

## Research Article

### Formation of *Porphyra yezoensis* Flavour Compounds through Hydrolysis and Maillard Reaction

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**Abstract:** *Porphyra yezoensis* has a unique flavour and nutritional value. In this study, flavour compounds were obtained by subjecting *P. yezoensis* to water extraction and hydrolysis with flavorzyme. Amino acids and reducing sugar were then to the hydrolysates to facilitate Maillard reaction. Hydrolysis with flavorzyme significantly increased the sensory score of *P. yezoensis* water extract ( $p < 0.05$ ), whereas Maillard reaction significantly increased the sensory score of *P. yezoensis* hydrolysates ( $p < 0.05$ ). Gas chromatography-mass spectrometry analysis showed that a total of 42 flavour compounds formed in Maillard reaction, which increased the flavour of *P. yezoensis* hydrolysates. These results indicated that the method can be used to prepare *P. yezoensis* essence.

**Keywords:** Flavour compound, hydrolysis, Maillard reaction, porphyra yezoensis

## INTRODUCTION

Maillard reaction involves the condensation of an amino group with a carbonyl group, usually on a reducing sugar (Desclaux *et al.*, 2006). Maillard reaction has utmost importance in the quality of food, especially heated food. This reaction leads to food browning, affects nutritive value, has toxicological implications (such as acrylamide formation), produces antioxidative components and largely influences flavour (Van Boekel, 2006).

*Porphyra yezoensis* is an economically important seaweed containing 25-50% protein and 25-40% carbohydrates on dry basis (Jiang, 2014). *P. yezoensis* can prevent hypertension, coronary heart disease and atherosclerosis, as well as protect the liver (Guo *et al.*, 2007; Qu *et al.*, 2010). Polysaccharides from *P. yezoensis* reportedly exert antioxidant activities (Zhou *et al.*, 2012). Moreover, *P. yezoensis* has nutritional value and a unique flavour (Zhou *et al.*, 2012). Maillard reaction is known to be the formation mechanism of flavour compounds, such as those from dairy, meat and chocolate. However, reports on the formation of *P. yezoensis* flavor compounds through Maillard reaction are limited (Chen and Robbins, 2000; Lee *et al.*, 2011; Newton *et al.*, 2012; Wang *et al.*, 2012).

In this study, we investigated the formation of flavour compounds from *P. yezoensis* through Maillard reaction. Amino acids in the *P. Yezoensis* Water Extract

(PYWE), hydrolysates from flavorzyme action (PYHF) and Maillard reaction liquid (PYML) were identified. Volatile flavour compounds that formed through Maillard reaction were then determined.

## MATERIALS AND METHODS

**Materials:** Dried *P. yezoensis* samples were purchased from a local farmers' market (Xipu, China). Flavorzyme was purchased from Novo Nordisk, Denmark. Glutamate, glycine, cysteine, xylose, glucose and thiamine were purchased from Sigma Aldrich, USA.

**Preparation of PYWE, PYHF and PYML:** Dried *P. yezoensis* materials were pulverised and sifted through a 60-mesh sieve to obtain fine powder. The dried powder was then soaked in distilled water to yield a 5% (w/v) suspension. The reactor was maintained in a thermostatic water bath at 80°C for 12 h. The extract was cooled to 55°C and filtered through a Whatman GF/A filter paper to obtain PYWE, to which 3% flavorzyme was added. The mixture was incubated in a thermostatic water bath at 55°C for 3 h to yield PYHF. Afterwards, 2% glutamate, 1% glycine, 0.4% cysteine, 4% xylose, 2% glucose and 0.2% thiamine were added to PYHF. The mixture was incubated in an oven at 110°C for 50 min to obtain PYML.

**Sensory evaluation:** The samples were evaluated by 18 panellists from the Department of Food Science and Technology, aged 20-25, using a 9-point hedonic scale, where 9 = extremely like; 7 = moderately like; 5 = neither like or nor dislike; 3 = moderately dislike; 1 = extremely dislike (Wu, 2013). Panellists were regular consumers of *P. yezoensis* and possess no allergies to *P. yezoensis*. All panellists were asked to evaluate for colour, odour and taste.

**Amino-acid determination:** Amino-acid determination was carried out using a high-performance liquid chromatography system (1100, Agilent, USA). Chromatographic conditions were as follows: dimensions, 4×125 mm; column type, C18; column temperature, 40°C; buffer flow rate, 1.0 mL/min; mobile phase A: 20 mmol sodium acetate; mobile phase B: sodium acetate: methanol: acetonitrile (1:2:2 [20 mmol]); and detection wavelength, 338 nm.

**Gas Chromatography (GC) -Mass Spectrometry (MS) analysis:** Analysis of volatiles was performed on a GC 7890A coupled with a Triple Quad 7000B (both Agilent, Palo Alto, CA, USA) and equipped with a Sniffer 9000 Olfactometer (Gerstel, KG, Germany). Separations in GC were performed on DB-5MS UI (30

m×0.250 mm×0.25 μm; J and W Scientific, Folsom, CA, USA). The carrier gas used was ultra-high-purity helium and the flow rate of column was 1.2 mL/min. Oven temperature was programmed as follows: 40°C for 3 min, increased at 5°C/min to 200°C and further increased at 15°C/min to 260°C, held for 5 min. Temperatures of injector and GC-MS interface were 250 and 280°C, respectively. Electron-impact mass spectra were generated at 70 eV, with m/z scan range of 35-550 amu at an ion source temperature of 230°C. Compounds were identified according to NIST 2.0 mass spectra libraries installed in GC-MS equipment (Wang *et al.*, 2012).

**Statistical analysis:** All data were presented as mean ± standard deviation. Statistical analysis was performed using Statgraphics Centurion XV version 15.1.02. Multifactor ANOVA with posterior multiple-range test was used to determine significant differences between groups.

## RESULTS AND DICUSION

**Amino-acid components of PYWE, PYHF and PYML:** Taste-delicious amino acids including aspartic acid, glutamate, glycine and alanine increase food taste

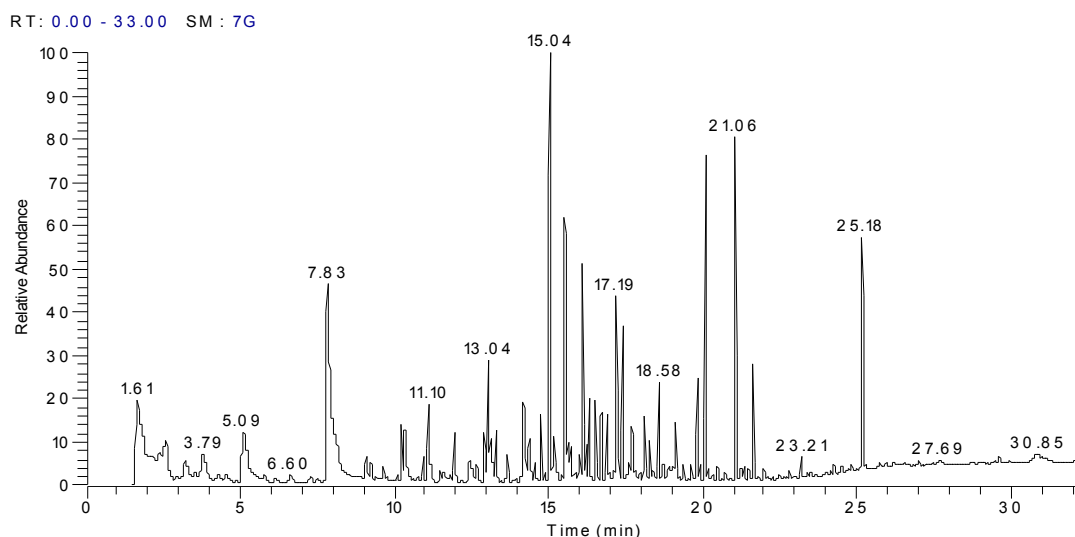


Fig. 1: Total-ion GC-MS chromatogram of volatile components of *Porphyra yezoensis* Maillard product

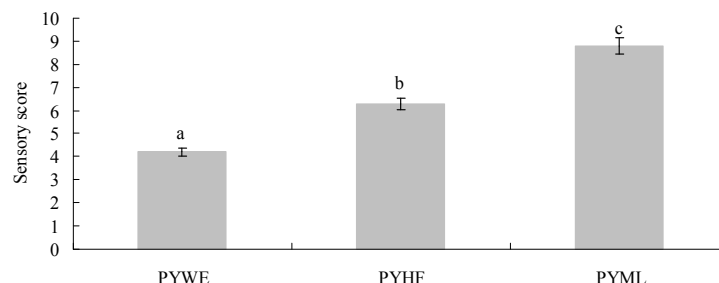


Fig. 2: Overall acceptance of *Porphyra yezoensis* Water Extract (PYWE), hydrolysates with flavorzyme (PYHF) and Maillard reaction liquid (PYML)

Table 1: The amino acids (mg/100 mL) in *Porphyra yezoensis* Water Extract (PYWE), hydrolysates with flavorzyme (PYHF), and Maillard reaction liquid (PYML)

Amino acids	PYWE	PYHF	PYML
Aspartic acid	5.83 <sup>a</sup>	8.08 <sup>b</sup>	8.20 <sup>b</sup>
Glutamate	17.55 <sup>a</sup>	24.72 <sup>b</sup>	27.49 <sup>c</sup>
Serine	0.53 <sup>a</sup>	0.75 <sup>b</sup>	0.19 <sup>c</sup>
Histidine	0.69 <sup>a</sup>	0.97 <sup>b</sup>	0.73 <sup>c</sup>
Glycine	1.03 <sup>a</sup>	1.43 <sup>b</sup>	5.37 <sup>c</sup>
Threonine	5.28 <sup>a</sup>	4.15 <sup>b</sup>	4.13 <sup>b</sup>
Arginine	8.32 <sup>a</sup>	11.61 <sup>b</sup>	5.66 <sup>c</sup>
Alanine	19.72 <sup>a</sup>	27.80 <sup>b</sup>	26.80 <sup>b</sup>
Tyrosine	3.48 <sup>a</sup>	4.98 <sup>b</sup>	4.70 <sup>b</sup>
Cysteine	1.72 <sup>a</sup>	2.40 <sup>b</sup>	3.80 <sup>c</sup>
Valine	2.69 <sup>a</sup>	3.80 <sup>b</sup>	0.92 <sup>c</sup>
Methionine	2.12 <sup>a</sup>	2.98 <sup>b</sup>	2.43 <sup>c</sup>
Tryptophan	0.66 <sup>a</sup>	0.92 <sup>b</sup>	1.05 <sup>c</sup>
Phenylalanine	4.37 <sup>a</sup>	6.16 <sup>b</sup>	0.29 <sup>c</sup>
Isoleucine	2.60 <sup>a</sup>	2.20 <sup>b</sup>	2.86 <sup>c</sup>
Leucine	6.59 <sup>a</sup>	9.27 <sup>b</sup>	1.91 <sup>c</sup>
Lysine	5.94 <sup>a</sup>	8.47 <sup>b</sup>	1.55 <sup>c</sup>
Proline	1.06 <sup>a</sup>	1.39 <sup>b</sup>	1.68 <sup>c</sup>
Total	86.80 <sup>a</sup>	122.1 <sup>b</sup>	99.81 <sup>c</sup>

and sensory score. Aspartic acid, glutamate, glycine and alanine contents of PYWE were 5.83, 17.55, 1.03 and 19.72 mg/100 mL, respectively, whereas those of PYHF were 8.08, 24.72, 1.43 and 27.80 mg/100 mL, respectively. Amino acid contents of PYHF were significantly higher than those of PYWE ( $p < 0.05$ ; Table 1). Aspartic acid, glutamate and alanine contents of PYML were 8.20, 27.49 and 26.80 mg/100 mL, respectively and were comparable to those of PYHF ( $p > 0.05$ ) but higher than those of PYWE ( $p < 0.05$ ; Table 1). The glycine content of PYML (5.37 mg/100 mL) was higher than those of PYHF and PYWE ( $p < 0.05$ ; Table 1). The greater number of delicious amino acids in PYML signified enhanced taste. The total number of amino acids in PYML was lower than that in PYHF but higher than that in PYWE ( $p < 0.05$ ; Table 1). This result indicated that a considerable quantity of amino acids was involved in Maillard reaction and increased the taste deliciousness of PYML.

**Volatile flavour components of PYML:** Glutamate, glycine, cysteine, xylose, glucose and thiamine were added to PYHF to facilitate Maillard reaction. The total-ion GC-MS chromatogram of volatile components of PYML is shown in Fig. 1 and the volatile components of PYML are listed in Table 2. A total of 42 volatile flavour components were generated through Maillard reaction. These volatile flavour components played an important role in the sensory score of PYWL. Similarly, Pan *et al.* (2004) obtained approximately 28 volatiles by the photo-oxidation of lipids applied to the surface of dried seaweed (*P. yezoensis* Ueda).

Table 2: The volatile flavor components in *porphyra yezoensis* Maillard reaction liquid

Number	Retention time (min)	Components	Content (%)
1	2.56	2-pentanamine	2.98
2	3.79	3-methylbutanal	2.13
3	4.52	(2Z, 4E)-hexa-2, 4-dienal	0.47
4	5.09	Pentanal	4.15
5	6.60	2-butenal	0.52
6	7.83	Hexanal	13.32
7	9.09	2-pentenal (E)	0.73
8	10.23	1-penten-3-ol	1.32
9	10.33	Heptanal	2.02
10	11.10	(E)-2-hexenal	2.16
11	11.57	(Z)-4-heptenal	0.62
12	11.95	1-pentanol	1.13
13	12.44	Octanal	0.86
14	12.67	1-octen-3-one	0.47
15	12.92	1, 1, 3-trimethyl-2-cyclohexanone	0.82
16	13.04	3-Octaone, 2-methyl	2.74
17	13.15	2, 5-dimethylpyrazine	0.83
18	13.30	6-methyl-5-hepten-2-one	0.94
19	13.64	1-hexanol	0.70
20	14.18	Nonanal	3.06
21	14.37	2-cyclohexen-1-ohlyne, 3, 3, 5-trimethyl	0.80
22	14.74	2-nonanal	1.06
23	15.04	1-octen-3-ol	7.42
24	15.17	(5Z)-octa-1, 5-dien-3-ol	0.78
25	15.60	1-hexanol,2-ethyl	1.19
26	16.10	Benzaldehyde	3.61
27	16.23	2-nonenal(z)	0.50
28	16.51	2-cyclohexen-1-ol3, 4, 4-trimethyl	1.46
29	16.71	3, 5-octadiene-2-one	1.01
30	16.90	Tran-2-cis-6-nonadien	0.97
31	17.19	1-methylcycloheptanol	3.08
32	17.25	(E)-2-octen-1-ol	1.77
33	17.40	1-cyclohexene-1-carboxaldehyde, 2, 2, 6-trimethyl	2.49
34	17.71	1, 3-cyclihexdiene-1-carboxaldehyde, 2, 6, 6-trimethyl	1.09
35	18.08	3-cyclohexene-1-ehanol	1.24
36	18.27	2, 6, 6-trimethyl-2-cyclohexene-1, 4-dione	0.58
37	18.58	8-heptadecence	1.41
38	19.11	Trans-2, 2-dimethyl-4-decene	1.00
39	19.83	2-undecyne	1.53
40	20.09	3-buten-2-one, 4-(2, 6, 6-trimethyl-2-cyclohexen-1-yl)	5.18
41	21.06	3-buten-2-one, 4-(2, 6, 6-trimethyl-1-cyclohexen-1-yl)	5.17
42	25.18	1, 2-benzenedicarboxylic acid, diethyl ester	5.10

**Sensory scores of PYWE, PYHF and PYML:** Sensory scores of PYWE, PYHF and PYML are shown in Fig. 2. Sensory scores of the samples followed the order PYWE < PYHF < PYML ( $p < 0.05$ ; Fig. 2). The highest sensory score of PYML was attributed to the high contents of delicious amino acids, including aspartic acid, glutamate, glycine and alanine, as well as to volatile flavour components.

## CONCLUSION

We prepared *P. yezoensis* essence by water extraction, hydrolysis with flavorzyme and Maillard reaction. The hydrolysis of *P. yezoensis* protein with flavorzyme increased the content of taste-delicious amino acids in PYWE, whereas Maillard reaction yielded 42 volatile flavour components, which increased the sensory score of PYML. Hence, the study successfully demonstrated a potential method for preparing *P. yezoensis* essence.

## ACKNOWLEDGMENT

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