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Composition of Transgenic *Volvariella volvacea* Tolerant to Cold Stress Is Equivalent to That of Conventional Control

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Transgenic *Volvariella volvacea* strains, which are tolerant to cold stress, were generated by the stable insertion of antifreeze protein gene isolated from budworm into the genome of a conventional variety V23 of *V. volvacea*. As a part of the safety assessment program, transgenic *V. volvacea* strains were compared to a nontransgenic near-isogenic *V. volvacea* strain V23 grown contemporaneously by applying the principle of substantial equivalence. Compositional analyses were conducted by measuring proximates, amino acids, fatty acids, minerals, vitamins, 5′-nucleotides, nucleic acid, and antinutrients such as tannin and cyanide in *V. volvacea* strains harvested at egg-shaped stage. Results of the comparisons indicate that these transgenic strains are compositionally equivalent to the conventional control.

KEYWORDS: Transgenic *Volvariella volvacea*; composition; substantial equivalence

INTRODUCTION

Volvariella volvacea, known as straw mushroom, is a commercially important edible mushroom cultivated in tropical and subtropical regions (*1*). The market demand for it has increased rapidly over recent years. *V. volvacea* is considered to be a highly nutritious food source, which has long been used for diet or as a source for the extraction of useful metabolites. Its valuable health-promoting properties, including immunomodulating, antitumor, and hypocholesterolaemic activities (*2*), have made it increasingly popular in the world. *V. volvacea* is a tropical/subtropical mushroom with optimal growth at 30−35 °C. It autolyzes or dies at temperatures below 4 °C. As a result, production is limited in temperate regions, and market demands are covered mainly by imported dried mushrooms from Southeast Asia (*3*). Our research group has performed a series of studies on the breeding of *V. volvacea*. More recently, we successfully acquired desirable transgenic strains with improved tolerance to cold stress by biotechnology breeding. The antifreeze protein (*afp*) gene, cloned from budworm, was introduced to the genome of a conventional *V. volvacea* variety by *Agrobacterium tumefaciens*-mediated transformation (*4, 5*). Expression of the gene product *afp* confers the recipient tolerance to cold stress (*6, 7*). Over two rounds of testing cultivation, transgenic *V. volvacea* has been found to have normal agronomic characteristics compared to conventional *V. volvacea* of the same genetic background while maintaining the cold stress tolerance. As a result, these transgenic *V. volvacea* strains are expected to be an excellent agronomic base trait for future market and breeding improvements.

As genetically modified crops become an increasingly important part of the commonly consumed food supply, public concern more for food safety has risen. Accordingly, many regulations have been implemented for safety assessment of such products, of which the concept of substantial equivalence has been accepted widely. The concept was developed by OECD (*8*) and further elaborated by FAO/WHO (*9, 10*), establishing that if no meaningful difference from the conventional counterpart was found, the transgenic crop is as safe and nutritious as its traditional counterpart that is generally accepted as safe on the basis of a history of human food use.

For safety assessment, compositional analysis of genetically modified products is a critical component, and information pertaining to the nutritional substantial equivalence to conventional counterpart is a basic necessity (*11*). Therefore, prior to availability on the market, these transgenic *V. volvacea* strains must undergo a thorough safety evaluation. This research was designed to evaluate the composition of transgenic *V. volvacea* relative to that of its conventional counterpart and determine if significant compositional changes are induced by the insertion of the *afp* gene into the *V. volvacea* genome or by the heterologous expression of *afp* following the guidelines of the OECD consensus document. This is the first report on compositional evaluation of transgenic *V. volvacea* strains in the world.

MATERIALS AND METHODS

Samples. Samples analyzed in this paper were *V. volvacea* V23 and transgenic *V. volvacea* strains named TV231, TV232, and TV233. V23 was purchased from the Institute of Edible Fungus, Shanghai Academy

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Table 1. Proximate Composition of Transgenic *V. volvacea* and Control (Mean ± SD; $n=3$)[a]

component[b]	content (g/100 g)				
	V23	TV231	TV232	TV233	literature range[c]
moisture	91.20 ± 1.52	91.15 ± 0.93	91.23 ± 0.94	91.22 ± 1.20	89.21–92.30
crude protein	28.06 ± 0.53 ab	27.38 ± 1.12 ab	26.63 ± 0.55 a	28.30 ± 0.72 b	21.00–29.50
carbohydrate	49.26 ± 0.40	50.08 ± 1.80	50.17 ± 0.92	50.06 ± 1.53	40.00–50.70
TDF	21.85 ± 0.07	21.95 ± 0.06	21.87 ± 0.14	21.75 ± 0.12	24.20–28.20
crude fat	3.15 ± 0.08	3.28 ± 0.12	3.21 ± 0.05	3.24 ± 0.08	2.24–3.60
ash	9.04 ± 0.36	8.93 ± 052	8.78 ± 0.41	8.84 ± 0.35	8.80–10.10

[a] In each row different small letters indicate significant difference ($p < 0.05$). [b] Grams per 100 g of dry weight (moisture based on fresh weight). [c] From refs *21*, *22*, and *23*.

of Agricultural Sciences (Shanghai, China). Transgenic strains TV231, TV232, and TV233 were produced by *A. tumefaciens*-mediated transformation of *V. volvacea* V23 with plasmid vector pLg-afp235. The binary vector contains the antifreeze protein gene from budworm, in which the product of *afp* gene expression, antifreeze protein, confers tolerance to cold. During transformation, the *afp* gene was used as both an aim gene to improve the tolerance of *V. volvacea* to cold stress and a marker gene for the selection of transformed cells, thereby producing transgenic *V. volvacea* without antibiotic marker gene.

Transgenic strains and the control V23 were cultivated under normal agronomic field conditions in the mushroom farm of our institution. Fresh fruit bodies of each strain were collected at egg-shaped stage and randomly divided into three samples. The samples were ground to a fine powder after lyophilizing and were maintained frozen until required compositional analysis. These were analyzed for moisture and vitamin C on the same day that the samples were collected. Samples analyzed for nucleic acid were stored at −80 °C.

Compositional Analyses. *Proximate Analysis.* The standard methods of the Association of Official Analytical Chemists (AOAC) were adopted to determine the levels of proximate compositions, including moisture, ash, carbohydrate, crude fat, total dietary fiber (TDF), and crude protein (*12*). Moisture content and ash were determined in an oven at 105 and 550 °C, respectively, until constant weight was attained. The crude protein content (N × 4.38) of the samples was estimated according to the macro-Kjeldhal method. Fat content was determined by using the Soxhlet extraction method. TDF was determined according to the enzymatic−gravimetric method. The carbohydrate content was calculated by subtracting the contents of crude protein, fiber, fat and ash from 100% of dry matter.

Amino Acid Composition. Mushroom powder (100 mg) was hydrolyzed with 10 mL of 6 M HCl at 110 °C under nitrogen atmosphere for 24 h. The hydrolysate was filtered and evaporated with a vacuum evaporator. After two washings with deionized water, the residue was dissolved in 10 mL of 0.01 N HCl and filtered with a 0.45 μm filter membrane. The amino acid amounts were measured by using a Hitachi L-8800 automatic amino acid analyzer (Hitachi, Japan), equipped with an ion-exchange column (4.6 × 60 mm) and a UV detector. The temperature of the separation column was at 57 °C. The buffer flow rate was 0.40 mL/min, and the pressure of the buffer pump was 12 Pa. The ninhydrin flow rate was 0.30 mL/min, and the pressure of the ninhydrin pump was 1.1 kPa.

Fatty Acids Analyses Composition. The fatty acids were converted to methyl esters according to the IUPAC method (*13*). Methyl heptadecanoate was used as the internal standard. Analyses of fatty acid methyl esters were carried out with Thermoquest trace gas chromatography (a Shimadzu-15A gas chromatograph (Kyoto, Japan)), equipped with an SP-2330 fused silica capillary column and flame ionization detector. The methyl esters were dissolved in *n*-heptane. The helium carrier gas flow was 1 mL/min. The initial oven temperature program was applied for 5 min at 100 °C and then raised by 10 °C min^{-1} to 200 °C for 5 min. The injection split ratio was 1:150. The injection and detector temperatures were 220 °C. Split flow ratio was 75 mL/min. Identification and quantification of fatty acid methyl esters were achieved by comparing the retention times of the peaks with those standards.

Minerals. Mineral elements (potassium, calcium, magnesium, iron, zinc, copper, manganese, and selenium) were measured by atomic absorption spectrophotometer (Varian). Mushroom powder was digested with a mixture of concentrated nitric acid, sulfuric acid, and perchloric acid (10:0.5:2, v/v) and analyzed according to AOAC method (*12*).

Vitamins. The content of ascorbic acid was determined by the 2,6-dichloroindophenol titrimetric method. Niacin was determined by using a spectrophotometer according to AOAC method (*14*). Thiamin and riboflavin were analyzed using a HPLC method in which the samples were extracted by heating in the presence of hydrochloric acid followed by takadiastase digestion. After neutralization, the extract was immediately injected into the chromatograph. Thiamin and riboflavin were detected separately (*15*). Total folate was analyzed according to the microbiological method (*16*).

5′-Nucleotides. Analysis of 5′-nucleotides was performed according to the method of Taylor et al. (*17*). Mushroom powder was extracted twice with deionized water. After evaporation, the combined filtrate was filtered and injected into the chromatograph. The mobile phase was 0.5 M KH_2PO_4/H_3PO_4 (pH 4.3) at a flow rate of 1 mL/min and UV detection at 254 nm. Each 5′-nucleotide was identified using the authentic 5′-nucleotide (Sigma) and quantified by the calibration curve of the authentic compound.

Nucleic Acid. Samples stored at −70 °C were extracted for nucleic acid (DNA and RNA) using the method described by Sambrook et al. (*18*). Nucleic acid was quantified by using an ND-1000 spectrophotometer (Nano Drop).

Antinutrients. Tannin was quantified in samples as described by Akindahunsi (*19*). Samples were extracted with 70% acetone. Standard tannic acid solution (50 mg/100 mL) was also prepared, and serial dilutions were made. The solutions were read at 725 nm after the addition of 0.5 mL of Folin and 2.5 mL of 20% $NaCO_3$. Cyanide content was estimated according to the standard method of AOAC (*20*), in which samples were soaked in a mixture of orthophosphoric acid and distilled water overnight to free bound cyanide. The resulting solution was distilled and the distillate titrated against 0.01 mol/L $AgNO_3$. Cyanide concentration was obtained in milligrams per kilogram.

Statistical Analysis. For each of the *V. volvacea* strains three samples were analyzed, and all of the assays were carried out in triplicate. The results are expressed as mean values and standard deviation (SD). Statistical analysis was carried out using one-way analysis of variance (ANOVA). This treatment was performed by using SPSS13.0 program. Statistical differences with p values under 0.05 were considered to be significant.

RESULTS AND DISCUSSION

The safety assessment of biotechnology-derived crops has relied on a comparative approach, in which the similarities and differences are identified between the biotech crops and their near-isogenic conventional counterpart and commercially available varieties that have a history of the same consumption. For each component measured in this study, transgenic *V. volvacea* strains were compared with their nontransgenic near-isogenic *V. volvacea* strain V23 and the range of values reported in the literature.

Proximate Compositions. The results of proximate compositions obtained from the four *V. volvacea* strains are shown in **Table 1**. The moisture was calculated on the basis of fresh

Table 2. Amino Acid Composition of Transgenic *V. volvacea* and Control (Mean ± SD; *n*=3)[a]

component	content (g/100 g of dry weight)				
	V23	TV231	TV232	TV233	literature range[b]
alanine	2.18 ± 0.05	2.17 ± 0.09	2.20 ± 0.10	2.14 ± 0.06	1.32–2.31
arginine	1.53 ± 0.03	1.50 ± 0.02	1.48 ± 0.02	1.51 ± 0.03	0.91–1.86
aspartic acid	2.93 ± 0.07 a	2.97 ± 0.08 a	2.87 ± 0.09 ab	2.78 ± 0.03 b	1.61–3.21
cystine	0.44 ± 0.02	0.45 ± 0.03	0.42 ± 0.02	0.45 ± 0.03	0.49–0.55
glutamic acid	5.33 ± 0.20	5.32 ± 0.15	5.41 ± 0.26	5.38 ± 0.31	2.47–7.80
glycine	1.30 ± 0.10	1.29 ± 0.11	1.33 ± 0.17	1.25 ± 0.10	0.86–1.57
histidine	1.17 ± 0.01	1.17 ± 0.03	1.16 ± 0.01	1.20 ± 0.02	0.20–1.21
isoleucine	1.30 ± 0.03	1.34 ± 0.12	1.37 ± 0.12	1.42 ± 0.05	0.79–1.62
leucine	1.76 ± 0.13 ab	1.91 ± 0.02 a	1.53 ± 10.17 b	1.67 ± 0.14 ab	1.01–1.96
lysine	2.21 ± 0.04	2.16 ± 0.09	2.20 ± 0.05	2.22 ± 0.10	0.79–3.31
methionine	0.51 ± 0.02	0.55 ± 0.04	0.53 ± 0.01	0.54 ± 0.03	0.32–1.24
phenylalanine	1.26 ± 0.07	1.26 ± 0.14	1.33 ± 0.06	1.26 ± 0.04	0.54–1.46
proline	1.41 ± 0.04	1.42 ± 0.04	1.44 ± 0.02	1.46 ± 0.06	0.97–1.61
serine	1.31 ± 0.05	1.19 ± 0.01	1.25 ± 0.11	1.32 ± 0.04	1.02–1.51
threonine	1.44 ± 0.10	1.38 ± 0.07	1.46 ± 0.04	1.47 ± 0.04	0.97–1.63
tryptophan	0.50 ± 0.02 a	0.61 ± 0.02 b	0.51 ± 0.02 a	0.62 ± 0.06 b	0.40–0.78
tyrosine	1.62 ± 0.03	1.58 ± 0.02	1.64 ± 0.03	1.58 ± 0.04	1.43–1.80
valine	2.61 ± 0.03	2.47 ± 0.11	2.61 ± 0.17	2.56 ± 0.04	1.55–2.31
TAA	30.81	30.74	30.76	30.81	
EAA	12.55	12.46	12.39	12.57	

[a] In each row different small letters indicate significant difference ($p < 0.05$). [b] From refs*25* and *26*.

Table 3. Fatty Acid Composition of Transgenic *V. volvacea* and Control (Mean ± SD; *n*=3)[a]

component	content (mg/g of dry weight)				
	V23	TV231	TV232	TV233	literature range[b]
myristic (14:0)	0.30 ± 0.02	0.28 ± 0.04	0.27 ± 0.02	0.31 ± 0.04	0.33–0.72
palmitic (16:0)	2.32 ± 0.07	2.31 ± 0.17	2.28 ± 0.10	2.25 ± 0.09	0.27–2.60
stearic (18:0)	1.94 ± 0.05 a	1.83 ± 0.04 b	1.91 ± 0.02 ab	1.85 ± 0.07 ab	1.96–3.04
oleic (18:1)	9.07 ± 0.05	9.14 ± 0.06	9.13 ± 0.03	9.12 ± 0.07	2.18–9.00
linoleic (18:2)	20.50 ± 0.08	20.61 ± 0.05	20.55 ± 0.06	20.62 ± 0.11	7.03–20.68
arachidonic (20:4)	1.26 ± 0.05	1.28 ± 0.03	1.26 ± 0.04	1.27 ± 0.04	1.47
eicosapentaenoic (20:5)	0.97 ± 0.03	1.00 ± 0.10	0.98 ± 0.07	1.02 ± 0.09	1.06
UFA	31.80	32.03	31.92	32.03	
UFA (%)[c]	87.46	87.87	87.74	87.90	

[a] In each row different small letters indicate significant difference ($p < 0.05$). [b] From refs *27* and *28*. [c] Percent total fatty acids.

weight. There were no statistically significant differences in moisture content between transgenic strains and the control V23. Contents of other chemical composition are presented on the basis of dry weight. Analysis of variance showed that protein values varied significantly among strains ($p < 0.05$), but mean content of each analyte was within the range reported previously for *V. volvacea* (*21*, *22*). No significant differences were observed in carbohydrate and crude fat contents between transgenic strains and the control. Contents of ash and TDF in transgenic strains were slightly lower than those reported for *V. volvacea* (*21*, *23*, *24*), but statistically identical to the control V23.

Amino Acid Contents. The amino acid contents of the four strains are presented in **Table 2**. Results obtained correlated with previous findings; 18 amino acids were detected in the four strains, and all amino acids were in the identical profile (*25*, *26*). Statistical analysis revealed that TV231 was significantly different from the control in tryptophan and that T233 was significantly different from the control in aspartic acid and tryptophan ($p < 0.05$), but these values were within published literature ranges (*26*, *27*). No significant differences were observed between TV232 and the control. All strains contained high percentages of essential amino acids (EAA), and there were no significant differences in content of total amino acid (TAA) and EAA among these strains.

Fatty Acid Contents. The contents of seven fatty acids detected in transgenic strains were comparable to that of the control (**Table 3**). All strains had the same profile of fatty acids and were characterized by a high concentration of unsaturated fatty acids (UFA). Analysis of results showed that no significant difference was observed between transgenic strains and the control in fatty acid composition except for stearic acid ($p < 0.05$), whereas the mean value of stearic acid agreed with previous results (*28*, *29*). There were no significant differences in content of UFA between three transgenic strains and the control.

Mineral Contents. Contents of eight minerals were determined in the four strains (**Table 4**). It can be seen from the table that all strains presented similar mineral profiles containing a high content of potassium and a low content of selenium. Analysis of the results indicated statistically significant differences in contents of potassium, iron, and zinc between three transgenic strains and the control ($p < 0.05$), but the mean mineral values of transgenic strains were consistent with the literature ranges reported for *V. volvacea* (*23*, *26*).

Vitamin Contents. Table 5 shows the vitamin contents in the four strains analyzed. Analysis results showed that no statistically significant difference was observed between three transgenic strains and the control. The vitamin contents obtained in this study were all within the range obtained from published data of *V. volvacea* with the exception of folates, which was lower than that of previous studies (*21*, *26*).

5′-Nucleotides. Analysis of 5′-nucleotides in the samples is presented in **Table 6**. Five 5′-nucleotides, including 5′-adenosine monophosphate (5′-AMP), 5′-cytosine monophosphate (5′-CMP), 5′-guanosine monophosphate (5′-GMP), 5′-inosine mono-

Table 4. Mineral Composition of Transgenic *V. volvacea* and Control (Mean ± SD; *n*=3)[a]

component	content (mg/100 g of dry weight)				
	V23	TV231	TV232	TV233	literature range[b]
potassium	1482.62 ± 3.44 a	1551.74 ± 4.28 b	1446.65 ± 0.52 c	1512.48 ± 0.40 d	1324.00–6144.00
calcium	451.49 ± 0.65	449.68 ± 1.53	449.30 ± 1.17	450.75 ± 1.62	339.00–446.00
magnesium	145.9 ± 0.58	146.30 ± 0.69	145.69 ± 0.54	145.25 ± 0.98	57.00–224.00
iron	17.27 ± 0.33 a	17.29 ± 0.72 a	16.03 ± 0.42 b	17.01 ± 0.73 ab	15.00–426.00
zinc	61.37 ± 0.04 a	62.18 ± 0.49 b	63.22 ± 0.56 c	61.08 ± 0.11 a	68.00–98.00
copper	15.07 ± 0.07	14.88 ± 0.21	14.90 ± 0.02	15.01 ± 0.12	15.50–16.42
manganese	3.08 ± 0.04	2.94 ± 0.15	3.12 ± 0.05	2.96 ± 0.11	3.50–5.90
selenium[c]	3.41 ± 0.09	3.44 ± 0.12	3.40 ± 0.12	3.36 ± 0.11	2.00–6.90

[a] In each row different small letters indicate significant difference ($p < 0.05$). [b] From refs *23* and *25*. [c] Selenium = grams per 100 g of dry weight.

Table 5. Vitamin Composition of Transgenic *V. volvacea* and Control (Mean ± SD; *n*=3)

component	content				
	V23	TV231	TV232	TV233	literature range[a]
ascorbic acid[b]	166.22 ± 2.54	168.53 ± 2.27	164.98 ± 1.42	164.19 ± 2.54	156.00–206.27
thiamin[c]	0.53 ± 0.02	0.55 ± 0.01	0.55 ± 0.01	0.52 ± 0.01	0.35–1.20
nicotinic acid[c]	67.02 ± 0.45	66.61 ± 0.78	66.71 ± 0.63	65.87 ± 0.43	64.90–91.90
riboflavin[c]	2.30 ± 0.01	2.29 ± 0.01	2.29 ± 0.00	2.27 ± 0.03	1.63–2.98
folates[d]	52.17 ± 1.09	52.7 ± 0.46	51.77 ± 0.75	52.60 ± 0.40	65.00

[a] From refs *21* and *26*. [b] Milligrams per 100 g of fresh weight. [c] Milligrams per 100 g of dry weight. [d] Grams per 100 g of dry weight.

Table 6. 5′-Nucleotide Composition of Transgenic *V. volvacea* and Control (Mean ± SD; *n*=3)

component	content (mg/g of dry weight)				
	V23	TV231	TV232	TV233	literature range[a]
5′-AMP	3.24 ± 0.01	2.92 ± 0.58	3.22 ± 0.01	3.25 ± 0.00	2.64–5.39
5′-CMP	16.44 ± 0.06	16.38 ± 0.06	16.40 ± 0.02	16.36 ± 0.04	12.82–28.49
5′-GMP	4.80 ± 0.01	4.71 ± 0.07	4.77 ± 0.05	4.79 ± 0.03	4.25–8.37
5′-IMP	0.25 ± 0.00	0.24 ± 0.00	0.25 ± 0.00	0.25 ± 0.00	0.16–0.69
5′-UMP	2.53 ± 0.02	2.54 ± 0.02	2.51 ± 0.01	2.53 ± 0.00	2.26–6.89
total	24.03	23.87	23.93	23.93	27.08–44.71

[a] From ref *30*.

Table 7. Nucleic Acid Composition of Transgenic *V. volvacea* and Control (Mean ± SD; *n*=3)

component	content (g/100 g of dry weight)				
	V23	TV231	TV232	TV233	literature range[a]
DNA	0.45 ± 0.02	0.45 ± 0.01	0.44 ± 0.01	0.46 ± 0.01	0.29
RNA	5.42 ± 0.08	5.36 ± 0.09	5.49 ± 0.16	5.37 ± 0.01	3.59
total nucleic acid	5.87	5.81	5.93	5.83	3.88

[a] From ref *31*.

phosphate (5′-IMP), and 5′-uridine monophosphate (5′-UMP), were detected in the four strains. There was no statistically significant difference in the contents of the five and total 5′-nucleotides between transgenic strains and the control. The mean values of 5′-nucleotides were within the range of previous studies of *V. volvacea* (*30*).

Nucleic Acid. According to the present study, the four strains proved to be a good source of nucleic acid (**Table 7**), which was in accordance with previous results for *V. volvacea*, but the contents of DNA or RNA obtained from the four samples were all higher than that reported for *V. volvacea* (*31*). Analysis of the results indicated no statistically significant difference was observed in contents of nucleic acid between transgenic strains and the control.

Antinutrient Composition. The results of the antinutrients analyzed are shown in **Table 8**. Tannin concentrations were generally low. These levels might not affect the nutritional potentials of the mushroom parts because they were all <10% of the total dry weight of the samples (*32*). The total cyanide content was very low in the four strains. Statistical analyses revealed there was no significant difference in antinutrients between transgenic strains and the control.

Table 8. Antinutrient Composition of Transgenic *V. volvacea* and Control (Mean ± SD; *n*=3)

component	V23	TV231	TV232	TV233
tannin[a]	3.25 ± 0.07	3.21 ± 0.06	3.23 ± 0.07	3.26 ± 0.03
cyanide[b]	0.18 ± 0.03	0.17 ± 0.04	0.18 ± 0.07	0.21 ± 0.03

[a] Percent dry weight. [b] Milligrams per kilogram of dry weight.

The results of compositional analyses showed significant differences were observed in detected components between transgenic strains and V23, but these detected components exhibited similar nutritional profiles with comparator V23 and were within the normal ranges reported for *V. volvacea*. Also, examination of the data indicated that there are no analytes that show a consistent trend of difference between transgenic *V. volvacea* strains and the control V23. Therefore, on the basis of the data presented in this study and the published literature, it can be calculated that the transgenic strains TV231, TV232, and TV233 are equivalent to the control V23 with respect to these important constituents.

ABBREVIATIONS USED

V. volvacea, *Volvariella volvacea*; afp, antifreeze protein; TDF, total dietary fiber; HPLC, high-performance liquid chro-

matography; ANOVA, one-way analysis of variance; EAA, essential amino acids; TAA, total amino acid; UFA, unsaturated fatty acid; 5′-AMP, 5′-adenosine monophosphate; 5′-CMP, 5′-cytosine monophosphate; 5′-GMP, 5′-guanosine monophosphate; 5′-IMP, 5′-inosine monophosphate; 5′-UMP, 5′-uridine monophosphate.

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