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Seafood flavourings characterization as prepared from the enzymatic hydrolysis of *Undaria pinnatifida* sporophyll by-product

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ABSTRACT

Protein by-products from *Undaria pinnatifida* (*U. pinnatifida*) sporophyll processing (i.e., after polysaccharide extraction) were hydrolysed using flavourzyme (enzyme activity = 7592 U/g). Optimal hydrolysis conditions were determined using response surface analysis (i.e., 7% flavourzyme for 18 h); a hydrolysate yield of 32.52 ± 0.46 g/100 g dry-solids was achieved with a degree of hydrolysis (DH) at 5.63 ± 0.27 g α-amino nitrogen/100 g total nitrogen. Five free amino acids (FAAs), alanine, glumatic acid, aspartic acid, proline, and glycine, were abundant in the best hydrolysate. Eighteen volatile flavour compounds were identified using gas chromatography/mass spectrometry, with hexanal, cedrol, nonanal, 2-heptenal, acetoin, and heptanal being the primary odorants. As indicated by the sensory panel, the hydrolysate from *U. pinnatifida* sporophyll by-products (HUPSB) exhibited an umami taste and a seaweed odour. It was concluded that the protein by-products of *U. pinnatifida* sporophyll processing could yield excellent seafood flavouring.

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Flavourzyme; Free amino acids; Seafood flavouring; Sporophyll; *Undaria pinnatifida*; Volatile flavours

Introduction

Protein hydrolysates from marine animal sources, such as fish, shrimp, clam, crab, and seafood by-products, have been widely used to produce seafood flavourings.[1] However, quality control of these protein-based flavouring production is often complicated by the need to remove excessive fats of the marine animal sources and to minimize lipid oxidation.[2] Such need is minimal in plant proteins, due to the much lower lipid contents of plant materials. For centuries, seaweed has been used in the preparation of soups and foods due to its pleasant flavour. Seaweed by-products, after agar extraction, are good sources of plant proteins, and they contain flavour-rich amino acids, such as aspartic acid, glutamic acid, arginine, and lysine, with a low fat content.[1] Various reports have indicated that these amino acids and short-chain peptides from vegetable proteins, meat muscle proteins, and seafood proteins can produce highly desirable taste profiles.[3–5] Indeed, glutamic acid and glutamic acid-rich oligo peptides from hydrolysed proteins generate an umami flavour. Furthermore, amino acids and peptides from hydrolysed vegetable proteins are precursors in a variety of Maillard reactions that produce an extensive range of volatile flavours.[3]

Undaria pinnatifida (*U. pinnatifida*) is a type of brown algae that normally grows in the seas of China, Korea, and Japan. Its structure can be categorized into blade (lamina), midrib, sporophyll, and root-like formations (haptera). Its Japanese name is wakame, which is also the name of the processed *U. pinnatifida* used as food.[6] In 2014, the annual production of *U. pinnatifida* was 20 tons (dry weight) in China.[7] Sporophyll, the reproductive organ of *U. pinnatifida* and a processing

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by-product of the seaweed food industry, is now being used as a feedstock in the functional food industry for the extraction of sulphated polysaccharide. However, the current processing is incomplete: after the removal of agar and sulphated polysaccharides, the residues of *U. pinnatifida* sporophyll are still rich in proteins, which can be further utilized to produce high-quality seafood flavourings.

Selective enzymatic hydrolysis under controlled conditions is an effective way to enrich volatile compounds and to improve the physicochemical characteristics and organoleptic quality of plant proteins. It produces desirable peptides and amino acids with less content of salt and carcinogenic compounds, such as mono- and dichloropropanols or 3-monochloropropane-1,2-diol (3-MCPD), than that of acid hydrolysis.[8] The hydrolysate contains amino acids and low molecular weight peptides with unique taste properties, e.g., sweet, salty, sour, bitter, and umami tastes.[9] A mixture of proteases called flavorzyme, with both exo- and endopeptidase activities, can be obtained by the controlled fermentation of *A. oryzae*.[10] It has been reported to change the volatile compounds profile of dry fermented sausage[11] by accelerating proteolysis. The objectives of this study were to: 1. produce a protein hydrolysate, which could be used as a precursor to produce seafood flavourings, from *U. pinnatifida* sporophyll by-products (i.e., HUPSB) using flavourzyme; 2. characterize the physicochemical properties and the volatile compounds profile of this protein hydrolysate, followed by a sensory evaluation to identify the key contributors to its flavouring attributes; and 3. apply the response surface methodology (RSM) to find the optimal hydrolysis conditions.

Materials and methods

Materials and chemicals

U. pinnatifida sporophyll by-products (UPSB) samples after agar and sulphated polysaccharide extraction were provided by Dalian Aquaculture Co., Ltd., located in Dalian, China. The chemical compositions of the sample were obtained according to AOAC (protein: AOAC954.01; lipid: AOAC2003.06; dietary fibre: AOAC992.16; ash: AOAC938.08; carbohydrate: AOAC979.06) (1997). UPSB on a dry weight basis contained 20.94 ± 0.20 g protein/100 g dry-solids, 3.20 ± 0.05 g lipid/100 g dry-solids, 19.06 ± 0.06 g ash/100 g dry-solids, 9.97 ± 0.19 g fibre/100 g dry-solids, and 46.83 ± 0.74 g carbohydrate/100 g dry-solids, respectively. Flavourzyme (E.C. 3.4.11.1; 7592 U/g) was purchased from Nanning Pangbo Biological Engineering Co., Ltd., located in Nanning, China. All reagents were of analytical grade, and used without further purification.

Preparation of the HUPSB

The preparation of the HUPSB followed previous research with some modification.[1,12,13] Around 10 g of UPSB was dispersed in 500 mL of purified water, and the pH of the dispersion was adjusted to pH 6.8 with 1 N HCl. The dispersion was then pre-incubated at 55°C for 10 min to ensure optimal flavourzyme activity. Vials of the dispersion were then incubated at 50°C for 1, 3, 6, 12, and 24 h with flavourzyme added at enzyme/substrate ratios (E/S) of 0%, 1%, 2%, 5%, and 10% (weight of enzyme/weight of UPSB). The hydrolysis was terminated at the end of the designated reaction time for each vial by heating at 100°C for 15 min. The reactant from each vial was then filtrated with a 240-mesh filter cloth. The resulting hydrolysate was collected and stored in glass bottles at 4°C prior to analysis. All tests were performed in triplicates.

Determination of the degree of hydrolysis (DH) and the yield of the HUPSB

The degree of hydrolysis (DH) was defined as the percentage ratio of the α-amino nitrogen to the total nitrogen. The total nitrogen was determined using the Kjeldahl Method. α-amino nitrogen was determined using the modified formol titration procedure as described by Gump et al. [14] and Filipe-Ribeiro et al. [15]. The DH can then be calculated using the general equation:

$$\text{Degree of hydrolysis (DH)} = \alpha - \text{amino nitrogen/total nitrogen} \times 100 \quad (1)$$

To calculate the percentage yield (g/100 g dry-solids), the hydrolysate (nearly 500 mL) was concentrated using a rotary evaporator at 40°C and 5 kPa, until the final volume reached 250 mL. Now, 50 mL of the concentrated hydrolysate was dried to constant weight in a baking oven at 90°C, and the final weight of the dried material was determined. The percentage yield was calculated as follows:

$$\text{Yield(g/100 g dry} - \text{solids)} = \text{weight of dried material in hydrolysate} \times 5/\text{weight of UPSB} \times 100 \quad (2)$$

Experimental design for enzymatic reaction optimization using RSM for HUPSB production

Optimal hydrolysis conditions were established using RSM. Two independent variables, enzyme concentration (x_1) and hydrolysis time (x_2), were investigated, each at five levels (x_1 = 0, 1, 2, 5, and 10% w/w; x_2 = 1, 3, 6, 12, and 24 h). A 2×5 randomized factorial design (CRD) was used to obtain the combination of x_1 and x_2 values that optimizes the DH and yield of the reaction, respectively. To find the optimal point, the response surface was established by a second-order polynomial model as follows:

$$y = a_0 + a_1x_1 + a_2x_2 + a_{12}x_1x_2 + a_{11}x_1^2 + a_{22}x_2^2 \quad (3)$$

where y represents the predicted response (yield and DH, respectively); x_1 and x_2 represent independent variables; a_0 represents an offset term; a_1 and a_2 represent linear effects; and a_{12} represents the interaction effect between the two variables. The model was used to quantify the effects of each independent variable. All analyses were performed using Design-Expert.8.05 (Stat Ease Inc, USA).

Analysis of free amino acids of the HUPSB

The analysis of free amino acids (FAAs) followed previously reported procedures with some modification.[2,13] The FAAs of the HUPSB were extracted by acetone, and derived with 2, 4-dinitrofluorobenzene. The FAA levels were determined by an LC-10 Avp Plus HPLC (Shimadzu Co., Tokyo, Japan) coupled with an Elite amino acid analysis column (Elite Analytical Instruments Co., Ltd., Dalian, Liaoning, China), according to the methods recommended by the instruction of the Elite-AAK amino acid analysis system (UV1201 ultraviolet visible detector, Elite Analytical Instruments Co., Ltd., Dalian, Liaoning, China). Amino acid mixture standards (Elite Analytical Instruments Co., Ltd., Dalian, Liaoning, China) including 19 different amino acids were used as calibration references. The levels of amino acids were estimated based on the peak areas calibrated with known concentrations of the standards using LC solution software (version 1.11 SP1, Shimadzu, Japan).

Compositional analysis of volatile compounds profile of the HUPSB

The composition of volatile compounds in HUPSB was analysed on a headspace solid-phase microextraction-gas chromatography-mass spectrometry (HS–SPME-GC–MS) system (5975C MS Agilent Technologies, Santa Clara, CA) following Sonklin et al.'s method with some modification.[3] Each sample (3 mL) was placed into a 20-mL vial and heated at 50 °C for 5 min in a GC–MS heating block for headspace analysis. Volatile compounds were absorbed onto an SPME fibre (50 × 30 µm DVB/Carboxen™/PDMS StableFlex™; Supelco, Bellefonte, PA) at 50°C for 20 min. After equilibrium, the SPME fibre was desorbed into the injector port at 260°C for 2 min in splitless mode. Helium was used as the carrier gas at a constant velocity of 1.5 mL/min. Volatile compounds were separated using a DB-Wax capillary column (30 m × 0.25 mm, 0.25 µm film thickness; Agilent Technologies,

Santa Clara, CA). The oven temperature profile was as follows: initial temperature of 35°C; raised to 70°C at 3°C/min; raised to 200°C at 10°C/min; raised to 260°C at 20°C /min; and held at 260°C for 5 min. Volatile compounds were detected using Mass Spectrometry Detection (MSD) (Single Quadrupole, scan range of m/z 29–350) at 260°C. The identification of compounds was based on the comparison of their retention time and mass spectrum with data in the NIST11 libraries at a quality match greater than 60%. A series of n-alkanes (C8-C20) was analysed by direct injection on the GC–MS to obtain retention index (RI) values. The RI data were compared with previously published literature values for references.

Sensory panel evaluation of the HUPSB

Descriptive sensory panel analysis of the HUPSB was performed following previous research with some modification.[16,17] A 15-member panel was randomly selected from a pool of graduate students and faculty members at the School of Food Science and Technology, Dalian Polytechnic University. The selected panellists were presented with food products having one of the following eight characteristic sensory attributes: (1) seaweed odour (kelp source), (2) crab odour (cooked crab), (3) shrimp odour (cooked shrimp), (4) caramel odour (cooked sugar), (5) umami taste (glutamic acid solution), (6) sweet taste (sugar solution), (7) salty taste (salt solution), and (8) bitter taste (quinine solution) for their training. The panellists were then served with the HUPSB in opaque, disposable plastic cups. For the HUPSB, the panellists were required to score each of the eight attributes on a scale of 0–10 anchored by the low intensity (0) and the high intensity (10).

Statistical analysis

All assays were performed at three repetitions. Data were subjected to analysis of variance (ANOVA), and the differences between means were evaluated by the least-significant difference test following Young.[18] The SPSS statistical program (Version 13.0) (SPSS Inc., 2001) was used for data analysis. Comparison that yielded $p < 0.05$ was considered significant.

Results and discussion

Physicochemical properties of the HUPSB

The UPSB is a suitable candidate to produce high-quality protein hydrolysate because it is high on proteins (21 g/100 g dry-solids) and low on lipids (3.2 g/100 g dry-solids). The total salt content of the HUPSB, 0.31 ± 0.01 g/100 g dry-solids, was not affected by the enzyme concentration or the hydrolysis time ($p > 0.05$). Figures 1a and 1b illustrate the influence of the hydrolysis time and enzyme concentration on the proteolytic reaction, represented by two response variables, yield and DH, which indicate the efficiency of the enzyme to cleave peptide bonds. In this study, the yield and DH were optimized using a statistical model established by RSM, which was effective in replacing traditional kinetic experiments to quantify the effects of each factor, and the interaction between factors.[19] Regression coefficients between the two response variables (yield and DH) and the two factors (i.e., enzyme concentration and hydrolysis time) are listed in Table 1. Correlation coefficient of determination (r^2) values for all of the response variables were higher than 0.80, indicating that the model adequately explained the correlations between the factors and the response variables (i.e., yield and DH). After RSM was applied, the following models were established for yield and DH, respectively:

$$\text{Yield} = 21.21 + 0.25x_1 + 1.00x_2 - 0.0015x_1x_2 - 0.011{x_1}^2 - 0.026{x_2}^2 \quad (4)$$

$$\text{DH} = 1.31 + 0.28x_1 + 0.42x_2 - 0.00065x_1x_2 - 0.022{x_1}^2 - 0.013{x_2}^2 \quad (5)$$

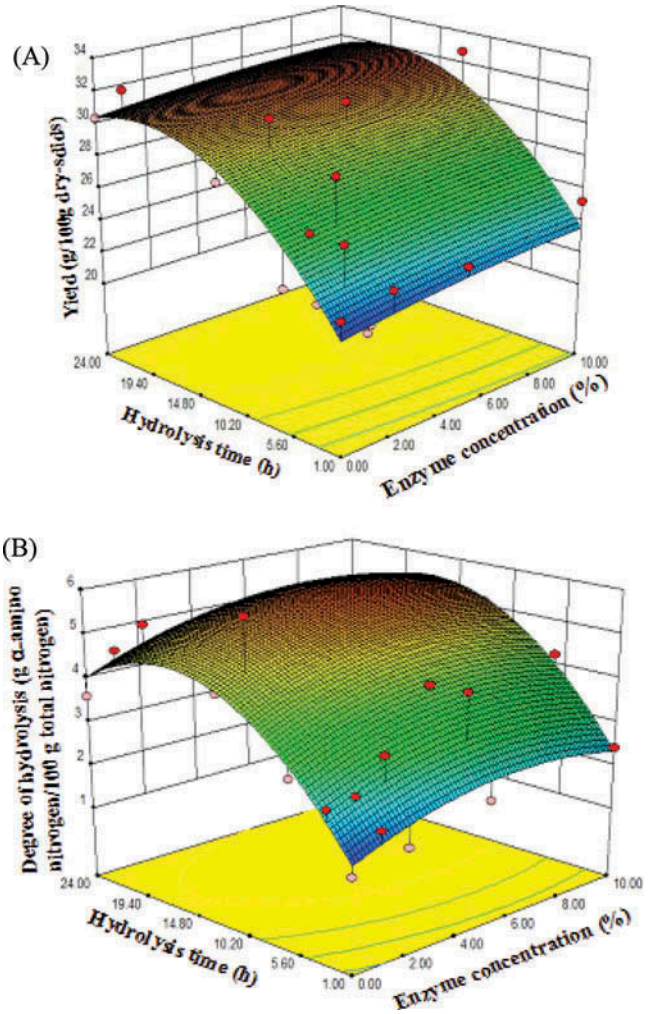

Figure 1. Response surfaces for the effect of enzyme concentration and hydrolysis time on (A) yield (g/100 g dry-solids) and (B) degree of hydrolysis of HUPSB (g α-amino nitrogen/100 g total nitrogen).

where x_1 represents enzyme concentration and x_2 represents the hydrolysis time. The interaction between the enzyme concentration (x_1) and the hydrolysis time (x_2) on the yield was significant ($p < 0.01$). The yield demonstrated a positive linear correlation with both the enzyme concentration (x_1) and hydrolysis time (x_2), but a negative correlation with the interaction of x_1^2 and x_2^2. The yields for all of the enzyme concentrations increased rapidly in the initial phase of hydrolysis (1–18 h) and then decreased after 18 h. The highest yield (31.93 g/100 g dry-solids) of the HUPSB was produced from an enzyme concentration of 7.31% flavourzyme and a hydrolysis time of 17.78 h. Regression coefficients indicated strong correlations between independent factors (the enzyme concentration and the hydrolysis time) and DH (Table 1). A positive value indicated that the DH values increased with increasing enzyme concentration, as reported by McCarthy et al. [3] Similar to the yield, the hydrolysis time also affected the DH of the HUPSB. The DH value decreased after 18 h (Fig. 1b). The DH dramatically increased when the enzyme concentration was increased from 0% to 7%. However, the DH values of the HUPSB using 10% flavourzyme were not significantly different, due to saturated enzyme/substrate or inhibitory effects of the end-products.[20]

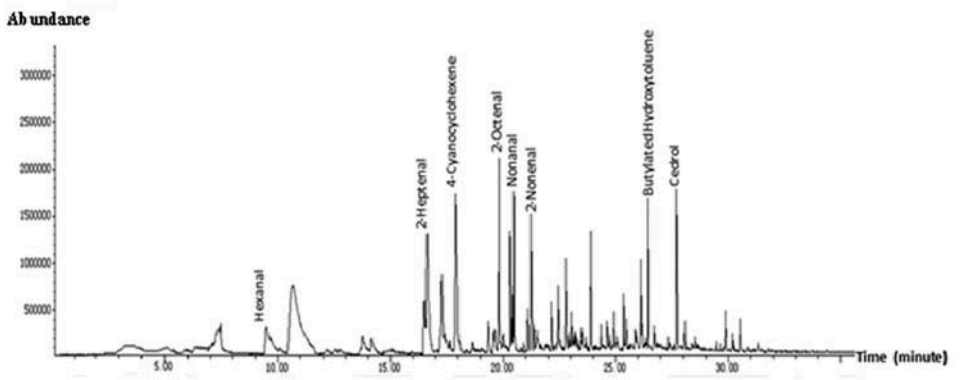

Figure 2. GC-MS chromatogram of HUPSB by 7% flavourzyme for 18 h.

Table 1. Regression coefficients of a quadratic model estimated by multiple linear regressions for the yield and DH of HUPSB.

	Regression coefficient	
Factors	Yield (g/100g dry-solids)	DH (g α-amino nitrogen/100 g total nitrogen)
Constant	21.21**	1.31**
Linear		
x_1	0.25	0.28*
x_2	1.00**	0.42**
Quadratic		
x_1^2	−0.011	−0.022
x_2^2	−0.026	−0.013**
Interaction		
x_1x_2	−0.0015	0.00065
Statistical analysis for the model		
r^2	0.8125	0.845
Adjusted r^2	0.7631	0.8042

** Significant at $p<0.01$; * Significant at $p<0.05$.

To visualize the results, response surface plots and a contour plot of yield and DH were used to determine the optimal hydrolysis conditions. Figures 1a and 1b show the three-dimensional response surface plots and contour plot for the independent factors (concentration and hydrolysis time) on yield and DH, respectively. Next, 7% flavourzyme concentration and 18 h hydrolysis time were selected as the optimal conditions for producing the HUPSB (Table 2); it is close enough to the model predicted value of 7.31% flavourzyme and 17.78 h, yet practical enough for experiments. The model predicted response values (yield 31.93 g/100 g dry-solids and DH 5.699 g α-amino nitrogen/100 g total nitrogen) were verified by experiments: the observed values were 32.52 ± 0.46 g/100 g dry-solids (yield) and 5.63 ± 0.27 g α-amino nitrogen/100 g total nitrogen (DH), respectively. The observed and predicted values are listed in Table 2. The experimental results were similar to the predicted values for yield and DH

Table 2. Predicted and experimental values of HUPSB.

	Yield (g/100 g dry-solids)		DH (g α-amino nitrogen/100 g total nitrogen)	
	Observed[a]	Predicted	Observed[a]	Predicted
x_1 = 7.31%, x_2 = 17.78 h	–	31.93[ns]	–	5.699[ns]
x_1 = 7%, x_2 = 18 h	32.52 ± 0.46[ns]		5.63 ± 0.27[ns]	

ns means of yield and DH compared by t-test are not significantly different ($p > 0.05$), a Observed yield and DH values are expressed as means ± SD (n = 3)

Table 3. Free amino acid composition in UPSB and HUPSB.

	Amino acid content(mg/100 g of protein)*	
Amino acids[a]	UPSB	HUPSB
Essential amino acid		
Threonine	320 ± 110	2000 ± 370
Tryptophan	0	270 ± 10
Valine	30 ± 0	100 ± 0
Tyrosine	70 ± 10	630 ± 130
Non-essential amino acid		
Aspartic acid	800 ± 70	3900 ± 580
Glutamic acid	1210 ± 130	3950 ± 380
Glycine	470 ± 50	2110 ± 360
Alanine	2760 ± 280	5080 ± 760
Arginine	250 ± 30	1460 ± 240
Serine	320 ± 30	1640 ± 290
Proline	430 ± 50	2240 ± 260
Total	6640 ± 80	23380 ± 230
Taste component		
Bitter[b]	500	2480
Umami[c]	2010	7850
Sweet[d]	4060	12420
Tasteless[e]	70	630
Total	6640	23380

[a]Amino acids in the seaweed by-products were in free amino acid form
[b]Bitter was calculated from the sum of valine + glycine + tryptophan.
[c]Umami was calculated from the sum of aspartic acid + glutamic acid.
[d]Sweet was calculated from threonine + serine + arginine + alanine + proline.
[e]Tasteless was calculated from tyrosine.
*Values are means and standard deviations of triplicate measurements.

using RSM. These results confirmed the suitability of the model to predict the optimal conditions for HUPSB.

Composition of FAA in the UPSB and the HUPSB and sensory evaluation

FAA composition is more meaningful in terms of determining the flavour attributes of the UPSB and HUPSB than the composition of protein amino acids because FAAs are active primary flavour components in the hydrolysate. The distribution and relative abundance of these active taste components affect the flavour quality.[21] The FAA composition of the UPSB and the HUPSB produced using 7% flavourzyme for 18 h was determined using HPLC, as shown in Table 3. Ten and eleven FAAs were found in the UPSB and HUPSB, respectively. Alanine was the most abundant (2760 ± 280 mg/100 g); other abundant amino acids found in the UPSB include glutamic acid (1210 ± 130 mg/100 g), aspartic acid (800 ± 70 mg/100 g), glycine (470 ± 50 mg/100 g), and proline (430 ± 50 mg/100 g).

All in all, eleven FAAs derived from the HUPSB were identified, including three hydrophobic (alanine, valine, and proline), four hydrophilic (glycine, serine, threonine, and tyrosine), two acidic (aspartic and glutamic acid), and one basic (arginine) amino acid. Among the identified amino acids, alanine was the most abundant (5080 ± 760 mg/100 g), followed by glutamic acid (3950 ± 380 mg/100 g), aspartic acid (3900 ± 580 mg/100 g), proline (2240 ± 260 mg/100 g), and glycine (2110 ± 360 mg/100 g). There was an over threefold increase in FAAs in the HUPSB (22930 mg/100 g) than those in the UPSB (6660 mg/100 g). Aspartic acid and glutamic acid are the major components in the taste sensation of umami, which means “delicious” in Japanese. Umami is described as savoury with a meat- or broth-like taste.[22] In the HUPSB, free glutamic acid and aspartic acid content is significantly elevated than that of the UPSB (7850 mg/100 g vs. 2010 mg/100 g). Umami and sweet taste are to be expected in the HUPSB than those of UPSB, due to flavourzyme hydrolysis. Among these FAAs, valine, histidine, and tryptophan contribute to bitterness, but their

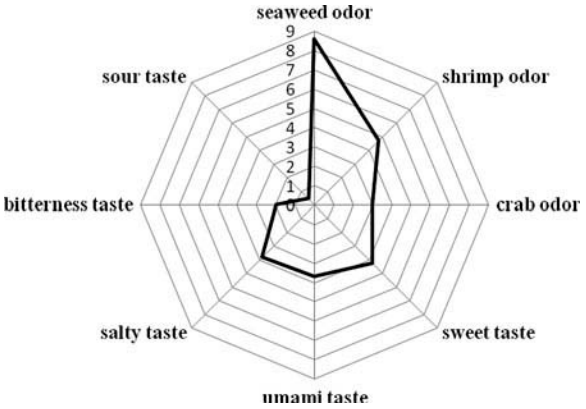

Figure 3. Sensory profiles of HUPSB by 7% flavourzyme for 18 h.

concentration (2480 mg/100 g) was much lower compared to the concentration of sweet FAAs (alanine, threonine, serine, arginine, and proline, at 12420 mg/100 g) in the HUPSB. These results suggest the flavourzyme hydrolysis is an effective way to improve the flavour of the UPSB. In addition, the FAA results were consistent with sensory panel evaluation. The panellists reported very low bitterness (2.0 ± 0.4 scores, Fig. 3). The absence of bitterness makes the HUPSB an ideal flavouring for taste enhancement.

Volatile compounds profile in the HUPSB and sensory evaluation

Eighteen volatile compounds in the HUPSB hydrolysed using 7% flavourzyme for 18 h were identified (Fig. 2) and are listed in Table 4. Major volatile compounds included hexanal, cedrol, nonanal, 2-heptenal, acetoin, and heptanal with % peak areas at 20.20 ± 1.58, 13.89 ± 0.21, 11.97 ± 1.11, 10.39 ± 0.11, 9.17 ± 1.29, and 6.97 ± 0.50, respectively. These volatile compounds of the HUPSB

Table 4. Volatile compounds in HUPSB by 7% flavourzyme for 18 h.

Volatile compound[a]	RI[a,b]	Relative content (%)	Door description[c]	Positive relation to
Acetoin	1352.5	9.17 ± 1.29	Butter, cream	
Hexanal	1534.7	20.20 ± 1.58	Resin, flower, green	Seaweed[A],crab[B]
Heptanal	1973.4	6.97 ± 0.50	Fat, citrus, rancid	crab[B]
2-Heptenal	2227.9	10.39 ± 0.11	Green	
2-Octanone	2360.2	3.23 ± 0.21	Sour	
4-Cyanocyclohexene	2453.3	1.06 ± 0.04	Scent	
D-Limonene	2487.4	2.15 ± 0.06	Lemon	
Benzyl alcohol	2520.7	0.81 ± 0.29	Sweety, fruit, flower	
2-Octenal	2574.6	5.29±0.27	Green, nut, fat	
Nonanal	2697.8	11.97 ± 1.11	Citrus, fatty	crab[B]
2-Nonenal	2830.9	3.22 ± 0.12	Orris, fat, cucumber	
2,4-Decadienal	3101	2.63 ± 0.05	Chicken like	
Tridecanal	3121.8	1.60 ± 0.04	Irritant	
Naphthalene, 1,7-dimethyl-	3300.5	1.16 ± 0.45	Fume like	
Pentadecane	3435	3.42 ± 0.12	Fume like	
Eicosane	3558.8	1.25 ± 0.01	Fume like	
Hexadecane	3582.7	1.60 ± 0.19	Fume like	
Cedrol	3629	13.89 ± 0.21	Warm fire like	

[a]All compounds were identified by comparison with mass spectra and retention index database.
[b]RI (retention index) calculated with a DB-Wax stationary phase using a series of alkanes between C_8 and C_{20} as reference standards.
[c]Odour descriptions were cited from www.flavornet.org and recent reports.
[A]Natta et al. (2014).
[B]Yu and Chen (2010).

are consistent with seaweed, green, flower, butter, fatty, and citrus odour characteristics. The sensory profile of the HUPSB was seaweed odour, shrimp odour, crab odour, sweet taste, salty taste, umami taste, bitter taste, and sour taste, with average scores of 8.6 ± 0.8, 4.7 ± 0.9, 3.0 ± 1.0, 4.3 ± 0.7, 3.8 ± 0.4, 3.7 ± 1.5, 2.0 ± 0.4, and 0.4 ± 0.1, respectively (Fig. 3). These sensory scores suggested the dominant flavour characteristics of the HUPSB is represented by a seaweed odour, followed by a shrimp odour, a sweet taste, and an umami taste. Hexanal compounds were reported as the major flavour compounds in seaweed by-products.[1] They are the most abundant compounds found in the HUPSB, consistent with the sensory evaluation of seaweed and crab odours. Hexanal and 2-heptanal were also associated with green odours in seaweed and crab.[1] The cedrol content is also quite high in the HUPSB, but our sensory panel did not report strong warm fire-like odour in the hydrolysate; it may be overwhelmed by more dominant seaweed and shrimp odours. Hexane and 2-heptenal, which have been detected in other marine species, such as shrimp, crab, and fish,[23,24] were also found in the HUPSB. Among the other volatile compounds found in the HUPSB, 4-cyanocyclohexene, 2-octenal, nonanal, 2-nonenal, and cedrol were detected in soybean.[3] Heptanal and nonanal, associated with citrus flavour, were also detected in crabs. These results were similar to hydrolysates from the protein by-products of seaweed (*Gracilaria sp.*).[1] Overall, the volatile compounds profile of the HUPSB suggests it to be a good base material for the production of seafood flavouring additives.

Conclusion

An RSM model was successfully developed to identify optimal hydrolysis conditions for a protein-based flavouring (i.e., HUPSB) production from seaweed UPSB. Using 7% flavourzyme for 18 h, a yield of 32.52 ± 0.46 g/100 g dry-solids and a DH of 5.63 ± 0.27 g α-amino nitrogen/100 g total nitrogen were obtained. The relatively high yield and high FAA contents suggest the high potential value of the HUPSB production. A comprehensive FAA profiling revealed that alanine, glutamic acid, aspartic acid, proline, and glycine were the most abundant FAAs in the hydrolysate, which is consistent with the predominant umami and sweet tastes of the HUPSB reported by a sensory evaluation panel. Volatile compounds profiling of the HUPSB confirmed that the primary odorants of the HUPSB are consistent with seaweed, green, sweet, fatty, and resin odour characteristics. The complex yet highly desirable odour and taste profile of the HUPSB, originating from its unique molecular makeup, indicate that the HUPSB has a great potential to serve as a flavour supplement or a savoury flavour source for various seafood flavouring products.

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