EFFECT OF COMBINED HIGH PRESSURE AND HEAT TREATMENTS ON FREE AMINO ACIDS IN BEEF MUSCLE

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Introduction

High pressure processing has developed in the last twenty years, because of its tenderizing effect on meat and extension of shelf life without loss of nutritional and flavor properties. High pressure treatment can also affect protease activities, accelerate meat conditioning and enhance the content of free amino acids (Homma, 1994, 1995; Ohmori, 1991; Jung et al, 2000; Suzuki, 1994). However, to improve the tenderization of post-rigor beef high pressure needs to combined with heat treatment. Zamri et al (2006) found that high pressure (200 MPa) combined with heat (60 and 70□) significantly decreased the hardness of chicken muscle. Our previous work showed that a pressure of 200 MPa combined with 60 and 70□ can lead to significant increases in tenderness of post-rigor beef muscle which was attributed to increased enzyme activity due to the combination of high pressure with slowly rising temperature (Ma and Ledward, 2004). But changes in the free amino acids in beef muscle subjected high pressure combined with heat treatment have not been reported, the objective of this paper was to describe the effect of high pressure and heat treatment on the amounts of the free amino acids in beef.

Materials and methods

Preparation of samples: The beef Longissimus dorsi was obtained from a 2 year old crossbreed (Chinese yellow cattle × Limusin) and its treatment was as described by Ma and Ledward (2004) .

High pressure and heat treatment: Samples were pressurized at 100 to 600MPa at room temperature and 200MPa and 600MPa at 40 and 60℃ for 20 min as described by Ma and Ledward (2004). Some samples were heated in water baths at 40 or 60℃ for 30 min.

Free amino acids analysis: 3.0 g of sample was homogenized at 12,000 rpm twice for 2 min with 20 mL of ice-cold 6.0% (v/v) perchloric acid in an ice bath using a homogenizer (IKA T18 basic, German). The homogenized sample was then incubated for 30 min in ice before centrifuging at 2000 × g for 15 min. The residue was re-extracted with 20 mL ice-cold perchloric acid and centrifuged as described above. The supernatants from the first and second extractions were combined and filtered through a Whatman No. 54 filter paper. The pH of the filtrate was adjusted to 7.0 using a 33% (w/v) KOH solution and centrifuged at 2000 × g for 10 min to remove precipitate of potassium perclorate. The supernatant was acidified to pH 2.2 with a 10 M HCl solution and then diluted to 50 mL with distilled water. Two milliliters of the extract was transferred into a clean tube and 1.0 mL of lithium citrate buffer (pH 2.2) was added. Samples were analyzed with an amino acid analyzer (Biochrom 20, Biochrom Ltd, Cambridge, U.K.).

Results and discussion

Changes in free amino acids of heated and/or pressurized beef muscle after storage for 1 week at 4℃ are presented in table 1.

On heat treatment at 40□ and ambient pressure it is seen that the total free amino acids markedly increased from 138mg/100g to 225mg/100g. Although our previous results showed that the protease activities decreased after 40℃ treatment, it should be noted that the optimum temperature of most proteases are around 40℃ (Zeece et al, 1989) and as the temperature rises from ambient to 40℃ enzymes are very active and consequently promote proteolysis, at the same time, the proteolytic susceptibility of denatured proteins would also be increased, the combined effect of all these factors would lead to the significant increase in total free amino acids observed. At 60℃, there is expected decrease in the amount of free amino acids in beef muscle since many of proteases will be denatured.

On pressure treatment at ambient temperature it is seen from table 1 that the total content of free amino acids in pressurized beef during storage at 4℃ for 1 week increased significantly when a pressure of 100 or 200 MPa was applied, the amino acid that marked increased were serine, glutamic acid, cystine, valine, methionine, leucine, phenylalanine and lysine, respectively. When the pressure was further increased to 400 or 600 MPa the total content of free amino acids decreased again. This result is similar to that of Ohmori et al (1991) who found that the beef rounds

Table 1 Change in the Free Amino Acid content of beef after pressure treatment for 20min at 20□, 40□, 60□ and storage for 1 week at 4□ (mg/100g)

Amino Acid	Treatments (temperature℃, pressure: MPa)										
			20℃				40℃			60℃	
	0.1MPa	100	200	400	600	0.1MPa	200	600	0.1MPa	200	600
Asp	-	-	-	-	-	-	-	-	-	-	-
Thr	40	41	48	44	48	49	51	44	36	57	49
Ser	3	9	7	4	3	9	6	4	3	6	4
Glu	1	6	6		2	11	3	1	1	5	2
Gly	11	13	11	12	11	13	12	10	9	13	11
Ala	25	42	37	30	27	40	35	31	25	34	26
Cys	4	12	11	2	12	5	4	10	7	12	9
Val	7	22	17	10	11	16	14	11	9	16	9
Met	6	25	20	8	18	14	16	16	12	17	10
Ile	8	18	14	7	9	9	12	9	8	12	7
Leu	4	25	21	8	5	12	17	8	3	19	10
Tyr	8	9	18	10	9	7	15	8	8	14	8
Phe	7	26	24	11	10	16	25	10	8	23	12
Lys	4	12	9	5	4	10	8	5	4	7	5
His	2	5	4	2	2	5	3	3	2	3	2
Arg	5	5	9	6	5	4	8	6	5	7	6
Pro	3	5	4	5	3	5	4	4	4	4	4
Try	-	-	-	-	-	-	-	-	-	-	-
Total	138	275	260	164	179	225	233	180	144	249	174

was pressurized at 1000 to 3000 atm and stored at 4℃ for 1 week the total content of free amino acids was greater than in samples treated at 0 and 5000 atm, especially in the case of those at 1000 atm.

At 40℃, pressure treatment at 200 MPa caused no obvious changes in the total content of free amino acids compared with heat treatment at 40℃ but treatment at 600 MPa caused a marked decrease. When treated at 60℃, 200 MPa increased the total content of free amino acids but higher pressures led to decreased amounts. This result is similar to the changes in tenderness seen when beef muscle subjected to 0.1 to 800 MPa at 60℃ (Ma and Ledward, 2004) where 200 MPa led to significant increases in tenderness but pressures of 400 MPa and higher caused significant decreases in tenderness. The possible explanation is that, as pointed out by Ma and Ledward (2004), the combination of high pressure with the slowly rising temperature make the proteases active and accelerate proteolysis.

Conclusions

From the results obtained in this paper, we can say that the proteolytic activities in muscle can be modulated by pressure and/or heat treatment to improve the content of free amino acids.

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