawson, 1999a). At the same time, minimized thawing times will reduce microbial growth, chemical deterioration and excessive water loss caused by dripping or dehydration. For this reason, increasingly more consumers are interested in refrigerated meat (Ngapo et al., 1999b).

On the basis of keeping fresh, how to improve the taste of the meat food is another key problem at present. Taste substances are mainly free amino acids, nucleotides and short peptides. These taste substances can be produced from protein hydrolysate (Aaslyng, Larsen, & Nielsen, 1999). Some enzymes that improve taste by releasing the taste amino acids, e.g. Savorase and Flavourzyme, have been developed (Imm & Lee, 1999); they are mainly used to hydrolyze meat before cooking. These taste enzymes are mainly composed of mesophilic protease, whose optimum temperature for protein hydrolysis is generally above 50 °C. At 0 °C, less than 1% of its activity at the optimum temperature

Table 2
The released amount of taste amino acids and essential amino acids from refrigerated meat treated with cold-adapted protease or mesophilic protease

Taste	Amino acid	The relative increased amount of free amino acid (times)					
		Pork		Marine fish		Shrimp meat	
		C-P[a]/CK[c]	C-P/M-P[b]	C-P/CK	C-P/M-P	C-P/CK	C-P/M-P
Umami	Glu	3.00	1.35	1.16	0.92	1.80	1.45
	Asp	4.30	2.17	13.0	3.15	2.00	1.50
Sweet	Ser	1.40	0.88	2.00	2.00	3.00	1.23
	Thr	2.60	1.80	1.60	0.78	6.00	1.05
	Ala	1.90	1.67	2.10	1.09	1.56	1.35
	Gly	2.00	1.75	1.19	1.00	1.15	1.82
Essential amino acid		5.22	1.23	2.25	1.13	3.83	1.69

[a] C-P, cold-adapted protease.
[b] M-P, mesophilic protease.
[c] CK, control.

remains. Therefore, they have relatively low proteolytic efficiency in refrigerated meat. The cold-adapted protease produced by deep-sea psychrophilic bacterium *Pseudoaltermonas* sp. SM9913 had an optimum temperature of 35 °C and still kept 10.5% of its highest activity at 0 °C. Therefore cold-adapted protease had higher catalytic efficiency at 0 °C than mesophilic protease. The results of the experiment showed that, cold-adapted protease can release more taste amino acids and essential amino acids than mesophilic protease during refrigerated storage. Therefore, as a new kind of taste enzyme, cold-adapted protease has good potential for the taste improvement of refrigerated meat.

4. Conclusion

Refrigerated meat treated with cold-adapted protease released more taste amino acids and essential amino acids than that treated with mesophilic protease. Therefore, cold-adapted protease has potential for improving the taste of refrigerated meat. Cold-adapted protease, hydrolysing meat protein, is a new alternative to the traditional mesophilic protease during refrigerated storage.

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