Development and Characterization of a Flavoring Agent from Oyster Cooker Effluent

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The general composition of concentrated oyster cooker effluent (OCE) was 80% moisture, 6.7% total nitrogen, 2.4% glycogen, and 8.5% ash. Optimum conditions for enzymatic hydrolysis of OCE were 50 °C, 2 h of reaction time, 0.1% amylase mixture (α-amylase plus glucoamylase), and 0.2% protease NP. Hydrolysis of OCE led to an increase in free amino acids, with taurine comprising ∼20% of the total. Inosine monophosphate was predominant (456 mg/100 g) among nucleotides and related compounds. Enzyme hydrolysis increased extractable nitrogen by ∼2-fold. Trimethylamine, trimethylamine oxide, and total creatinine levels were not affected by enzyme treatment. Predominant aroma-active components of enzyme-hydrolyzed OCE included 2-acetyl-1-pyrroline and 3-(methylthio)propanal. Results of this study may help alleviate the wastewater disposal problem currently caused by OCE.

Keywords: *Aroma; enzymatic hydrolysis; flavor; gas chromatography−olfactometry; oyster; oyster byproduct; oyster cooker effluent*

INTRODUCTION

The oyster industry is the largest commercial shellfish aquaculture industry in Korea, with an annual production in excess of 220000 metric tons. Eighty percent of the annual production is cooked and processed into canned oyster products. This process generates a large quantity of oyster cooker effluent (OCE) containing appreciable amounts of soluble components, such as peptides, amino acids, glycogen, and other organic compounds. About 40000 tons of OCE (6 °Brix) is generated per year in Korea. In most processing plants, OCE is discarded into waterways, causing pollution. OCE has potential as a feedstock for production of a value-added oyster flavoring; however, only a few seafood processors have realized the potential of converting this byproduct into a marketable flavoring agent.

The potential for recovery of water-soluble organic components of discharge streams from shrimp processing plants has been demonstrated (Perkins and Meyers, 1997). The process for the recovery of organic materials from wastewater not only solves the pollution problem but also maximizes the use of food-processing wastes for ultimate human consumption. Wash water from clam processing plants has been converted into a potentially marketable natural clam flavoring agent (Reddy et al., 1989; Joh and Hood, 1979; Burnette et al., 1983) and a protein concentrate (Hang et al., 1980). Also, oyster shucking liquid waste, containing protein, nonproteinaceous nitrogen compounds, and other organic materials, was concentrated and evaluated for human consumption as oyster soup (Shiau and Chai, 1990). However, the above studies focused on the utilization of oyster-processing byproducts by use of simple extraction and concentration steps only and did not consider the use of commercially available enzymes as processing aids to improve yields and the quality of final products.

Enzymatic hydrolysis has been used to produce flavorants from seafood-processing byproducts (In, 1990; Kim et al., 1994a; Baek and Cadwallader, 1995, 1999). In general, proteolytic enzymes have been employed in these processes. In addition to proteases, use of amylases in the hydrolysis of OCE has potential because this material contains appreciable amounts of glycogen (Kim et al., 1996). A protease/amylase process has particular appeal because during thermal processing amino acids and peptides released by protease action can react with reducing sugars liberated by amylase action to generate cooked meat aroma. This technology has been used for the production of meat and savory flavors (Dziezak, 1986).

The objectives of this study were to determine the chemical composition of OCE and to evaluate the use of enzymatic hydrolysis of OCE by combined protease/amylase action to produce a hydrolysate suitable for further processing into a seafood flavoring agent.

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MATERIALS AND METHODS

Materials. Oyster (*Crassostrea gigas*) cooker effluent concentrate (OCE) was obtained from a local oyster canning company. Protease NP and amylase mixture (α-amylase BAN and glucoamylase AMG) were purchased from Pacific Chemical Co. (Seoul, Korea) and Novo (Novo Nordisk A/S, Denmark), respectively.

Proximate Analysis. Total nitrogen (TN) was determined by a semimicro-Kjeldahl method. Ash, fat, moisture, and salt contents were measured according to standard methods (AOAC, 1990). Glycogen was determined by using the method described by Caroll et al. (1956). Trimethylamine oxide (TMAO) and trimethylamine (TMA) were analyzed using the method described by Hashimoto and Okaichi (1957). Total creatinine was determined according to the method described by Suyama et al. (1977).

Protein Hydrolysis. Hydrolysis of OCE was accomplished using a semipilot scale system equipped with a pH and temperature controller. The reaction mixture, consisting of 2 L of OCE and 4 g of protease NP, was incubated at 50 °C in the pH range of 6.8−7.0 for 4 h with stirring (250 rpm), and then 20 mL of reaction mixture was withdrawn at indicated time intervals. The reaction mixture was mixed with 10 mL of 30% trichloroacetic acid (TCA) solution and centrifuged. The supernatant was analyzed for soluble tyrosine equivalents, and the precipitate was analyzed for TN as described above. The amount of soluble tyrosine equivalents was determined according to the procedure of Lowry et al. (1951).

Glycogen Hydrolysis. The reaction mixture, composed of 2 L of OCE and 2 g of amylase mixture (α-amylase BAN and glucoamylase AMG), was incubated at 50 °C in the pH range of 6.8−7.0 for 4 h with stirring (250 rpm). The amount of reducing sugar liberated at time intervals was measured using a 3,5-dinitrosalicylic acid method (Bernfelt, 1955). The amount of reducing sugar was expressed as glucose equivalents.

Preparation of Hydrolyzed Oyster Cooker Effluent (HOCE). Preparation of HOCE was conducted using a semipilot system in the pH range of 6.8−7.0. The reaction mixture, composed of 2 L of OCE, 4 g of protease NP, and 2 g of amylase mixture, was incubated at 50 °C for 2 h with stirring (250 rpm). Reaction was stopped by heating at 95−100 °C for 10 min, and then the product was freeze-dried.

Nucleotides. Ten grams of HOCE was homogenized with 25 mL of chilled 10% $HClO_4$ for 15 min. The homogenate was centrifuged at 10000*g* (4 °C) for 10 min, and the residue was re-extracted twice more in the same manner. The combined supernatant was neutralized with 5.0 N KOH and centrifuged at 10000*g* for 10 min and the final volume made up to 100 mL with neutralized $HClO_4$. Aliquots were filtered through a Millipore filter (0.45 μm) for analysis. Nucleotide analysis was performed by a high-performance liquid chromatographic (HPLC) method similar to that reported by Ryder (1985). An HPLC system (LKB, Bromma, Sweden) with a C_{18} reverse phase column (Cosmosil, $5C_{18}$-MS, Nacalai Tesque, Inc.) was used at room temperature. The mobile phase was 0.04 M KH_2PO_4−0.06M K_2HPO_4, pH 7.0, and flow rate was 1 mL/min. Signal was detected with a model 2151 UV detector, and peaks were recorded using a model 2221 integrator.

Free Amino Acids. Free amino acids were determined using a modification of the method described by Gerritsen et al. (1965). One gram of HOCE was mixed with 80 mL of 1% picric acid and then homogenized. After the volume was made up to 100 mL with deionized water, the mixture was centrifuged at 3000*g*. Aliquots of supernatant were loaded onto a Dowex 2X8-200 (Sigma Chemical Co.) column (1.5 × 15 cm) to remove picric acid. The picric acid-free effluent was concentrated under vacuum in a rotary evaporator. The volume of the concentrate was made up to 5 mL with lithium citrate buffer, pH 2.2. Free amino acid compositions were identified and quantified by an automated amino acid analyzer (LKB 4150 plus, Pharmacia & Upjohn, Inc.).

Vacuum Simultaneous Steam Distillation−Solvent Extraction (V-SDE). HOCE (200 g) plus deodorized distilled water (1 L) and internal standard 3-heptanol, 9.72 μg) was extracted with stirring for 4 h using redistilled dichloromethane (150 mL) under reduced pressure in a modified SDE apparatus (catalog no. K-523010-0000, Kontes, Vineland, NJ) as described by Cadwallader et al. (1994). After extraction, V-SDE extracts were kept at −20 °C overnight to facilitate water removal. The solvent was evaporated under a gentle nitrogen stream to ~10 mL and dried over 3 g of anhydrous sodium sulfate. The extract was further concentrated to 100 μL. Triplicate extractions were carried out.

Table 1. Percent Chemical Composition of OCE[a]

sample	moisture	total N	crude lipid	ash	NaCl	glycogen
OCE	80.3 (±0.09)	6.69 (±0.15)	trace	8.48 (±0.11)	7.62 (±0.12)	2.42 (±0.09)

[a] Results are mean values of three determinations. Numbers in parentheses represent standard deviations.

Gas Chromatography−Mass Spectrometry (GC-MS). The GC-MS system consisted of an HP 5890 series II GC/HP 5972 mass selective detector (MSD) (Hewlett-Packard Co., Palo Alto, CA). Two microliters of each extract was injected (splitless mode; injector temperature = 200 °C; 60 s valve delay) into a fused silica capillary column (DB-Wax, 60 m length × 0.25 mm i.d. × 0.25 μm film thickness; J&W Scientific, Folsom, CA). Helium was used as carrier gas at a constant flow of 1 mL/min. Oven temperature was programmed from 40 to 200 °C at 3 °C /min with initial and final hold times of 5 and 60 min, respectively. MSD conditions were as follows: capillary direct interface temperature, 280 °C; ionization energy, 70 eV; mass range, 33−350 amu; electron multiplier voltage, 1824 V (200 V above autotune); scan rate, 2.2 scans/s. Duplicate analyses were performed on each extract.

Aroma Extract Dilution Analysis (AEDA). The gas chromatography−olfactometry (GC-O) system used for AEDA has been previously described (Ullrich and Grosch, 1987; Guth and Grosch, 1994; Baek et al., 1997). Serial dilutions (1:3) of HOCE were prepared using dichloromethane as a diluent. From each dilution, 1 μL was injected into a fused silica capillary column (DB-Wax, 30 m length × 0.32 mm i.d. × 0.25 μm film thickness; J&W Scientific). GC conditions were the same as for GC-MS except that the oven temperature was programmed from 40 to 200 °C at 8 °C /min with initial and final hold times of 5 and 30 min, respectively. GC-O was performed by one experienced panelist familiar with oyster aroma.

RESULTS AND DISCUSSION

Proximate Composition of OCE. The proximate composition of OCE is given in Table 1. OCE contained 6.7% TN and 2.4% glycogen. Furthermore, 50% of the TN was estimated to be protein, in the form of heat-denatured proteins extracted during the oyster cooking process (data not shown). Ash content was high and consisted mostly of NaCl. The presence of protein and glycogen in OCE increased its viscosity and imparted an unacceptable appearance. Therefore, subsequent studies focused on the reduction of OCE viscosity and an increase in flavor components through hydrolysis facilitated by the combined use of a protease and an amylase mixture.

Reaction Conditions for Protein Hydrolysis in OCE. Figure 1 shows the effect of incubation time on hydrolysis of OCE protein at 50 °C with 0.2% protease NP. As a result of proteolytic activity, soluble tyrosine equivalents increased with reaction time with a corresponding decrease in protein. Only slight degradation of protein was observed after 2 h. This trend was similar to that previously observed for hydrolysis of crab-processing byproduct (CPB) (Kim et al., 1994), except

Table 2. Free Amino Acid Profile (Milligrams per 100 mL) of HOCE[a]

amino acid	HOCE	amino acid	HOCE
phosphoserine	71.2 (±1.98)	cystathionine	6.83 (±0.10)
taurine	1090 (±10.9)	isoleucine	21.0 (±0.85)
urea	957 (±13.4)	leucine	46.4 (±1.84)
aspartic acid	50.4 (±1.48)	tyrosine	82.0 (±1.91)
threonine	216 (±4.95)	β-alanine	431 (±7.07)
serine	32.0 (±1.13)	phenylalanine	20.8 (±0.85)
glutamic acid	454 (±5.66)	β-aminoisobutyric acid	2.96 (±0.18)
proline	47.0 (±1.34)	ammonia	24.7 (±2.05)
glycine	821 (±13.4)	D,L-allohydroxylysine	3.20 (±0.13)
alanine	779 (±11.3)	ornithine	15.5 (±0.92)
α-aminoisobutyric acid	19.7 (±0.99)	lysine	42.6 (±1.20)
valine	37.3 (±0.28)	histidine	77.8 (±2.55)
cysteine	5.44 (±0.04)	arginine	160 (±8.49)
methionine	87.6 (±1.77)		
total			5602.43 (±28.6)

[a] Results are mean values of three determinations. Numbers in parentheses represent standard deviations.

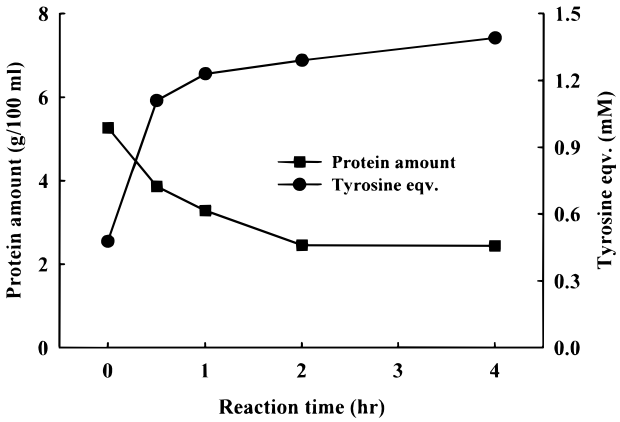

Figure 1. Progress curves for the hydrolysis of OCE by Protease NP (0.2%) at 50 °C. Protein amount was determined by a semimicro-Kjeldahl method after precipitation by 30% TCA solution.

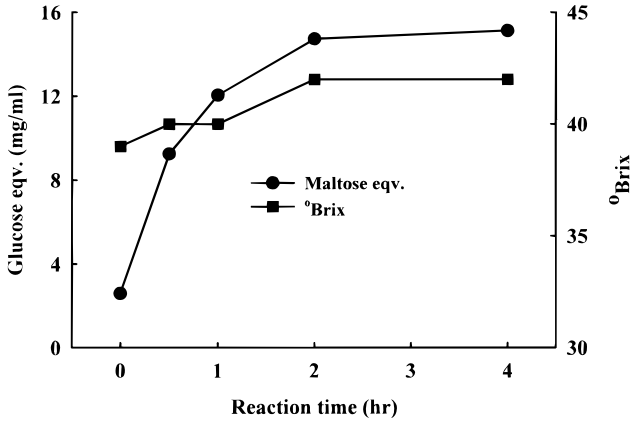

Figure 2. Progress curves for the hydrolysis of glycogen in OCE by amylase mixture (0.1%) at 50 °C.

that with CPB further protein degradation continued for up to 4 h. The difference observed between OCE and CPB might be due to protein solubility; that is, the proteins in OCE were soluble, whereas those in CPB were mostly insoluble.

Reaction Conditions for Glycogen Hydrolysis in OCE. In a preliminary experiment, hydrolytic activities of α-amylase and glucoamylase against OCE were determined at pH 7.0 and at 50 °C. The hydrolytic activity of α-amylase against OCE was 10 times higher than that of glucoamylase under these conditions (data not shown). Therefore, an α-amylase and glucoamylase solution was mixed at the ratio of 1:10 for the hydrolysis of glycogen in OCE. A progress curve for the hydrolysis of glycogen in OCE by the mixture of α-amylase and glucoamylase (1:10) at pH 7.0 and 50 °C is shown in Figure 2. Glycogen was nearly completely hydrolyzed after 2 h. Increase in soluble solids (°Brix) showed a similar trend, with an increase observed up to 2 h of reaction time, possibly due to the conversion of glycogen (2.42%) to glucose. Hydrolysates prepared by the combined protease/amylase enzyme system showed a noticeable increase in sweetness and no bitterness.

Free Amino Acid Profile of HOCE. Table 2 indicates profiles of free amino acids in HOCE, and total content was estimated to be 5600 mg/100 mL; the predominant amino acid was taurine, which comprised 19.5% of total free amino acids. Glycine, alanine, glutamic acid, and β-alanine were also predominant components.

The muscle tissue of crustaceans has been shown to contain high levels of taurine, proline, glycine, and alanine (Konosu and Kasai, 1961). On the other hand, the free amino acid pool of crustaceans contains appreciable levels of arginine, glycine, and proline, along with lesser amounts of alanine, glutamic acid, and taurine (Konosu et al., 1978). Previous studies have shown that cooker effluents produced from seafoods contain high levels of taurine in the free amino acid pool. Taurine was found at 42% in oyster, 57% in pen-shell, and 50% in blue-mussel cooker effluents (Kim et al., 1994b). Also, skipjack cooking effluent contained a high level of taurine, which comprised ∼11.6% of the total free amino acids (Choi et al., 1996). In the present study, glycine, alanine, glutamic acid, and arginine comprised ∼40% of total free amino acids. These amino acids are recognized as being important in the tastes of fish and shellfish (Choi et al., 1996).

Nucleotides and Their Related Compounds in HOCE. Level of nucleotides and their related compounds was estimated to be 483.7 mg/100 mL of HOCE (Table 3). ATP and ADP were not detected in HOCE. IMP comprised ∼94% of total nucleotides and their related compounds. Previously, percentages of ATP, ADP, and AMP in total nucleotides and their related compounds were estimated to be 86% in oyster, 63% in pen-shell, and 40% in blue-mussel cooker effluent (Kim et al., 1994b). It is possible that ATP, ADP, and AMP were destroyed by heat during the concentration of OCE.

IMP and GMP are known to impart umami tastes (Shallenberger, 1992). Also, these compounds in the

Table 3. Nucleotides and Related Compounds (Milligrams per 100 mL) in HOCE[a]

nucleotide	HOCE
ATP	
ADP	
AMP	8.5 (±1.93)
IMP	456 (±13.5)
inosine	17 (±1.05)
hypoxanthine	2.2 (±0.32)
total	483.7 (±13.7)

[a] Results are mean values of three determinations. Numbers in parentheses represent standard deviations.

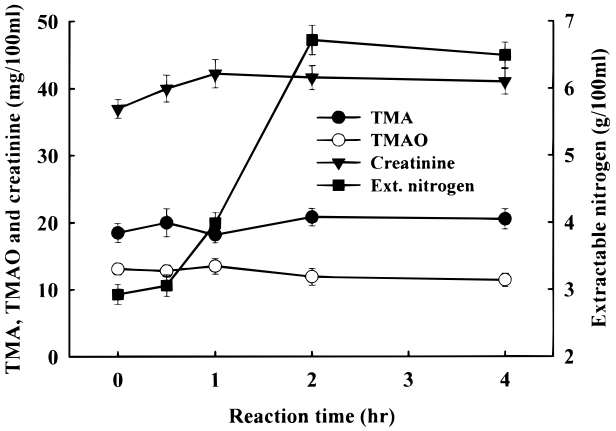

Figure 3. Change in levels of TMA, TMAO, total creatinine, and extractable nitrogen during hydrolysis of OCE. Reaction was performed with 0.2% Protease NP and 0.1% amylase mixture at 50 °C with stirring at 250 rpm.

presence of monosodium glutamate are known to have a synergistic effect on flavor intensity (Yamaguchi et al., 1968). Both IMP and glutamic acid were found at ~0.46% in a total HOCE.

TMA, TMAO, and Extractable Nitrogen Content. The contents of TMA and TMAO did not change during enzyme reaction; however, extractable nitrogen content increased (Figure 3). Total creatinine increased slightly up to 1 h of reaction time and thereafter did not change. After 2 h, the amount of extractable nitrogen content increased by 2-fold, with no further change observed up to 4 h. The increase of extractable nitrogen was mostly the result of proteolytic activity, and ~84% of extractable nitrogen was determined to be free amino acids. Nucleotides and their related compounds comprised ~7% of the extractable nitrogen. The amount of TMA, TMAO, and total creatinine comprised only ~1% of extractable nitrogen.

OCE contained 47.2 and 3.8 mg/100 mL of TMAO and TMA, respectively, which was greater than that found in pen-shell and blue-mussel cooker effluents (Kim et al., 1994b). OCE contained a 12-fold greater level of TMAO than TMA; however, HOCE in the present study had similar levels of TMA and TMAO. This might be explained by the possible degradation of TMAO to TMA during enzymatic reaction.

Aroma Components of HOCE. Fifteen aroma-active compounds were detected by AEDA of HOCE (Table 4). 2-Acetyl-1-pyrroline (2-AP) (**4**), 3-(methylthio)-propanal (methional) (**7**), and one unknown compound having marine shell-like aroma (**2**) were detected as the most potent aroma-active compounds. 2-AP and methional have been reported to be important aroma components of cooked crustaceans (Baek and Cadwallader, 1997). Two unknown compounds having meaty (**11**) and dried anchovy (**12**) aroma notes probably play roles in the overall aroma of HOCE because of their high $\log_3$ FD factors. 2,3-Butanedione (**1**), 2-acetyl-2-thiazoline (**13**), and one unknown compound having an oyster-like aroma (**15**) had relatively high $\log_3$ FD factors. Like 2-AP and methional, 2,3-butanedione and 2-acetyl-2-thiazoline were also commonly detected aroma-active components of cooked crustaceans (Baek and Cadwallader, 1997). Seven aroma-active compounds including 2-ethyl-3,5-dimethylpyrazine (**8**) and (*E*,*Z*)-2,6-nonadienal (**10**) were detected with relatively low $\log_3$ FD values. 2-Ethyl-3,5-dimethylpyrazine and (*E*,*Z*)-2,6-nonadienal have been found in crayfish (Baek and Cadwallader, 1996, 1998) and crustaceans (Baek and Cadwallader, 1997).

Table 4. Aroma-Active Compounds in HOCE

no.	compound name	RI[a]	aroma description	concn (ppb)	$\log_3$ FD[b]
1	2,3-butanedione	981	buttery	302	4
2	unknown	1160	marine shell		6
3	unknown	1242	fresh, metallic		3
4	2-acetyl-1-pyrroline	1339	popcorn	2.1	6
5	unknown	1430	popcorn		3
6	unknown	1439	sweet, popcorn		3
7	3-(methylthio)propanal	1457	soy sauce	98	6
8	2-ethyl-3,5-dimethyl-pyrazine	1469	nutty	5.8	3
9	unknown	1499	nutty		3
10	(*E*,*Z*)-2,6-nonadienal	1596	cucumber		3
11	unknown	1683	meaty		5
12	unknown	1726	dried anchovy		5
13	2-acetyl-2-thiazoline	1777	popcorn		4
14	unknown	2025	oyster		3
15	unknown	2145	oyster		4

[a] Retention index. [b] $\log_3$ (flavor dilution factor).

Conclusion. This study has established the chemical composition, including protein and glycogen, of OCE and of its hydrolyzed product (HOCE). Levels and types of taste- and aroma-active components of HOCE indicate the potential use of HOCE as a value-added seafood flavoring agent. These findings will help facilitate recovery and utilization of flavor and taste compounds from processing byproducts of oyster canning operations and help to reduce the volume of waste currently produced by the oyster industry.

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