J Ind Microbiol Biotechnol (2005) 32: 487–489
DOI 10.1007/s10295-005-0024-9

ORIGINAL PAPER

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Debittering effect of *Monascus* carboxypeptidase during the hydrolysis of soybean protein

Received: 20 July 2005 / Accepted: 25 July 2005 / Published online: 23 September 2005

Abstract The actions of pepsin and the admixture of pepsin and *Monascus pilosus* carboxypeptidase 1 (MpiCP-1) on the hydrolysis of soybean protein were studied. The results showed that the pepsin hydrolyzate of soybean protein was much more bitter and contained relatively smaller amounts of total free amino acids than the hydrolyzate obtained with the admixture of pepsin and MpiCP-1. In addition, hydrophilic and hydrophobic amino acids were present in almost equal proportions in the pepsin hydrolyzate, while mainly hydrophobic amino acids made up the hydrolyzate obtained with the admixture of pepsin and MpiCP-1. These results suggest that MpiCP-1 suppresses and reverses the development of the bitterness taste that results from the pepsin hydrolysis of soybean protein by releasing mainly hydrophobic amino acids from the C-termini of the bitter components.

Keywords *Monascus* · Carboxypeptidase · Debittering effect · Soybean protein

Introduction

Soybean protein is well known as a vegetable protein with a high nutritional value, however, it has a number of unpleasant properties, including allergenicity, an unpleasant odor, insolubility, acid instability, heat instability, and indigestibility. As a result, various enzymatic methods have been developed to modify soybean protein in attempts to reduce or eradicate these properties [2, 3, 8, 9, 14]. However, the enzymatic hydrolysis of soybean protein frequently leads to the production of a bitter taste, which is due to the presence of strongly hydrophobic bitter peptides that arise as natural degradation products of the proteolytic reaction [2, 3, 7, 8, 10, 14].

Arai et al. [2] reported that the combination of aspergillopeptidase A and *Aspergillus* acid carboxypeptidase was capable of having both a deodorization and a debittering effect to produce a bland soybean protein hydrolyzate. Umetsu reported that wheat carboxypeptidase was also able to eliminate the bitter taste in the peptic hydrolyzate of soybean protein [13]. Liu et al. [11] reported that a carboxypeptidase 1 that had isolated from *Monascus pilosus* (MpiCP-1) was in fact a serine carboxypeptidase with a broad specificity similar to that of other fungal carboxypeptidases [4], such as *Aspergillus* acid carboxypeptidase [2]. In the investigation reported here, we investigated the action of MpiCP-1 during the hydrolysis of soybean protein.

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Materials and methods

Substrate

Soybean protein isolate (SPI) was provided by the Fuji Oil Company, Osaka, Japan. The substrate solution was prepared as follows: 5 g of SPI was shaken with 1000 ml of distilled water (DW) at 30°C for 2 h and then centrifuged at 10,000 *g* for 15 min. The supernatant was used as the substrate.

Enzymes

Pepsin (porcine gastric mucous membrane, 3900 U/mg of solid) was purchased from the Sigma Chemical Co. (St. Louis, Mo.). MpiCP-1 was purified according to the method described by Liu et al. [11].

Enzymatic hydrolysis

A 190-ml sample of the substrate was incubated with 0.01 g pepsin (39,000 U) in 10 ml of distilled water, and a second 190-ml sample of the substrate was incubated with 0.01 g pepsin (39,000 U) and 10 ml MpiCP-1 (500 μg, 2 U) at 37°C for 24 h in a water incubator with shaking. At defined intervals of time (0, 1, 2, 4, 8 and 24 h), 20-ml aliquots of each sample were removed from the reaction mixture and immediately boiled for 10 min to stop the enzymatic reaction; these were subsequently used for analyzing for free amino acids and the sensory properties.

Analysis of free amino acids

To a given volume of the enzymatic hydrolyzate, an equal volume of 20% trichloroacetic acid was added. The resulting mixture was allowed to stand for 30 min at room temperature and then filtered. The presence of free amino acids in the filtrate was determined using a Pico-Tag amino acid analyzer system. An amino acid standard solution (Wako pure chemical industries, Osaka, Japan) was used to calibrate for free amino acids.

Sensory analysis of bitterness

The sensory analysis of bitterness was performed using the methods of Carpenter et al. [5] and Einstein [6] with a slight modification. Twenty-five people from the Department of Agriculture Faculty at the University of the Ryukyus participated in a set of screening tests, which consisted of a triangle test and a ranking test, using caffeine solutions. Ten of the participants were selected to form a sensory panel. After being trained, the panelists tasted each sample and rated the intensity of its bitterness on a rating line with a six-point scale (from left to right: (0) non-bitter; (1) slightly bitter; (2) distinctly bitter; (3) very bitter; (4) extremely bitter; (5) very extremely bitter). Each degree represented a caffeine concentration of 0 (0), 0.25 (1), 0.50 (2), 0.75 3), 1.00 (4), and 1.25 m *M* (5), respectively. The bitterness intensity of each sample was expressed as the average of those obtained from all panelists.

Results

The results shown in Table 1 indicate that both total free amino acids and bitterness indeed increased with increasing incubation time in both the pepsin hydrolyzate and the hydrolyzate of the admixture of pepsin and MpiCP-1. However, the slope of their increase varied. Relative to the pepsin treatment, the treatment with the admixture of pepsin and MpiCP-1 resulted in a significantly rapid increase in the amounts of total free amino acids but a significantly slow increase in bitterness.

Table 1 The changes in total free amino acid levels and bitterness during the enzymatic hydrolysis of soybean protein

Enzyme	Incubation time (h)	Amino acid (μmol/100 ml)	Bitterness (ranking)
Pepsin	0	0	1.2
	1	0.0612	1.5
	2	0.0840	1.7
	4	0.1089	1.9
	8	0.1357	2.2
	24	0.2572	2.3
Pepsin + MpiCP-1	0	0	1.2
	1	0.1811	1.4
	2	0.2499	1.5
	4	0.3541	1.6
	8	0.5809	1.7
	24	0.7969	1.8

Thes results shown in Table 2 indicate that the pepsin treatment produced smaller amounts of free amino acids with a ratio of 1:1 for hydrophilic to hydrophobic amino acids, while the admixture treatment with pepsin and MpiCP-1 produced the larger amounts of free amino acids with a hydrophilic:hydrophobic ratio of 1:2.

These results suggested that MpiCP-1 seemed to suppress and reverse the development of the bitterness resulting from the pepsin hydrolysis of soybean protein by releasing mainly hydrophobic amino acids from the C-termini of the bitter components.

Discussion

As shown in Tables 1 and 2, when soybean protein is subjected to pepsin hydrolysis alone, much bitterness is produced and relatively small amounts of free amino

Table 2 Levels of free amino acids in the enzymatic hydrolyzates of soybean protein after incubation of 24 h

Amino acid (μmol/100 ml)	Pepsin		Pepsin + MpiCP-1	
	Amount	Ratio[a]	Amount	Ratio[a]
Asp	0.0029	1	0.0209	1
Glu	0.0127		0.0089	
Arg	0.0208		0.0191	
Lys	0.0036		0.0300	
His	0.0168		0.0282	
Ser	0.0702		0.1744	
Thr	0		0.0055	
Gly	0.0258	1	0.0068	2
Ala	0.0037		0.0122	
Pro	0.0015		0.0022	
Tyr	0.0011		0.0327	
Val	0.0064		0.0464	
Met	0.0020		0.0200	
CysCys	0.0256		0.0098	
Ile	0.0050		0.0223	
Leu	0.0571		0.2473	
Phe	0.0022		0.1105	
Total	0.2572		0.7969	

[a]Arough approximation of the ratio between hydrophilic and hydrophobic amino acids

acids are liberated, which were in almost equal proportions of hydrophilic:hydrophobic amino acids. On the other hand, the combination treatment of pepsin and MpiCP-1 produced little bitterness and liberated relatively larger amounts of amino acids, which were mainly hydrophobic one (for example, leucine, phenylalanine, valine, tyrosine, isoleucine, methionine, and alanine). The scope of this investigation did not enable us to draw any conclusion as to why the amounts of some free amino acids in this hydrolyzate were lower than those in the pepsin hydrolyzate. However, these results do show that there should be a correlation between bitterness and the amounts of hydrophobic amino acids liberated from soybean protein during hydrolysis procedures, and they are similar to the observations of Arai et al. [2] using aspergillopeptidase A and *Aspergillus* acid carboxypeptidase.

Pepsin, which is a well-known acid proteinase [1], selectively splits the peptide bonds of hydrophobic and aromatic amino acids to produce peptides bearing the amino acids mentioned above at both the C-termini and N-termini. In practice, most of the bitter peptides isolated from the peptic hydrolyzate of soybean protein were shown to bear hydrophobic amino acid residues, in particular, leucine at the C-termini [3, 7, 10, 14].

MpiCP-1, like most of the other known fungal carboxypeptidases [4], such as *Aspergillus* acid carboxypeptidase [2], is a serine carboxypeptidase with broad substrate specificity [11]. Besides acidic amino acids at the C-termini [11], MpiCP-1 especially prefers peptides containing the hydrophobic and aromatic amino acids. As, based on our data, the bitter peptides that resulted from the pepsin hydrolysis of soybean protein are apparently the appropriate substrates of MpiCP-1 [12], we suggest that MpiCP-1 might be able to lessen the bitterness of the pepsin hydrolyzate of soybean protein by decomposing the C-terminal structures of the bitter peptides, as has been shown for *Aspergillus* acid carboxypeptidase [2] and wheat carboxypeptidase [13].

When the soybean protein was treated with the admixture of pepsin and MpiCP-1, the protein may first have been hydrolyzed by pepsin to form peptides, which contributed to the bitterness, and then further degraded by MpiCP-1 to smaller peptides and free amino acids, which would account for the decreased bitterness [12]. MpiCP-1 may greatly suppress and reverse the development of the bitterness resulting from the pepsin hydrolysis of soybean protein by releasing mainly hydrolyphobic amino acids (for example, leucine, phenylalanine, valine, tyrosine, isoleucine, methionine, and alanine) from the C-termini of the bitter peptides.

The information obtained from the present investigation may provide a clue to the elucidation of the applicability of *Monascus* carboxypeptidase to the modification of soybean protein with its debittering effect.

Acknowledgements We would like to thank Dr. Toki Taira for his help in the use of several pieces of equipment, Mrs. Hanifah N. Lioe for her some valuable advice on taste test, and the panelists for their whole-hearted participation in thetaste test. The panelists are all part of the Faculty of Agriculture, University of the Ryukyus, Okinawa, Japan.

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