Aroma Extract Dilution Analysis of a Beeflike Process Flavor from Extruded Enzyme-Hydrolyzed Soybean Protein

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Aroma-active compounds from a beeflike process flavor, produced by extrusion of enzyme-hydrolyzed vegetable protein (E-HVP), were analyzed using aroma extract dilution analysis. The number of aroma-active compounds and the aroma intensity were increased by the addition of aroma precursors prior to extrusion. The most intense compound was 2-methyl-3-furanthiol having a cooked rice/vitamin-like/meaty aroma note. Several sulfur-containing furans, such as 2-methyl-3-(methylthio)-furan, 2-methyl-3-(methyldithio)furan, and bis(2-methylfuryl)disulfide, were detected with high flavor dilution (FD) factors. Some pyrazines, such as 2-ethyl-3,5-dimethylpyrazine, 2,6-diethylpyrazine, and 3,5-diethyl-2-methylpyrazine, also had high FD factors. It is hypothesized that sulfur-containing amino acids and thiamin were important precursors in aroma formation in process flavor from E-HVP.

Keywords: *Aroma extract dilution analysis; process flavor; enzyme-hydrolyzed vegetable protein; aroma-active compound; extrusion*

INTRODUCTION

Hydrolyzed vegetable protein (HVP) has been used for >100 years to impart meatlike flavor to prepared foods and represents one of the earliest forms of process flavor (*1*). HVP is primarily composed of amino acids liberated by the hydrolysis of vegetable protein with HCl at 110−140 °C. Enzyme-hydrolyzed vegetable protein (E-HVP), which is an alternative to the traditional HVP, is produced using proteases under a more neutral pH and lower temperature. These two types of HVP have different sensory profiles due to the differences of the contents of free amino acids and the composition of volatile components (*2*).

Process flavor is important food flavorings (*1*, *3*). Process flavor is defined as a group of flavors or flavoring ingredients that are produced from precursors via some type of processing technique such as thermal processing (*4*). Precursors play an important role in the generation of process flavor. The Maillard reaction between reducing sugars and amino acids is known to generate flavors similar to those of cooked foods. Meat flavor can be produced through process flavor technology.

Extruders used for ready-to-eat breakfast cereals and snacks can be also used as flavor reactors (*5*). Extrusion is performed at high temperature for a short time process, affecting the flavor and aroma profiles of food products. The addition of precursors during extrusion, as well as the process parameters, affects the quality characteristics of final food products.

Aroma extract dilution analysis (AEDA) has been widely used to evaluate potent odorants in various food systems (*6*−*9*). AEDA is a dilution method in which a flavor extract is serially diluted and evaluated by gas chromatography−olfactometry (GCO). The highest dilution at which an odorant is detected is the so-called flavor dilution (FD) factor for that compound. FD factors have been used to rank the importance or intensities of odorants in the flavor extract.

The objective of this study was to evaluate aroma-active compounds from a beeflike process flavor developed by extrusion of E-HVP using AEDA.

MATERIALS AND METHODS

Sample. Beeflike process flavor was prepared by extrusion of E-HVP (Nong Shim Co., Kunpo, Korea) with amino acids (L-alanine, L-cystine, and methionine), fructose, thiamin, beef tallow, organic acids, and flavor enhancers using a twin-screw extruder (Bühler Brothers Co. Ltd., Biex DNDL, Uzwil, Switzerland). The extruder was operated at a feed rate of 10 kg/h with a screw speed of 180 rpm, a pressure of 5 bar, and a temperature of 165 °C. E-HVP without added precursors also was extruded under the same conditions.

Extraction of Volatiles. Each sample (30 g) plus 1 L of deodorized distilled water was extracted using vacuum simultaneous steam distillation/solvent extraction as described by Cadwallader et al. (*10*). 3-Heptanol (9.72 μg) was used as internal standard. Dichloromethane (100 mL) was used as extracting solvent. Extraction was carried out for 2.5 h. The boiling point was maintained at 58−60 °C. Residual water was removed from the extract by freezing out and drying over 3 g of anhydrous sodium sulfate. Extract was concentrated to 50 μL under a gentle N_2 stream prior to analysis. Extractions were performed in duplicate.

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10.1021/jf000609j CCC: $20.00
Published on Web 01/11/2001

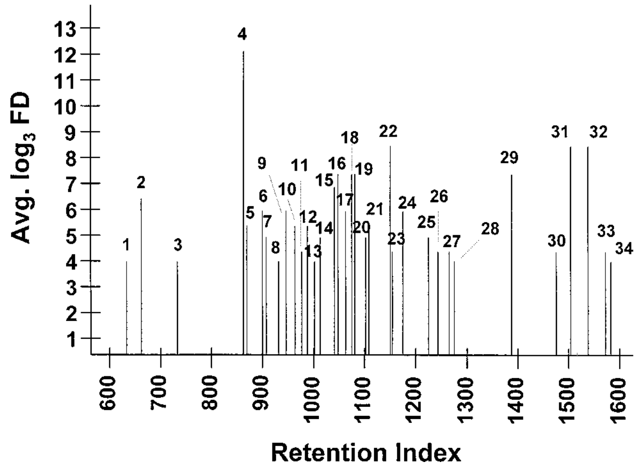

Figure 1. FD chromatogram of a beeflike process flavor from extruded enzyme-hydrolyzed soybean protein with added precursors

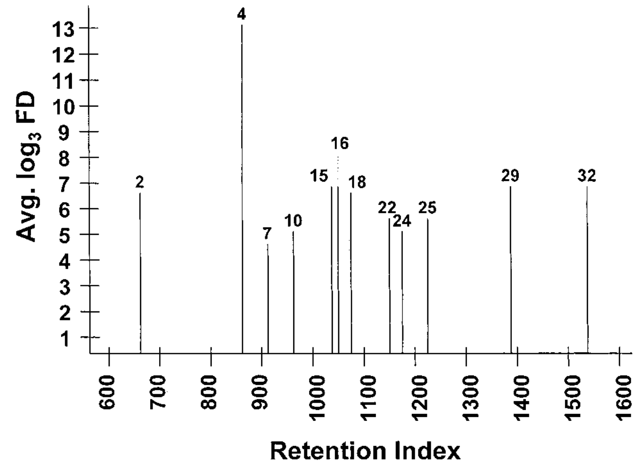

Figure 2. FD chromatogram of process flavor from extruded enzyme-hydrolyzed soybean protein without added precursors

Gas Chromatography—Mass Spectrometry (GC-MS). Volatile compounds were analyzed using an HP 5890 Series II GC/HP 5972 mass selective detector system (Hewlett-Packard Co., Palo Alto, CA). One microliter of extract was injected into DB-Wax (or DB-5ms) (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 linear velocity of 25 cm/s. Oven temperature was programmed from 40 to 200 °C at a rate of 3 °C/min with initial hold times of 5 and 60 min. GC-MS conditions were as follows: splitless mode; injector temperature, 200 °C; 60 s valve delay; 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.

AEDA. The GCO system consisted of a Varian 3300 GC (Varian Instrument Group, Walnut Creek, CA) equipped with a flame ionization detector and a sniffing port. Each extract was serially diluted (1:3) using dichloromethane as diluent. One microliter was injected into a DB-5ms column (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 a rate of 6 °C/min with initial and final hold times of 5 and 30 min, respectively. GCO was performed by two expert panelists familiar with the aroma of beeflike process flavor. FD factors were obtained by averaging FD factors determined by panelists and expressed as average $\log_3$ FD. Standard deviations of the FD factors were 0 and 0.72 for most of the aroma-active compounds.

Compound Identification. Positive identifications were based on the comparison of GC retention indices (RI) (*11*), Wiley mass spectral database (*12*), and aroma properties of unknowns with those of authentic standard compounds. When standards were not available, compounds were identified on the basis of comparison of mass spectra with those in the Wiley mass spectral database or by comparison of RI values (*13*) and aroma properties with literature data.

RESULTS AND DISCUSSION

A total of 34 aroma-active compounds with average $\log_3$ FD >4 were detected in the beeflike process flavor from extruded E-HVP with added precursors (Figure 1). Only 12 compounds were detected by extrusion of E-HVP without added precursors (Figure 2). Table 1 lists the aroma-active compounds, their RI values on the DB-5ms column, and their aroma descriptions. The addition of precursors significantly increased both the number of aroma-active compounds and the aroma intensity of the process flavor.

Of the detected aroma-active compounds, 2-methyl-3-furanthiol (2-MF, **4**), having a cooked rice/vitamin-like/meaty aroma note, was the most intense compound in process flavors from extruded E-HVP made with or without the added precursors. This compound can be formed via thermal degradation of thiamin (*14*) or by Maillard reaction between ribose and cysteine (*15*). The formation mechanism of 2-MF in the Maillard reaction (ribose−cysteine model system) has been proposed to occur between a ribose Amadori product and cysteine capable of liberating hydrogen sulfide (*16*). 2-MF also can be formed from the reaction of inosine-5′-monophosphate, which produces ribose, and hydrogen sulfide under acidic conditions (*17*). However, Bolton et al. (*18*) have proposed that thiamin degradation was the primary formation pathway of 2-MF, which results from direct cyclization of 5-hydroxy-3-mercaptopentan-2-one. 2-MF has been identified as the most potent aroma-active compounds in cooked beef and has a low odor detection threshold value (0.005−0.01 ppb) (*7*). 2-MF also has been reported to be present in yeast extracts (*19*) and processed tuna (*20*).

Other sulfur-containing furans, such as 2-methyl-3-(methylthio)furan (**9**), 2-methyl-3-(methyldithio)furan (**24**), and bis(2-methylfuryl) disulfide (**32**), were detected with high $\log_3$ FD factors. Compound **9** was tentatively identified as 2-methyl-3-(methylthio)furan on the basis of published RI and mass spectral data (*19*). 2-Methyl-3-(methylthio)furan was detected with a relatively high $\log_3$ FD factor in beeflike process flavor with added precursors, whereas this compound was not detected in the process flavor without added precursors. 2-Methyl-3-(methyldithio)furan and bis(2-methylfuryl) disulfide were detected at higher $\log_3$ FD factors in the process flavor with added precursors than in the process flavor without added precursors. 2-Methyl-3-(methylthio)furan and 2-methyl-3-(methyldithio)furan were proposed to be formed via either Maillard reaction of ribose and cysteine, involving the reaction with methanethiol, or the reaction of 2-MF with methanethiol (*16*, *21*). All of these sulfur-containing furans have been previously identified in yeast extracts (*19*). Bis(2-methylfuryl) disulfide is a dimer of 2-MF, and it is known that 2-methyl-3-furanthiol oxidizes easily to bis(2-methylfuryl) disulfide (*22*).

Several aroma-active compounds (**16**, **21**, **30**, and **31**) having yeast extract-like aroma notes were detected with relatively high $\log_3$ FD factors. Of these, **31** was tentatively identified as 3-[(2-methyl-3-furyl)dithio]-2-butanone on the basis of published mass spectral and

Table 1. Aroma-Active Compounds Detected in a Beeflike Process Flavor from Extruded Enzyme-Hydrolyzed Soybean Protein

no.	RI	compound name	identification method	aroma description
1	613	2,3-butanedione	RI, MS, odor	buttery
2	661	unknown		gravy, roasted garlic
3	729	unknown		metallic, rancid, earthy, musty
4	863	2-methyl-3-furanthiol	RI, MS, odor	cooked rice, vitamin-like, meaty
5	869	unknown		woody, burnt wood
6	900	3-mercapto-2-penanone	RI, MS, odor	spicy, catty
7	906	3-(methylthio)propanal	RI, MS, odor	soy sauce
8	923	unknown (*m/e* 125, 110)		spicy
9	938	2-methyl-3-(methylthio)furan	RI, MS, odor	yeast extract
10	966	dimethyl trisulfide	RI, MS, odor	rotten cabbage, garlic
11	977	unknown		spicy
12	990	2-methyltetrahydrothiophene-3-one	RI, MS, odor	spicy, catty
13	1001	unknown		nutty
14	1004	unknown (*m/e* 101, 60)		burnt
15	1045	phenylacetaldehyde	RI, MS, odor	floral
16	1052	unknown (*m/e* 142, 114, 85, 57)		yeast extract
17	1074	2-ethyl-3,5-dimethylpyrazine	RI, MS, odor	nutty
18	1081	2,6-diethylpyrazine	MS, odor	sesame oil
19	1185	2-methoxyphenol	RI, MS, odor	burnt, smoky
20	1102	unknown (*m/e* 127, 71)		spicy, catty
21	1105	unknown		yeast extract, vitamin-like
22	1150	3,5-diethyl-2-methylpyrazine	RI, MS, odor	nutty, roasty
23	1151	unknown		spicy, catty
24	1169	2-methyl-3-(methyldithio)furan	RI, MS, odor	yeast extract
25	1220	dimethyl tetrasulfide	RI, MS, odor	sour, cooked cabbage
26	1239	unknown		spicy, catty
27	1269	unknown		nutty, roasted peanut
28	1276	unknown		floral
29	1393	unknown (*m/e* 192, 180, 165, 113)		spicy, burnt, meaty, roasty
30	1474	unknown (*m/e* 202, 179, 144, 113)		yeast extract, vitamin-like
31	1502	3-[(2-methyl-3-furyl)dithio]-2-butanone	RI, MS, odor	yeast extract, vitamin-like
32	1535	bis(2-methylfuryl) disulfide	RI, MS, odor	yeast extract
33	1571	3-[(2-methyl-3-furyl)dithio]-2-pentanone	RI, MS, odor	spicy, catty
34	1585	unknown		burnt, rubber-like

RI data (*23*). Although the other compounds were not identified, these compounds were hypothesized to be sulfur-containing furans on the basis of their mass spectra and odor properties.

In addition to sulfur-containing furans, other sulfur-containing compounds such as 3-(methylthio)propanal (**7**), dimethyl trisulfide (**10**), 2-methyltetrahydrothiophene-3-one (**12**), and dimethyl tetrasulfide (**25**) were detected with moderate $\log_3$ FD factors. These compounds were considered to be either thermally generated aroma-active compounds from sulfur-containing amino acids or Maillard reaction products (*24*).

Some pyrazines, such as 2-ethyl-3,5-dimethylpyrazine (**17**), 2,6-diethylpyrazine (**18**, tentative identification), and 3,5-diethyl-2-methylpyrazine (**22**), had high $\log_3$ FD factors. 2-Ethyl-3,5-dimethylpyrazine was detected only in beeflike process flavor with added precursors, and 2,6-diethylpyrazine and 3,5-diethyl-2-methylpyrazine showed higher $\log_3$ FD factors by adding precursors. In addition to sulfur-containing compounds, thermally generated pyrazines were thought to impart a beeflike flavor to extruded E-HVP with added precursors.

In our study, thiamin, methionine, cystine, fructose, and flavor enhancer were added to E-HVP during extrusion, which played an important role in the formation of a large amount of sulfur-containing compounds as well as pyrazine compounds of extruded E-HVP.

Some aroma-active compounds with spicy and catty odors (**6**, **12**, **20**, **23**, **26**, and **33**) were detected, which were thought to give undesirable aroma notes to the process flavor. On the basis of published data, compounds **6** and **33** were tentatively identified as 3-mercapto-2-pentanone (*25*) and 3-[(2-methyl-3-furyl)dithio]-2-pentanone (*23*), respectively. It is proposed that these undesirable odors can be reduced, while desirable beefy/meaty aroma can be retained or enhanced, by controlling extrusion conditions or changing precursor system. Parameters such as type of precursors, temperature, water content, and pH affect aroma properties in thermally processed foods (*26*, *27*).

Numerous studies have been performed on the generation of beeflike process flavor using various precursor systems (*28*). Amino acids, peptides, carbohydrates, ribonucleotides, lipid, and thiamin have been widely used for the development of meat flavor via process flavor technology. Evaluation of aroma-active compounds in beeflike process flavor has demonstrated that beeflike flavor could be successfully developed by extrusion of E-HVP with appropriate precursors. It was hypothesized that sulfur-containing amino acids and thiamin were important precursors in process flavor of E-HVP.

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Received for review May 16, 2000. Revised manuscript received October 16, 2000. Accepted October 26, 2000. This work was supported by a grant from the Yulchon Foundation.

JF000609J