J Food Sci Nutr
Vol 16, p 55～61 (2011)
DOI: 10.3746/jfn.2011.16.1.055

J Food Science and Nutrition

Chemical and Textural Properties in Commercial Fermented Soybean Curds of Sufu

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Abstract

A survey aiming to find out the chemical and textural properties of commercial fermented soy bean curd called sufu was conducted. Sixteen brands of plain sufu produced in the Northern or the Southern part of China were collected and examined for their crude protein, crude fat, texture profiles, free amino acids, and free fatty acid contents. Twenty-one free amino acids were extracted and derivatized using a commercial kit followed by separation and analyzed by the gas chromatography-mass spectrometry (GC-MS). Similarly, ten free fatty acids were extracted using alumina, eluted, separated and analyzed. The content ranges of crude fat and protein were 22～36% and 31～38%, respectively. In texture profile analysis, ranges of the texture parameters were 131～493 g (hardness), 0.4～0.5 (cohesiveness), -137 to -50 gs (adhesiveness), 0.6～1 (springiness), 47～220 g (gumminess) and 32～177 g (chewiness). Twenty-one different free amino acids, especially alanine, glycine, α-aminobutyric acid, valine, leucine, allo-isoleucine, aspartic acid, glutamic acid and lysine in large amount, as well as ten fatty acids in total, notably linoleic acid (9-octadecanoic acid), oleic acid (9,12-octadecadienoic acid), linolenic acid (9,12,15-octadecadienoic acid), hexadecanoic acid and octadecanoic acid were found. This information provides important quality reference ranges for product developers and manufacturers to optimize and produce the plain sufu.

Key words: fermented soybean curd, sufu, free amino acid, free fatty acid, texture

INTRODUCTION

Fermented soybean curd or sufu, originated in China, is a soft creamy cheese-type product made from soybean curd by the fermentation of mould (1,2). This product with its nutrition and characteristic flavor has been widely consumed by the natives as an appetizer or seasoning for many centuries. The maturation period for sufu production usually takes three to six months for component hydrolysis resulting in textural changes and flavour intensity increases. During that stage, hydrolysis converts macromolecules to small molecules such as peptides, amino acids, amines, ammonia, triglyceride, fatty acids making it a good source of digestive amino acids and fatty acids in the Chinese diet from the nutritional point of view. Furthermore, both protein and fat fractions have effects on its texture, and on development of the sufu flavor (3).

There are only a handful of publications describing the texture profile, amino acid profile and fatty acid profile in sufu, because during the fermentation, many changes take place. It is rather complicated to monitor individual amino acid, fatty acid and many other parameters at the same time. Even as such, the knowledge of chemical and biological properties of sufu is important to understand the fundamentals of food fermentation and further investigation in the sufu system. Therefore, the primary objective in this study was to determine the various qualities in the commercial plain sufus. The investigation covered from the basic crude protein, crude fat and texture profile to an in-depth monitoring of individual free amino acid and free fatty acids hoping the data will be eventually served as references for research-purposed laboratory-scale sufu production, control and improvement.

MATERIALS AND METHODS

Sufu sampling

Sixteen brands (D～F, H～T) of white commercial sufu were purchased from both the Northern and Southern China. Among them, brand F and H were produced in Beijing; brand E was manufactured in Hunan province; brand K, R and S were produced in Hong Kong; brand D, J, L, M, O, P, Q and T were manufactured in several cities of Guangdong province; brand I and N were produced in Taiwan. Brands from the South were a few more because plain sufu is mainly consumed in Southern

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China, and manufacturers centralize in the South. Samples were all well-chosen randomly and all of them represented sufu of different origins.

Crude protein analysis

Crude protein of sufu samples was determined according to Kjeldahl method AOAC No. 987.02 (4) using Kjeltec Systems with Digester 2006 and Distiller 1002 (FOSS Tecator AB, Hoganas, Sweden). Protein content was calculated by multiplying the nitrogen content by a factor of 6.25 (5) and expressed as the concentration of protein in the freeze-dried sufu samples (protein% dry mass weight).

Crude fat analysis

Crude fat was measured by a Soxtec System HT 1043 Extraction Unit (FOSS Tecator AB) according to AOAC No. 39.1.08 (6).

Free amino acid analysis

A "EZ: Faast GC-MS for free amino acid analysis" kit purchased from Phenomenex (EZ: Faast TM KGO-7166, Phenomenex®, Torrance, CA, USA) which contained all amino acid standards and reagents used in the SPE (Solid Phase Extraction) and derivatization steps was used to analyze the free amino acids. Sample pretreatment was required before using the kit. Briefly, two grams of fresh sufu was manually grinded and sonicated for 30 min with 10 mL of distilled water in a capped plastic bottle in order to dissolve the free amino acid in water (7). The sample was centrifuged at 16000×*g*, 4°C for 15 min after 5 mL of water was added. Supernatant was filtered through a 0.45-μm pore size HA membrane filter (Millipore DURAPORE® Cat No.: HVLP-02500, 0.45 μm HV, Millipore Ireland, Cork, Ireland), collected and stored at -70°C before further analysis (8).

Free amino acid gas chromatography-mass spectrometry (GC-MS) conditions

The derivatized free amino acids present in the standards and samples were separated and identified by the GC-MS. A 10 m×0.25 mm Zebron TM ZB-AAA gas chromatography column comes with the EZ: Faast TM was installed in the GC. The helium carrier gas was set at a constant flow of 1.1 mL/min. Temperature of GC oven increased from 110°C to 320°C at the rate of 30°C/min. Injection port temperature was 250°C with split ratio of 15:1. MS temperatures were set to 240°C for ion source, 180°C for the quadrupole and 130°C for MSD auxiliary unit. The scan range was 45～450 m/z. Sample injection volume was 2 μL each time.

Free fatty acid determination

The method was adapted from Deeth et al. (9). Briefly, one gram of fresh sufu was grinded and mixed with five mL of diethyl ether containing 100 μg of each C5:0 and C17:0, 0.1 mL of 4N-H_2SO_4 and 2.5 g of granular anhydrous sodium sulfate. After stood for 1 hr, 5 mL of hexane was added. The mixture was centrifuged at 2000×*g* for 5 min at 4°C and the supernatant passed through twice a Pasteur pipette containing 1 g of neutral alumina oxide (ALOX 90N, 71-077-2.0 NA Chemicals, Art.) (9). Two mL of diisopropyl ether containing 6% of formic acid was added to the alumina followed by centrifugation at 2000×*g* for 5 minutes at room temperature to free the fatty acid (9). The extracts were injected into the GC-MS.

Free fatty acid GC-MS conditions

The free fatty acids present in standards and extracted from sufu samples were analyzed using Agilent GC-MS system as before. The flow rate of helium was set at 1.5 mL/min during the run and at 1.0 mL for the post run. A 30-m HP-5MS (0.25 mm i.d., 0.25 μm df, Agilent Technologists, Santa Clara, CA, USA) column was installed in the GC. Oven temperature program was: 110°C for 3 min, and raised to 310°C at the ramp rate of 35°C /min for 5 min. Temperature of the post run was set to 310°C and held for 8 min. Injection port temperature was 280°C in splitless mode. For the MS, the ion source was set at 230°C, quadrupole was at 150°C and the MSD auxiliary unit was held at 310°C. The scan range was set to 70～500 m/z (3.5 scans/s). Sample injection volume was 2 μL.

Compound identification and quantification

The presence of each amino acid and fatty acid were confirmed by their retention time and mass spectrum with that of the authentic standard. For quantification, 3-point calibration curve was derived for each authentic standard.

Texture profile analysis (TPA)

In this investigation, one sufu cube randomly picked from the aging sample jars was placed on the base-plate of TA-XTi Texture Analyzer (Stable Micro Systems, Surrey, England). Each sample was compressed by an alumina cylinder probe to 30% of its original height. The parameters of the instrument were set as follows: pretest speed at 1.0 mm/sec, test speed at 1.0 mm/sec, post- test speed at 1.0 mm/sec, 5 sec of delay between the two bites, trigger force at 5.0 g and data acquisition rate at 200 pps. The data was analyzed using Texture Expert Version 1.22 Software (Stable Micro Systems) to measure hardness, cohesiveness, adhesiveness, springiness and gumminess as described by Bourne (10). All experiments were performed in triplicate.

Statistical analysis

Concentrations of compounds from the three samples were analyzed by one-way analysis of variance (ANOVA) and compared by the Tukey test at $p<0.05$ level of significance using SPSS 10.0 (SPSS Inc., Chicago, IL, USA). Concentrations of compounds from both fresh and deep-fat fried samples were analyzed by student *t*-test at p=0.05 level of significance.

RESULTS AND DISCUSSION

Crude protein and fat profiles of the sixteen different commercial brands were compared by one-way ANOVA and found out that at significant level of $p<0.05$, there was evidence that different brands of sufu were inconsistent. The mean protein and fat content (%, dry wt basis) in the sufus were 35±1 and 27±1, respectively. Products from southern China had higher protein content (S. China> N. China> Taiwan> Hong Kong: 36%> 35% > 34%> 33%). For fat content, products from Taiwan (35%) had the highest quantity followed by Hong Kong (28%), S. China (26%) and N. China (23%) (Table 1).

Texture profiles of the sixteen different commercial brands and their one-way ANOVA results (Table 1) showed that hardness, cohesiveness, gumminess and chewiness of the sixteen commercial brands were statistically the same from one brand to another, respectively at significant level of 0.05. For adhesiveness, only brand L and brand T were different from brand Q, while for springiness, only brand I was not the same as brand N. Overall the texture profiles were quite similar for the sixteen brands of sufu. As shown in the texture profile analysis of Table 1, the mean values for the hardness, cohesiveness, adhesiveness, springiness, gumminess and chewiness were 254±47 g, 0.4±0.0, -75±12 g, 0.7±0.0, 85±20 g and 59±17 g, respectively. Among the different regions, products from both N. China and Taiwan had the hardest of over 300 g. Products from Hong Kong (182 g) were the softest compared with that from S. China (236 g). Cohesiveness was similar among products from N. and S. China and Hong Kong (0.4). Products of Taiwan also had cohesiveness of 0.4. For adhesiveness, products from Hong Kong and Taiwan were similar (-82 g) and were weaker than that from N. China (-74 g) and S. China (-71 g). For springiness, product of Taiwan has the highest value of 0.8 while the rest of the products were slightly lower. Highest and lowest gumminess were found in products from N. China (128 g) and Hong Kong (62 g). Texture profiles were relatively stable among the different commercial sufus. It might be because the texture properties were basically affected by the composition of protein, fat and water etc. (11). After the hydrolysis of protein and fat diminished, the major chemical activities might have taken place for the small molecules, such as the catabolism of free amino acids and free fatty acids, namely the deamination, transamination and decarboxylation of amino acids (12,13), the esterification and microbial β-oxidation of fatty acids (14). Although the amounts of small molecules determined might be different resulted from the above activities, the amount ratio (protein-fat-water-etc.) might be quite stable in the maturated sufu after

Table 1. Crude protein, fat and texture profiles of different sufu brands

	Crude protein (% dry weight±SE)	Crude fat (% dry weight±SE)	Texture profile (mean±SE)					
			Hardness/g	Cohesiveness	Adhesiveness/gs	Springiness	Gumminess/g	Chewiness/g
D	38±1^{ac}	25±2^{acdf}	357±7	0.4±0.0	-83±9	0.7±0.0	125±9	91±7
E	34±1	22±1^{cf}	493±243	0.5±0.1	-67±8	0.7±0.1	220±132	177±130
F	38±1^{c}	24±2^{adf}	306±47	0.4±0.0	-72±9	0.7±0.1	95±20	66±23
H	33±1^{be}	24±1.2^{adf}	218±13	0.4±0.0	-83±7	0.7±0.1	69±6	47±8
I	34±1	33±1^{b}	387±130	0.4±0.1	-105±5	0.6±0.1^{a}	115±35	64±15
J	35±1	28±1^{dg}	131±9	0.4±0.0	-55±5	0.7±0.0	47±3	34±3
K	35±0^{acde}	26±0^{adf}	206±10	0.4±0.0	-84±12	0.6±0.0	72±8	44±6
L	37±0^{acd}	26±1^{defg}	300±33	0.4±0.0	-50±12^{a}	0.6±0.1	87±10	55±11
M	34±1	25±0^{adf}	204±22	0.5±0.0	-54±3	0.7±0.0	73±2	49±2
N	33±1^{bde}	36±0^{b}	223±10	0.4±0.0	-58±7	1±0^{b}	61±7	61±9
O	35±1^{acde}	28±2de	158±33	0.4±0.0	-61±11	0.6±0.1	50±12	32±9
P	37±1^{acd}	27±1^{defg}	256±66	0.4±0.0	-75±26	0.6±0.0	81±19	51±14
Q	36±1^{acde}	23±1^{adf}	283±39	0.4±0.0	-137±43^{b}	0.7±0.1	84±13	57±8
R	31±1^{b}	32±1^{beg}	177±23	0.4±0.0	-67±4	0.6±0.0	60±9	38±7
S	34±0^{ab}	27±1^{defg}	163±27	0.4±0.0	-94±18	0.7±0.0	53±9	37±6
T	36±0^{acde}	25±1^{adg}	199±45	0.4±0.0	-52±8^{a}	0.7±0.0	68±19	46±12
Mean	35	27	254	0.4	-75	0.7	85	59

Data are presented as mean±SE for 3 replicates. The values with different lowercase letters differ with each other at the significant level of $p<0.05$. The mean values with no lowercase letter mean that this property in this brand is nearly the same as that in all other brands.

complete hydrolysis. If the initial content of protein in soybean was close in different brands, under similar production conditions, the final amount ratio of protein-fat-water-etc. should be quite consistent, making the texture profile uniform. It is also implied that hydrolysis took only a short period in aging. After that, hydrolysis was nearly done and other chemical activities play more important role. Aroma compounds such as flavor esters transformed from fatty acids were mainly formed in this post-hydrolysis period.

The corresponding mass charge fragment ions of the derivatized common free amino acids in standards, used to confirm the identification of the amino acid peaks and quantify their amounts, were listed in Table 2. Thirty-three standard amino acids in total, including the internal standard, were positively separated in 8 min. The results of every single free amino acid in all sixteen different brands of commercial samples were listed in Table 3. Twenty-one free amino acids in total were positively identified and quantified. The total free amino acid contents ranged from 100 nmol/g in brand I to 194 nmol/g in brand N among 16 brands. Among the sixteen brands, ALA, GLY, ABA, VAL, LEU, aILE, ASP, GLU and LYS were higher in amount than others, while the amount of ALA was especially the highest. The mean total amount of free amino acids was 139 nmole/g fresh sample. Among the regions, products of Taiwan had the highest free amino acid contents (147 nmole/g fresh sample) and were followed by S. China (142), Hong Kong (141) and N. China (123) (Tables 2 and 3).

The corresponding mass fragment ions of the derivatized common free fatty acids in standards were listed in Table 4. Thirty fatty acid standards in total (including the internal standard) were positively separated in 10 min. Results of free fatty acids in all sixteen different brands of commercial samples were listed in Table 5. Ten free fatty acids in total positively identified and quantified in all sixteen different commercial sufu samples. The total free fatty acid contents ranged from 299 mg/100 g in brand E to 1203 mg/100 g in brand L. The mean total amount of free fatty acids was 747 mg/100 g of fresh sample. Among the sixteen brands, fatty acids $C_{18:2(n-6)}$, $C_{18:1(n-9)}$, $C_{16:0}$, $C_{18:0}$ and $C_{18:3}$ were higher in amount than other fatty acids, while that of

Table 2. Retention times, elution order, names, abbreviations and ion fragments of the 33 amino acid standards

Rt (min)	Elution order	Chemical name	Abbreviation	Mass fragment ions
1.19	1	Alanine	ALA	130, 88
1.26	2	Sarcosine	SAR	130, 217
1.30	3	Glycine	GLY	116, 207
1.40	4	α-Aminobutyric acid	ABA	144, 102
1.50	5	Valine	VAL	158, 116
1.57	6	β-Aminoisobutyric acid	β-AiB	116, 158
1.63	7	Norvaline (internal standard)	NORV (I.S.)	158, 72
1.71	8	Leucine	LEU	172, 86
1.74	9	allo-Isoleucine	aILE	172, 130
1.81	10	Isoleucine	ILE	172, 130, 69
1.98	11	Threonine	THR	101, 160
2.03	12	Serine	SER	203, 146
2.09	13	Proline	PRO	156, 243
2.19	14	Asparagine	ASN	69, 155
2.55	15	Thioproline	TPR	88, 174, 147
2.76	16	Aspartic acid	ASP	216, 130
2.78	17	Methionine	MET	203, 277
2.93	18	4-Hydroxyproline	4HYP	172, 86
3.12	19	Glutamic acid	GLU	84, 230, 170
3.14	20	Phenylalanine	PHE	148, 206, 190
3.43	21	α-Aminoadipic acid	AAA	98, 244
3.70	22	α-Aminopimelic acid	APA	198, 258, 286
3.77	23	Glutamine	GLN	84, 187
4.17	24	Ornithine	ORN	156, 70
4.21	25	Glycyl-proline	GPR	70, 300
4.44	26	Lysine	LYS	170, 128
4.63	27	Histidine	HIS	81, 282, 168
4.80	28	Hydroxylysine	HLY	129, 169
4.91	29	Tyrosine	TYR	107, 206
5.14	30	Proline-hydroxyproline	PHP	156, 184
5.20	31	Tryptophan	TRP	130
5.70	32	Cystathionine	CTH	203, 272
5.92	33	Cystine	C-C	174, 248, 216

Table 3. Free amino acid contents of sufu brands

Brand	Free amino acid concentration (nmol/g fresh weight of sufu) (mean±SE for 3 replicates)										
	ALA	GLY	ABA	VAL	LEU	aILE	PRO	ASN	ASP	MET	GLU
D	29±1	13±2	4.4±0.7^{ad}	14±1	22±0	13±0^{acd}	1±0^{a}	0.1±0.0^{ac}	3.9±0.6^{a}	2.3±0.2	5.9±0.0^{ae}
E	26±1	13±0	8.8±0.2^{be}	13±0	20±1	11±0	2.1±0.8^{a}	0.5±0.2^{c}	1.8±0.2^{a}	1.6±0.5	3.8±0.9^{ae}
F	12±0^{ad}	18±1^{a}	10±1^{be}	12±2	18±4	11±2	2.5±1.2^{ac}	0.03±0.00^{a}	6.4±0.2^{a}	1.9±0.4	2.7±0.0^{a}
H	36±2^{bc}	17±1^{a}	10±0^{be}	15±1^{ac}	21±0	12±0^{acd}	1.5±0.5^{a}	0.02±0.00^{a}	3.5±0.3^{a}	2.4±0.0	5.9±0.2^{ae}
I	12±1^{ad}	10±1	0.4±0.0^{ac}	7±1^{b}	16±1^{ac}	6±1^{bd}	5.9±0.0^{b}	0.1±0.0^{ac}	9.4±1.9	1.5±0.1	15±2^{b}
J	20±7^{ace}	12±2	7.1±1.2^{de}	10±2^{bc}	14±2^{a}	9±1^{bcd}	5.8±0.9^{b}	0.1±0.0^{a}	9±2	1.3±0.3^{a}	1.9±0.1^{a}
K	32±3^{bc}	15±2	10±1^{be}	14±2	21±2	12±1^{acd}	0.8±0.3^{a}	0.1±0.0^{ac}	6±2^{a}	2.2±0.2	4.4±1.0^{ae}
L	32±1^{bc}	13±0	10±0^{be}	14±1	20±1	13±1^{acd}	0.9±0.3^{a}	0.2±0.0^{ac}	7±2^{a}	2±0	5.6±1.3^{ae}
M	14±1^{cd}	13±2	2±0^{ac}	11±1	16±1^{ac}	10±1^{d}	5.4±0.6^{bc}	0.16±0.05^{ac}	17±4^{b}	1.5±0.1	2.1±0.0^{a}
N	16±1^{cd}	18±2^{a}	0.2±0.0^{c}	13±2	19±2	12±1	1.2±0.0^{a}	0.1±0.0^{ac}	17±3^{b}	1.8±0.0	75±3^{c}
O	40±2^{b}	15±1	12±1^{b}	18±2^{a}	27±2^{bc}	16±2^{a}	0.8±0.2^{a}	0.2±0.0^{ac}	7±1	2.8±0.2^{b}	6.3±0.4^{ae}
P	34±7^{be}	15±3	10±2^{be}	16±3^{ac}	26±3^{c}	15±2^{ad}	2.1±0.6^{a}	0.9±0.2^{b}	4.3±0.6^{a}	2.8±0.4^{b}	20±0^{b}
Q	37±2^{be}	11±0	12±1^{b}	16±1^{ac}	24±1	14±1^{acd}	1±0^{a}	0.1±0.0^{ac}	8.7±1.5	2.6±0.3	37±0^{d}
R	35±3^{be}	14±3	11±1^{be}	16±2^{ac}	23±2	14±1^{acd}	0.8±0.3^{a}	0.09±0.05^{a}	6±0^{a}	2.4±0.3	6.1±0.8^{ae}
S	39±3^{b}	8±0^{b}	11±1^{b}	15±1^{ac}	23±2	13±1^{acd}	1.7±0.6^{a}	0.05±0.01^{a}	4.4±0.5^{a}	2.1±0.0	9±1^{e}
T	34±3^{be}	17±1^{a}	11±0^{b}	16±0^{ac}	24±1	14±0^{acd}	0.7±0.0^{a}	0.1±0.0^{ac}	7±0^{a}	2.7±0.1	7±1de
Mean	28	14	8	14	21	12	2.1	0.18	7.4	2.1	13

	PHE	AAA	GLN	ORN	LYS	HIS	TYR	PHP	TRP	C-C	Total FAA
D	3.0±0.1	0.27±0.00^{ac}	1.0±0.1	1.7±0.0^{a}	7.0±0.7	0.4±0.1	0.3±0.0^{ac}	1.3±0.0	0.7±0.1^{ac}	0.8±0.0	127^{ac}
E	2.5±0.4	0.26±0.01^{ac}	0.8±0.0^{a}	1.9±0.2^{a}	5.3±2.0	0.5±0.1	0.2±0.0^{a}	1.3±0.1	0.5±0.0^{cf}	0.9±0.0	116^{ae}
F	2.7±0.3	0.25±0.01^{a}	0.8±0.0^{a}	1.6±0.0^{a}	6.5±1.2	0.4±0.0	0.8±0.1^{acd}	1.4±0.1	0.7±0.1^{cd}	0.8±0.1	111^{ae}
H	2.9±0.0	0.27±0.01^{ac}	0.7±0.0^{a}	2.0±0.3^{a}	7.7±0.1	0.4±0.0	0.5±0.1^{ac}	1.3±0.1	0.6±0.1^{ce}	0.9±0.0	142
I	2.6±0.1	0.24±0.00^{a}	0.7±0.0^{a}	2.8±0.2^{a}	4.4±0.3	0.4±0.0	3.0±0.1^{e}	1.3±0.0	1.1±0.1^{abde}	0.7±0.0	100^{a}
J	2.4±0.2^{a}	0.25±0.00^{a}	0.8±0.0^{a}	2.0±0.2^{a}	5.5±0.9	0.5±0.1	1.4±0.3^{bdf}	1.3±0.0	1.1±0.1^{abd}	0.7±0.0	105^{ae}
K	2.9±0.2	0.26±0.00^{ac}	0.8±0.1^{a}	1.3±0.2^{a}	7.4±0.9	0.5±0.1	0.9±0.3^{acd}	1.3±0.0	0.4±0.0^{c}	0.9±0.1	133^{ace}
L	2.8±0.0	0.29±0.01^{ac}	0.8±0.0^{a}	1.3±0.3^{a}	7.0±0.2	0.5±0.1	0.3±0.0^{ac}	1.3±0.0	0.5±0.1^{cf}	0.8±0.0	133^{ac}
M	2.5±0.1	0.25±0.00^{a}	0.8±0.1^{a}	1.4±0.1^{a}	6.2±0.5	0.5±0.0	0.3±0.1^{ac}	1.3±0.0	1.3±0.1^{b}	0.7±0.0	109^{ae}
N	2.8±0.1	0.24±0.00^{a}	1±0	5.4±1.6^{b}	6.3±0.5	0.4±0.0	2.1±0.1^{eg}	1.3±0.1	1.2±0.1^{ab}	0.7±0.0	194^{bd}
O	3.2±0.2	0.42±0.01^{b}	0.8±0.0^{a}	1.5±0.4^{a}	8.1±0.8	0.3±0.0	0.5±0.0^{acf}	1.4±0.1	0.6±0.1^{ce}	0.9±0.1	164^{ade}
P	3.4±0.3^{b}	0.26±0.00^{a}	1.3±0.2^{b}	1.9±0.2^{a}	8.0±1.6	0.4±0.1	0.5±0.1^{acf}	1.4±0.1	1.0±0.1^{abdef}	0.9±0.1	165^{ade}
Q	3.1±0.1	0.36±0.06^{bc}	0.8±0.0^{a}	1.6±0.0^{a}	7.2±0.8	0.4±0.0	1.4±0.4^{bdg}	1.5±0.1	0.7±0.1^{cd}	0.8±0.1	181^{cd}
R	3.0±0.1	0.29±0.03^{ac}	0.7±0.0^{a}	1.6±0.3^{a}	7.3±1.3	0.4±0.0	1.0±0.2^{acd}	1.4±0.1	0.6±0.0^{ce}	0.8±0.0	146
S	3.0±0.1	0.26±0.01^{a}	0.8±0.0^{a}	1.2±0.2^{a}	7.8±0.4	0.4±0.0	1.1±0.2^{cd}	1.3±0.0	0.8±0.1^{ac}	0.8±0.0	144
T	3.1±0.1	0.31±0.01^{ac}	0.7±0.0^{a}	1.7±0.1^{a}	8.2±0.2	0.5±0.1	1.0±0.1^{acd}	1.5±0.0	0.8±0.2^{ac}	0.9±0.0	152
Mean	2.9	0.28	0.8	1.9	6.9	0.4	1.0	1.4	0.8	0.8	139

Data are presented as mean±SE for 3 replicates. The values with different superscript letters differ with each other at the significant level of $p<0.05$. The mean values with no superscript letter mean that this amino acid in this brand is nearly the same as that in all other brands.

$C_{18:2(n-6)}$ was especially the highest in all fatty acids. Products from S. China had the highest free fatty acid contents (830 mg/100 g fresh sample) and were followed by Hong Kong (706). Products from N. China and Taiwan were comparable in the free fatty acids content at 636 and 639 mg/100 g fresh sample, respectively (Tables 4 and 5). Especially, $C_{18:2(n-6)}$, $C_{18:3}$ and $C_{18:1(n-9)}$, which were found in relatively higher amount, were not only the essential fatty acids, but also the precursors for the desired flavor of sufu. Chung (12) found numerous special esters in volatile components of commercial sufus. Those esters were converted from the above three fatty acids esterified with alcohol and might contribute sour, meaty, coconut-like and sweaty flavor to sufu odor (12,15). It is believed that, if more of these desired fatty acids are released during fermentation, the taste and odor of sufu will be much more attractive.

To date, there are no uniform criteria existing for determining whether the quality and maturation of sufu is good or not in the sufu industry for its production. Although the basic production procedures of sufu are the same, the production method, raw materials, production seasons and the fermentation period usually vary from one manufacture to another. Thus, any deviation in the process of production may result in very different products. It is hoped that the information found in this investigation is able to provide some important quality reference ranges for product developers and manufacturers to optimize and produce their fermented soybean curds.

Table 4. Retention times, elution order, names, abbreviations and ion fragments of the 30 free fatty acids

Rt (min)	Elution order	Chemical name	Abbreviation	Mass fragment ions
1.42	1	Butanoic acid	$C_{4:0}$	73, 88
1.65	2	n-Valeric acid	$C_{5:0}$ (I.S.)	73, 87, 93
2.07	3	Hexanoic acid	$C_{6:0}$	73, 87, 80
2.83	4	Hexanoic acid	$C_{7:0}$	87, 101
3.85	5	Octanoic acid	$C_{8:0}$	73, 101
4.64	6	Nonanoic acid	$C_{9:0}$	73, 115
5.27	7	Decanoic acid	$C_{10:0}$	73, 129, 143
5.78	8	Undecanoic acid	$C_{11:0}$	73, 129, 85
6.22	9	Dodecanoic acid	$C_{12:0}$	73, 129, 157
6.60	10	Tridecanoic acid	$C_{13:0}$	73, 129, 171
6.95	11	Tetradecanoic acid	$C_{14:0}$	73, 129, 185
7.27	12	Pentadecanoic acid	$C_{15:0}$	73, 129, 199
7.53	13	cis-9-Hexadecenoic acid	$C_{16:1}$	83, 97, 70
7.58	14	Hexadecanoic acid	$C_{16:0}$	73, 129, 256
7.86	15	Heptadecanoic acid	$C_{17:0}$ (I.S.)	73, 129, 270
8.08	16	Linoleic acid	$C_{18:2(n-9)}$	81, 95, 109
8.09	17	Oleic acid	$C_{18:1(n-9)}$	83, 97, 111
8.10	18	Linolenic acid	$C_{18:3}$	79, 93, 108
8.13	19	Octadecanoic acid	$C_{18:0}$	73, 129
8.39	20	Nonadecanoic acid	$C_{19:0}$	73, 129, 298
8.53	21	Arachidonic acid	$C_{20:4}$	79, 91, 105
8.55	22	cis-5,8,11,14,17-Eicosapentaenoic acid	$C_{20:5}$	79, 91, 105
8.64	23	Arachidic acid	$C_{20:0}$	73, 129, 312
8.88	24	Heneicosanoic acid	$C_{21:0}$	73, 326, 129
9.02	25	cis-4,7,10,13,16,19-Docosahexaenoic acid	$C_{22:6}$	79, 91, 105
9.09	26	Erucic acid	$C_{22:1}$	83, 97, 111
9.14	27	Docosanoic acid	$C_{22:0}$	73, 340, 129
9.40	28	Tricosanoic acid	$C_{23:0}$	73, 129, 354
9.65	29	Nervonic acid	$C_{24:1}$	83, 97, 111
9.70	30	Tetracosanoic acid	$C_{24:0}$	73, 368, 129

Table 5. Free fatty acid contents of different sufu brands

Brand	Free fatty acid concentration (mg/100 g fresh weight of sufu) (mean±SE for 3 replicates)										
	$C_{4:0}$	$C_{8:0}$	$C_{14:0}$	$C_{16:1}$	$C_{16:0}$	$C_{18:2(n-6)}$	$C_{18:1(n-9)}$	$C_{18:3}$	$C_{18:0}$	$C_{20:0}$	Total FFA
D	0.7 ± 0.1^{a}	0.9 ± 0.1	0.7 ± 0.0	0.7 ± 0.0	70 ± 3^{ac}	489 ± 10^{acef}	78 ± 6^{ac}	6 ± 0^{a}	35 ± 5^{ade}	1.5 ± 0.2	683^{ac}
E	0.9 ± 0.2^{a}	0.8 ± 0.0^{a}	0.6 ± 0.1	0.6 ± 0.1	51 ± 0^{a}	172 ± 3^{b}	52 ± 8^{a}	5 ± 0^{a}	16 ± 0^{ce}	1.0 ± 0.1^{a}	299^{c}
F	0.6 ± 0.1^{a}	0.8 ± 0.1	0.8 ± 0.1	0.7 ± 0.0	96 ± 0	391 ± 6^{cfi}	133 ± 9	8 ± 0	41 ± 6	1.8 ± 0.0	674^{ad}
H	2.3 ± 0.2^{ac}	0.9 ± 0.1	1.1 ± 0.2	0.9 ± 0.1	165 ± 30^{c}	558 ± 13^{cdef}	138 ± 23	11 ± 2	56 ± 5^{bd}	1.8 ± 0.1	936^{ab}
I	1.5 ± 0.1^{a}	0.9 ± 0.0	1.2 ± 0.3	0.9 ± 0.2	88 ± 6^{ac}	312 ± 12^{cfgh}	113 ± 7	7 ± 1^{ac}	34 ± 3^{ade}	1.6 ± 0.1	560^{ac}
J	1.7 ± 0.4^{a}	0.8 ± 0.0^{a}	0.6 ± 0.1	0.8 ± 0.1	57 ± 4^{a}	690 ± 13^{di}	126 ± 28	9 ± 1	25 ± 4^{ef}	1.9 ± 0.4	913^{ab}
K	1.3 ± 0.1^{a}	0.9 ± 0.1	0.8 ± 0.0	0.7 ± 0.1	93 ± 0	243 ± 33^{bghj}	83 ± 11^{ac}	6 ± 0^{a}	54 ± 0^{bd}	2.0 ± 0.4	484^{cd}
L	4.8 ± 0.3^{a}	1.3 ± 0.0^{b}	1.1 ± 0.1	0.8 ± 0.0	190 ± 18^{b}	726 ± 19^{di}	206 ± 12^{cd}	15 ± 2^{b}	60 ± 7^{bd}	2.4 ± 0.2^{b}	1203^{b}
M	2.3 ± 0.3^{ac}	0.9 ± 0.0^{a}	1.1 ± 0.1	0.8 ± 0.0	162 ± 45^{c}	568 ± 65^{cdefi}	194 ± 38	14 ± 2^{bd}	52 ± 9^{bd}	1.7 ± 0.1	997^{ab}
N	1.7 ± 0.1^{ac}	0.9 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	101 ± 9	357 ± 45^{abghj}	193 ± 48	8 ± 2^{acd}	53 ± 6^{bd}	1.7 ± 0.2	718^{ad}
O	4.2 ± 0.2^{bc}	0.9 ± 0.1	1.1 ± 0.2	0.8 ± 0.0	117 ± 12	632 ± 13^{edi}	155 ± 18	6 ± 0^{a}	51 ± 6^{bdf}	1.9 ± 0.3	968^{ab}
P	0.8 ± 0.1^{a}	0.9 ± 0.0	0.8 ± 0.0	0.7 ± 0.1	99 ± 28	395 ± 46^{cfg}	111 ± 7	9 ± 2	32 ± 0^{ade}	1.4 ± 0.1	649^{ac}
Q	1.8 ± 0.4^{a}	0.8 ± 0.0^{a}	0.7 ± 0.0	0.7 ± 0.0	84 ± 18^{ac}	278 ± 54^{bghj}	90 ± 22	8 ± 1^{acd}	31 ± 1^{ade}	1.4 ± 0.1	497^{cd}
R	1.6 ± 0.3^{a}	1.1 ± 0.3	0.8 ± 0.2	0.7 ± 0.1	62 ± 6^{a}	285 ± 11^{bghj}	76 ± 12^{ac}	6 ± 0^{a}	32 ± 3^{ade}	1.5 ± 0.3	467^{cd}
S	2.1 ± 0.4^{a}	0.9 ± 0.0^{a}	1.1 ± 0.2	1.0 ± 0.2	134 ± 24	716 ± 6^{d}	233 ± 6^{bd}	13 ± 2^{bc}	65 ± 1^{b}	2.0 ± 2.0	1168^{b}
T	5.1 ± 1.1^{b}	0.9 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	107 ± 2	425 ± 26^{fj}	149 ± 0	9 ± 1	34 ± 4^{ade}	1.4 ± 0.1	732^{ad}
Mean	2.1	0.9	0.9	0.8	105	452	133	9	42	1.7	747

Data are presented as mean±SE for 3 replicates. The values with different superscript letters differ with each other at the significant level of $p<0.05$. The mean values with no superscript letter mean that this fatty acid in this brand is nearly the same as that in all other brands.

ABBREVIATIONS

TPA, texture profile analysis; SD, standard; SE, standard error; FAA, free amino acid; FFA, free fatty acid; SPE, solid phase extraction; GC/MS, gas chromatography-mass spectrometry; MSD, mass spectrometry detector.

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(Received February 22, 2011; Accepted March 8, 2011)