Isolation and Identification of the Volatile Components of an Extruded Autolyzed Yeast Extract

Henry V. Izzo and Chi-Tang Ho*

Department of Food Science, Cook College, New Jersey Agricultural Experiment Station, Rutgers University, New Brunswick, New Jersey 08903

The volatiles generated from an extruded autolyzed yeast extract (AYE) were isolated and analyzed by GC and GC–MS. The volatile profile of the extruded AYE was compared with that of the unextruded ingredient. It was found that the same volatiles were formed in both cases and that the only difference was in the amounts formed. In both systems a total of 9 sulfur-bearing volatiles, 5 volatiles characteristic of sugar degradation, and 39 nitrogen-containing heterocyclics were identified. The overall profile was dominated by alkylpyrazines, and extrusion was found to enhance the formation of pyrazines considerably.

INTRODUCTION

The group of unicellular fungi, known to us as yeasts, has played an important role in the food industry for centuries. Traditionally, the utilization of yeasts in food processes has been mostly for their metabolic power to convert carbohydrates into ethanol and carbon dioxide during fermentation (Dziezak, 1987). It is this characteristic that has made yeast an integral component of the beer, wine, and baking industries. Today, however, yeasts are commanding additional attention for some of their more unique food applications. One such use that has been manifested over the past several years, and is increasing in popularity, is the exploitation of yeast derivatives known as autolyzed yeast extracts (AYE) as savory flavoring agents.

AYEs are FDA approved as natural food flavorings and are fast becoming a popular interjection into a variety of savory food applications (Blake, 1982). The production of autolyzed yeasts has been described in detail (Reede and Peppler, 1973; Peppler, 1982). The general basis of this production is the self-destruction and solubilization of the yeast cell. This solubilization results in the extraction of various peptides, amino acids, nucleotides, nucleosides, and saccharrides. The resulting extract can then be further processed into a liquid, paste, powder, or granular preparation (Acraman, 1966).

Given the relative increase in the use of AYEs as savory flavoring agents in the food industry, it is interesting to note that few research papers have been produced regarding the volatile composition of AYE (Davidek et al., 1979; Hajslova et al., 1980; Ames and MacLeod, 1985; Werkoff et al., 1990). These papers have focused mostly on one aspect of AYE, which is simply the volatile profiles of the primary ingredient. However, there are other technical dimensions of AYE which must be considered. Given the relative composition of AYE, in particular with reference to its carbohydrate and protein composition, it is clear that this ingredient, upon heating, will undergo the much researched Maillard reaction. Since AYEs are a unique composite source of Maillard reactants, the possibility of enhancing the volatile composition of AYE by heating has great potential.

A traditional way of producing a so-called "reaction flavor" is by heating reducing sugars and amino acids contained in a pressurized vessel during a batch process. A new and unique way to accomplish this is by utilizing cooker extruders as continuous flavor reactors for the sole purpose of modifying or enhancing the volatile profile of the precursors. The use of extruders as ingredient modifiers is an interesting prospect (Cheftel, 1986).

The purpose of this work was to compare the volatile composition of a commercial powdered AYE with the volatiles that were generated as a result of the single-screw extrusion of this material.

EXPERIMENTAL PROCEDURES

Materials. Commercial powdered autolyzed yeast extract was purchased from Universal Foods Corp. (Milwaukee, WI) and is sold under the trade name Flavormate 945. This material was obtained from a primary grown strain of bakers' yeast, *Saccharomyces cerevisiae*, and was essentially a spray-dried powder of the extract. Reagent grade methylene chloride was used as the primary extraction solvent for the volatile isolation. It was obtained from Fisher Scientific Co. (Fair Lawn, NJ) and was redistilled before use. The standard compound 2,6-dimethylpyrimidine was obtained from Aldrich Chemical Co. (Milwaukee, WI). Crystalline sodium hydroxide and crystalline anhydrous sodium sulfate were purchased from Fisher Scientific.

Extrusion of AYE. Extrusion was accomplished by utilizing a Type 2003 Brabender 3/4-in. single-screw extruder. This unit was equipped with a screw having a length to diameter ratio of 20:1 and a circular die with an inner diameter of 0.6 cm. The sample was extruded at an equilibrated melt temperature of 115 °C. The unit screw speed was 140 rpm. These set conditions dictated a total throughput of approximately 24 g/min. The total feed moisture at the inlet of the cooker extruder was 5.5%. The resulting extrudates generated were stored at −4 °C under nitrogen in sealed 1-qt Mason jars until analysis.

Volatile Isolation. The extrudate was ground in the presence of dry ice in a bench top grinder (Micromill, Cleveland, OH) and passed through a 24-mesh sieve to ensure uniform particle size distribution. The moisture content of the resulting powder was determined according to the AOAC air oven method (AOAC, 1984). Forty grams (dry basis) of ground extrudate was weighed into a 200-mL Erlenmeyer flask, and 80 mL of redistilled methylene chloride was added. To this was added 1 mL of 0.2314 mg/mL 2,6-dimethylpyrimidine as an internal standard. The extraction took 6 h, and the resulting liquid fraction was recovered by vacuum filtration through Whatman No. 2 filter paper. The solid residue was then subjected to another extraction identical to the first. Lastly, the two liquid organic extracts were combined. The resulting total extract was then subjected to the Nickerson–Likens extraction distillation method to remove any water-soluble pigments which could interfere with further analysis. The

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resulting clarified extract was then subjected to acid/base fractionation by extracting in a separatory funnel with 3 volumes of 80 mL of aqueous NaOH (5% w/w). This step aided in the removal of an unidentified acidic preservative probably used as antifungal or antimicrobial agent in the AYE. The resulting organic extract was dried over anhydrous sodium sulfate, and the sample was then concentrated with a Kuderna-Danish concentrating apparatus to a volume of 5 mL. The concentrated extract was quantitatively transferred to a glass sample vial and finally concentrated to a volume of 0.2 mL to facilitate GC and GC–MS analysis. The volatiles from the unextruded AYE were isolated in an identical fashion.

Gas Chromatography (GC). Separation of the resulting volatiles was accomplished on a Varian 3400 gas chromatograph equipped with an FID and nonpolar fused silica capillary column [50 m × 0.32 mm (i.d.), 1.05-μm thickness, HP-1; Hewlett-Packard]. For each sample 0.8 μL was injected with a GC split ratio of 50:1. The GC was operated with an injector temperature of 270 °C, a detector temperature of 300 °C, and a helium carrier flow rate of 0.8 mL/min. The program for the separation was as follows: initial column temperature of 40 °C and a temperature increase of 2 °C/min from 40 to 260 °C with a 40-min isothermal hold. Volatiles were quantified by use of the internal standard 2,6-dimethylpyrimidine. Linear retention indices for the volatiles were determined through the use of a C_5–C_{26} *n*-paraffin standard (Alltech Associates) according to the method of Majlat et al. (1974).

Gas Chromatography–Mass Spectrometry (GC–MS). The samples were also analyzed by gas chromatography–mass spectrometry. This was accomplished by utilizing a Varian 3700 gas chromatograph coupled with a Finnigan MAT 8320 high-resolution mass spectrometer. The volatiles were separated using the same temperature program that was used for GC analysis. The mass spectrometer ionization was set at 70 eV, and the source temperature was 250 °C with a filament emission current of 1 mA. Spectra obtained were identified by utilizing an on-line computer library (NBS) or the eight-peak mass spectra series (MSDC, 1984).

RESULTS AND DISCUSSION

The volatiles from both extruded and unextruded AYE were analyzed as described in the previous section. Table I lists the compounds identified by GC–MS analysis and presents their retention indices as well as semiquantitative data. It was found that a total of 53 volatile compounds were identified in the extruded AYE system. These included 9 sulfur-bearing volatiles, 5 volatiles characteristic of sugar degradation, 7 non-pyrazine nitrogen-containing compounds, and finally a total of 32 pyrazine compounds. Interestingly, all volatiles appeared in both extruded and unextruded AYE and varied only in amounts of each compound formed. It is hypothesized that the unextruded AYE may have developed some aromas during the production steps, such as mild heat exposure during spray drying. This would result in the manifestation of some extent of the Maillard reaction but not an overabundance of volatile production. This phenomenon could have also been attributed to artifact production during isolation. In a sense, the generation of the same volatiles in both systems would be expected since we are simply utilizing identical precursors and inducing the same reactions but to different extents.

Sulfur-Bearing Volatiles. These volatiles were most likely formed as a result of the generation of hydrogen sulfide due to the thermal degradation of sulfur-bearing amino acids or from the thermal degradation of thiamin (MacLeod and Seyyedain-Ardebili, 1981). Three sulfides were formed: dimethyl disulfide, dimethyl trisulfide, and dimethyl tetrasulfide. These volatiles play an important role in meats and vegetables by providing sulfury notes. They were most likely produced by the thermal degradation of methionine, which results in the formation of methylthio radicals. These resulting species can then join with other radicals as well as undergo disproportionation, forming sulfides. 2-Methylthiophene and 2-ethylthiophene were identified in the AYE systems and are responsible for the mild sulfurous odors of cooked meats (MacLeod, 1986). The formation of 3,5-dimethyl-1,2,4-trithiolane as well as 4,6-dimethyl-1,2,3,5-tetrathiane from AYE was probably due to the reactions of hydrogen sulfide with acetaldehyde, which are the thermal degradation products of cysteine or cystine (Zhang and Ho, 1989; Zhang, 1991). Finally, two thiazoles were detected: 2-acetylthiazole and 4-methyl-2-acetylthiazole. It is possible that

Table I. Volatile Compounds from Extruded and Unextruded Autolyzed Yeast Extract

compound	Ik (DB-1)	amount, ppm U-AYE[a]	amount, ppm E-AYE[b]
pyrazine	710	–[d]	–
dimethyl disulfide	731	–	–
pyrrole	733	–	–
2-methylthiophene	758	–	–
2-methyltetrahydrofuran-3-one	776	–	–
2-methylpyridine	787	–	–
methylpyrazine	798	–	0.554
furfural	802	–	–
2-methylpyrrole	*[c]	–	–
trimethyloxazole	*	–	–
2-furfuryl alcohol	835	–	–
2-ethylthiophene	844	–	–
2-acetylfuran	882	–	–
2,6-dimethylpyrazine	884	–	–
ethylpyrazine	888	–	3.014
2,3-dimethylpyrazine	891	–	0.236
dimethyl trisulfide	949	–	–
2-ethyl-5-methylpyrazine	974	–	0.723
2-methyl-6-methylpyrazine	980	0.028	2.063
2-ethyl-3-methylpyrazine	982	–	0.652
2-acetylthiazole	989	–	–
6-methyl-2-vinylpyrazine	992	0.023	0.031
propenylpyrazine	*	–	–
2-acetylpyridine	1026	–	–
2-acetylpyrrole	*	–	–
2,5-dimethyl-3-ethylpyrazine	1059	0.163	1.073
2,3-diethylpyrazine	1063	–	–
2,3-dimethyl-5-ethylpyrazine	1065	0.077	0.547
2-methyl-6-propylpyrazine	1069	–	–
6-ethyl-2-vinylpyrazine	1076	–	0.124
indoline	*	–	–
4-methyl-2-acetylthiazole	1085	–	–
5-methyl-2-acetylpyrazine	1088	–	–
6-methyl-2-acetylpyrazine	1093	–	0.044
3,5-dimethyl-1,2,4-trithiolane	1101	–	–
2-methyl-5-butylpyrazine	1114	–	0.048
2-methyl-6-butylpyrazine	1120	–	–
2-methyl-3-butylpyrazine	1125	–	–
2,5-diethyl-3-methylpyrazine	1136	–	0.049
2,6-diethyl-3-methylpyrazine	1138	–	0.134
2,3-diethyl-5-methylpyrazine	1140	–	0.147
furfuryl butyrate	*	–	–
2-ethyl-6,7-dihydro-5*H*-cyclopentapyrazine	*	–	–
2-methyl-6-propenylpyrazine	1161	–	–
ethylacetylpyrazine	1170	–	–
2,3-dimethyl-5-butylpyrazine	1198	–	0.066
dimethyl tetrasulfide	1186	–	–
methylpentylpyrazine	*	–	–
2,3-dimethyl-6,7-dihydro-5*H*-cyclopentapyrazine	1202	–	–
2-methyl-5,6,7,8-tetrahydroquinoxaline	*	–	–
2-methylquinoxaline	*	–	–
dimethylpentylpyrazine	1336	–	–
4,6-dimethyl-1,2,3,4-tetrathiane	1389	–	–

[a] U-AYE, unextruded autolyzed yeast extract. [b] E-AYE, extruded autolyzed yeast extract. [c] *, unable to be detected by GC. [d] –, trace component, less than 0.001 ppm.

these compounds were formed from the Maillard reaction of cysteine with reducing sugars (Zhang and Ho, 1991).

Volatiles from Sugar Degradation. The five volatiles that were derived from sugar degradation are furfural, 2-furfuryl alcohol, 2-acetylfuran, 2-methyltetrahydrofuran-3-one, and furfuryl butyrate. The general structure of all of these compounds stems from the fragmentation of the sugar moiety and the resulting cyclization of the hydroxy ketone compounds, forming furans with various substitutions (Hodge, 1953). The formation of furfuryl butyrate could be due to the reaction of furfuryl alcohol with butyric acid, which would be derived from a lipid backbone.

Nitrogen-Containing Heterocyclics. The major volatile fraction in the extruded AYE was found to be the pyrazine compounds. This seems likely since the AYE system is composed mostly of peptides and free amino acids. Of the non-pyrazine nitrogen-containing volatiles, an oxazole, two pyridines, three pyrroles, and indoline were identified. The formation of the trimethyloxazole could have originated from the cyclization of the Schiff base moiety before hydrolysis (Ho and Hartman, 1982). Of the two pyridines identified, 2-acetylpyridine has been known to exhibit the flavor of stale popcorn and methylpyridine has been known to have an objectionable odor (Shibamoto, 1989). These compounds were probably formed from the interaction between amino acid and sugars followed by cyclization so as to include a single nitrogen in the ring. The three pyrroles identified were most likely formed by the Strecker degradation of dicarbonyls with amino acids, resulting in the final cyclization of the aminocarbonyl. Pyrroles have been known to impart pleasant crackerlike and bakery notes to foods (Shibamoto, 1989). Finally, indoline, being bicyclic, could have been formed by the condensation of nitrogen-containing ring structures with with aldehydes, diketones, or other alkyl-bearing moieties.

As mentioned previously, the greatest majority of the compounds identified in the extruded yeast extract systems were alkylpyrazines. Pyrazines are described as being roasted or toasted in flavor character and are highly desirable in foods. They have been identified in a wide variety of food sources including roasted beef (Hartman et al., 1983). These compounds also quantitatively dominated the aroma profile of extruded AYE. A total of 32 alkylpyrazines were identified in the extruded AYE system. Of these pyrazines, 24 were present in major amounts and were able to be quantified by gas chromatography. The major pyrazines identified in the extruded AYE system were methylpyrazine, ethylpyrazine, 2-ethyl-6-methylpyrazine, 6-methyl-2-vinylpyrazine, and 2,5-dimethyl-3-ethylpyrazine. It seems that the well-established Strecker degradation mechanism is very significant during extrusion.

Methylpyrazine has been identified as a constituent of roasted meat (Maga, 1982) and has been described as having burnt and roasted characteristics. It is possible that this pyrazine was produced by the condensation of a two-carbon aminocarbonyl fragment and a three-carbon aminocarbonyl fragment.

Ethylpyrazine was found as the major pyrazine in extruded AYE. Since this pyrazine is present in the largest amount, one can hypothesize that sugar fragmentation occurred between the second and third carbons of the glucose moiety. Ethylpyrazine is described as buttery or rumlike and has been identified in grilled, boiled, and pressure-cooked beef (Maga, 1982).

Of the remaining major pyrazines 2-ethyl-6-methylpyrazine and 6-methyl-2-vinylpyrazine both could have been formed from the condensation of a three-carbon fragment and a four-carbon fragment. And finally, 2,5-dimethyl-3-ethylpyrazine was formed from the combination of two four-carbon fragments.

The formation of higher carbon number substituted pyrazine compounds, such as methylpentylpyrazine and dimethylpentylpyrazine, is hypothesized to be due to the intervention of aldehydes. The presence of aldehydes, originating either from amino acid degradation or from lipid degradation, could facilitate addition to the metastable dihydropyrazine compound, resulting in longer chain substituted pyrazines (Huang et al., 1987).

Even in the unextruded systems we see that pyrazines were the major volatile, quantitatively dominating the volatile profile. These pyrazines, however, were more substituted than the pyrazines that were generated in major amounts in the extruded AYE. It seems that the role of sugar fragmentation is enhanced during the extrusion process, giving us smaller aminocarbonyl fragments which in turn generate pyrazines of less substitutions.

Conclusion. It seems that by extruding AYE we have enhanced its roast aroma character. This may prove to be desirable since many food applications today require that the final aroma profile have a very good meaty aroma as well as a roasted characteristic. Ordinarily these aromas are only obtained after the food has been heated at high temperatures for prolonged time periods. By applying the high-temperature short-time technology of extrusion, we have reduced this reaction time considerably. Given the advent of today's quick-cooked convenience foods, the production of savory meat flavors with a strong roasted aroma component should prove to be highly desirable. Therefore, further research is warranted into the modification and enhancement of the roast aroma of AYE by extrusion processing.

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Registry No. Pyrazine, 290-37-9; dimethyl disulfide, 624-92-0; pyrrole, 109-97-7; 2-methylthiophene, 554-14-3; 2-methyltetrahydrofuran, 3188-00-9; 2-methylpyridine, 109-06-8; methylpyrazine, 109-08-0; furfural, 98-01-1; 2-methylpyrrole, 636-41-9; trimethyloxazole, 20662-84-4; 2-furfuryl alcohol, 98-00-0; 2-ethylthiophene, 872-55-9; 2-acetylfuran, 1192-62-7; 2,6-dimethylpyrazine, 108-50-9; ethylpyrazine, 13925-00-3; 2,3-dimethylpyrazine, 5910-89-4; dimethyltrisulfide, 3658-80-8; 2-ethyl-5-methylpyrazine, 13360-64-0; 2-ethyl-6-methylpyrazine, 13925-03-6; 2-ethyl-3-methylpyrazine, 15707-23-0; 2-acetylthiazole, 24295-03-2; 6-methyl-2-vinylpyrazine, 13925-09-2; propenylpyrazine, 43039-96-9; 2-acetylpyridine, 1122-62-9; 2-acetylpyrrole, 1072-83-9; 2,5-dimethyl-3-ethylpyrazine, 13360-65-1; 2,3-diethylpyrazine, 15707-24-1; 2,3-dimethyl-5-ethylpyrazine, 15707-34-3; 2-methyl-6-propylpyrazine, 29444-46-0; 6-ethyl-2-vinylpyrazine, 32736-90-6; indoline, 496-15-1; 4-methyl-2-acetylthiazole, 7533-07-5; 5-methyl-2-acetylpyrazine, 22047-27-4; 6-methyl-2-acetylpyrazine, 22047-26-3; 3,5-dimethyl-1,2,4-trithiolane, 23654-92-4; 2-methyl-5-butylpyrazine, 29461-04-9; 2-methyl-6-butylpyrazine, 32184-46-6; 2-methyl-3-butylpyrazine, 15987-00-5; 2,5-diethyl-3-methylpyrazine, 32736-91-7; 2,6-diethyl-3-methylpyrazine, 18138-05-1; 2,3-diethyl-5-methylpyrazine, 18138-04-0; furfuryl butyrate, 623-21-2; 2-ethyl-6,7-dihydro-5*H*-cyclopentapyrazine, 38917-60-1; 2-methyl-6-propenylpyrazine, 55138-64-2; ethylacetylpyrazine, 38890-44-7; 2,3-dimethyl-5-butylpyrazine, 15834-78-3; dimethyl tetrasulfide, 5756-24-1; methylpentylpyrazine, 97485-50-2; 2,3-dimethyl-6,7-dihydro-5*H*-cyclopentapyrazine, 38917-63-4; 2-methyl-5,6,7,8-tetrahydroquinoxaline, 38917-65-6; 2-methylquinoxaline, 7251-61-8; dimethylpentylpyrazine, 97485-51-3; 4,6-dimethyl-1,2,3,5-tetrathiane, 96504-25-5.