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Survey of nutrients and quality assessment of crab paste by ^{1}H NMR spectroscopy and multivariate data analysis

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Crab paste is a traditional and popular food for people in the coastal area of China. Visual inspection is currently the only method of quality assessment. We developed a new analytical method of a comprehensive survey of nutrients and quality assessment for crab paste based on a combination of ^{1}H NMR spectroscopy with multivariate data analysis. The aqueous extract of crab paste was dominated by amino acids, sugars, carboxylic acids, nucleotides and amines (including 19 first-time-reported compounds such as choline, uracil and guanosine). Two grades of crab paste had significant compositional differences in terms of amino acids, lactate, *N*-acetylglutamate, choline, dimethylamine, uridine, 1-methylnicotinamide and 2-pyridienmethanol. These results provided important information on the grade-dependence of crab-paste composition, and demonstrated that NMR-MDA was effective not only for the comprehensive survey of nutrients, but also for quality assessment of crab paste.

crab paste, *Portunus trituberculatus*, composition, nuclear magnetic resonance (NMR), multivariate data analysis (MDA), quality assessment

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Crab paste is a traditional and popular food in the coastal area of China. It is a processed product of the swimming crab *Portunus trituberculatus*. This is achieved by saturation with salt and liquor, which maintains the characteristic flavor, taste, and nutrients of the raw materials [1]. The nutrient analysis has revealed that the swimming crab is rich in proteins with complete amino acids, unsaturated fatty acid and inorganic elements [2]. However, a quality standard of crab paste based on the nutritional analysis has not been developed. There is very scarce information on the nutrient profiles, which determine the taste, appearance, and nutritional quality. This is because comprehensive analysis of the chemical compositions of seafood products is difficult because of the complexity of the matrix and limited analytical capacity of conventional chemistry methods. In fact, the current method to classify the quality grades of crab paste is dependent mainly on visual inspection.

To develop a more accurate, rapid and reliable analytical method to assess seafood quality, we think nuclear magnetic resonance (NMR) spectroscopy is suitable as an effective non-invasive analytical tool that can provide comprehensive information on the profiles of organic chemical metabolites. More recently, a combination of ^{1}H NMR spectroscopy with multivariate data analyses has been established to analyze the chemical components of food [3]. NMR technique is intrinsically reproducible with rich information about structure and rapidity without or simple preparation of complex samples. Thus, it can be used to meet the increasing demands of analyzing complex samples such as food with good accuracy and consistency based on the entire chemical composition of the samples [4,5]. Multivariate data analytical techniques such as orthogonal projection to latent structure discriminant analysis (OPLS-DA), a supervised multivariate data analytical tool, can be used to provide group data clus-

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tering by analyzing NMR-derived data [6]. Such technique combinations have been applied successfully to assess the quality of orange juice [7], beer [8], instant coffee [9], green tea [10], pine-mushroom [11], Cheonggukjang [12], soy sauce [13], milk [14], wine [15], beef [16] and honey [17].

In the present study, we systematically analyzed the chemical compositions of crab paste using high-resolution NMR spectroscopy coupled with multivariate data analyses. The study objectives were to: (1) validate the potential of a metabolomic approach in the survey of nutrients of marine products; (2) investigate all of the detectable components in aqueous extracts from crab paste; and (3) define the statistically significant metabolomic features that could be used to differentiate between two grades of crab paste.

1 Materials and methods

Acetonitrile, $NaH_2PO_4 \cdot 2H_2O$ and $K_2HPO_4 \cdot 3H_2O$ were purchased from Guoyao Chemical Co. Ltd. (Shanghai, China), and all were of analytical grade. D_2O (99.9%) and sodium 3-trimethylsilyl [2,2,3,3-d_4] propionate (TSP) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Phosphate buffer (K_2HPO_4/NaH_2PO_4, 0.1 mol L^{-1}, pH 7.4), containing 10% D_2O (v/v) and 0.005% TSP (w/v), was prepared in H_2O [18].

Grade-3A and grade-2A samples of crab (*Portunus trituberculatus*) paste (six bottles in each group) were purchased from a supermarket in Ningbo, China. According to the ingredients marked on the packaging, sucrose and glutamate, as food additives, were supplemented into crab paste. All samples were stored at −20°C for subsequent analyses during the shelf-life.

Raw ground materials of crab paste (500 mg) were extracted twice with 300 μL of acetonitrile and 300 μL of phosphate buffer by intermittent sonication on a wet ice bath for 10 min (3-s sonication with a 3-s break). After centrifugation for 10 min at 12000 r/min and 4°C, the combined supernatants were lyophilized after removing acetonitrile *in vacuo*. Each of the aqueous extracts was reconstituted into 600 μL phosphate buffer. After centrifugation for 10 min at 12000 r/min and 4°C, 550 μL of the supernatant of each extract was pipetted into an NMR tube (ST500-7; outer diameter, 5 mm; Norell, Landisville, NJ, USA) for NMR analyses.

^{1}H NMR spectra of crab paste extracts were recorded at 298 K on a Bruker Avance III 600-MHz Spectrometer (operating at 600.13 MHz for ^{1}H), equipped with an inverse detection cryogenic probe (Bruker BioSpin, Berlin, Germany). For each sample, a standard one-dimensional (1D) NMR experiment with water pre-saturation was carried out using the first increment of a nuclear Overhauser effect spectroscopy (NOESY) pulse sequence (recycle delay (RD) -90°-t_1-90°- t_m-90°-acquisition) with recycle delay of 2 s, t_1 of 3 μs and a mixing time (t_m) of 100 ms. Typically, a 90° pulse was set to ~10 μs and water suppression achieved with weak continuous-wave irradiation during the RD and t_m. D_2O and TSP provided a field frequency lock and chemical shift reference (^{1}H, δ 0.00), respectively. Sixty-four transients were collected into 32k data points for each spectrum with a spectral width of 20 ppm.

All free induction decays were multiplied by an exponential function with a 1-Hz line-broadening function before Fourier transformation. Spectra were referenced to TSP at 0.00 ppm. To facilitate NMR signal assignments, a range of two-dimensional (2D) NMR spectra were acquired and processed as described previously [18,19] for selected samples, including ^{1}H-^{1}H COSY, ^{1}H-^{1}H TOCSY, ^{1}H J-resolved, ^{1}H-^{13}C HSQC, and ^{1}H-^{13}C HMBC.

After corrections for phase and baseline distortion (Bruker BioSpin), ^{1}H NMR spectral regions δ 9.20 − 0.83 were integrated into regions with an equal width of 0.004 ppm (2.4 Hz) using the AMIX software package (V3.8, Bruker BioSpin). The region δ5.2–4.7 was removed to avoid the effects of imperfect suppression of water. Each bucketed region was normalized to the total sum of the spectral integrals to compensate for the overall concentration differences before statistical data analysis.

Multivariate data analyses were carried out with SIMCA-P$^+$ software (V11.0; Umetrics, Stockholm, Sweden). Principal component analysis (PCA) was carried out with mean-centered data to generate an overview. Subsequently, OPLS-DA was done with unit variance scaling to further analyze the ^{1}H NMR spectral data [20,21]. OPLS-DA results were visualized in the forms of the scores and loading plots. Each point in the scores represented each bottle of crab paste. Interpretation of the OPLS-DA model was facilitated by back-scaled transformation of the loadings [6], with incorporated color-coded correlation coefficients (r) of the metabolites responsible for the differentiation. The color plot was generated with MATLAB scripts (http://www.mathworks.com/) with certain in-house modifications. The hot-colored (e.g., red) variables have higher significance (positive/negative) in discriminating between classes than the cold-colored (e.g. blue) variables. In the present study, the absolute value of a correlation coefficient, $|r| > 0.754$ ($r >$ 0.754 and $r < -0.754$) was chosen as the cutoff value for the statistical significance based on the discrimination significance at $P < 0.05$ according to the test for the significance of Pearson's product-moment correlation coefficient. To check the validity of the model and to avoid over-fitting of the PLS model, a sixfold cross-validation method was applied and the cross-validation parameter Q^2 and R^2X calculated [22]. Q^2 indicated the predictability of the model, whereas R^2X represented the total explained variables for the X matrix. A permutation test, an additional cross-validation tool, also was carried out for the OPLS-DA model by randomizing the order of Y variables for a specified number of times (200 permutations). Q^2 in the permutated plot described the predictability and quality of the model, whereas R^2 described how well the data fitted the model. If Q^2 from the

true model was higher than each of Q^2 obtained from permutation model, then the model had high predictive ability [23,24].

It is well-known that the integral of a given ^{1}H NMR signal is linearly proportional to the number of protons which give such a signal. Therefore, the concentration of components was calculated by equating the peak areas of selected NMR signals (least overlapping ones) and a reference compound (i.e. TSP in our case) with known concentration. Although the values of relaxation time, T_1, for components and reference were different, and the concentrations measured here were semi-quantitative, the comparison of component concentration between samples was reasonable because the inter-sample T_1 variations were, in general, small for the same component (or reference). Classical statistical analysis (one-way ANOVA), using SPSS 13.0 software (SPSS, Chicago, IL, USA) with a Tukey post-hoc test, was carried out on the obtained component concentration.

2 Results and discussions

2.1 ^{1}H NMR analyses of extracts of crab paste

Figure 1 shows a representative ^{1}H NMR spectrum of crab-paste extract. The resonances were assigned according to literature data [25,26] and confirmed with a series of 2D NMR experiments. Forty-three components from aqueous crab-paste extract are tabulated in Table 1 together with the corresponding ^{1}H and ^{13}C NMR biochemical shifts as well as signal multiplicities. The spectra showed that, apart from those signals of compounds including most of the amino acids, betaine, trimethylamine-N-oxide and hypoxanthine previously reported in the swimming crab [2,27], more components were observed, for the first time, in the present study. These included lactate, acetate, N-acetylglutamate, succinate, dimethylamine, trimethylamine, asparagine, choline, choline-O-sulfate, phosphorylcholine, glucose, uracil, guanosine, uridine, fumarate, adenosine, formate, 2-pyridinemethanol, and 1-methylnicotinamide.

No typical resonances and coupling patterns from serine, cystine, 3-methylhistidine or α-amino butyric acid were observed, which were not in accordance with previous results in the swimming crab [2,27]. This is probably because of their lower abundances and because the NMR method was less effective for detecting certain low-concentration compounds than MS [19,28]. In fact, these two amino acids were less abundant even in swimming crabs than other amino acids [2]. Thus, levels of serine and cystine may have been too low to be detectable by NMR in our samples.

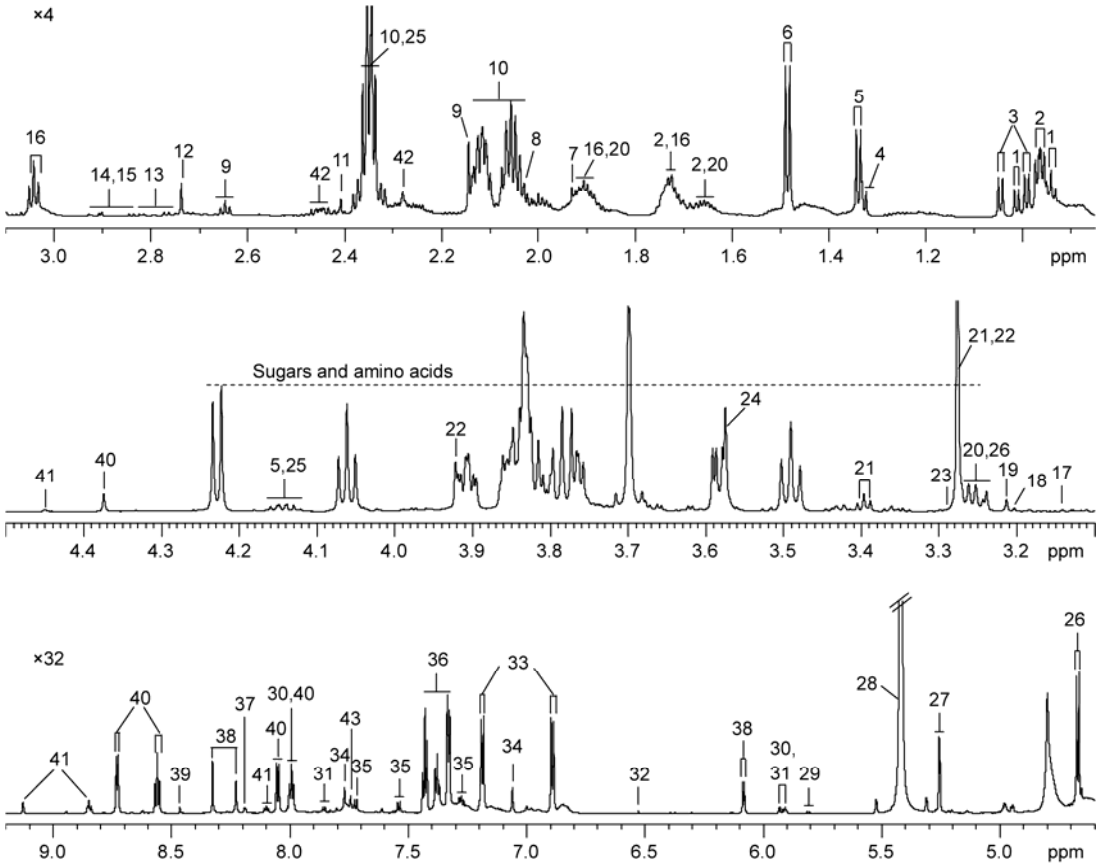

Figure 1 Representative 600-MHz ^{1}H NMR spectrum of crab-paste extract. Compared with region δ3.1–4.5, the spectral regions δ0.9–3.1 and δ4.6–9.2 were vertically expanded for 4 and 32, respectively (see Table 1 for resonance assignment).

Table 1 ^{1}H NMR biochemical shifts and signal assignments of compounds found in crab-paste extract[a)]

No.	Compounds	Assignment	δ^{1}H (multiplicity)	δ^{13}C	Assigned with
1	Isoleucine	δCH_3	0.94(t, 7.3 Hz)	13.8	JRE, COSY, TOCSY, HSQC, HMBC
		$\beta'CH_3$	1.01(d, 7.0 Hz)	17.8	
		γCH_2	1.26(m), 1.45(m)	27.2	JRE, TOCSY, HSQC, HMBC
		βCH	1.98(m)	38.4	JRE, COSY, TOCSY, HSQC, HMBC
		αCH	3.69	62.2	TOCSY, HMBC
2	Leucine	$\delta'CH_3$	0.96(d, 5.9 Hz)	23.6	JRE, COSY, TOCSY, HSQC
		δCH_3	0.97(d, 6.5 Hz)	24.8	
		γCH	1.66(m)	26.7	JRE, COSY, TOCSY, HSQC, HMBC
		βCH_2	1.72(m)	42.4	
		αCH	3.74	56.1	COSY, TOCSY, HMBC
3	Valine	$\gamma'CH_3$	0.99(d, 6.9 Hz)	19.4	JRE, COSY, TOCSY, HSQC, HMBC
		γCH_3	1.04(d, 6.9 Hz)	20.7	
		βCH	2.27(m)	31.7	JRE, COSY, TOCSY, HMBC
		αCH	3.62(d)	63.0	JRE, COSY, TOCSY, HSQC, HMBC
4	Threonine	γCH_3	1.33(d, 7.3 Hz)	22.9	JRE, COSY, TOCSY
		βCH	4.26	68.9	TOCSY, HSQC, HMBC
		αCH	3.58	63.0	COSY, TOCSY, HMBC
5	Lactate	βCH_3	1.34(d, 6.8 Hz)	20.0	JRE, COSY, TOCSY, HSQC
		αCH	4.14(q)	71.0	JRE, COSY, TOCSY, HSQC, HMBC
		COOH		185.0	HMBC
6	Alanine	βCH_3	1.48(d, 6.8 Hz)	19.0	JRE, COSY, TOCSY, HSQC, HMBC
		αCH	3.80	53.2	COSY, TOCSY, HSQC, HMBC
		COOH		178.6	HMBC
7	Acetate	CH_3	1.94(s)	26.2	JRE, HSQC
		COOH		183.9	HMBC
8	N-acetylglutamate	$\delta COOH$		185.4	HMBC
		γCH_2	2.26	35.9	COSY, TOCSY, HSQC, HMBC
		βCH_2	1.98, 1.83	33.3	
		αCH_2	4.06	74.4	
		COOH		181.5	HMBC
		CH_3	2.03(s)	26.4	JRE, HSQC
9	Methionine	δCH_3	2.14(s)	16.7	JRE, COSY, TOCSY, HSQC, HMBC
		γCH_2	2.65(t)	31.5	
		βCH_2	2.15	32.5	COSY, TOCSY, HMBC
		αCH	3.85	56.6	HMBC
10	Glutamate	δCO		183.8	HMBC
		γCH_2	2.36(dt, 3.8, 11.6 Hz)	35.9	JRE, COSY, TOCSY, HSQC, HMBC
		βCH_2	2.05(m), 2.12(m)	29.7	
		αCH	3.77(m)	57.2	
		COOH		177.4	HMBC
11	Succinate	CH_2	2.41(s)	36.3	JRE, HSQC
12	Dimethylamine	CH_3	2.74(s)	37.5	JRE, HSQC, HMBC
13	Aspartate	βCH_2	2.71(dd), 2.81(dd)	39.0	JRE, COSY, TOCSY, HSQC, HMBC
		αCH	3.90	54.7	COSY, TOCSY, HSQC, HMBC
		$\gamma COOH$		180.3	HMBC
		COOH		177.3	HMBC
14	Trimethylamine	CH_3	2.91(s)	47.5	JRE, HSQC, HMBC
15	Asparagine	βCH_2	2.83(dd), 2.91(dd)	38.2	JRE, COSY, TOCSY, HSQC, HMBC
		αCH	3.96	54.1	COSY, TOCSY, HSQC, HMBC

(*To be continued on the next page*)

(*Continued*)

No.	Compounds	Assignment	δ^1H (multiplicity[a)])	δ^{13}C	Assigned with
		COOH		177.1	HMBC
16	Lysine	εCH_2	3.03(t, 7.5 Hz)	41.9	JRE, COSY, TOCSY, HSQC, HMBC
		δCH_2	1.73(m)	29.0	
		γCH_2	1.48(m)	24.0	
		βCH_2	1.91(m)	32.6	
		αCH	3.76	56.1	COSY, TOCSY, HMBC
17	Choline-O-sulfate	$N\text{-}CH_3$	3.14(s)	55.5	JRE, HSQC, HMBC
		βCH_2	3.69	65.9	COSY, TOCSY, HMBC
		αCH_2	4.33	#[b]	COSY, TOCSY
18	Phosphorylcholine	$N\text{-}CH_3$	3.20(s)	54.0	JRE, HSQC, HMBC
		βCH_2	3.57	76.1	COSY, HMBC
		αCH_2	4.24	#	COSY
19	Choline	$N\text{-}CH_3$	3.21(s)	56.4	JRE, HSQC, HMBC
		βCH_2	3.54	69.9	COSY, TOCSY, HSQC, HMBC
		αCH_2	4.08	#	COSY, TOCSY
20	Arginine	εC		159.2	HMBC
		δCH_2	3.25(t, 9.2 Hz)	43.1	JRE, COSY, TOCSY, HSQC, HMBC
		γCH_2	1.68(m)	26.6	
		βCH_2	1.92(m)	30.4	
		αCH	3.77	57.1	COSY, TOCSY, HMBC
21	Taurine	CH_2SO_3	3.26(t)	50.6	JRE, COSY, TOCSY, HSQC, HMBC
		CH_2NH_2	3.41(t, 6.5 Hz)	38.2	
22	Betaine	CH_3	3.27(s)	55.9	JRE, HSQC, HMBC
		CH_2	3.91(s)	68.7	
		COO^-		171.8	HMBC
23	Trimethylamine-N-oxide	CH_3	3.29	62.2	HSQC, HMBC
24	Glycine	αCH_2	3.57(s)	44.3	JRE, HSQC
		COOH		175.3	HMBC
25	Proline	δCH_2	3.35, 3.43	48.9	COSY, TOCSY, HSQC, HMBC
		γCH_2	2.01	26.4	
		βCH_2	2.07, 2.35	31.3	
		αCH	4.15(dd)	63.8	JRE, COSY, TOCSY, HSQC, HMBC
		COOH		177.3	HMBC
26	α-Glucose	C_1H	4.67(d, 7.8 Hz)	98.3	JRE, COSY, TOCSY, HSQC
		C_2H	3.26	#	COSY, TOCSY
		C_3H	3.50	#	TOCSY
27	β-Glucose	C_1H	5.25(d, 3.9 Hz)	94.6	JRE, COSY, TOCSY, HSQC
		C_2H	3.56	#	COSY, TOCSY
		C_5H	3.83	#	TOCSY
		C_6H	3.78	#	
28	Sucrose	G_1H	5.42(d, 3.8 Hz)	94.7	JRE, COSY, TOCSY, HSQC, HMBC
		G_2H	3.58(dd)	73.5	
		G_3H	3.77(t)	75.0	
		G_4H	3.49(t, 9.3 Hz)	71.8	
		G_5H	3.82(q)	74.9	
		G_6H	3.81(q)	62.8	
		F_1H	3.70(s)	64.0	
		F_2		106.1	HMBC
		F_3H	4.23(d, 9.2 Hz)	79.2	JRE, COSY, TOCSY, HSQC, HMBC

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(*Continued*)

No.	Compounds	Assignment	δ^1H (multiplicity[a)])	δ^{13}C	Assigned with
		F_4H	4.06(t, 8.5 Hz)	76.6	
		F_5H	3.91(m)	83.7	
		F_6H	3.83	65.0	
29	Uracil	C_1		170.2	HMBC
		C_2		156.1	
		C_3H	7.56(d, 7.6 Hz)	144.6	JRE, COSY, TOCSY, HSQC, HMBC
		C_4H	5.81(d, 7.6 Hz)	103.4	
30	Guanosine	C_4		153.7	HMBC
		C_8H	8.03(s)	140.4	JRE, HSQC, HMBC
		$C_{1'}H$	5.91(d)	90.2	JRE, COSY, TOCSY, HSQC, HMBC
		$C_{5'}H$	4.74	76.0	COSY, TOCSY, HMBC
31	Uridine	C_1		169.8	HMBC
		C_2		154.9	
		C_3H	7.86(d, 8.1 Hz)	144.2	JRE, COSY, TOCSY,HSQC, HMBC
		C_4H	5.91(d)	105.0	JRE, COSY, TOCSY, HSQC
		$C_{1'}H$	5.93(d)	91.6	JRE, COSY, TOCSY, HSQC
		$C_{2'}H$	4.36	76.3	COSY, TOCSY, HMBC
32	Fumarate	COOH		176.9	HSQC
		CH	6.53(s)	137.9	JRE, HMBC
33	Tyrosine	αCH	3.95	58.7	COSY, HSQC, HMBC
		βCH_2	3.06, 3.20	38.4	COSY, TOCSY, HSQC, HMBC
		Ring C_1		129.2	HMBC
		Ring $C_{2,6}H$	7.19(d, 8.4 Hz)	133.3	JRE, COSY, TOCSY, HSQC, HMBC
		Ring $C_{3,5}H$	6.90(d, 8.4 Hz)	118.5	
		Ring C_4		157.6	HMBC
34	Histidine	C_4H	7.76(d)	139.1	JRE, COSY, TOCSY, HSQC, HMBC
		C_5		134.6	HMBC
		C_6H	7.06(d)	119.7	JRE, COSY, TOCSY, HSQC, HMBC
35	Tryptophan	Ring C_2H	7.34(s)	127.6	JRE, HSQC
		Ring C_3		110.2	HMBC
		Ring C_4H	7.54(d, 8.0 Hz)	114.5	JRE, COSY, TOCSY, HSQC, HMBC
		Ring C_5H	7.27(t, 7.5 Hz)	124.5	
		Ring C_6H	7.19	121.8	COSY, TOCSY, HSQC, HMBC
		Ring C_7H	7.73(d, 7.9 Hz)	121.0	JRE, COSY, TOCSY, HSQC, HMBC
		Ring C_8		138.7	HMBC
		Ring C_9		129.1	
36	Phenylalanine	COOH		177.1	HMBC
		αCH	4.00	58.7	HSQC, HMBC
		βCH_2	3.13 3.29	39.3	
		Ring C_1		137.8	HMBC
		Ring $C_{2,6}H$	7.33(dd, 1.5 Hz, 8.6 Hz)	131.9	JRE, COSY, TOCSY, HSQC, HMBC
		Ring $C_{3,5}H$	7.43(t, 7.5 Hz)	131.6	
		Ring C_4H	7.38(t, 7.5 Hz)	130.1	
37	Hypoxanthine	C_2H	8.19(s)	148.6	JRE, HSQC
		C_4		121.9	HMBC
		C_5		155.6	
		C_6		160.5	
		C_8H	8.20(s)	145.2	JRE, HSQC
38	Adenosine	C_2H	8.25(s)	150.5	JRE, HSQC

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No.	Compounds	Assignment	δ^1H (multiplicity[a])	δ^{13}C	Assigned with
		C_4		151.0	HMBC
		C_5		126.6	
		C_6		163.0	
		C_8H	8.34(s)	142.3	JRE, HSQC, HMBC
		$C_{1'}H$	6.08(d, 5.7 Hz)	90.7	JRE, COSY, TOCSY, HSQC, HMBC
		$C_{2'}H$	4.8	76.3	COSY, TOCSY, HMBC
		$C_{3'}H$	4.45	73.0	COSY, TOCSY, HSQC, HMBC
		$C_{4'}H$	4.30	#	COSY
		$C_{5'}H$	3.85	#	TOCSY
39	Formate	CH	8.48(s)	173.6	JRE, HSQC
40	2-pyridinemethanol	C_2		154.3	HMBC
		C_3H	8.73(dd, 6.1 Hz)	147.9	JRE, COSY, TOCSY, HSQC, HMBC
		C_4H	7.99(td, 6.9 Hz)	129.6	
		C_5H	8.57(td, 7.8 Hz)	148.8	
		C_6H	8.05(dd, 8.0 Hz)	128.4	
		C_7H	4.38(s)	49.3	JRE, HSQC, HMBC
41	1-methylnicotinamide	CH_3	4.45(s)	50.8	JRE, HSQC, HMBC
		C_2H	9.13(d)	148.2	JRE, COSY, TOCSY, HSQC, HMBC
		C_3		139.2	HMBC
		C_4H	8.84(d)	147.3	JRE, COSY, TOCSY HSQC, HMBC
		C_5H	8.1(d)	130.3	
		C_6H	8.85(d)	148.5	
42	U1		2.29(s)	14.7	JRE, HSQC, HMBC
			2.45	33.5	HSQC
			2.11	#	HMBC
				170.5	HMBC
43	U2		7.74(s)	138.8	JRE, HSQC
					HMBC

a) Multiplicity: s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublets; dt, doublet of triples; m, multiplet. U: unidentified signal; #: the signal was not determined.

Conversely, asparagine and N-acetylglutamate, which were not observed previously in the swimming crab, were found in these salt- and liquor-saturated products.

Similar to the amino acids, the nucleotide derivatives adenosine monophosphate (AMP), adenosine diphosphate (ADP), inosine monophosphate (IMP) and inosine were not observed in the present study although they had been reported in the swimming crab [27]. We also noted that compounds such as choline, 2-pyridinemethanol and 1-methylnicotinamide have rarely been reported in various crabs [29–31], except that lactate and succinate have been found in *Callinectes sapidus* and *Paralithodes camtschaticus* [27]. However, acetate, fumarate, formate, glucose, choline, phosphorylcholine, uracil and hypoxanthine were, in general, found in foods such as soy sauce [13] and beef [16]. Nucleotides such as uracil, guanosine, uridine and adenosine have been reported in tea leaves and might be important precursors for the biosynthesis of umami-taste nucleotides [32]. 2-pyridinemethanol, as one of the volatile flavor compounds, has been found in sesame seed oil [33]. Dimethylamine and trimethylamine have been observed in fish [34] and soy sauce [13].

2.2 Compositional differences between grade-3A and grade-2A crab paste

To investigate the compositional differentiation of two grades of crab paste, aqueous acetonitrile extracts from samples of grade-3A and grade-2A crab paste were analyzed with ^{1}H NMR followed by multivariate data analyses. The NMR spectra of two different extracts showed clear differences in signal intensities (data not shown). PCA was initially carried out on mean-centered NMR data to visualize inherent patterns and clustering within the data (data not shown). Subsequently, the combination of PC1 and PC2 generated well-separated clusters in terms of their components. The major components contributing to the classification of crab paste were obtained by the OPLS-DA of normalized NMR data.

This scores plot (Figure 2(a)) from OPLS-DA showed

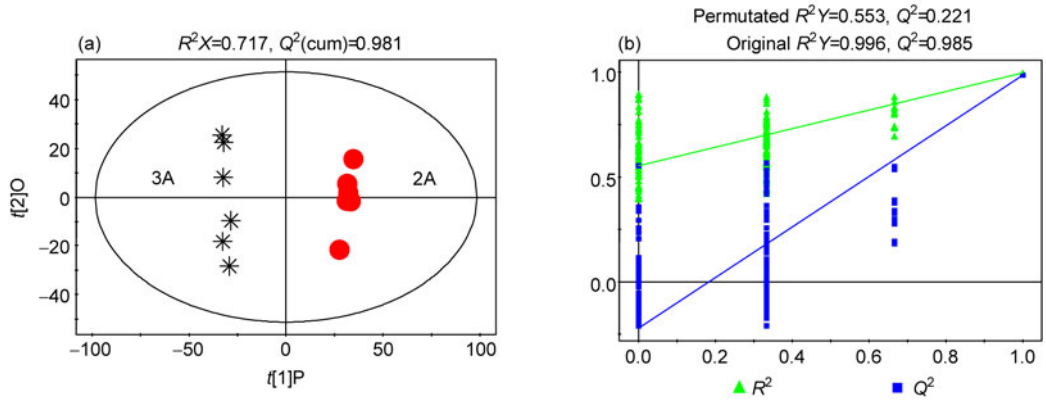

Figure 2 OPLS-DA cross-validated scores plot (a) for grade-3A (∗) and grade-2A (●) crab-paste extracts and permutation test (b) of the OPLS-DA model. The regression line for R^2 and Q^2 is represented with a green line and blue line, respectively.

good model quality as indicated by the values of R^2X and Q^2. Furthermore, a permutation test using the PLS-DA model with the same number of components was undertaken to additionally validate the OPLS-DA model (Figure 2(b)). The components that could be used for discrimination were clearly distinguishable in the loading plot (Figure 3) corresponding to OPLS-DA scores. These revealed the significantly changed components, and the correlation coefficients as well as quantitative information about the relevant components are listed in Table 2. When the absolute value of the correlation coefficient (r) was >0.754, the relevant components made a statistically significant contribution to the classification.

One of the most prominent findings in the current investigation was significantly higher levels of leucine, lysine, histidine, tyrosine, tryptophan, phenylalanine, lactate, dimethylamine, 1-methylnicotinamide and 2-pyridinemethanol, together with significantly lower levels of threonine, alanine, glutamate, N-acetylglutamate, proline, choline, uridine and unassigned compound U2 in the extracts from grade-3A crab paste compared with those in grade-2A crab paste. For example, the mean concentrations of leucine, lysine and tryptophan in grade-3A crab paste showed a ≈70% increase compared with the cases in grade-2A crab paste (Table 2). Significant differences were not observed for components such as isoleucine, valine, methionine, aspartate, asparagine, arginine, glycine, taurine, acetate, succinate, fumarate, formate, trimethylamine, betaine, trimethylamine-N-oxide, choline-O-sulfate, phosphorylcholine, glucose, sucrose, uracil, hypoxanthine, guanosine, and adenosine between two grades of crab paste. The levels of these components were also subjected to ANOVA, and similar conclusions obtained.

Based on the observations mentioned above, we concluded that alterations in these components were probably related to the discrimination of two grades of crab paste. These considerable differences between two grades may

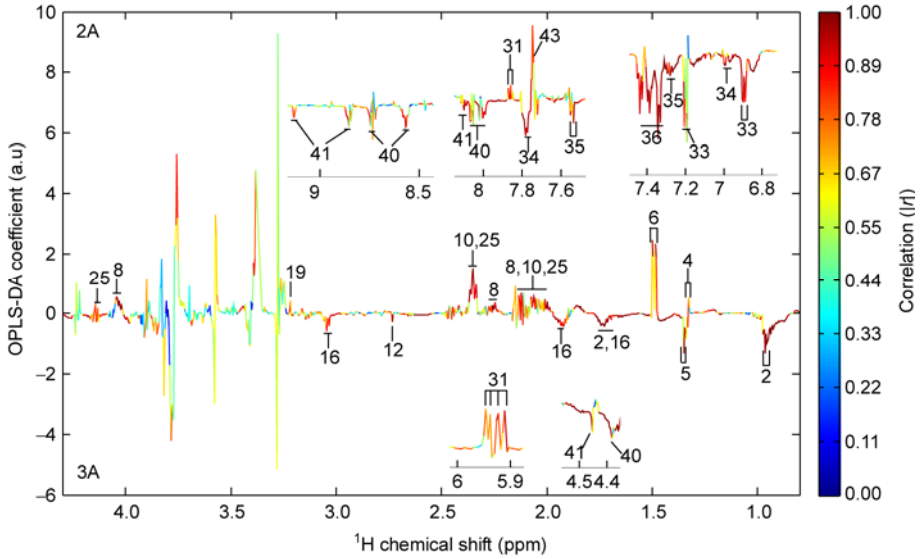

Figure 3 Coefficient plot obtained from OPLS-DA of ^{1}H NMR spectra of grade-3A and grade-2A of crab paste.

Table 2 Correlation coefficients from OPLS-DA and the content of components of crab-paste extracts from grade 3A and grade 2A

Component	Delta (ppm)	Coefficient [a] 2A/3A	Mean ± SD (mg/g) [b]	
			3A	2A
Leucine	0.97(d)	–0.991	8.94±0.66 [c]	5.25±0.46
Valine	1.04(d)	–0.470	1.87±0.15	1.50±0.12
Threonine	1.33(d)	0.863	–	–
Lactate	1.34(d)	–0.948	3.86±0.46 [c]	1.96±0.18
Alanine	1.48(d)	0.954	5.91±0.47 [c]	6.90±0.62
N-acetylglutamate	2.03(s)	0.905	–	–
Glutamate	2.36(dt)	0.988	–	–
Dimethylamine	2.74(s)	–0.994	–	–
Lysine	3.03(t)	–0.915	6.85±0.58 [c]	4.05±0.41
Choline	3.21(s)	0.900	–	–
Proline	4.15(dd)	0.795	–	–
Glucose[d]	4.67(d)	0.401	2.05±0.13	2.31±0.15
Uridine	5.93(d)	0.944	–	–
Fumarate	6.53(s)	0.357	0.02±0.00	0.02±0.00
Histidine	7.06(d)	–0.968	–	–
Tyrosine	7.19(d)	–0.972	3.39±0.26 [c]	2.22±0.26
Tryptophan	7.27(t)	–0.968	1.96±0.14 [c]	1.13±0.11
Phenylalanine	7.33(dd)	–0.982	4.81±0.38 [c]	3.05±0.29
Adenosine	8.33(s)	0.642	1.35±0.09	1.43±0.12
2-pyridinemethanol	8.57(td)	–0.953	1.49±0.12 [c]	1.15±0.10
1-methylnicotinamide	9.13(d)	–0.905	0.21±0.02 [c]	0.14±0.01

a) Coefficients from OPLS-DA results; the positive and negative signs indicate positive and negative correlation in the concentrations, respectively. An absolute value of correlation coefficient, $|\gamma|>0.754$ was used as the cutoff value for the evaluation of significant differences ($P<0.05$). –, The concentration was not determined because of signal weakness or overlapping. b) The average concentration and standard deviation (mean ± SD; mg/g crab paste) were obtained from six parallel samples. c) Significant difference by one-way ANOVA analyses ($P<0.05$) 3A vs. 2A. d) Sum of α-glucose and β-glucose concentrations.

result from the raw materials, production system, and/or environmental parameters. In fact, the reasons for the differences in compositional profiles as a function of grade could not be fully elucidated in the present study. However, these differences would clearly affect the quality and food safety of crab paste. For example, we noted that grade-3A crab paste had remarkably higher levels of essential amino acids, including leucine, lysine, histidine, tryptophan and phenylalanine than grade-2A crab paste. The score for these essential amino acids is widely used for evaluation of protein quality [30], so our findings suggest that grade-3A crab paste may have higher protein quality compared with grade-2A crab paste. Conversely, grade-2A crab paste had higher levels of alanine and glutamate (two of the most important free amino acids with respect to the taste of aquatic products [35]) than grade-3A crab paste. Such observations indicated that grade-2A crab paste may taste better than grade-3A crab paste from a flavor viewpoint. Although the levels of threonine, N-acetylglutamate, proline, tyrosine, lactate, choline, uridine, 2-pyridinemethanol and 1-methylnicotinamide in crab paste differed significantly, the differences in terms of quality remain to be evaluated.

The grade-3A crab paste contained significantly higher level of dimethylamine than grade-2A crab paste. This amine compound is, in general, associated with the metabolism of microorganisms [36], and bacteria such as *Staphylococcus* spp., *Kocuria rosea*, *Corynebacterium* spp. have been detected in bottled crab paste [1]. Therefore, dimethylamine level may be used as a quality indicator of fish "freshness" and as an indicator of the potential for such material to spoil [34].

Although the values of various components remain to be fully understood for assessment of seafood quality, the present study suggested that NMR-based metabolomic analyses are probably an effective way for the survey of nutrients and classification of quality of marine-based food products. The present study also strongly suggested that compositional differentiations must be taken into consideration if assessment of seafood quality is carried out.

3 Conclusions

The present study showed that NMR-based metabolomic technology is a powerful tool to analyze the complex aqueous composition of crab paste, which is largely dominated by amino acids, organic acids, nucleotides, amines and sugars. The present study also suggested that it is possible to

discriminate between two grades of crab paste using OPLS-DA of ^{1}H NMR spectra of aqueous extractions from crab paste. Also, the NMR-based method of compositional profiling may offer a potentially objective criterion for the quality classification of crab paste instead of merely depending on visual inspection. These results provide important values for the survey of nutrients of other marine products.

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1 Ma C, Xu Z, Yang X S. Study on microbiological quality and safety characteristics of bottled crab paste in cold storage (in Chinese). J Shanghai Fish Univ, 2008, 17: 222–226
2 Su X R, Li T W, Oyang F, et al. Study on the nutritive compositions of *Portunus trituberculatus* (in Chinese). Acta Nutr Sin, 1996, 18: 342–346
3 Xiao C N, Dai H, Liu H B, et al. Revealing the metabonomic variation of rosemary extracts using ^{1}H NMR spectroscopy and multivariate data analysis. J Agric Food Chem, 2008, 56: 10142–10153
4 Nicholson J K, Lindon J C, Holmes E. 'Metabonomics': Understanding the metabolic responses of living systems to pathophysiological stimuli via multivariate statistical analysis of biological NMR spectroscopic data. Xenobiotica, 1999, 29: 1181–1189
5 Tang H R, Wang Y L. Metabonomics: A revolution in progress. Prog Biochem Biophys, 2006, 33: 401–417
6 Cloarec O, Dumas M E, Trygg J, et al. Evaluation of the orthogonal projection on latent structure model limitations caused by chemical shift variability and improved visualization of biomarker changes in ^{1}H NMR spectroscopic metabonomic studies. Anal Chem, 2005, 77: 517–526
7 Gall G L, Puaud M, Colquhoun I J. Discrimination between orange juice and pulp wash by ^{1}H nuclear magnetic resonance spectroscopy, identification of marker compounds. J Agric Food Chem, 2001, 49: 580–588
8 Duarte I, Barros A, Belton P, et al. High resolution nuclear magnetic resonance spectroscopy and multivariate analysis for the characterization of beer. J Agric Food Chem, 2002, 50: 2475–2481
9 Charlton A J, Farrington H H, Brereton P. Application of ^{1}H NMR and multivariate statistics for screening complex mixtures, quality control and authenticity of instant coffee. J Agric Food Chem, 2002, 50: 3098–3103
10 Tarachiwin L, Ute K, Kobayashi A, et al. ^{1}H NMR based metabolomic profiling in the evaluation of Japanese green tea quality. J Agric Food Chem, 2007, 55: 9330–9336
11 Cho I H, Kim Y S, Choi H K. Metabolomic discrimination of different grades of pine-mushroom (*Tricholoma matsutake* Sing) using ^{1}H NMR spectrometry and multivariate data analysis. J Pharm Biomed Anal, 2007, 43: 900–904
12 Choi H K, Yoon J H, Kim Y S, et al. Metabolomic profiling of Cheonggukjang during fermentation by ^{1}H NMR spectrometry and principal components analysis. Process Biochem, 2007, 42: 263–266
13 Ko B K, Ahn H J, van den Berg F, et al. Metabolomic insight into soy sauce through ^{1}H NMR spectroscopy. J Agric Food Chem. 2009, 57: 6862–6870
14 Sacco D, Brescia M A, Sgaramella A, et al. Discrimination between southern Italy and foreign milk samples using spectroscopic and analytical data. Food Chem, 2009, 114: 1559–1563
15 Son H S, Hwang G S, Kim K M, et al. Metabolomic studies on geographical grapes and their wines using ^{1}H NMR analysis coupled with multivariate statistics. J Agric Food Chem, 2009, 57: 1481–1490
16 Jung Y, Lee J, Kwon J, et al. Discrimination of the geographical origin of beef by ^{1}H NMR-based metabolomics. J Agric Food Chem, 2010, 58: 10458–10466
17 Schievano E, Peggion E, Mammi S. ^{1}H nuclear magnetic resonance spectra of chloroform extracts of honey for chemometric determination of its botanical origin. J Agric Food Chem, 2010, 58: 57–65
18 Xiao C N, Hao F H, Qin X R, et al. An optimized buffer system for NMR-based urinary metabonomics with effective pH control, chemical shift consistency and dilution minimization. Analyst, 2009, 134: 916–925
19 Dai H, Xiao C N, Liu H B, et al. Combined NMR and LC-MS analysis reveals the metabonomic change in *Salvia miltiorrhiza* Bunge induced by water depletion. J Proteome Res, 2010, 9: 1460–1475
20 Trygg J, Wold S. Orthogonal projections to latent structures (O-PLS). J Chemom, 2002, 16: 119–128
21 Vandenberg R A, Hoefsloot H C J, Westerhuis J A, et al. Centering, Scaling, and transformations: Improving the biological information content of metabolomics data. BMC Genomics, 2006, 7: 142–156
22 Trygg J, Holmes E, Lundstedt T. Chemometrics in metabonomics. J Proteome Res, 2007, 6: 469–479
23 Slupsky C M, Rankin K N, Wagner J, et al. Investigations of the effects of gender, diurnal variation and age in human urinary metabolomic profiles. Anal Chem, 2007, 79: 6995–7004
24 Westerhuis J A, Hoefsloot J C, Smit S, et al. Assessment of PLSDA cross validation. Metabolomics, 2008, 4: 81–89
25 Fan T W M. Metabolite profiling by one- and two-dimensional NMR analysis of complex mixtures. Prog Nucl Magn Reson Spectrosc, 1996, 28: 161–219
26 Fan T W M, Lane A N. Structure-based profiling of metabolites and isotopomers by NMR. Prog Nucl Magn Reson Spectrosc, 2008, 52: 69–117
27 Carr W E S, Netherton III J C, Gleeson R A, et al. Stimulants of feeding behavior in Fish: Analyses of tissues of diverse marine organisms. Biol Bull, 1996, 190: 149–160
28 Dai H, Xiao C N, Liu H B, et al. Combined NMR and LC-DAD-MS analysis reveals comprehensive metabonomic variations for three phenotypic cultivars of *Salvia Miltiorrhiza* Bunge. J Proteome Res, 2010, 9: 1565–1578
29 Naczk M, Williams J, Brennan K, et al. Compositional characteristics of green crab (*Carcinus maenas*). Food Chem, 2004, 88: 429–434
30 Chen D W, Zhang M, Shretha S. Compositional characteristics and nutritional quality of Chinese mitten crab (*Eriocheir sinensis*). Food Chem, 2007, 103: 1343–1349
31 Wu X G, Zhou B, Cheng Y X, et al. Comparison of gender differences in biochemical composition and nutritional value of various edible parts of the blue swimmer crab. J Food Comp Anal, 2010, 23: 154–159
32 Koshiishi C, Crozier A, Ashihara H. Profiles of purine and pyrimidine nucleotides in fresh and manufactured tea leaves. J Agric Food Chem, 2001, 49: 4378–4382
33 Shimoda M, Shiratsuchi H, Nakada Y, et al. Identification of sensory characterization of volatile flavor compounds in sesame seed oil. J Agric Food Chem, 1996, 44: 3909–3912
34 Wu T H, Bechtel P J. Ammonia, dimethylamine, trimethylamine, and trimethylamine oxide from raw and processed fish by-products. J Aquat Food Prod Technol, 2008, 17: 27–38
35 Niwa K, Furuita H, Aruga Y. Free amino acid contents of the gametophytic albdes from the green mutant conchocelis and the heterozygous conchocelis in *Porphyra yezoensis* Ueda (Bangiales, Rhodophyta). J Appl Phycol, 2003, 15: 407–413
36 Wang Y L, Lawler D, Larson B, et al. Metabonomic investigations of aging and caloric restriction in a life-long dog study. J Proteome Res, 2007, 6: 1846–1854