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Effects of Food Processing on the Nutrient Composition of *Pyropia yezoensis* Products Revealed by NMR-based Metabolomic Analysis

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Abstract The laver product is processed from fresh thallus of *Pyropia yezoensis* by washing, cutting, roasting, and seasoning. The nutrient composition of raw materials, semi-finished products, and finished products of *P. yezoensis* was systematically characterized using NMR spectroscopy and multivariate data analysis. The results showed that the nutrient composition of *P. yezoensis* was dominated by 11 amino acids, 11 carboxylic acids, four choline metabolites, and four sugars. The seasoning unsurprisingly caused a significant elevation in the levels of sucrose, glucose, and glutamate in the finished products, up to 38.67 ± 4.91 mg/g, 4.22 ± 0.55 mg/g, and 17.60 ± 1.93 mg/g, respectively. However, other food processing procedures such as washing and roasting may be also responsible for widespread changes of nutrient composition including amino acids, carboxylic acids, choline metabolites, laminitol, floridoside, and isofloridoside from raw materials to finished products of seaweed. Typically, the levels of choline-O-sulfate and isofloridoside were respectively decreased to 5.93 ± 0.86 mg/g and 0.67 ± 0.09 mg/g after food processing. These findings offer essential information for the effects of food processing on the nutrient composition of seaweed products and demonstrate that NMR-based metabolomic strategy is of important values for understanding the effects of food processing on the quality and taste of seaweed products.

Keywords: *Pyropia yezoensis nutritional value, Pyropia yezoensis processing, nuclear magnetic resonance (NMR) of Pyropia yezoensis components, multivariate data analysis*

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1. Introduction

Fresh or processed laver *Pyropia yezoensis* is traditionally and extensively consumed as popular food-stuff in China, Japan and Korea [1,2]. The laver product is processed from fresh thallus of *P. yezoensis* by washing, cutting, roasting, and seasoning. These processing procedures are able to result in great nutrition changes. For instance, the mechanical rupture of seaweed tissues causes the leakage of nutrient substances. The high temperature roasting results in the degradation of proteins and polysaccharides. Importantly, a body of evidence has elucidated that the boiling changes the biochemical compositions and mineral contents of seaweeds [3]. The drying also causes the marked changes of the nutritional compositions of seaweed *Sargassum hemiphyllum*. Freeze-dried seaweed has the highest content of total amino acids, total polyunsaturated fatty acids and total vitamin C, compared with sun-dried and oven-dried seaweed [4].

Many studies have been conducted for the analyses of nutrient composition of fresh *P. yezoensis*. A series of primary metabolite or secondary metabolite contents have been elucidated such as proteins [5], polysaccharide [6,7], porphyran [8,9], vitamins [10] and minerals [11]. It has been indicated that fresh *P. yezoensis* is abundant in proteins, sulfated galactan, choline, taurine, inositol, eicosapentanoic acid and minerals such as Zn, Cu, Mn and Se [5,11]. The contents of free amino acids are dependent on the genotype of *P. yezoensis* blades and the progression of harvest number [12,13]. However, little information is available regarding the effects of processing on the nutrient composition of *P. yezoensis*.

The NMR-based metabolomic analysis has been extensively used for the characterization of complex foodstuffs. This is because the advantages of metabolomics in food analysis relate to it enabling direct sample analysis and holistic detection of large numbers of compounds in a single experiment. Such a strategy has been already successfully applied to green tea [14], pine-mushroom [15], Cheonggukjang [16], soy sauce [17], and crab paste [18]. It is particularly interesting to note that the

metabolomics approach has been proved powerful for detecting the metabolome of *Porphyra haitanensis* and its alterations in response to high temperature stress [19].

In this study, the nutrient composition of raw materials, semi-finished products, and finished products of *P. yezoensis* was systematically analyzed using the NMR-based metabolomics strategy coupled with multivariate data analysis. The objective of this study was to understand the effects of food processing on the nutrient composition of *P. yezoensis* products.

2. Materials and Methods

2.1. Chemicals and Reagents

Acetonitrile, $K_2HPO_4 \cdot 3H_2O$, and $NaH_2PO_4 \cdot 2H_2O$ (all of analytical grade) were purchased from Guoyao Chemical Co., Ltd. (Shanghai, China). Sodium 3-trimethylsilyl [2,2,3,3-d_4] propionate (TSP) and deuterated water (D_2O, 99.9% D) were purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA). Phosphate buffer (K_2HPO_4/NaH_2PO_4, 0.1M, pH 7.4), containing 10% D_2O (v/v) and 0.005% TSP (w/v), was prepared in H_2O [20].

2.2. Sample Collection and Extraction

All samples of *P. yezoensis* were provided by ShenXian Laver Co., Ltd. (Lianyungang, Jiangsu Province, China). Raw material, semi-finished product, and finished product of *P. yezoensis* were obtained from the same thalli of *P. yezoensis*. The seaweed processing was described briefly as follows. (1) The raw materials were prepared by the following procedures: the fresh thalli of *P. yezoensis* were washed thoroughly with tap water, and cut coarsely, then dried in the air oven at 60°C for 90 min and preserved at 4°C. (2) The semi-finished products were obtained from the above raw materials after roasting at 160°C for 60 sec. (3) The finished products were obtained from the semi-finished products after seasoning procedure using a mixture of monosodium glutamate, soy sauce, and sugar as the seasoning paste, and subsequent roasting at 90°C for 60 sec. All these three products were manufactured based on standard procedure, and for sale to the customers.

The raw material, semi-finished product and finished product were sampled randomly and divided into ten portions, respectively. Each portion (about 0.05 g) was extracted with 600 µL of 50% aqueous acetonitrile by shaking with a TissueLyser (Qiagen/Retsch, Germany) at 20 Hz for 90 sec. The resultant extracts were denoted as A, B and C, sequentially. The supernatant for each sample was collected respectively after 10 min of centrifugation (12,000 rpm, 4°C). The remaining solid residues of each sample were further extracted once using the same extraction procedure. After centrifugation for 10 min (12,000 rpm, 4°C), the resultant two supernatants were combined and lyophilized following removal of acetonitrile *in vacuo*. The extracts were then reconstituted into 600 µL of phosphate buffer. Following centrifugation, 550 µL of the supernatant from each extract was transferred into a 5 mm NMR tube.

2.3. NMR Analysis

All ^{1}H NMR spectra were recorded at 298 K on a Bruker Avance III 800 MHz spectrometer operating at 800.20 MHz for ^{1}H, equipped with an inverse detection cryogenic probe (Bruker, Biospin, Germany). A standard one-dimensional NMR experiment was recorded using the first increment of NOESY pulse sequence (recycle delay-90°-t_1-90°-t_m-90°-acquisition). Typically, the 90° pulse length was adjusted to approximately 10 µs and t_1 was set to 6 µs. The water signal was suppressed by irradiation during the recycle delay (2 s) and mixing time (100 ms). Sixty-four transients were collected into 32 K data points for each spectrum with a spectral width of 20 ppm. Prior to Fourier transformation, an exponential window function with a line-broadening factor of 0.5 Hz was applied to all free induction decays. To facilitate NMR signal assignments, a range of 2D NMR spectra were acquired and processed as previously described [21,22] for selected samples including the ^{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 spectra.

2.4. NMR Data Processing and Multivariate Data Analysis

The ^{1}H NMR spectral region δ 5.55-0.80 was divided into bins with an equal width of 3.2 Hz using the AMIX software package (V3.8, Bruker-Biospin, Germany) after manual phase- and baseline-corrections as well as calibration to TSP at 0.0 ppm. Regions δ4.96-4.67 and δ2.09-2.07 were removed to avoid the effects of imperfect water suppression and residual acetonitrile signal, respectively. Each bucketed region was then normalized to the sum of total spectral integrals to compensate for the inter-sample differences in sample volume/concentrations.

Multivariate data analysis was carried out on the normalized NMR data using SIMCA-P$^+$ software (V.12, Umetrics, Umeå, Sweden). Initially, principal component analysis (PCA) was conducted to generate an overview of group clustering and to identify outliers, using a mean-centered scaling. Subsequently, orthogonal projection to latent structure with discriminant analysis (OPLS-DA) was performed using the unit-variance scaled NMR data as *X*-matrix and the group information as *Y*-matrix. To avoid over-interpreting, only two components were calculated for OPLS-DA models with obtained R^2 and Q^2 values as initial indicators for model quality. Each of OPLS-DA model qualities was evaluated by 7-fold cross-validation and further ensured by variance analysis of the cross-validated residuals (CV-ANOVA) method with $p < 0.05$ considered as valid [23] The loadings plots from these models were generated using an in-house developed MATLAB script following back-transformation [24], where signals were color-coded with correlation coefficients (r) to indicate significantly altered compounds. The color code stands for the absolute value of Pearson's product-moment correlation coefficients (|r|), which indicates the weight of each variable contributing to the differentiation between groups. Here, the value of |r| greater than 0.602, was considered to be statistically significant ($n = 10$, $p < 0.05$).

Nutrient component quantification of the seaweed was carried out by equating the integrals of selected component NMR signals (non-overlapping ones) relative to that of internal reference (TSP) with known concentration. All the obtained nutrient component concentrations were also subjected to classical statistical

analyses (one way-ANOVA) using SPSS 13.0 software with a Tukey post-test.

3. Results and Discussion

3.1. ^{1}H NMR Spectra of Aqueous *P. yezoensis* Extracts

To have a complete view of nutrient composition of *P. yezoensis*, a detailed study of ^{1}H NMR spectra of *P. yezoensis* extracts obtained from raw material, semi-finished product and finished product was performed. As shown in Figure 1, 32 compounds were identified in the seaweed and its products according to a series of 2D NMR experiments and literature data [19,25,26]. The ^{1}H and ^{13}C signal information of the compounds is tabulated in Table S1. The aqueous extracts of *P. yezoensis* and its products were dominated by 11 amino acids (isoleucine, leucine, valine, threonine, alanine, glutamate, glutamine, aspartate, asparagine, β-alanine, and glycine), 11 carboxylic acids (6-deoxy-ascobate, lactate, 2-hydroxy-5-aminovalerate, 2-oxo-5-aminovalerate, acetate, malate, succinate, citrate, taurine, γ-amino-n-butyric acid, and isethionate), four choline metabolites (choline, choline-O-sulfate, betaine, and betaine aldehyde), four sugars (glucose, sucrose, floridoside, and isofloridoside), laminitol, and dimethylsulphoniopropionate (DMSP). Although these above compounds have been unambiguously assigned, some compounds detected have not been identified in this study (e.g., unknowns in Table S1). In these assigned compounds, a large number of nutrient components such as choline-O-sulfate, betaine, betaine aldehyde, floridoside, isofloridoside, laminitol, and DMSP were reported for the first time in *P. yezoensis* extracts, apart from amino acids and choline already reported previously [11,12,13]. In fact, the nutrient composition of *P. yezoensis* is highly similar to the metabolites of *P. haitanensis*, another species of genus *Pyropia*, which has been reported recently [19]. In addition, some known compounds including eicosapentanoic acid, inositol, porphyosin, and a sulfated galactan [5] in *P. yezoensis* were not observed in this study. The possible reason is that some of them remain in the residue during extraction or their concentrations were below the detection limit of NMR spectrometer.

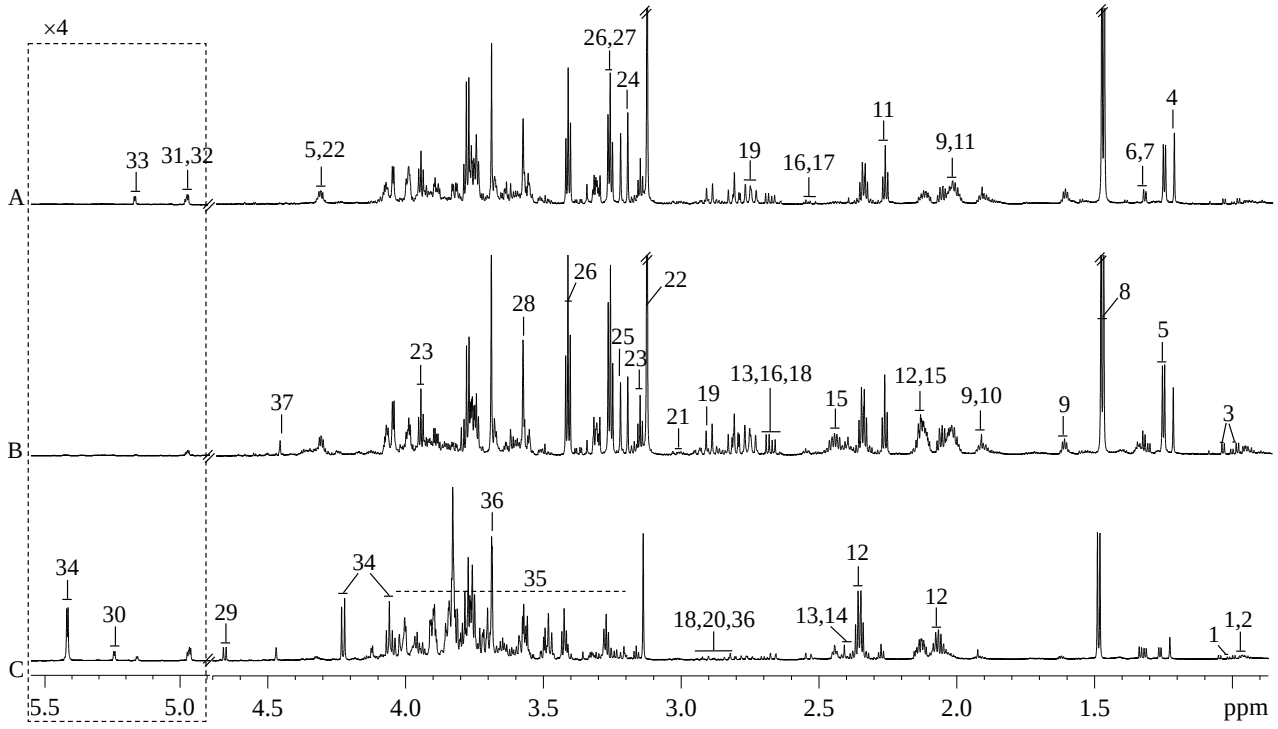

Figure 1. Three typical 800 MHz ^{1}H NMR spectra of raw materials (A), semi-finished products (B), and finished products (C) of *P. yezoensis*. Compared to the chemical shift range at □ 0.9-4.7, the spectra in the regions □ 4.9-5.55 were displayed at 4-fold magnification. Resonance assignments are given in Table S1. Keys: 1, isoleucine; 2, leucine; 3, valine; 4, laminitol; 5, 6-deoxy-ascobate; 6, lactate; 7, threonine; 8, alanine; 9, 2-hydroxy-5-aminovalerate; 10, acetate; 11, 2-oxo-5-aminovalerate; 12, glutamate; 13, malate; 14, succinate; 15, glutamine; 16, citrate; 17, β-alanine; 18, aspartate; 19, dimethylsulphoniopropionate; 20, asparagine; 21, γ-amino-n-butyric acid; 22; choline-O-sulfate; 23, isethionate; 24, choline; 25, betaine; 26, taurine; 27, betaine aldehyde; 28, glycine; 29, β-glucose; 30, α-glucose; 31, L-isofloridoside; 32, D-isofloridoside; 33, floridoside; 34, sucrose; 35, sugar and amino acids α-CH resonance; 36, unidentified signal 1; 37, unidentified signal 2

It is clear from the visual inspection of ^{1}H NMR spectra (Figure 1 A, and B) that *P. yezoensis* raw material extracts has higher signals of 6-deoxy-ascobate and choline-O-sulfate, but lower signals of choline, floridoside, and isofloridoside relative to the semi-finished product. The NMR profiling of finished product extracts (Figure 1C) was drastically different from those of above two groups. An increased intensity in the signals of two sugars (glucose and sucrose) were observed in the finished products relative to the other two groups, accompanied by a decreased intensity in the signals of laminitol, 6-deoxy-ascobate, 2-hydroxy-5-aminovalerate, 2-oxo-5-aminovalerate, choline-O-sulfate, and isethionate. These observations indicate that the nutrient composition of seaweed product strongly depends on the processing procedures. To obtain further details on the statistically significant compositional differences between seaweed and its products due to the food processing, multivariate data analyses were performed on the NMR data from three groups of samples.

Table S1. Summary of the nutrient composition identified in the aqueous extracts of *P. yezoensis* and its products

No.	Metabolite	Group	δ^1H (multiplicity)[a]	δ^{13}C
1	Isoleucine	βCH, γCH, γCH', γ'CH_3, δCH_3	1.98(#), 1.28(#), 1.48(#), 0.94(t), 1.01(d)	38.4, 27.5
2	Leucine	βCH_2, δCH_3, δ'CH_3	1.72(#), 0.97(d), 0.96(d)	42.8, 27.0, 23.9
3	Valine	βCH, γCH_3, γ'CH_3	2.28(m), 0.99(d), 1.04(d)	32.0, 20.9, 19.1
4	Laminitol	CH_3, 1-C, 2-CH, 3-CH, 4-CH	1.23(s), 3.33(d), 3.58(#), 4.09(#)	18.4, 79.5,74.5, 74.3, 76.1
5	6-Deoxy-ascobate	6-CH_3, 5-CHO, 4-CHO, CO, COOH	1.26(d), 4.32(dd), 4.08(#)	22.2, 71.0, 67.1, 161.5, 178.2
6	Lactate	αCH, βCH_3, COOH	4.11(#), 1.33(#)	183.1
7	Threonine	αCH, βCH, γCH_3	3.59(#), 4.26(#), 1.33(d)	63.4, 69.3, 22.3
8	Alanine	αCH, βCH_3, COOH	3.79(q), 1.49(d)	53.4, 19.1, 178.8
9	2-hydroxy-5-aminovalerate	αCH, βCH, βCH', γCH_2, δCH_2, COOH	4(m), 1.93(m), 2.02(m), 1.62(m), 3.99(m)	71.9, 59.3, 28.3, 22.3, 176.7
10	Acetate	CH_3, COOH	1.93(s)	26.8, 184.3
11	2-Oxo-5-aminovalerate	αCH_2, βCH_2, γCH_2, COOH	2.27(t), 2.03(m), 3.32(d)	36.2, 22.3, 68.8, 183.5
12	Glutamate	αCH, βCH_2, γCH_2, δCO, COOH	3.77(m), 2.06(m), 2.13(m), 2.36(dt)	57.6, 29.8, 36.4, 184.3, 177.5
13	Malate	αCH, βCH_2	2.4(dd), 2.67(dd)	70.9, 45.9
14	Succinate	CH_2	2.41(s)	37.5
15	Glutamine	αCH, βCH_2, γCH_2, $CONH_2$, COOH	3.77(t), 2.14(m), 2.45(m)	57.2, 29.2, 33.8, 180.3, 176.8
16	Citrate	αCH_2, βCOH, γCOOH, δCH_2, COOH	2.54(d), 2.67(d)	48.6, 78.2, 182.1, 48.6, 184.7
17	β-alanine	αCH_2, βCH_2NH, COOH	3.18(#), 2.56(t)	39.6, 35.9, 181.2
18	Aspartate	αCH, βCH_2, γCOOH	3.91(dd), 2.68(dd), 2.82(dd)	55.2, 39.4, 180.3
19	Dimethylsulphoniopropionate	αCH_2, βCH_2, S-CH_3, COOH	2.75(t), 3.46(t), 2.93(s)	33.9, 43.3, 27.8, 179.9
20	Asparagine	αCH, βCH_2, γ$CONH_2$, COOH	4.00(dd), 2.87(dd), 2.96(dd)	54.2, 36.2, 180.3, 177.2
21	γ-amino-n-butyric acid	αCH_2, βCH_2, γCH_2NH_2	2.3(t), 1.91(m), 3.01(t)	55.7, 42.0
22	Choline-O-sulfate	αCH_2, βCH_2, N-CH_3	4.33(#), 3.69(#), 3.14(s)	69.0, 55.8
23	Isethionate	CH_2, OCH_2	3.16(t), 3.96(t)	55.7, 59.8
24	Choline	CH_2OH, N-CH_2, N-CH_3	4.07(#), 3.53(#), 3.21(s)	58.4, 70.2, 56.7
25	Betaine	CH_2, N-CH_3	3.92(s), 3.23(s)	68.7, 56.8
26	Taurine	N-CH_2, S-CH_2	3.27(t), 3.42(t)	50.5, 38.2
27	Betaine aldehyde	N-CH_3, CH_2	3.27(s), 3.43(d)	55.0, 79.9
28	Glycine	αCH_2, COOH	3.58(s)	175.8
29	β-Glucose	C_1H, C_2H, C_3H, C_4H, C_5H, C_6H	4.67(d), 3.25(t), 3.49(dd), 3.38(dd), 3.41(t), 3.73(dd), 3.83(dd)	99.2, 77.6, 79.0, 56.1, 72.8, 63.1
30	α-Glucose	C_1H, C_2H, C_3H, C_4H, C_5H, C_6H	5.25(d), 3.54(#), 3.71(#), 3.43(#), 3.84(#), 3.73(#)	95.4, 72.2, 76.0, 72.8, 74.5, 64.2
31	L-isofloridoside	C_1H, C_2H, C_3H, C_4H, C_5H, C_6H, $C_{1'}$H, $C_{2'}$H, $C_{3'}$H	4.96(d), 3.85(#), 3.89(#), 4.00(#), 3.91(#), 3.75(d), 3.82(#),3.50(dd)	101.4, 71.4, 72.1, 72.2, 73.8, 63.9, 71.2, 71.6, 65.4
32	D-isofloridoside	C_1H	4.97(d)	#
33	Floridoside	C_1H, C_2H, C_3H, C_4H, C_5H, C_6H, $C_{1'}$H, $C_{2'}$H	5.17(d), 3.85(#), 3.90(#), 4.01(#), 4.10(#), 3.75(d), 3.83(#)	100.8, 71.2, 72.1, 72.2, 73.8, 63.9, 64.1, 81.4
34	Sucrose	G_1H, G_2H, G_3H, G_4H, G_5H, G_6H, F_1H, F_2, F_3H, F_4H, F_5H, F_6H	5.42(d), 3.58(dd), 3.77(t), 3.49(t), 3.82(q), 3.81(q), 3.70(s), 4.23(d), 4.06(t), 3.91(m), 3.83(#)	94.7, 73.5, 75.0, 71.8, 74.9, 62.8, 64.0, 106.1, 79.2, 76.6, 83.7, 65.0
35	Sugar and amino acids α-CH resonance	αCH resonances	3.46-4.03	#
36	U1		3.7(s), 2.91(d), 2.75(d)	62.2, 128.3, 36.0
37	U2		4.47(s)	#

[a] Multiplicity: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; q, quartet; m, multiplet; U, unidentified signal; #: signals or multiplicities were not determined.

3.2. *P. yezoensis* compositional alterations with processing

PCA of the normalized NMR data obtained from *P. yezoensis* raw material and its product extracts was performed to give an overview of the dataset (Figure 2). No outliers were observed for all samples. The raw material, semi-finished product and finished product of *P. yezoensis* were clearly clustered into three groups with the first two principal components (PC1 and PC2), cumulatively explaining 96.3% of variables in the data set. This suggests that these three groups may have distinct compositional differences due to food processing. Moreover, the nutrient composition of raw materials and semi-finished products is more similar than that of finished products.

To further investigate which nutrient composition was significantly affected by food processing, an OPLS-DA model was constructed with compositional profile data as *X*-matrix and group information as *Y*-matrix. The cross-validated scores plots from OPLS-DA (Figure 3, left) showed that samples from three groups of seaweed clearly clustered into respectively separate groups with good model quality indicated by the high values of R^2X and Q^2. The validity of two OPLS-DA models was further evaluated with the CV-ANOVA approach ($p < 0.05$). Both of these models passed the rigorous test of CV-ANOVA with extremely low r value as listed in Figure 3.

Compared with semi-finished product, raw material exhibited a greater variation along the orthogonal axes (Figure 3a, left), probably due to more differentiation among individual raw materials than semi-finished products. OPLS-DA loading plots (Figure 3, right) were obtained to display the significantly altered compounds contributing to the group discrimination. Relative to raw materials, semi-finished products had higher levels of amino acids (isoleucine, leucine, valine, threonine, glutamine, and aspartate), carboxylic acids (lactate, 6-deoxy-ascobate, 2-oxo-5-aminovalerate, taurine and isethionate) as well as choline-O-sulfate. These alterations were also associated with relatively lower concentrations of alanine, choline, isofloridoside, and floridoside. After further processing of semi-finished products, a significant increase in the levels of glutamate, glucose, floridoside, and sucrose was observed in the finished products. A significant decrease was simutaneously observed in the finished products in the levels of amino acids (alanine, glutamine, aspartate, and glycine), carboxylic acids (6-deoxy-ascobate, 2-hydroxy-5-aminovalerate, 2-oxo-5-aminovalerate, taurine, and isethionate), choline metabolites (choline, choline-O-sulfate, and betaine) as well as laminitol. The dynamic changes of representative compounds with the food processing are shown in Figure 4. Their corresponding correlation coefficients, which are above the cutoff value 0.602, are listed in Table S2 in the Supporting Information. The concentrations of some compounds were obtained and subjected to classical statistical analysis (one-way ANOVA) as summarized in Table 1. The semi-quantitative results were consistent with the OPLS-DA ones. For example, the mean concentrations of floridoside and isofloridoside in semi-finished products were, respectively, 0.16 ± 0.02 mg/g and 0.55 ± 0.08 mg/g, an decrease of 86% and 59% from the cases in the raw materials accordingly (Table 1). After the seasoning and roasting of semi-finished products, finished products had the highly elevated sucrose, glucose, and glutamate, up to 38.67 ± 4.91 mg/g, 4.22 ± 0.55 mg/g, and 17.60 ± 1.93 mg/g, respectively. However, they displayed the greatly depleted choline-O-sulfate and 6-deoxy-ascobate, from 10.05 ± 0.66 mg/g and 2.59 ± 0.15 mg/g to 5.93 ± 0.86 mg/g and 1.31 ± 0.41 mg/g, respectively.

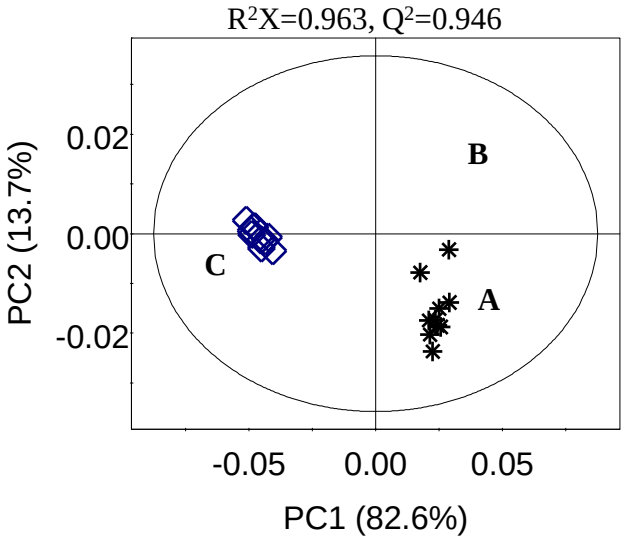

Figure 2. (Color online) PCA scores plot for extracts from raw materials (A, star), semi-finished products (B, circles), and finished products (C, diamond) of *P. yezoensis*. PC1 and PC2 represent 82.6% and 13.7% of the total variance, respectively

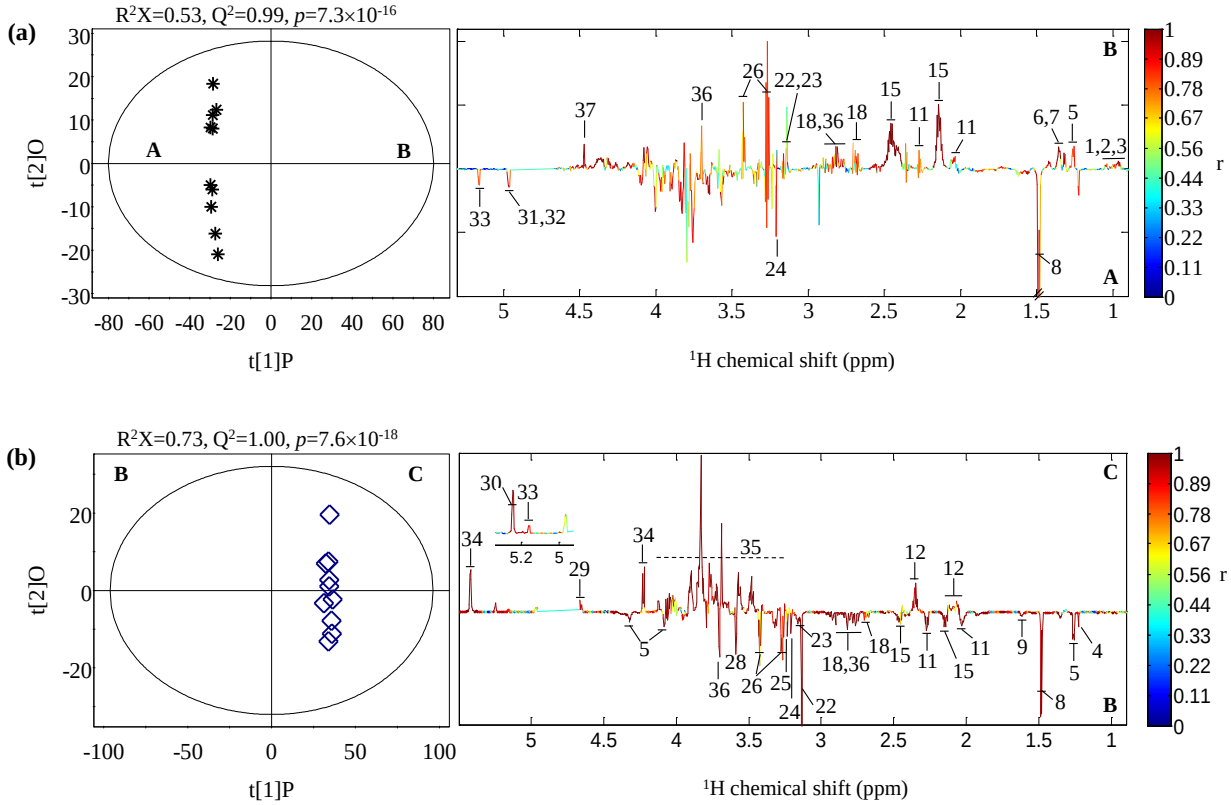

Figure 3. (Color online) OPLS-DA scores (left) and coefficient-coded loadings plots (right) for the models discriminating the raw material (A, star), semi-finished product (B, circle), and finished product (C, diamond) extracts of *P. yezoensis*. (see Table S1 for compound identification key)

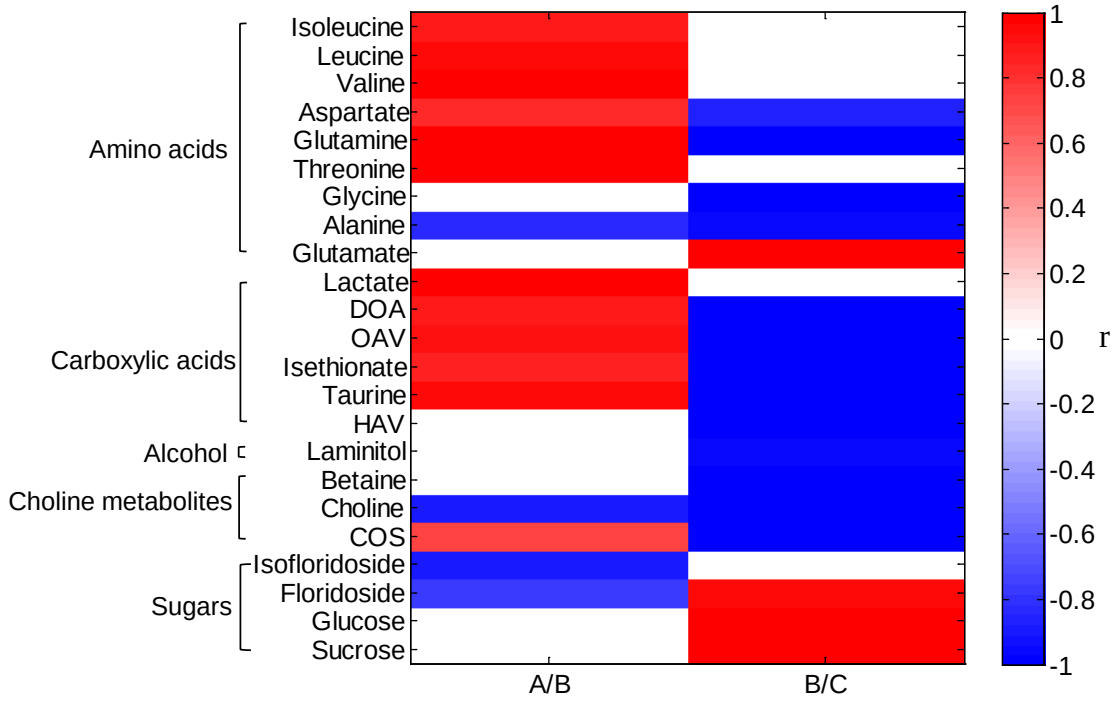

Figure 4. (Color online) Dynamic alterations of key nutrient components of raw material (A), semi-finished product (B) and finished product (C) extracts of *P. yezoensis*. The color indicates a correction coefficient as scaled on the right-hand side. The warm colors (e.g., red) denote an increase in the levels of compounds in B and C against the levels in A and B, respectively, and the cool colors (e.g., blue) indicate a decrease. DOA, 6-deoxy-ascobate; OAV, 2-oxo-5-aminovalerate; HAV, 2-hydroxy-5-aminovalerate; COS, choline-O-sulfate

Table S2. The significantly altered compounds with corresponding correlation coefficients in the *P. yezoensis* extracts from raw materials, semi-finished products, and finished products, respectively

Compounds	Correlation coefficients (r[a])	
	A/B	B/C
Isoleucine	+0.89	-0.95
Leucine	+0.96	-0.98
Valine	+0.98	-0.99
Aspartate	+0.82	-0.87
Glutamine	+1.00	-1.00
Taurine	+0.95	-1.00
Threonine	+0.98	–
Glycine	–	-0.98
Alanine	-0.83	-0.95
Glutamate	–	+0.98
Lactate	+0.99	–
6-Deoxy-ascobate	+0.88	-1.00
2-Oxo-5-aminovalerate	+0.92	-1.00
Isethionate	+0.86	-0.99
2-Hydroxy-5-aminovalerate	–	-0.99
Laminitol	–	-0.94
Betaine	–	-1.00
Choline	-0.88	-0.97
Choline-O-sulfate	+0.72	-0.99
Isofloridoside	-0.90	–
Floridoside	-0.78	+0.94
Glucose	–	+0.97
Sucrose	–	+1.00

[a] Correlation coefficients, positive and negative signs indicate positive and negative correlation in the concentrations, respectively. $p = 0.05$, df (degree of freedom) = 9, r = 0.602 was used as the corresponding cutoff value of correlation coefficient for the statistical significance based on the discrimination significance. ‘‘–’’ means the correlation coefficient |r| is less than cutoff value.

The above compositional alterations of *P. yezoensis* due to processing were significant, although Yoshie and coworkers have shown that there are no significant changes in any of the components during processing [27]. This divergence of results may be resulted from the different samples, detection technique, processing method and/or target compounds. Moreover, it is probable that nutrient composition of *P. yezoensis* is changed by a variety of procedures of food proceeding in this study. For example, the washing and cutting procedures mechanically damage the seaweed tissues, leading to the leakage of some free nutrients such as alanine, isofloridoside, and floridoside. Furthermore, roasting at 160°C may also cause the breakdown of proteins, which contributes to an increase in the levels of amino acids such as isoleucine, leucine, valine, threonine, glutamine, and aspartate in the semi-finished products.

For the finished products, a significant increase in the levels of glutamate, glucose and sucrose is highly correlated with various seasonings including monosodium glutamate, sugar, and soy sauce. It is intriguing to note that soy sauce contains a variety of compounds including amino acids, organic acids, and sugars [17], however, the supplement of soy sauce did not lead to the increased levels of these compounds in the finished products. On the contrary, four amino acids (alanine, glutamine, aspartate, and glycine), as well as five carboxylic acids (6-deoxy-ascobate, 2-hydroxy-5-aminovalerate, 2-oxo-5-aminovalerate, taurine, and isethionate) were greatly depleted in it. Hence, it was speculated that roasting may play some roles in causing the aforementioned compositional changes. Nevertheless, this study strongly suggests the great effects of food processing on the composition of *P. yezoensis* products, which is worthy to be fully considered. This is because all of these nutrient compositional alterations may lead to the changed quality and taste of seaweed products. For example, the score for the essential amino acids such as leucine, isoleucine, and valine is widely used for evaluation of protein quality [28]. Taurine, as an organic acid containing an amino group in man, involves in numerous physiological processes such as membrane stabilization, detoxification, and

antioxidation [29]. Therefore, the food processing-caused alterations of nutrient levels probably highly affect the seaweed quality. In addition, many compounds are saporous substances. Free amino acids specially account for the taste of the seaweed products. For an instance, aspartate and glutamate exhibit interesting properties in flavor development, and glutamate is a main component in the taste sensation of umami [30]. Such significantly altered levels of amino acids may lead to the changed flavor of seaweed product.

4. Conclusion

^{1}H NMR spectroscopy analysis on *P. yezoensis* showed that its nutrient composition was largely dominated by 11 amino acids, 11 carboxylic acids, four choline metabolites, and four sugars. The different nutrient concentrations in seaweed extracts varied with processing procedures. Although seasoning unsurprisingly elevated the levels of glucose, sucrose, and glutamate in the finished products, other processing procedures such as washing and roasting may be also responsible for a significant alteration in the levels of amino acids, carboxylic acids, choline metabolites, laminitol, floridoside, and isofloridoside of seaweed from raw materials to finished products. It is therefore essential to take these factors into consideration and make appropriate choices for the quality and taste of seaweed products. Furthermore, this study demonstrates that NMR-based metabolomic strategy is of important values for understanding the effects of food processing on the nutrient composition of seaweed products.

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Statement of Competing Interests

The authors have no competing interests.

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