3044 J. Agric. Food Chem. 2007, 55, 3044–3050

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Aroma Components of Acid-Hydrolyzed Vegetable Protein Made by Partial Hydrolysis of Rice Bran Protein

ARPORN JARUNRATTANASRI,[†] CHOCKCHAI THEERAKULKAIT,*[,†] AND KEITH R. CADWALLADER[‡]

Department of Food Science and Technology, Kasetsart University, Bangkok, 10900, Thailand, and Department of Food Science and Human Nutrition, University of Illinois, 1302 West Pennsylvania Avenue, Urbana, Illinois 61801

Hydrolyzed vegetable protein (HVP) was prepared from rice bran protein concentrate (RBPc) by partial hydrolysis with aqueous 0.5 N HCl at 95 °C for 12 or 36 h (H-RBPc-12 and H-RBPc-36, respectively). Aroma components of the RBPc and the HVPs were characterized by gas chromatography–olfactometry, gas chromatography–mass spectrometry, aroma extract dilution analysis, and calculation of odor activity values (OAVs). The predominant odorants in RBPc were 3-methylbutanal, hexanal, 2-aminoacetophenone, (*E*)-2-nonenal, phenylacetaldehyde, and β-damascenone. Among these, the odor of 2-aminoacetophenone, present at 59 ng/g in RBPc, was reminiscent of the typical odor of RBPc. Most of the predominant odorants had higher $\log_3$FD factors in the H-RBPc-36 as compared to H-RBPc-12. Aroma impact compounds of H-RBPc-12 and H-RBPc-36 were 2-methoxyphenol (guaiacol), 4-hydroxy-2,5-dimethyl-3(*2H*)furanone, 3-hydroxy-4,5-dimethyl-2(*5H*)-furanone (sotolon), vanillin, 3-methylbutanal, (*E*)-2-nonenal, 4-vinyl-2-methoxyphenol (*p*-vinylguaiacol), and β-damascenone. Guaiacol had the highest OAV values of 2770 and 17650 in H-RBPc-12 and H-RBPc-36, respectively.

KEYWORDS: Acid hydrolysis; rice bran protein concentrate; hydrolyzed vegetable protein; aroma; flavor

INTRODUCTION

Rice bran (RB) is the outer brown layer, including the rice germ that is removed during the milling of brown rice. Fujimaki (*1*) reported that although a large quantity of RB is available throughout the world, little is actually consumed by humans because of its unpleasant flavor. Over 270 aroma compounds were detected in RB, of which approximately 170 compounds were identified (*1*, *2*). These included minor amounts of *p*-vinylguaiacol and *p*-vinylphenol that were reported to impart unpleasant medicinal odors.

The number and complexity of compounds that contribute to the flavor of various protein sources are considerable. The biggest contribution to flavor is made by free amino acids and peptides. The most common reaction causing protein fragmentation is hydrolysis (*3*), which yields peptides and free amino acids. These compounds can influence perceived flavor or can serve as flavor precursors, such as in the Strecker degradation of amino acids. In addition, amino acids and peptides may undergo many transformations, such as reaction with reducing sugars (Maillard reaction) to form aroma compounds and pigments (*4*). Chemical hydrolysis remains one of the most popular forms of protein modification (*5*). Peptide bond hydrolysis, by either a strong acid or a base, may be used to yield smaller peptides with a more uniform molecular size or free amino acids. Hydrolyzed vegetable protein (HVP) produced commercially by acid hydrolysis has been used to produce various types of flavors by the Maillard reaction. Enzyme-hydrolyzed vegetable protein (EVP), where the protein source is hydrolyzed by proteolytic enzymes, is an alternative to HVP. However, the acidic hydrolysate is dark brown with a strong savory flavor, whereas the enzymatic hydrolysate is usually lighter in color and has a much less pronounced meaty or savory flavor (*3*). Hydrochloric acid (HCl) is commonly used in the production of protein hydrolysates because it works quickly and yields a fully hydrolyzed product with a highly acceptable savory profile (*5*). Currently, the formation of potentially carcinogenic chlorohydrins (e.g., 3-chloro-1,2-propanediol and 1,3-dichloro-2-propanol) in HVP from the use of concentrated HCl for hydrolysis has been a serious concern in many countries (*6*). Collier et al. (*6*) reported 0.9–5.7% of total chloropropandiols by weight of substrate in HVP produced under strong hydrolysis conditions (5.5 M HCl, 107 °C). To avoid the formation of chlorohydrins, mild hydrolysis reactions such as the use of proteolytic enzyme (*3*) or mild acid hydrolysis conditions (*7*) should be used. The use of mild acid hydrolysis conditions (low concentration of HCl and low temperature) leads to partial hydrolysis, which generates a mixture of free amino acids and small and large peptides. The size of the peptide, the position

* To whom correspondence should be addressed. Tel: +66-2-502-5032. Fax: +66-2-562-5020. E-mail: fagicct@ku.ac.th.
[†] Kasetsart University.
[‡] University of Illinois.

10.1021/jf0631474 CCC: $37.00 © 2007 American Chemical Society
Published on Web 03/17/2007

of the amino acids within the peptide, and the resistance of the peptide bond to further hydrolysis are important in flavor formation (*8*). Ho et al. (*9*) studied amino acids and peptides as flavor precursors in a model Maillard reaction by heating amino acids or peptides separately with glucose at 180 °C under pH 4−5 for 2 h. The reaction of glucose with glycine or triglycine produced more pyrazines than when glucose was reacted with diglycine or tetraglycine.

One of the most important flavor precursor components in defatted rice bran (DRB) is protein. DRB may be used as a raw material for the production of HVP (*10*). Hamada (*11*) demonstrated that peptides, which are generated from DRB by enzymatic hydrolysis, contained substantial amounts of glutamic acid and could serve as a flavor-enhancing ingredient after further deamination. Jarunrattanasri et al. (*12*) also produced HVP from DRB by first preparing a rice bran protein concentrate (RBPc) prior to hydrolysis with 4.0 N HCl at 95 °C for 72 h. However, HVP prepared from RBPc by acid hydrolysis using mild reaction conditions has not been previously reported. Use of a mild acid hydrolysis of DRB protein concentrate may add value to underutilized RB through its conversion, via acid hydrolysis of protein and the subsequent Maillard reaction of amino acids and peptides with carbonyl compounds in RBPc, to an aroma or flavoring ingredient.

In the analysis of flavor, gas chromatography−mass spectrometry (GC-MS) can be used to selectively analyze aroma compounds once their spectral and chromatographic properties are known. However, the task of determining which compounds in a sample are odor-active requires a bioassay. Teranishi (*13*) concluded that the constituent(s) that contribute to the characteristic sensory properties of the food product should first be investigated. Gas chromatography−olfactometry (GCO) is a method that reveals odorants in terms of their pattern of smell activity, thus eliminating odorless compounds from consideration. The purpose of our study was to identify and quantify the predominant aroma components that are generated by partial acid hydrolysis of RBPc.

MATERIALS AND METHODS

Chemicals. Analytical-grade reference compounds were obtained from Aldrich Chemical Co. (St. Louis, MO) except for (*E*)-β-damascenone, which was provided by Firmenich Co. (Princeton, NJ). 2-Acetyl-1-pyrroline was a gift from Dr. R. Buttery (Agricultural Research Service, U.S. Department of Agriculture, WRRC, Albany, CA). *trans*-4,5-Epoxy-(*E*)-2-decenal was synthesized using a previously published procedure (*14*). (*Z*)-2-Nonenal was synthesized from (*Z*)-2-nonen-1-ol (Bedoukian Research Inc., Danbury, CT) by oxidation with Dess−Martin periodinane (0.3 M in dichloromethane; Aldrich Chemical Co.) using a published procedure (*15*). Odorless distilled water was prepared by boiling glass-distilled water in an open flask until its volume was reduced by one-third of the original volume.

DRB was prepared from freshly milled RB from jasmine rice (*Oryza sativa*, Variety Khao Dawk Mali 105) by defatting twice with hexane (RB to hexane at a ratio of 1:3, w/v), followed by air drying overnight in a fume hood, grinding in a sample mill, and sieving through an 80 mesh screen (*16*). DRB was packed in polyethylene bags and stored at 5 °C until needed. The alkali extraction procedure followed by isoelectric precipitation was used to prepare RBPc from DRB (*17*).

Hydrolysis. RBPc (containing 100 g of protein) plus 500 mL of aqueous 0.5 N HCl was placed in a 1 L amber glass bottle. After purging with purified nitrogen gas for 10 min, the bottle was sealed with a PTFE-linned cap and then incubated for either 12 or 36 h at 95 °C. The hydrolysate was adjusted to pH 6.0 with aqeuous 1.0 N NaOH, then filtered through Celite (0.1% w/v added to hydrolysate prior to filtration), freeze-dried, and stored at −5 °C until analysis (*18*). The degree of hydrolysis (DH) was defined as the percentage of peptide bonds cleaved (*19*). DH values were determined by analyzing free and total amino acids (*19*) and calculated as follows (*20*):

$$\%\mathrm{DH} = \frac{\text{number of free amino groups}}{\text{total number of amino acid residues}} \times 100$$

Proximate Analysis. The percent moisture (method 935.29), crude fat (method 945.16), crude protein (method 990.03), and ash (method 923.03) of samples were determined according to AOAC procedures (*21*). Carbohydrate was determined by difference (*22*).

Determination of Free and Total Amino Acids. Free and total amino acids were determined using a Beckman 6380 Amino Acid Analyzer (Beckman Instruments Inc., Palo Alto, CA) with a 10 cm ion exchange column and lithium citrate buffer supplied by Beckman. Detection was via postcolumn derivatization using ninhydrin. Calibration was achieved using mixed external free amino acid standards. The analytical conditions were the same as previously published (*12*).

Isolation of Volatile Compounds. RBPc or HVP (20 g) was dissolved in 100 mL of odorless distilled water and spiked with 25 μL of internal standard solution (5.00 μg/μL of 2-ethyl butyric acid in methanol as acidic fraction internal standard and 4.31 μg/μL of 2-methyl-3-heptanone in methanol as neutral/basic fraction internal standard). The solution was extracted (with shaking for 30 min) two times with 50 mL of diethyl ether. The solvent extract was subjected to a high vacuum distillation (approximately 5×10^{-5} Torr operating vacuum level) cleanup step as previously described (*23*) for 3 h to further remove nonvolatile residue, with the sample kept at room temperature for the first 1 h and then warmed to 45 °C using a water bath. The solvent extract was evaporated to 20−25 mL using a Vigreux column in a 40 °C water bath.

To separate the acidic volatiles from the neutral/basic volatiles, the extract was washed three times with aqueous 5% Na_2CO_3 solution (3 × 20 mL), and the organic layer containing the neutral/basic volatiles was collected. The aqueous layer was then acidified to pH 3 with 10% aqueous HCl and extracted with diethyl ether (3 × 15 mL). The organic layer containing the acidic volatiles was collected. Each fraction was then concentrated under a gentle stream of nitrogen gas to 10 mL, dried over 2 g of anhydrous sodium sulfate, and further concentrated to 250 and 500 μL under a nitrogen gas stream for neutral/basic and acidic fractions, respectively. Samples were prepared in duplicate and kept at −70 °C until analysis.

GC-MS. GC-MS was performed on a 6890 GC /5973 mass selective detector (MSD) (Agilent Technologies, Inc., Palo Alto, CA). One microliter of each extract was injected using cool on-column mode (38 °C initial temperature, with +3 °C oven temperature tracking) into a DB-FFAP (30 m × 0.25 mm i.d. × 0.25 μm film; J&W Scientific, Folsom, CA) or HP5-MS (30 m × 0.25 mm i.d. × 0.5 μm film; Agilent Technologies, Inc.) fused silica capillary column. The GC oven temperature was programmed from 35 to 225 °C at rate of 4 °C/min with initial and final hold times of 5 and 30 min, respectively. Helium was used as the carrier gas at a constant flow of 1 mL/min. The mass spectrometer was operated in electron impact mode with an electron energy of 70 eV and an emission current of 50 mA. The mass spectrometer scanned from *m*/*z* 29 to *m*/*z* 400 at 1.9 scans/s. The MSD interface temperature was 280 °C.

GCO. GCO was performed on an Agilent 6890 GC equipped with a flame ionization detector (FID) and olfactory detection port (OPD2, Gerstel, Germany). Two microliters of each was injected using a direct on-column mode (38 °C initial temperature, with +3 °C oven temperature tracking) into a DB5-MS or DB-FFAP (15 m × 0.32 mm i.d. × 0.5 μm film thickness, J&W Scientific) fused silica capillary column. The oven was held at 35 °C for 5 min, ramped at 10 °C/min to 225 °C, and then held for 10 min. The column effluent was spilt 1:1 between the FID and an olfactory port using deactivated fused silica tubing (1 m × 0.15 mm i.d). Helium was used as the carrier gas at a constant flow of 2.2 mL/min. The aroma-active compounds were evaluated by aroma extract dilution analysis (AEDA) (*24*) on 1:3 dilution series of the aroma extracts. The ratio of the concentration of the odorant in the initial extract to its concentration in the most dilute extract in which the odor was detected by GCO was the flavor dilution

Table 1. Proximate Composition[a] (% by Weight) of RB, DRB, and RBPc

sample	moisture	crude fat	protein	carbohydrate (by difference)	ash
RB	8.90 b	19.54 b	12.35 c	51.34 a	7.57 b
DRB	12.09 a	5.26 c	13.97 b	60.10 a	8.58 a
RBPc	5.02 c	22.35 a	53.78 a	16.61 b	2.24 c

[a] Means ($n = 3$) in the same column with different letters are significantly different ($p \leq 0.05$).

Table 2. Protein Content[a] and Percent DH[a] of RBPc and Acid-Hydrolyzed RBPcs Prepared at 12 (H-RBPc-12) and 36 h (H-RBPc-36)

sample	% protein (wet basis)	% DH
RBPc	53.8 a	
H-RBPc-12	23.4 c	13.3 b
H-RBPc-36	23.9 b	24.0 a

[a] Means ($n = 3$) in the same column with different letters are significantly different ($p \leq 0.05$).

Table 3. Free and Total Amino Acids of RBPc and Free Amino Acid of Acid-Hydrolyzed RBPcs Prepared at 12 (H-RBPc-12) and 36 h (H-RBPc-36)

		free amino acid (% dry basis)[a]			
amino acid	MW	RBPc	H-RBPc-12	H-RBPc-36	total amino acids of RBPc (% dry basis)
alanine	89.09	0.010 c	0.327 b	0.865 a	4.095
arginine	174.20	ND*b*	0.751 a	0.807 a	5.913
aspartic	133.10	0.020 c	1.390 b	2.201 a	4.865
glutamic	147.13	0.034 c	0.494 b	3.989 a	8.840
glycine	75.07	0.005 c	0.446 b	0.879 a	3.531
histidine	155.15	ND	0.072 b	0.172 a	1.932
isoleucine	131.18	0.006 c	0.048 b	0.203 a	1.948
leucine	131.18	ND	0.138 b	0.471 a	4.699
lysine	146.19	0.010 c	0.101 b	0.279 a	2.830
methionine	149.21	0.002 b	0.066 b	0.246 a	1.114
phenylalanine	165.19	ND	0.083 b	0.236 a	2.564
proline	115.13	ND	ND	0.452 a	2.595
serine	105.09	0.005 c	0.156 b	0.438 a	3.185
threonine	119.12	0.004 b	0.065 b	0.241 a	2.268
tyrosine	181.19	ND	0.069 b	0.251 a	2.344
valine	117.13	ND	ND	0.070 a	3.131

[a] Means ($n = 3$) in the same row with different letters are significantly different ($p \leq 0.05$). [b] ND, not detected.

(FD) factor (*24*). GCO was then performed by two experienced analysts. Results of AEDA are reported as average $\log_3$FD factors (*25*).

Compound Identification. Compounds were positively identified by comparing their mass spectra and retention indices (RI) on both DB-FFAP and HP5-MS columns to those of authentic reference compounds analyzed under identical conditions. When mass spectra were unavailable, compounds were tentatively identified by comparison of their RI values and odor properties on both DB-FFAP and HP5-MS columns to those of authentic reference compounds. RI values were determined by analyzing a series of *n*-alkanes (C6−C28 or C6−C18 for DB-FFAP or HP5-MS, respectively) as described by van Den Dool and Kratz (*26*).

Compound Quantification. The concentration of an aroma component was calculated by internal standard methodology by GC-MS using a DB-FFAP column as described above. Prior to GC-MS, reference standards (at three different levels) plus internal standards were diluted in deodorized water and subjected to solvent extraction, high vacuum distillation, and compound class fractionation. Because water was used as the matrix and not mimic matrices based on RBPc or the hydrolysates, the values reported herein are considered relative concentrations. The relative concentration of a compound was calculated as previously described by Zhou et al. (*25*).

Statistical Analyses. Three sample replications were performed. Data were analyzed by least significant differences procedures to separate means, and differences were reported as significant at $p = 0.05$ (*27*).

RESULTS AND DISCUSSION

Chemical Composition of RB and RBPc. Protein contents of full fat RB and its protein concentrate (RBPc) were 12.35 and 53.78%, respectively (**Table 1**). Crude fat increased slightly from 19.54 to 22.35% in RB and RBPc, respectively (**Table 1**), which could be explained by the retention of residual protein−lipid complexes in RBPc that were not fully extracted in the defatting step. Cheftel et al. (*28*) pointed out that lipid oxidation followed by protein−lipid covalent interactions can take place in some foods and feeds. The DH of acid-hydrolyzed RBPcs as a function of hydrolysis time is shown in **Table 2**. Free and total amino acid contents (% dry basis) of RBPc and free amino acids of HVPs prepared with 0.5 N HCl for 12 (H-RBPc-12) and 36 h (H-RBPc-36) are given in **Table 3**. RBPc was obtained by isoelectric precipitation at pH 4.5; therefore, in RBPc, the major protein is acidic. This agrees with the results of free and total amino acid analysis in that glutamic acid was in highest abundance in RBPc (**Table 3**). Juliano (*29*) and Wang et al. (*16*) also reported that glutamic acid was most abundant among the total amino acids in RB. Therefore, glutamate residues should provide the main acidic character to the protein in RBPc. Glutamic acid also was in highest abundance of free amino acid in H-RBPc-36. Jarunrattanasri et al. (*12*) also found glutamic acid in highest abundance in HVP prepared from RBPc by hydrolysis with 4.0 N HCl at 95 °C for 72 h. On the other hand, aspartic acid was the predominant free amino acid in H-RBPc-12. The above results are reasonable since glutamic acid, arginine, and aspartic acid are the major amino acids among the total amino acids in RBPc.

Aroma-Active Compounds. Aroma compounds of intermediate and low volatility were isolated by liquid−liquid solvent extraction, followed by a high vacuum distillation cleanup step. A total of 27 odorants were detected by GCO and AEDA. Seven, 19, and 21 volatile compounds with $\log_3$FD factors ≥ 2 were detected in RBPc, H-RBPc-12, and H-RBPc-36, respectively (**Table 4**). Previously, 30 odorants were reported in HVP produced from RB protein made by hydrolysis with concentrated (4 N) HCl (*12*). Twenty-one of those compounds were also detected in the present study in H-RBPc-12 and H-RBPc-36. Most of the predominant odorants identified in the present study had higher $\log_3$FD factors in H-RBPc-36 as compared with H-RBPc-12. (*E*)-2-Nonenal (no. 28) and *trans*-4,5-epoxy-(*E*)-2-decenal (no. 33) were an exception and had higher $\log_3$FD factors in H-RBPc-12 than in H-RBPc-36. Results showed that odorants originated from lipid oxidation, lignin degradation, and Maillard reactions as discussed below.

Acidic Fraction. Among the acidic odorants, hexanoic acid (no. 8) and vanillin (no. 18) had the highest $\log_3$FD factors in RBPc and imparted sweaty and vanilla-like notes, respectively. Vanillin was also a predominant odorant of both HVPs. In a previous study, vanillin was not reported as a predominant odorant in HVP produced from RBPc by hydrolysis with concentrated (4 N) HCl (*12*). Other acidic odorants found with high FD factors in H-RBPc-12 and H-RBPc-36 were 2-methoxyphenol (guaiacol, no. 9), 4-hydroxy-2,5-dimethyl-3(*2H*)-

Table 4. Potent Odorants in RBPc and Acid-Hydrolyzed RBPcs Prepared at 12 (H-RBPc-12) and 36 h (H-RBPc-36)

no.[a]	odorants	odor	RI[b]		Log_3FD[c]		
			FFAP	DB5	RBPc	H-RBPc-12	H-RBPc-36
		acidic fraction					
3	acetic acid[d]	vinegar	1449	–[e]	–	2	3
6	3-methylbutyric acid[d]	sweaty	1667	888	1	2	1
8	hexanoic acid[d]	sweaty	1839	–	2	1	1
9	2-methoxyphenol (guaiacol)[d]	smoky	1867	1096	1	4	5
11	4-hydroxy-2,5-dimethyl-3(2*H*)furanone[f]	burnt sugar	2028	1097	–	4	6
12	octanoic acid[d]	sweaty	2046	–	–	–	2
13	4-methylphenol (*p*-cresol)[d]	stable, dung	2089	1098	–	2	3
14	4-vinyl-2-methoxyphenol (*p*-vinylguaiacol)[d]	clove	2175	1365	–	7	7
15	3-hydroxy-4,5-dimethyl-2(5*H*)furanone (sotolon)[f]	seasoning	2198	1105	–	4	6
16	2,6-dimethoxyphenol (syringol)[d]	smoky	2273	1367	–	2	3
17	phenylacetic acid[d]	honey	2565	1292	–	3	4
18	vanillin[d]	vanilla	2571	1415	2	4	6
		neutral/basic fraction					
19	2-methylbutanal[d]	malty	915	659	1	1	2
20	3-methylbutanal[d]	malty	922	649	1	1	2
22	hexanal[d]	grass	1087	799	2	1	1
23	1-octen-3-one[f]	mushroom	1303	979	1	3	3
24	2-acetyl-1-pyrroline[d]	cooked rice	1340	922	1	3	3
25	dimethyl trisulfide[f]	cabbage	1381	972	1	–	1
26	3-(methylthio)propanal (methional)[d]	cooked potato	1455	906	1	3	5
27	(*Z*)-2-nonenal[f]	fatty, hay	1513	–	1	3	1
28	(*E*)-2-nonenal[d]	lipstick, waxy	1543	1171	3	4	3
29	(*E*,*Z*)-2,6-nonadienal[f]	cucumber	1596	1156	1	1	3
30	phenylacetaldehyde[d]	rosy	1652	1050	3	1	1
31	β-damascenone[f]	applesauce	1830	1384	3	6	7
32	2-phenylethanol[d]	floral	1920	1139	–	3	3
33	*trans*-4,5-epoxy-(*E*)-2-decenal[f]	unripe, metallic	2017	1389	–	5	3
34	2-aminoacetophenone[d]	tortilla	2225	1315	4	2	2

[a] Numbers correspond to those **Table 5**. [b] Retention index calculated from GCO data. [c] Average Log_3FD ($n = 2$) determined on DB-FFAP column. [d] Compound positively identified by comparison of its RI values, odor properties, and mass spectrum with reference compound. [e] Not available. [f] Compound tentatively identified by comparison of its RI values and odor properties with reference compound.

furanone (no. 11), 3-hydroxy-4,5-dimethyl-2(*5H*)furanone (sotolon, no. 15), and phenylacetic acid (no. 17).

Both guaiacol (no. 9) and 2,6-dimethoxyphenol (syringol, no. 16) imparted strong and potentially undesirable smoky notes to both HVPs. 4-Vinyl-2-methoxyphenol (*p*-vinylguaiacol, no. 14) contributed a spicy, clovelike note at high log_3FD factors of 7 in both of HVPs, but it was not detected in RBPc. Phenols and guaiacols are the major contributors to wood smoke aroma (*30*). The presence of guaiacol has been previously reported in cooked brown rice (*31*), in both acid- and enzyme-hydrolyzed soy protein (*32*). and in soybean-based enzyme hydrolysates (*33*). Maga (*34*) pointed out that in wood smoke phenol and its derivatives are formed as a result of lignin degradation.

4-Hydroxy-2,5-dimethyl-3(*2H*)furanone (no. 11) imparted a burnt sugar note at high log_3FD factors of 4 and 6 in H-RBPc-12 and H-RBPc-36, respectively. While this compound is a predominant odorant in both HVPs, it was less important in an HVP made using concentrated (4 N) HCl (*12*). Schieberle (*35*) stated that 4-hydroxy-2,5-dimethyl-3(*2H*)furanone will usually be formed in some heat-processed foods via the Maillard reaction, and it has been detected in several foods, e.g., roasted almond, wheat bread crust, and popcorn. In particular, hexoses can be rapidly converted into 4-hydroxy-2,5-dimethyl-3(*2H*)-furanone (*35*). This is supported by Toledo et al. (*36*) who demonstrated that 4-hydroxy-2,5-dimethyl-3(*2H*)furanone was formed only from arginine and rhamnose under acidic conditions in a model citrus juice system, whereas at pH 6−8, 4-hydroxy-2,5-dimethyl-3(*2H*)furanone was formed from glucose and fructose. 4-Hydroxy-2,5-dimethyl-3(*2H*)furanone was also formed from rhamnose and alanine at pH 3.5 after refluxing for 5 h (*37*).

Sotolon (no. 15) with high log_3FD factors of 4 and 6 in H-RBPc-12 and H-RBPc-36, respectively, was responsible for a seasoning or currylike odor. This compound was reported as an important odorant of an HVP made by hydrolysis of RBPc with concentrated (4 N) HCl (*12*). Sotolon was previously reported to contribute to the burnt/sweet aroma of cane sugar (*38*).

Similar to compounds imparting smoky notes, some other odorants such as 3-methylbutyric acid (no. 6), hexanoic acid (no. 8), and octanoic acid (no. 12) may be considered undesirable because they imparted sweaty odors.

Neutral/Basic Fraction. Predominant neutral/basic odorants in RBPc were (*E*)-2-nonenal (no. 28), phenylacetaldehyde (no. 30), β-damascenone (no. 31), and 2-aminoacetophenone (no. 34). 2-Aminoacetophenone may be a characteristic aroma component of RBPc, since not only did it have the highest log_3-FD factor in RBPc but its odor is very similar to that of RBPc. 2-Aminoacetophenone has been previously reported as a volatile constituent of scented rice (*31*), and it contributes to the off-flavor of dried milk (*39*) and renet casein powders (*40*). Buttery and Ling (*41*) suggested that 2-aminoacetophenone may be formed by breakdown of tryptophan during lime treatment of corn during the preparation of masa dough. It has been clearly demonstrated that the most likely pathway of formation of 2-aminoacetophenone is from the degradation of tryptophan and/or indole-3-acetic acid, which are present in wine musts (cited by ref *42*). In the present study, tryptophan was not detected among the free or total amino acids in **Table 3** since it is present in only trace amounts as a free amino acid in RBPc (*43*); furthermore, tryptophan is readily degraded during the acid hydrolysis step (*44*).

Table 5. Concentrations and Odor Activity Values (OAVs) of Selected Volatile Components of RBPc and of Acid-Hydrolyzed RBPcs Prepared at 12 (H-RBPc-12) and 36 h (H-RBPc-36)

		RI[b]			concentration (ng/g)[d]			OAV[e]		
no.[a]	compound	FFAP	HP5	threshold (ng/g in water)[c]	RBPc	H-RBPc-12	H-RBPc-36	RBPc	H-RBPc-12	H-RBPc-36
					acidic fraction					
1	ethyl acetate[f]	896	612	12200	60227 (4818) A	9537 (674) B	10657 (753) B	5.0	<1	<1
2	ethanol[f]	939	–[g]	100000	–	432 (30)	–	–	<1	–
3	acetic acid[f]	1452	–	50000	670 (63) C	3516 (248) B	4501 (318) A	<1	<1	<1
4	propanoic acid[f]	1534	–	2190	–	76 (5.6) B	126 (8.0) A	–	<1	<1
5	butanoic acid[f]	1624	–	240	–	86 (6.4) B	132 (9.0) A	–	<1	<1
6	3-methylbutyric acid[f]	1666	–	250	–	247 (18)	–	–	~1.0	–
7	pentanoic acid[f]	1733	–	2100	978 (81) A	188 (13) B	225 (16) B	<1	<1	<1
8	hexanoic acid[f]	1839	–	3000	7965 (643) A	1092 (77) B	1306 (93) B	2.7	<1	<1
9	2-methoxyphenol (guaiacol)[f]	1871	1092	2.5	–	6934 (490) B	44130 (3120) B	–	2770	17650
10	phenol[f]	2008	1113	5900	–	72 (4.9) B	123 (9.0) A	–	<1	<1
11	4-hydroxy-2,5-dimethyl-3(2*H*)furanone[h]	–	–	60	–	–	–	–	–	–
12	octanoic acid[f]	2055	–	3000	971 (69) B	1106 (78) B	2987 (212) A	<1	<1	~1.0
13	4-methylphenol (*p*-cresol)[f]	2087	1075	55	–	8 (1.5) B	12 (1.8) A	–	<1	<1
14	4-vinyl-2-methoxyphenol (*p*-vinylguaiacol)[f]	2205	1366	20	–	1644 (116) B	3174 (225) A	–	82.2	159
15	3-hydroxy-4,5-dimethyl-2-(5*H*)furanone (sotolon)[h]	–	–	0.001	–	–	–	–	–	–
16	2,6-dimethoxyphenol (syringol)[f]	2276	1357	1850[i]	–	–	50210 (3550)	–	–	27.1
17	phenylacetic acid[f]	2561	1254	10000	–	483 (34) A	548 (39) A	–	<1	<1
18	vanillin[f]	2579	1409	25	1897 (134) B	2226 (157) B	2772 (197) A	75.9	89.0	110
					neutral/basic fraction					
19	2-methylbutanal[f]	920	659	1	–	15 (4.0) B	59 (14) A	–	15.0	59.0
20	3-methylbutanal[f]	927	649	0.35	118 (8.5) B	126 (19) B	366 (26) A	337	360	1050
21	2,3-pentanedione[f]	1068	<600	30	–	–	22 (4.5)	–	–	<1
22	hexanal[f]	1089	799	4.5	3576 (253) A	347 (24) B	163 (14) B	495	77.1	36.2
23	1-octen-3-one[h]	–	–	0.005	–	–	–	–	–	–
24	2-acetyl-1-pyrroline[f,j]	1347	922	0.1	–	0.45 (0.14) B	0.76 (0.16) A	–	4.5	7.6
25	dimethyl trisulfide[h]	–	–	0.01	–	–	–	–	–	–
26	3-(methylthio)propanal (methional)[h]	1466	–	0.2	–	–	71 (15)	–	–	357
27	(*Z*)-2-nonenal[h]	–	–	0.004	–	–	–	–	–	–
28	(*E*)-2-nonenal[f]	1545	1161	0.15	74 (5.2) B	197 (14) A	92 (17) B	493	1310	613
29	(*E*,*Z*)-2,6-nonadienal[h]	–	–	0.01	–	–	–	–	–	–
30	phenylacetaldehyde[f]	1659	–	4	268 (19) A	50 (14) B	62 (14) B	67.0	12.5	15.5
31	β-damascenone[h]	–	–	0.002	–	–	–	–	–	–
32	2-phenylethanol[f]	1925	–	1000	–	182 (12)	–	–	<1	–
33	*trans*-4,5-epoxy-(*E*)-2-decenal[h]	–	–	0.12	–	–	–	–	–	–
34	2-aminoacetophenone[f]	2242	–	0.2	59 (14) A	2 (0.6) B	8 (1.6) B	295	10.0	40.0

[a] Numbers correspond to those **Table 4**. [b] Retention index calculated from GC-MS data. [c] Ref *45*. [d] Mean (standard deviation, $n = 2$). Letters A–C mean that means with different letters in the same row are significantly different ($p \leq 0.05$). [e] Odor activity value calculated by dividing compound concentration by its odor detection threshold. [f] Compound positively identified by comparison of its RI values and mass spectrum with reference compound. [g] Not available. [h] Compound tentatively identified (from **Table 4**). [i] Ref *46*. [j] Quantified by SIM using $m/z = 83$ and 111.

In both HVPs, the predominant neutral/basic odorants were 3-(methylthio)propanal (methional, no. 26), (*E*)-2-nonenal (no. 28), β-damascenone (no. 31), and *trans*-4,5-epoxy-(*E*)-2-decenal (no. 33). β-Damascenone was found at high $\log_3$FD factors of 3, 6, and 7 (the highest value) in RBPc, H-RBPc-12, and H-RBPc-36, respectively. This compound has an extremely low odor threshold in water (**Table 5**) and, therefore, should be an important odorant in RBPc and especially in both HVPs. β-Damascenone has been reported as an important aroma component of soybean-based EVP (*33*).

Hexanal (no. 22) had $\log_3$FD factors of 2, 1, and 1 in RBPc, H-RBPc-12, and H-RBPc-36, respectively. Hexanal has been reported as a volatile component of RB (*29*). Aaslyng et al. (*32*) reported that hexanal probably causes an undesirable odor in enzymatic HVP.

Quantification of Volatile Components and Their OAVs. Concentrations and odor activity values (OAVs) of selected volatile compounds are shown in **Table 5.** Positively identified odorants detected by AEDA at high $\log_3$FD factors were present in RBPc, H-RBPc-12, and H-RBPc-36 at levels above their odor detection thresholds. For some odorants (e.g., nos. 9, 14, 18, 24, 30, and 34), the OAVs were in good agreement with the determined $\log_3$FD factors. Some volatile compounds, despite being present at high and intermediate levels, were not detected by AEDA. These included ethyl acetate (no. 1), ethanol (no. 2), propionic acid (no. 4), butanoic acid (no. 5), pentanoic acid

(no. 7), and phenol (no. 10) in the acidic fraction and 2,3-pentanedione (no. 21) in the neutral/basic fraction. With the exception of ethyl acetate (OAV of 5 in RBPc), these compounds were all present at levels below their odor detection thresholds.

Hexanal (no. 22) and (*E*)-2-nonenal (no. 28) had the highest OAVs in RBPc, followed by 3-methylbutanal (no. 20) and 2-aminoacetophenone (no. 34). Hexanal (no. 22) decreased and 3-methylbutanal (no. 20) increased as a result of acid hydrolysis of RBPc into H-RBPc-12 and H-RBPc-36. Aldehydes, such as 2- and 3-methylbutanal (nos. 19 and 20, respectively) and phenylacetaldehyde (no. 30), identified in the present study, are produced during the Maillard reaction from Strecker degradation of amino acids and have been previously identified as components of HVP (*12*, *32*). The odorant with the highest OAVs in H-RBPc-12 and H-RBPc-36 was guaiacol (no. 9), which increased as a function of hydrolysis time. Some compounds (nos. 11, 15, 23, 25, 27, 29, 31, and 33) with low odor detection thresholds were not quanitified because they were present at levels below GC-MS detection limits.

In conclusion, the overall aromas of the partial acid hydrolysates produced from RB protein are comprised of a complex and unique combination of odorants generated during the hydrolysis procedure through lipid oxidation, lignin degradation, and Maillard reactions. On the basis of the results of AEDA and/or on the calculation of OAVs, the predominant odorants in RBPc were 3-methylbutanal (no. 20), hexanal (no. 22), (*E*)-2-nonenal (no. 28), phenylacetaldehyde (no. 30), β-damascenone (no. 31), and 2-aminoacetophenone (no. 34). In particular, 2-aminoacetophenone (no. 34) with a corn torilla-like odor note may be an important contributor to the characteristic odor of RBPc. Meanwhile, guaiacol (no. 9), 4-hydroxy-2,5-dimethyl-3(2*H*)furanone (no. 11), sotolon (no. 15), vanillin (no. 18), 3-methylbutanal (no. 20), (*E*)-2-nonenal (no. 28), β-damascenone (no. 31), and *p*-vinylguaiacol (no. 14) were found to be predominant odorants of H-RBPc-12 and H-RBPc-36. Most of the predominant odorants had higher $\log_3$FD factors in H-RBPc-36 than in H-RBPc-12. The presence of guaiacol (no. 9), and to a lesser extent 2,6-dimethoxyphenol (syringol, no. 16), may have an undesirable effect on the overall aroma of hydrolyzed RB protein since they imparted atypical smoky notes.

ACKNOWLEDGMENT

Special thanks to Patum Rice Mill and Granary Public Co. Ltd., Thailand, for supplying the RB for this research.

LITERATURE CITED

(1) Fujimaki, M.; Tsugita, T.; Kurata, T. Fraction and identification of volatile acids and phenols in the steam distillate of rice bran. *Agric. Biol. Chem.* **1977**, *41*, 1721−1725.
(2) Tsugita, T.; Kurata, T.; Fujimaki, M. Volatile components in the steam distillate of rice bran: Identification of neutral and basic compounds. *Agric. Biol. Chem.* **1978**, *42*, 643−651.
(3) Weir, G. S. D. Proteins as a source of flavor. In *Biochemistry of Food Proteins*; Hudson, B. J. F., Ed.; Elsevier Applied Science: London and New York, 1992; pp 363−408.
(4) Ames, J. M. The Maillard reaction. In *Biochemistry of Food Proteins*; Hudson, B. J. F., Ed.; Elsevier Applied Science: London and New York, 1992; pp 150−154.
(5) Nagodawithana, T. W. *Savory Flavors*; Esteekay Associates, Inc.: Milwaukee, WI, 1995; pp 401−434.
(6) Collier, P. D.; Cromie, D. D. O.; Davis, A. P. Mechanism of formation of chloropropanols present in protein hydrolysates. *J. Am. Oil Chem. Soc.* **1991**, *68*, 785−790.
(7) Shih, F. F. Modification of food proteins by non-enzymatic methods. In *Biochemistry of Food Proteins*; Hudson, B. J. F., Ed.; Elsevier Applied Science: London and New York, 1992; pp 235−248.
(8) Izzo, H. V.; Ho, C. T. Peptide-specific Maillard reaction products: A new pathway for flavor chemistry. *Trends Food Sci. Technol.* **1992**, *3*, 253−257.
(9) Ho, C. T.; Oh, Y. C.; Zhang, Y.; Shu, C. K. Peptides as flavor precursors in model Maillard reactions. In *Flavor Precursors: Thermal and Enzymatic Conversions*; Teranishi, R., Takeoka, G. R., Guentert, M., Eds.; American Chemical Society: Washington, DC, 1992; pp 193−202.
(10) Prakash, J. Rice bran protein: Properties and food uses. *Crit. Rev. Food Sci. Nutr.* **1996**, *36*, 537−552.
(11) Hamada, J. S.; Spanier, A. M.; Bland, J. M.; Diack, M. Preparative separation of value-added peptides from rice bran proteins by high-performance liquid chromatography. *J. Chromatogr. A* **1998**, *827*, 319−327.
(12) Jarunrattanasri, A.; Cadwallader, K. R.; Theerakulkait, C. Aroma and amino acid composition of hydrolyzed vegetable protein from rice bran. In *Process and Reaction Flavors: Recent Developments*; Weerasinghe, D. K., Sucan, M. K., Eds.; American Chemical Society: Washington, DC, 2006; pp 83−97.
(13) Teranishi, R. Challenges in flavor chemistry: an overview. In *Flavor Analysis: Development in Isolation and Characterization*; Mussinan, C. J., Morello, M. J., Eds.; American Chemical Society: Washington, DC, 1998; pp 1−6.
(14) Lin, J.; Fay, L. B.; Welti, D. H.; Blank, I. Synthesis of *trans*-4,5-epoxy-(*E*)-2-decenal and its deuterated analog used for the development of a sensitive and selective quantification method based on isotope dilution assay with negative ion chemical ionization. *Lipids* **1999**, *34*, 1117−1126.
(15) Meyer, S.; Schreiber, S. L. Acceleration of the Dess-Martin oxidation by water. *J. Org. Chem.* **1994**, *59*, 7549−7552.
(16) Wang, M.; Hettiarachchy, N. S.; Qi, M.; Burk, W.; Siebenmorgen, T. Preparation and functional properties of rice bran protein isolate. *J. Agric. Food Chem.* **1999**, *47*, 411−416.
(17) Gnanasambandam, R.; Hettiarachchy, N. S. Protein concentrates from unstabilized rice bran: Preparation and properties. *J. Food Sci.* **1995**, *60*, 1066−1074.
(18) Wattanapat, R.; Nakayama, T.; Beuchart, L. R. Characteristics of acid hydrolysates from defatted peanut flour. *J. Food Sci.* **1995**, *60*, 443−445.
(19) Adler-Nissen, J. Determination of the degree of hydrolysis of food protein hydrolysates by trinitrobenesulfonic acid. *J. Agric. Food Chem.* **1979**, *27*, 1256−1262.
(20) Hajós, G. Y.; Mietsch, F.; Halász, A. Change of degree of hydrolysis in the course of enzymatic peptide modification. *Acta Aliment.* **1988**, *17*, 265−270.
(21) *Official Methods of Analysis*, 15th ed.; Association of Official Analytical Chemists: Washington, DC, 1990.
(22) Mattila, P.; Salo-Väänanen, P.; Könkö, K.; Aro, H.; Jalava, T. Basic composition and amino acid contents of mushroom cultivated in Finland. *J. Agric. Food Chem.* **2002**, *50*, 6419−6422.
(23) Karagül-Yüceer, Y.; Drake, M. A.; Cadwallader, K. R. Aroma-active components of nonfat dry milk. *J. Agric. Food Chem.* **2001**, *49*, 2948−2953.
(24) Grosch, W. Detection of potent odorants in food by aroma extract dilution analysis. *Trends Food Sci. Technol.* **1993**, *4*, 68−73.
(25) Zhou, Q.; Wintersteen, C. L.; Cadwallader, K. R. Identification and quantification of aroma-active components that contribute to the distinct malty flavor of buckwheat honey. *J. Agric. Food Chem.* **2002**, *50*, 2016−2021.
(26) van Den Dool, H.; Kratz, P. D. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. *J. Chromatogr.* **1963**, *11*, 463−471.
(27) *Statistix 7: User's Manaul*; Analytical Software: Tallahassee, FL, 2000; p 359.

(28) Cheftel, J. C.; Cuq, J. L.; Lorient, D. Amino acids, peptides, and proteins. In *Food Chemistry*; Fennema, O. R., Ed.; Marcel Dekker, Inc.: New York, 1985; pp 245−369.

(29) Juliano, B. O. Rice bran. In *Rice: Chemistry and Technology*; Juliano, B. O., Ed.; The American Association of Cereal Chemists, Inc.: St. Paul, Minnesota, 1985; pp 647−688.

(30) Cadwallader, K. R. Wood smoke flavor. In *Flavor Technology*; Hui, Y. H., Nollet, L. M. L., Boylsten, T., Beatriz, M., Chen, F., Peris-Tortajada, M., Eds.; DEStech Publications Inc.: Lancaster, PA, 2007; in press.

(31) Jezussek, M.; Juliano, B. O.; Schieberle, P. Comparison of key aroma compounds in cooked brown rice varieties based on aroma extract dilution analyses. *J. Agric. Food Chem.* **2002**, *50*, 1101−1105.

(32) Aaslyng, M. D.; Martens, M.; Poll, L.; Nielsen, P. M.; Flyge, H.; Larsen, L. M. Chemical and sensory characterization of hydrolyzed vegetable protein, a savory flavoring. *J. Agric. Food Chem*. **1998**, *46*, 481−489.

(33) Wu, Y. F. G.; Cadwallader, K. R. Characterization of the aroma of a meatlike process flavoring from soybean-based enzyme-hydrolyzed vegetable protein. *J. Agric. Food Chem*. **2002**, *50*, 2900−2907.

(34) Maga, J. A. Contribution of phenolic compounds to smoke flavor. In *Phenolic Compounds in Food and Their Effects on Health I: Analysis*, *Occurrence and Chemistry*; Ho, C. T., Lee, C. Y., Huang, M. I., Eds.; American Chemical Society: Washington, DC, 1992; pp 170−179.

(35) Schieberle, P. Formation of furaneol in heated-processed foods. In *Flavor Precursors: Thermal & Enzymatic Conversions*; Teranishi, R., Takeoka, G. R., Guentert, M., Eds.; American Chemical Society: Washington, DC, 1992; pp 164−174.

(36) Toledo, E. H.; Naim, M.; Zehavi, U.; Rouseff, R. L. 4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone formation in buffers and model solution of citrus juice. *J. Agric. Food Chem*. **1997**, *45*, 1314−1319.

(37) Shaw, P. E.; Berry, R. E. Hexose-amino acid degradation studies involving formation of pyrroles, furans, and other low molcular weight products. *J. Agric. Food Chem*. **1977**, *25*, 641−644.

(38) Tokitomo, Y.; Kobayashi, A.; Yamanishi, T.; Muraki, S. A studies on the “sugary flavor” of raw cane sugar. III Key compound of sugary flavor. *Proc. Jpn. Acad*. **1980**, *56B*, 457−462.

(39) Karagül-Yüceer, Y.; Cadwallader, K. R.; Drake, M. A. Volatile flavor components of stored nonfat dry milk. *J. Agric. Food Chem.* **2002**, *50*, 305−312.

(40) Karagül-Yüceer, Y.; Vlahovich, K. N.; Drake, M. A.; Cadwallader, K. R. Characteristic aroma components of rennet casein. *J. Agric. Food Chem.* **2003**, *51*, 6797−6801.

(41) Buttery, R. G.; Ling, L. C. Importance of 2-aminoacetophenone to the flavor of masa corn flour products. *J. Agric. Food Chem.* **1994**, *42*, 1−2.

(42) Mattivi, F.; Vrhovsek, U.; Versini, G. Determination of indole-3-acetic acid, tryptophan and other indoles in must and wine by high-performance liquid chromatography with fluorescence detection. *J. Chromatogr. A* **1999**, *855*, 227−235.

(43) Luh, B. S.; Barber, S.; deBarber, C. B. In *Rice Volume II: Utilization*; Luh, B. S., Ed.; Van Nostrand Reinhold: New York, 1991; pp 313−362.

(44) Fountoulakis, M.; Lahm, H. W. Review: Hydrolysis and amino acid composition analysis of proteins. *J. Chromatogr. A* **1998**, *826*, 109−134.

(45) Rychlik, M.; Schieberle, P.; Grosch, W. *Compilation of Odor Thresholds, Odor Qualities and Retention Indices of Key Food Odorants*; Deutsche Forschungsanstalt für Lebensmittelchemie and Institut für Lebensmittelchemie der Technischen, Universität München: Garching, Germany, 1998.

(46) Wasserman, A. E. Organoleptic evaluation of three phenols present in wood smoke. *J. Food Sci.* **1966**, *31*, 1005−1010.

Received for review November 1, 2006. Revised manuscript received February 11, 2007. Accepted February 17, 2007. We express sincere gratitude to the Kasetsart University Research and Development Institute (KURDI), Kasetsart University, Thailand, and Southeast Asian Ministers of Education Organization Regional Center for Graduate Study and Research in Agriculture (SEARCA) for financial support of this research.

JF0631474