ISSN 1330-9862
(FTB-1585)

preliminary communication

Evolution of Proteolytic Tasty Components During Preparation of Douchiba, a Traditional Chinese Soy-Fermented Appetizer

*Likang Qin[1,2] and Xiaolin Ding[1]**

[1]Key Laboratory of Food Science and Safety, Ministry of Education, Southern Yangtze University, Wuxi, 214036 Jiangsu Province, PR China

[2]Department of Food Quality and Safety, School of Life Science, Guizhou University, Guiyang, 550025 Guizhou Province, PR China

Received: November 28, 2005
Accepted: March 1, 2006

Summary

Douchiba, a traditional Chinese soy-fermented appetizer, has been abundantly produced and widely consumed in Guizhou province of Southwest China. In this study, analysis of low-molecular mass peptide fractions, hydrophobic bitter peptide fractions, and free amino acid (FAA) profiles was conducted to understand the changes in tasty components of douchiba during five consecutive stages of its manufacture: steamed soybean (SS), 5-day incubated douchi qu (DQ), 6-month fermented douchi (DC), semi-finished douchiba (sm-DCB), and 6-month ripened douchiba as a finished product (DCB). Results indicated that the ratio of potentially taste-active oligopeptides (500–1000 Da) accounted for 13.98 and 2.54 % of low-molecular mass peptide fractions and hydrophobic bitter peptide fractions at DCB stage, respectively. The evolution patterns of total free amino acids (TFAA) increased significantly ($p<0.05$) from SS to DCB by about 11 times and amounted to 20.14 % of crude protein. At the end of the ripening period (6 months), Arg, Glu, Phe, Leu, and Lys were the most abundant FAA, adding up to 64.37 % of TFAA. The most abundant tasty FAA class was bitter FAA, about 8- and 3-fold higher than the content of sweet and monosodium glutamate (MSG)-like FAA, respectively. The final values of all bitter and MSG-like FAA at DCB stage were significantly higher than their respective thresholds. However, the final characteristic taste of douchiba was predominated by saltiness, followed by moderate umami taste and slight bitterness, possibly as a result of the balance and interaction among different tasty components.

Key words: douchiba, peptides, free amino acids, tasty components

Introduction

Fermentation, one of the oldest techniques in food preservation and manufacture, can contribute directly to many advantageous properties of products by biochemical modification of microorganisms (*1*). The most pronounced one is the improvement and diversification of texture, taste and aroma acceptable to more consumers. Currently, fermented food is accounting for 30–40 % of the total worldwide food consumption (*2*).

Soybean (*Glycine max* (L.) Merr.), one of the most widely grown crops in the world, is rich in proteins (40–50 %), lipids (20–30 %), and carbohydrates (26–30 %) (*3*), and consequently has been generally considered as

*Corresponding author; Phone: ++86 510 85 864 457; Fax: ++86 510 85 879 957; E-mail: likangqin@126.com, qlk6509@yahoo.com.cn

one of the best non-meat sources of high-quality protein and cholesterol-free edible oil (*4*). Recently, with a variety of potential anti-carcinogens and other therapeutic agents elucidated (*5–10*), soybean and its fermented products have received increasing attention as foodstuff of functional foods. Originally important only in traditional oriental diet, soy-fermented foods such as natto, tempeh, sufu, soy sauce, soy nuggets (douchi), soy paste, *etc.* are now being increasingly valued in occidental foods. For example, the sale of soy foods in the United States increased from $2.77 billion in 2000 to more than $3.5 billion in 2002, and is currently at approximately 10–11 % increase per year (*11*). Undoubtedly, the main reason for this increase is the higher consumers' interest in their unique texture, taste and aroma, together with the increased consumer awareness of soy foods as healthy foods (*11*).

Douchi is soy-fermented food originated from China as early as 206 B.C. It was introduced into the world as Buddhism spread, and consequently it has spread as different food around the world such as natto and hamanatto in Japan, tempeh and tao-tji in Indonesia, chungkookjang in Korea, kinema in India, tau-nua in Thailand, and soumbala in Africa. Like douchi, douchiba is a unique traditional soy-fermented appetizer widely consumed in China. However, it has unique taste and aroma characteristics due to the quality of soybeans used in China, longer and complicated manufacture as compared to douchi. Traditionally, the typical manufacture of douchiba is performed in December and its manufacture can be roughly divided into the following four major stages: firstly, whole yellow soybeans, preferably small-seeded cultivars with high protein content grown in Guizhou province, are soaked for 24–36 h at ambient temperature to take up sufficient water. The soaked beans are steamed or boiled under atmospheric pressure for about 10 h until they are tender enough to be mashed by fingers, then drained and cooled to about 40 °C. The cooked soybeans are wrapped with locally available leaves in a sackcloth, packed tightly into bamboo baskets, and left to incubate naturally for 5–6 days in a warm place (20–40 °C) until the beans are covered with a stringy, mucilaginous coating and obtain a typical douchi-like flavour. Secondly, the mature douchi qu (DQ) is mixed with 4–8 % (wet mass) salt and/or a small amount of spices before being put into large earthenware jars or crocks for fermentation under natural conditions for several months (December to June). Thirdly, the aged bean called douchi (DC) is taken out, smashed by wooden or stone tool, sun-dried for about two weeks to decrease water concentration, and moulded into rectangular shape (each piece weighing 500–750 g). Finally, the resultant semi-finished douchiba (sm-DCB) is put into jars or crocks again and air-proofed with the clay slurry to ripen for approximately 6 months in shady and cool place. The finished DCB has a strongly palatable taste and aroma, a high salt content, and a blackish-brown colour. Due to its unique taste and aroma characteristics, it was selected as the tribute to emperors of Qing Dynasty (1644–1911) and has earned the reputation of being one of the best soy-fermented condiments in Guizhou, China.

It is well known that proteolysis is one of the major and complex biochemical events which take place during preparation of soy-fermented condiments, and its degradation products, peptides and amino acids, not only have a considerable influence on the nutritional values, but also contribute directly to the taste characteristics, in some cases even act indirectly as precursors of aromatic substances of products (*12,13*). Consequently, proteolytic peptide profile and free amino acid composition of many soy-fermented foods, such as Chinese sufu (*14*), Korean doenjang (*15*), Nigerian daddawa (*16*), and Nepalese kinema (*17*), have been evaluated to serve as a typicality and quality index of these products. It is well established that the water-soluble fraction contains the majority of taste compounds such as salts, amino acids and low-molecular mass peptides produced during proteolysis (*15*). However, there is no report about scientific assessment of douchiba's proteolytic tasty components.

In order to establish a scientific basis for the optimization of manufacture technology and standardization of quality control, this study was conducted to track the proteolytic tasty component evolution by the assessment of low-molecular mass peptide fractions, hydrophobic bitter peptide fractions, and free amino acid (FAA) profiles in water-soluble fraction during consecutive stages of douchiba manufacture.

Materials and Methods

Source of douchiba samples

Three batches of douchiba samples were provided by Weiquan Food Ltd., a well-known douchiba manufacturer located in Guizhou province, following the traditional method described briefly in the introduction section and technical parameters shown in Fig. 1. From the same batch preparation cycle of douchiba, five representative samples at five consecutive stages of douchiba, namely steamed soybean (SS), douchi qu (DQ), douchi (DC), semi-finished douchiba (sm-DCB) and finished douchiba (DCB), were taken. Once collected, the samples were vacuum-packed and transported to the laboratory at 4–6 °C in portable coolers (within 2 h). Then the samples were smashed, freeze-dried by a model 18 LFD lyophilizer (Labconco, USA), and stored in airtight bottles at –18 °C until analysis.

Extraction of peptide fractions in water-soluble fraction

The low-molecular mass peptide fractions were extracted from the water-soluble fraction obtained according to the procedure described by Gibbs *et al.* (*3*). Briefly, water-soluble fraction samples were mixed with 50 % acetonitrile solution and 0.1 % trifluoroacetic acid (TFA). The mixture was sonicated for 5 min, then vortexed for 2 min, and centrifuged at 20 000 rpm and 0 °C for 10 min using a CR22G high-speed refrigerated centrifuge (Hitachi, Japan). The supernatant was collected, filtered through a 0.45-μm filter, lyophilized, and stored in closed container for the molecular mass distribution determination.

The hydrophobic bitter peptide fractions were also extracted from the water-soluble fraction with 2-butanol

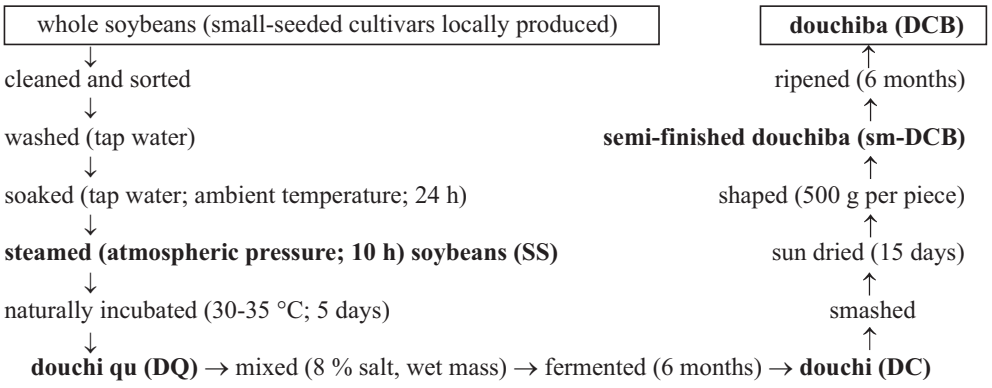

Fig. 1. Schematic representation of douchiba preparation. The five sampling points are marked with bold face

according to the procedure described by Kukman *et al.* (*18*), freeze-dried, and stored in a closed container for the molecular mass distribution determination.

Gel permeation high-performance liquid chromatography

The molecular mass distribution of the low-molecular mass peptide fractions and hydrophobic bitter peptide fractions was determined by gel permeation high-performance liquid chromatography (GP-HPLC) on a TSK gel 2000 SWXL column (300×7.8 mm i.d., particle size 5 μm) with a separation range of the molecular mass (M_r) of 189–12 500 Da using a Waters 600 analytical HPLC system. The mobile phase was acetonitrile/distilled water/trifluoroacetic acid (45:55:0.1, by volume) at a flow-rate of 0.5 mL/min. Peptides were monitored using a UV detector at 220 nm with an absorbance range of 0.02 AU. Calibration was performed with five proteins or peptide standards (cytochrome *c* 12 500 Da, aprotinin 6500 Da, bacitracin 1450 Da, glycocoll-glycocoll-tyrosine-arginine 451 Da, glycocoll-glycocoll-glycocoll 189 Da) and the molecular mass (M_r) corresponding to various segments of the chromatogram was calculated on the basis of the calibration curve log M_r *vs.* t (min): log M_r=7.33–2.41·t, R^2=0.9922. The area under the curve was determined in four segments (M_r<300, 500–1000, 1000–2000, and 2000–5000 Da for low-molecular mass peptide fractions; and M_r>1000, 600–550, 200–150, and <150 Da for hydrophobic bitter peptide fractions) and was expressed as the percentage of the total area of the four segments combined.

Free amino acids analyses

For determination of individual free amino acids, 1 g of lyophilized and pulverized samples (fine) was precipitated using 50 mL of 12 % trichloroacetic acid (TCA) for 3 h at ambient temperature to remove large peptides and then centrifuged at 12 000 rpm for 30 min. The supernatant was filtered through 0.45-μm filter. The sample solution prepared in 10 μL was analyzed by an Agilent HP1100 HPLC (Agilent, USA) equipped with an Agilent Zorbax 80A Extend-C_{18} column (150 × 4.6 mm i.d., particle size 5 μm), an *o*-phthalaldehyde (OPA) forward-column derivatisation autosampler, and a UV detector. The mobile phases were: A, 20 mM sodium acetate (pH=7.2) with 0.5 % tetrahydrofuran; B, 20 mM sodium acetate (pH=7.2)/methanol/acetonitrile (1:2:2, by volume). The linear elution gradient was A:B (by volume) from 100:0 to 50:50 for 0–17 min, 50:50 to 0:100 for 17–20 min, and 100:0 for 20–24 min. The flow rate was 1.0 mL/min. The temperature was controlled at 40 °C. The amino acids were detected at 338 nm except for proline, which was detected at 262 nm. Each amino acid was identified by comparing the samples with a standard (Sigma) analyzed under the same conditions and quantified by the calibration curve of the authentic compound.

Amino acid grouping

According to the taste characteristics as described by Tseng *et al.* (*19*), amino acids were grouped as MSG-like (Asp+Glu), sweet (Ala+Gly+Ser+Thr), bitter (Arg++His+Ile+Leu+Met+Phe+Trp+Try+Val), and tasteless (Cys++Lys+Pro).

Statistical analysis

Data were analyzed using the SPSS statistical package version 10.0 (SPSS Inc., Chicago, IL, USA). Analysis of variance (ANOVA) was performed to test the differences in low molecular mass peptides, hydrophobic bitter peptides and free amino acids among five consecutive stages of douchiba manufacture. The least significant difference (LSD) test at a p-value<0.05 was applied for comparison of mean values.

Results and Discussion

Peptide profiles in water-soluble fraction

Several groups had previously shown that the peptide content of soy-fermented products was greater than that of unfermented soybeans, but little was known about the chromatographic profiles of those extracts (*13*). In this study, the molecular mass (M_r) distribution of douchiba's low-molecular mass peptide fractions was analyzed (Fig. 2). The M_r of low-molecular mass peptide fractions was almost less than 5000 Da. During three consecutive stages of douchiba manufacture, the amount of segment M_r<300 Da in low-molecular mass peptide fraction, which was composed mainly of FAA and a small peptide with two or three amino acid residues, increased markedly (p<0.05) from 47.20 to 76.59 %. In contrast, oligopeptide (500–1000 Da), medium-molecular

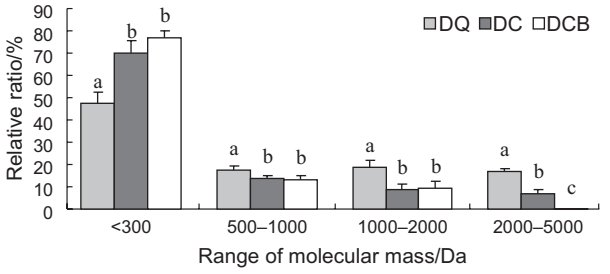

Fig. 2. Molecular mass distribution of the low-molecular mass peptide fractions during consecutive stages of douchiba manufacture. Elution profiles were determined on TSK gel 2000 SWXL column (300×7.8 mm i.d.) at 220 nm detection using a Waters 600 HPLC system. Data were expressed as mean±SD (*N*=3), and means with different letter within a given peptide fraction are significantly different ($p<0.05$)

(1000–2000 Da) and high-molecular peptide (2000–5000 Da) decreased significantly ($p<0.05$) from 17.80, 18.51, and 16.57 % at DQ stage to 13.98, 8.61, and 7.14 % at DC stage, respectively. Up to DCB stage, the high-molecular peptide disappeared. There were many reports that these peptides could elicit different taste characteristics such as umami, bitter, sweet, sour, salty and astringent. Thus, peptide fractions expressing different taste characteristics were given special attention (*18*).

It has been known for a long time that enzymatic hydrolysis of proteins frequently leads to the production of a bitter taste due to the presence of strongly hydrophobic bitter peptides arising as natural degradation products of the proteolytic reaction (*18*). Therefore, the hydrophobic bitter peptide fractions during two manufacture stages (DC and DCB) were analyzed. Their molecular mass (M_r) distribution is shown in Fig. 3. Surprisingly, the ratio of four segments in the hydrophobic bitter peptide fractions at both DC and DCB stages had a significant difference ($p<0.05$), but their molecular mass distribution patterns were similarly predominated by the fractions with M_r=200–150 Da and less than 150 Da, reaching in total 88.85 and 96.75 %, respectively, while hydrophobic oligopeptide (600–550 Da) and medium molecular peptide (>1000 Da) only accounted for 9.51 or 2.54, and 1.54 or 0.25 % of hydrophobic bitter peptide fractions, respectively. This result might be explained by

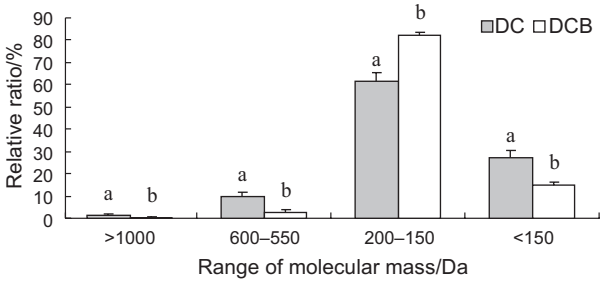

Fig. 3. Molecular mass distribution of the hydrophobic bitter peptide fractions during two stages of douchiba manufacture. Elution profiles were determined on TSK gel 2000 SWXL column (300×7.8 mm i.d.) at 220 nm detection using a Waters 600 HPLC system. Data were expressed as mean±SD (*N*=3), and means with different letter within a given peptide fraction are significantly different ($p<0.05$)

the fact that in peptides with a longer chain length that allow conformations such as hair-pin loops or clusters, the hydrophobic chains were generally directed towards the interior part of the molecule, therefore these peptides were not extracted with 2-butanol (*18*).

Changes in the free amino acids

The content evolutions of individual free amino acid (FAA), total free amino acid (TFAA), and five major FAA profiles during consecutive stages of DCB manufacture are shown in Table 1. As expected, FAA was released throughout the whole preparation period. The concentrations of most FAA increased significantly ($p<0.05$) from SS to sm-DCB stage and then decreased slightly up to DCB stage (ripened for 6 months), while the highest levels of Ala, His, Cys, Pro, and Arg were found at SS (Ala), DC (His, Cys, and Pro) or DCB (Arg) stage. Basically, coinciding with the evolution patterns of most FAA, the content of TFAA increased significantly ($p<0.05$) from SS to sm-DCB and then decreased slightly up to DCB, reaching a final value of 9.08 or 7.65 g/100 g of dry mass at sm-DCB or DCB stage, respectively, which was approximately 12 or 10 times of FAA content at SS stage. The profiles of five major FAA, Arg, Glu, Phe, Leu, and Lys, in the descending ratio order, remained essentially constant, and were found in large quantities throughout the four fermentation periods of DQ, DC, sm-DCB and DCB, adding up to 61.41, 57.02, 59.55, and 64.37 % of TFAA, respectively. However, the five major FAA profiles at SS stage were different, with large proportions of Ala, Glu, Arg, Pro, and Tyr, reaching in total 62.82 % of TFAA at this stage. This might be related to unequal breakdown among several subunits of soybean protein (*13*).

In respect to the changes in FAA or TFAA contents of other soy-fermented food during preparation, similar behaviour or patterns were reported by other authors, despite the presence of slight difference resulting from microflora or soybean cultivars. For instance, the final values of TFAA in soy-daddawa (*16*), and red and white sufu (8 % salt, wet mass) ripened for 80 days (*14*) were 84.32, 88.00, and 104.00 mg/100 g of dry mass, respectively; the content of FAA in soybean was only 0.2 % of the total dry mass, but processing of soybean with *Bacillus subtilis* during kinema fermentation led to a 60-fold increase in FAA, which accounted for approximately 26 % of total amino acids (*17*). The five major FAA profiles in the descending ratio order found in soybean (*19*), kinema (*17*), and red and white sufu (8 % salt, wet basis) ripened for 80 days (*14*) turned out to be very similarly involved in Glu, Arg, Phe, Leu, Lys, Asp, and Ala.

As for content evolution of grouping free amino acids, it was conducted on the basis of their taste characteristics as described by Tseng *et al.* (*20*) and is shown in Table 2. Similar to the patterns of most FAA, the content evolutions of three FAA classes, MSG-like, sweet and bitter FAA, increased significantly ($p<0.05$) from SS to sm-DCB stage and then decreased slightly to DCB stage, but for tasteless FAA class, the peak content level appeared at DC stage. During consecutive stages of douchiba manufacture, the most abundant tasty FAA class was bitter FAA class, being about 8- and 3-fold higher than the amount of sweet and MSG-like FAA at DC

Table 1. Evolution of free amino acids (FAA) during consecutive stages (SS, DQ, DC, sm-DCB, and DCB) of douchiba manufacture

FAA	m[a]/(g/100 g)					Threshold value[b]
	SS	DQ	DC	sm-DCB	DCB	
Asp	0.04±0.01a	0.07±0.01b	0.19±0.01c	0.52±0.01d	0.40±0.01e	1000
Glu[c]	0.08±0.01a	0.29±0.02b	1.10±0.04c	1.60±0.07d	1.18±0.01e	300
Ala	0.20±0.04a	0.02±<0.01b	0.03±0.00b	0.02±<0.01b	0.01±0.00b	600
Gly	0.03±0.01a	0.03±0.00a	0.19±0.02b	0.29±0.02c	0.29±<0.01c	1300
Ser	0.01±0.00a	0.02±<0.01a	0.08±0.02b	0.16±0.03c	0.08±<0.01b	1500
Thr	0.02±<0.01a	0.03±<0.01a	0.10±0.02b	0.28±0.04c	0.22±0.03d	2600
Arg[c]	0.08±0.02a	0.18±0.02a	0.54±0.03b	1.53±0.04c	1.88±0.11d	500
His	0.04±<0.01a	0.04±0.02a	0.25±0.03b	0.16±0.03c	0.11±0.01d	200
Ile	0.02±<0.01a	0.04±0.02a	0.24±0.03b	0.51±0.04c	0.37±0.03d	900
Leu[c]	0.02±0.01a	0.11±0.03b	0.50±0.04c	0.91±0.04d	0.64±0.04e	1900
Met	0.01±<0.01a	0.02±<0.01a	0.16±0.02b	0.19±0.02c	0.18±<0.01c	300
Phe[c]	0.04±0.01a	0.14±0.02b	0.63±0.05c	0.77±0.04d	0.66±0.04c	900
Trp	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Tyr	0.06±<0.01a	0.07±<0.01a	0.45±0.03b	0.53±0.03c	0.35±0.02d	n.d.
Val	0.03±0.01a	0.06±0.02a	0.36±0.04b	0.61±0.02c	0.51±0.03d	400
Cys	0.01±<0.01a	0.02±<0.01a	0.07±<0.01b	0.05±<0.01c	0.06±0.01bc	
Lys[c]	0.01±<0.01a	0.07±0.02b	0.63±0.04c	0.65±0.02c	0.57±0.02d	500
Pro	0.07±0.00a	0.08±<0.01a	0.43±0.02b	0.35±0.03c	0.14±0.01d	3000
TFAA	0.78±0.06a	1.27±0.08b	5.96±0.13c	9.08±0.09d	7.65±0.27e	
5-mFAA[d]	0.49±0.02a	0.78±0.04b	3.40±0.05c	5.41±0.07d	4.93±0.18e	
(5-mFAA/TFAA)/%	62.82±0.55a	61.41±1.82b	57.02±0.35c	59.55±0.24d	64.37±0.23e	

[a]Each value is expressed as mean±SD (N=3). Means with different letter(s) within a row are significantly different ($p<0.05$)
[b]Threshold value is expressed as mg(FAA)/L(water) (*21*)
[c]Five major FAA found throughout four fermentation periods (DQ, DC, sm-DCB and DCB)
[d]Total content value of the five major FAA
n.d.: not determined

Table 2. Evolution of taste characteristics of free amino acids (FAA) during consecutive stages (SS, DQ, DC, sm-DCB, and DCB) of douchiba manufacture

Taste characteristics	m[a]/(g/100 g)				
	SS	DQ	DC	sm-DCB	DCB
MSG-like	0.12±0.02a	0.36±0.01b	1.29±0.03c	2.07±0.06d	1.57±0.02e
Sweet	0.26±0.04a	0.10±<0.01b	0.40±0.05c	0.75±<0.01d	0.60±0.04e
Bitter	0.30±0.04a	0.64±0.07b	3.14±0.03c	5.21±0.09d	4.71±0.22e
Tasteless	0.10±0.01a	0.17±0.02b	1.13±0.04c	1.05±0.05d	0.77±0.01e

[a]Each value is expressed as mean±SD (N=3). Means with different letter within a row are significantly different ($p<0.05$)

stage, respectively. With regard to the content of individual tasty FAA, the final values of all bitter and MSG-like FAA at DCB stage, 0.11–1.88 and 0.40–1.18 g/100 g of dry mass, were significantly higher than their respective thresholds, 300–1000 and 200–1900 mg/L water (*21*), respectively, but in four sweet FAA, concentrations of Ala, Ser and Thr, *i.e.* 0.01, 0.08, and 0.22 g/100 g of dry mass, were lower than their respective thresholds, 600, 1500, and 2600 mg/L water (*21*), respectively.

It should be noticed, in particular, that though douchiba products ripened for 6 months contained much bitter FAA than MSG-like and sweet FAA, their sensory properties in practical consumptions presented a characteristic taste profile: predominant saltiness, intense umami, slight bitterness, sourness and astringency. Perhaps this may be explained by the fact that the final characteristic taste of douchiba was definitively determined by balance and interaction among different taste components. The predominantly salty taste may be directly related to the addition of Na^+ (NaCl) at much higher concentrations (4–8 %, wet mass); more intense umami taste than expected is possibly the result of the synergistic action (*15*) of relatively abundant low-molecular mass peptide fractions as mentioned above, in addition to the most typical umami taste or palatable taste imparted by Asp and Glu (*20*); the decrease of unpleasant bitterness,

derived from bitter FAA and hydrophobic bitter peptide fractions, may be attributed to the diminishing or masking effect of saltiness, umami taste, sourness, and sweetness (*15*); sourness and astringency may be directly related to the presence of a few of organic acids and phenolics, respectively. Therefore, further study is needed for the identification of key umami taste peptides in DCB and the interaction among the tasty components to eliminate or suppress the bitter taste and to enhance the umami and sweet taste.

Conclusions

Each type of soy-fermented food has its characteristic profile of peptides and free amino acids that results from the balance of the degradation of the peptides to free amino acids and the degradation and inter-conversion of the different free amino acids. Douchiba, a traditional Chinese soy-fermented appetizer, was analyzed in this initial study. From the results obtained it can be concluded that proteolytic peptides, in particular potentially taste-active oligopeptides (500–1000 Da), and free amino acids, such as Arg, Glu, Phe, Leu, and Lys, were abundant and are recognized as important contributors to taste of the ripened douchiba. The final taste is determined by balance and interaction among different taste components.

Acknowledgements

This study is part of two broader research projects (Programme *QKH 2005–2023* and *QJK 2004–204*) supported by the Natural Science Foundation and the Bureau of Education of Guizhou Province, China, respectively.

References

1. J.Y. Je, P.J. Park, W.K. Jung, S.K. Kim, Amino acid changes in fermented oyster (*Crassostrea gigas*) sauce with different fermentation periods, *Food Chem. 91* (2005) 15–18.
2. G. Campbell-Platt, Fermented foods: A world perspective, *Food Res. Int. 27* (1994) 253–257.
3. B.F. Gibbs, A. Zougman, R. Masse, C. Mulligan, Production and characterization of bioactive peptides from soy hydrolysate and soy-fermented food, *Food Res. Int. 37* (2004) 123–131.
4. R.N. Tharanathan, S. Mahadevamma, A review: Grain legumes a boon to human nutrition, *Trends Food Sci. Technol. 14* (2003) 507–518.
5. C.C. Hu, C.H. Hslao, S.Y. Huang, S.H. Fu, C.C. Lai, T.M. Hong, H.H. Chen, F.J. Lu, Antioxidant activity of fermented soybean extract, *J. Agric. Food Chem. 52* (2004) 5735–5739.
6. K.Y. Park, K.O. Jung, S.H. Rhee, Y.H. Choi, Antimutagenic effects of *doenjang* (Korean fermented soypaste) and its active compounds, *Mutat. Res. 523–524* (2003) 43–53.
7. H. Fujita, T. Yamagami, K. Ohshima, Long-term ingestion of Touchi-extract, an α-glucosidase inhibitor, by borderline and mild type-2 diabetic subjects is safe and significantly reduces blood glucose levels, *Nutr. Res. 23* (2003) 713–722.
8. Y. Suauki, K. Kondo, H. Ichise, Y. Tsukamoto, T. Urano, K. Umemura, Dietary supplementation with fermented soybeans suppresses intimal thickening, *Nutrition, 19* (2003) 261–264.
9. S. Watanabe, S. Uesugi, Y. Kikuchi, Isoflavones for prevention of cancer, cardiovascular diseases, gynecological problems and possible immune potentiation, *Biomed. Pharmacother. 56* (2002) 302–312.
10. G. Moon, M. Lee, Y. Lee, Main component of soy sauce representing antioxidative activity, *Int. Cong. Ser. 1245* (2002) 509–510.
11. S. Min, Y. Yu, S. Martin, Effect of soybean varieties and growing locations on the physical and chemical properties of soymilk and tofu, *J. Food Sci. C, 70* (2005) 8–12.
12. B.Z. Han, F.M. Rombouts, M.J.R. Nout, A Chinese fermented soybean food, *Int. J. Food Microbiol. 65* (2001) 1–10.
13. J.L. Kiers, A.E.A. Van Laeken, F.M. Rombouts, M.J.R. Nout, *In vitro* digestibility of *Bacillus* fermented soya bean, *Int. J. Food Microbiol. 60* (2000) 163–169.
14. B.Z. Han, F.M. Rombouts, M.J.R. Nout, Amino acid profiles of sufu, a Chinese fermented soybean food, *J. Food Compos. Anal. 17* (2004) 689–698.
15. S.H. Kim, K.A. Lee, Evaluation of taste compounds in water-soluble extract of a *doenjang* (soybean paste), *Food Chem. 83* (2003) 339–342.
16. B.O. Omafuvbe, O.O. Shonukan, S.H. Abiose, Microbiological and biochemical changes in the traditional fermentation of soybean for 'soy-daddawa' – Nigerian food condiment, *Food Microbiol. 17* (2000) 469–474.
17. P.K. Sarkar, L.J. Jones, G.S. Craven, S.M. Somerset, C. Palmer, Amino acid profiles of *kinema*, a soybean-fermented food, *Food Chem. 59* (1997) 69–75.
18. I.L. Kukman, M. Zelenik-Blatnik, V. Abram, Isolation of low--molecular-mass hydrophobic bitter peptides in soybean protein hydrolysates by reversed-phase high-performance liquid chromatography, *J. Chromatogr. A, 704* (1995) 113–120.
19. I.M. Vasconcelos, E.A. Siebra, A.A.B. Maia, R.A. Moreira, A.F. Neto, G.J. Campelo, J.T.A. Oliveira, Composition, toxic and antinutritional factors of newly developed cultivars of Brazilian soybean (*Glycine max*), *J. Sci. Food Agric. 75* (1997) 419–426.
20. Y.H. Tseng, Y.L. Lee, R.C. Li, J.L. Mau, Non-volatile flavor components of *Ganoderma tsugae*, *Food Chem. 90* (2005) 409–415.
21. H. Kato, M.R. Rhue, T. Nishimura: Role of Free Amino Acids and Peptides in Food Taste. In: *Flavor Chemistry Trends and Developments*, R. Teranishi, R.G. Buttery, F. Shahidi (Eds.), American Chemical Society, Washington DC, USA (1989) pp. 158–174.