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Key Odor Impact Compounds in Three Yeast Extract Pastes

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Three types of yeast extract pastes from two different suppliers were compared. Compounds responsible for the key odors include 2-methyl-3-furanthiol, 2-methyl-3-methyldithiofuran, methional, 1-octen-3-one, dimethyltrisulphide together with a number of pyrazines, thiophenes, and aliphatic compounds. The three types of yeast extract paste differed in the intensity of their main odors and, in particular, those caused by furans, furanthiols, and heterocyclic sulfur compounds. Not only do pastes from different suppliers differ in terms of odor volatiles, but so do different treatments and batches of yeast extract from one supplier. The results suggest that normal variations in the concentrations of precursors and processing conditions may cause variations in the flavor of the end product.

KEYWORDS: yeast extract; volatile; odor compound; GCO; GC-MS; precursor

INTRODUCTION

Yeast extracts are the concentrates of the soluble fraction of yeast (*1*, *2*). They are a natural source of a number of volatile aroma compounds and are widely used as flavoring agents (*1*, *3*) and as a precursor for processed meat flavors (*4*). Different types of yeast extracts from different manufacturing sources have been shown to vary in their volatile profile (*2*, *5*−*9*). Most of these investigations focused on the detection and identification of the volatile compounds in yeast extract. For instance, Werkhoff et al. (*10*, *11*) reported a comprehensive study on a yeast extract, focusing on sulfur-containing volatiles generated by its thermal treatment. Other investigations reported the identification of volatile compounds that are products of amino-carbonyl interactions and suggested their possible contribution to the overall odor of yeast extract (*7*, *12*). Only two research papers (*9*, *13*) provided information on the key odorants in yeast extract samples.

A range of yeast extracts is available commercially with varying aroma characteristics (*8*), but not much is reported about the chemical basis for their odor differences, and these warranted further investigation. In this study, the key odor compounds in three yeast extracts are compared and some of the precursors believed to contribute to their formation are quantified. Unlike many previous studies, the samples used in this study were "pure" yeast extracts; that is, they did not contain added spices and were manufactured under factory processing conditions rather than laboratory conditions. The aim of this work was to identify the volatile compounds originating from the yeast extract itself and to identify the key odor impact compounds. Furthermore, it was intended to identify those odor compounds responsible for differences in odor that are observed between very similar products and to suggest possible reasons for these differences that would merit further investigation.

MATERIALS AND METHODS

Materials. *Yeast Extract.* Three types of yeast extract paste samples manufactured from *Saccharomyces cerevisiae* were obtained from commercial sources. All three samples were commercially produced for use in savory food products. Type A (batch X, 75.0% total solids, and batch Y, 76.9% total solids) and type B (82.7% total solids) paste samples were manufactured from the same type of liquor (yeast autolysate). For both types of paste, the yeast autolysis step was followed by a heat-processing step, which involved heating the yeast autolysate under vacuum at 70 °C to form the liquor (ca. 45% total solids), and a final evaporation step (using same conditions) to form the respective pastes. For type B paste, the liquor was thermally evaporated for a longer period of time to achieve the higher total solids content. The type C (80.6% total solids) sample was a similar yeast extract paste from another commercial source. All three pastes, when diluted, had a pH of 5.1.

Standards. Authentic standards of volatile compounds were obtained from Sigma-Aldrich Co. Ltd. (Poole, United Kingdom) except for (methylthio)acetaldehyde, 1-methylthio-3-pentanone, *cis*- and *trans*-3,5-dimethyl-1,2-dithiolan-4-one, 2-nonen-4-one, and *trans*-β-damascenone, which were gifts from Quest International (Naarden, The Netherlands). Standard solutions of authentic compounds were prepared at a concentration of ca. 10 ng μL^{-1} in pentane [high-performance liquid chromatography (HPLC) grade, Rathburn Chemicals Ltd., Walkerburn, United Kingdom] or diethylether (analytical grade, BDH Laboratory Supplies, Poole, United Kingdom) depending on their solubility. For the purpose of calculating linear retention indices, solutions of alkanes (Sigma-Aldrich) were prepared at a concentration of ca. 20 ng μL^{-1} as follows: (a) C_9-C_{28} in pentane (for use on the polar column), (b) C_8-C_{24} in pentane (for use on the nonpolar column), and (c) C_9-C_{20} in ethanol (A.R. 99.7%, Hayman Ltd., Witham, England). Alkane solutions

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a and b were used for direct injection while analyzing simultaneous distillation extracts while alkane solution c was used during headspace analyses.

In order to identify sulfur-substituted furans such as 2-methyl-3-methyldithiofuran and 2-methyl-3-methyltrithiofuran, authentic 2-methyl-3-furanthiol and dimethyltrisulfide (Sigma-Aldrich) were dissolved in hexane (1 mg mL^{-1}). The solution was shaken with aqueous $CuSO_4$ (3 M), washed with distilled water, and dried with Na_2SO_4 (anhydrous) and analyzed by gas chromatography−mass spectrometry (GC-MS) and GC−odor assessment (OA). The disulfides and trisulfides in the resulting mixture of products were used to determine the mass spectra, linear retention indices (LRIs), and odors of these compounds.

Collection of Volatile Compounds. Two methods of volatile collection were employed, namely, simultaneous distillation extraction (SDE) and dynamic headspace concentration. Volatile compounds were extracted from type A (batch Y), type B, and type C yeast extract samples (60 g of paste in 200 mL of HPLC grade water) using SDE as described by Likens and Nickerson (*14*) to detect the less volatile compounds. The volatiles were extracted into a 1:1 mixture of pentane and diethylether (20 mL). Bromobenzene (Aldrich, Poole, United Kingdom) was added to the solvent mix as an internal standard (20 μL; 1000 ng mL^{-1} solution in pentane) prior to extraction. After 15 min of priming the system, the volatiles from the yeast extract were extracted for 2 h and dried as described previously (*15*). The extract was then quantitatively transferred to another pear-shaped flask (50 mL) and made up to 15 mL with the pure solvent mixture. After the mixture was mixed well, an aliquot (0.5 mL) of the extract was pipetted into a 1 mL graduated v-vial with solid screw cap (Wheaton Science Products, NJ) and stored in the freezer at −18 °C. For GC-OA, the extract thus obtained was used in both undiluted form and diluted 10-fold with pentane:ether (1:1). The remaining volume of extract was concentrated to about 1 mL by evaporation using a gentle stream of nitrogen (600 mL min^{-1}) and stored at −18 °C in sealed 2 mL HP vials. These extracts were analyzed for volatile compounds present using GC-MS and GC-IR. The extraction was carried out in duplicate for types A−C paste samples.

Dynamic headspace concentration was used for collecting volatiles from the headspace of yeast extract solution as described previously (*16*). The yeast extract solution (40 g of paste in 100 mL of HPLC grade water, Sigma-Aldrich) was placed in a 250 mL Erlenmeyer flask containing a Teflon-coated magnetic flea (2.5 cm long) to continuously stir the solution during the course of the collection. It was heated for 30 min in a water bath at 60 °C. A stream of nitrogen gas (50 mL min^{-1}) swept the volatiles released from the samples onto a conditioned glass-lined stainless steel trap containing 2.6 mg of Tenax GC (SGE Ltd., Milton Keynes, United Kingdom). After collection, the trap was purged with nitrogen (50 mL min^{-1} for 5 min) to remove any residual moisture. Alkanes (C_9−C_{20}, ca. 20 ng in 1 μL of ethanol) were injected onto the Tenax trap prior to collection to aid in calculating the LRIs (*16*). The excess ethanol was removed by flushing the trap with nitrogen at 50 mL min^{-1} for 5 min. Two collections were performed each on type A (batch Y) and type B paste samples, and the volatiles collected were analyzed using GC-MS. For GC-OA, the volatiles were collected from solutions of type A (batch X and Y) yeast extract pastes at a concentration of 0.5 g dry weight in 100 mL of HPLC grade water. For each collection method, blank samples were prepared by following the collection procedure using water instead of yeast extract solution.

GC-MS. For the analyses of SDE extracts and standard solutions, a HP 6890 Series GC interfaced to a HP 5973 mass selective detector (Manchester, United Kingdom) operated at 70 eV in the electron impact mode over the range 35−550 amu was used. The samples were injected in the splitless mode automatically by the HP 7683 Series injector maintained at 250 °C, and data analysis was performed by HP Chemstation software. Analyses were performed on a polar CPWax52CB column (50 m × 0.32 mm i.d., Chrompack Ltd., London, United Kingdom), the oven temperature was maintained at 30 °C for 3 min, increased to 220 °C at 4 °C min^{-1}, and maintained at 220 °C for 25 min. Some analyses were also performed on a nonpolar CPSil8CB column (50 m × 0.32 mm i.d., Chrompack Ltd.) using an oven program starting at 35 °C for 3 min, increased to 220 °C at 4 °C min^{-1}, and finally maintained at 220 °C for 25 min.

Volatiles collected by dynamic headspace concentration from type A (batch Y) and type B pastes were analyzed on a polar CPWax52CB capillary column using a HP 5890 Series II GC, fitted with a "Unijector" (SGE Ltd.) and connected to a HP 5970 mass selective detector operated in the electron impact mode at 70 eV, over the range 35−450 amu as described previously (*16*). The volatiles were desorbed in the "Unijector" (250 °C, 5 min) and were refocused on a 10 cm region of the column that had been precooled for 5 min using liquid nitrogen. After desorption, the liquid nitrogen was removed, and the oven was heated rapidly to 60 °C and maintained at this temperature for 5 min before increasing to 220 °C at a rate of 4 °C min^{-1} and maintained at 220 °C for 30 min.

The identities of volatile compounds were confirmed by comparison of the LRI, mass spectra, and odor with those of authentic compounds, when possible. When authentic samples were not available, tentative identifications were derived by comparing the mass spectra of unknown compounds with those in the Wiley mass spectral library computer software (6th edition, 1998, Hewlett-Packard) and other published spectra.

GC-OA. GC was performed on SDE extracts (undiluted extract and 10^{-1} dilute extract) using the 5890 Series II gas chromatograph (Hewlett-Packard) fitted with a "split/splitless" injector operating in the purged splitless sampling mode, a flame ionization detector (FID), and a sniffing port. One microliter of the extract with 0.3 μL of 20 ng μL^{-1} alkane solution and 0.5 μL of pentane (to ensure complete transfer) with 0.5 μL of airspace separating each segment were injected manually into the injector using a 5 μL syringe (SGE Ltd.). The volatiles in the samples were thermally desorbed on to a polar CPWax52CB column at 225 °C and analyzed by GC-OA as described previously (*16*). The oven temperature program was the same as described for GC-MS on SDE extracts. The odors of the volatiles eluting from the GC column were described and scored on a scale of 1 (very weak) and 5 (very strong) for a period of 60 min. Four different assessors performed GC-OA on each SDE extract in both the undiluted state and diluted by a factor of 10, with the exception of type C paste, where only two assessors were used. Only those odors detected in the diluted extract (mean score ≥ 0.25) as well as in the undiluted extract (mean score ≥ 1.2) are reported.

Descriptors from all assessors were used to help identify the compounds responsible for key odors. The odor scores were analyzed by the GenStat statistical package REML procedure for analysis of variance of unbalanced designs. The analyses were carried out both for all assessors and for the two assessors common to all treatments.

GC-OA analyses were also conducted on the volatiles from two batches (batch X and Y) of type A paste, collected by dynamic headspace concentration. The volatile desorption system and oven temperature program were the same as described for GC-MS of volatiles collected by dynamic headspace concentration. The odors of the volatiles eluting from the column were described and scored on a five-point scale by four assessors. The mean odor score from all four assessors was calculated.

Identification of the compounds responsible for key odors was achieved by matching the odor description and LRIs with the compounds identified by GC-MS. LRIs were calculated according to the method of Van den Dool and Kratz (*17*). Identities of compounds responsible for odors were confirmed by conducting GC-OA of the authentic compounds, when available, at comparable concentrations to those found in the samples.

GC-IR. The volatile compounds collected by SDE were analyzed by GC-IR to serve as an additional compound identification tool. Vapor-phase Fourier transform infrared spectroscopy was performed using a HP 5890 Series II GC connected to an HP 5965B infrared detector. The sample was injected on to a polar CPWax52CB column in the splitless mode. The effluent from the column was passed through the infrared detector and then transferred, at a split ratio of 10:1, to a HP 5970 Series mass selective detector operated in the electron impact mode over the range 35−450 amu. The sample extracts had to be concentrated from 1 mL to 50 μL in order to be able to detect some compounds' spectra. Standards were injected at a concentration of 100 ng in 1 μL solvent in order to obtain good infrared spectra.

Determination of Selected Precursors. *Analysis of Thiamin.* Thiamin was analyzed by converting it to its fluorescent derivative, thiochrome, and quantified using HPLC (*18*). Recovery samples were prepared by adding a known amount of reference thiamin (30.3 μg) to the yeast extract paste samples prior to the extraction procedure. The percentage recovery was calculated by comparing spiked and unspiked samples. All analyses were carried out in duplicate.

Analysis of Ribonucleotides. Ribonucleotides in yeast extract samples were analyzed using HPLC by adapting the method previously described (*19*). Perchloric acid extraction was followed by centrifugation (Heraeus Megafuge 1.0, Kalkberg, Germany) at 3900*g* for 6 min and filtration through Whatman 541 filter paper. The filter paper was washed with HPLC grade water to extract any adhering ribonucleotides. After the filtrate was adjusted to pH 5.5 using 6 M potassium hydroxide (BDH Laboratory Supplies), the resulting potassium perchlorate precipitate was removed by centrifugation for 15 min at 3900*g* and filtration. The filtrate was collected into a 10 mL volumetric flask (Volac, John Poulten Ltd., Essex, United Kingdom) and made to volume by washing the filter paper with water (HPLC grade). This final extract was used for analysis.

All of the samples were analyzed in duplicate. Blanks were prepared by following the extraction procedure without any sample. The peaks were identified based on the retention time of the respective standards and also by spiking the sample with the authentic standards. Standard solutions (10 mM) were prepared by dissolving an appropriate amount of the respective ribonucleotides (5′-IMP, 5′-GMP, 5′-UMP, 5′-CMP, 5′-AMP, 5′-ATP, 5′-ADP, 3′-AMP, 3′-GMP, 2′-AMP, adenosine, inosine, and guanosine) in 0.02 M KH_2PO_4 buffer at pH 5.5.

Recoveries for selected ribonucleotides were determined by comparing unspiked samples with others spiked with 5′-UMP (2.92 mg), 5′-IMP (3.94 mg), 5′-ATP (0.90 mg), 5′-ADP (9.2 mg), and guanosine (1.02 mg) prior to the extraction procedure.

Separation and resolution of the peaks were carried out using gradient elution with two chromatography programs and different mobile phase compositions. Program 1 enabled the separation and quantification of 5′-ATP and 5′-ADP along with all of the ribonucleotides except 5′-IMP and 5′-GMP, which coeluted in this program. Program 2 was used to mainly separate and quantify 5′-IMP and 5′-GMP. In both programs, the solvents were filtered through a 45 μm membrane filter (Millipore) and degassed using helium for 30 min before use.

Program 1: Solvent A was HPLC grade methanol (Lab-Scan Analytical Sciences, Dublin, Ireland). Solvent B contained 0.1 M aqueous KH_2PO_4 (BDH Laboratory Supplies) with 1.95 mM tetrabutylammonium hydrogen sulfate (Aldrich Chemical Co. Inc., Milwaukee, WI) adjusted to pH 7.0 with 6 M potassium hydroxide. The program began with 100% B (0% A) for 15 min, followed by 100−93% B (0−7% A) in 3 min, and maintained at this ratio for 10 min. At the end of this isocratic period, the mobile phase was brought back to 100% B using a reverse gradient over a period of 24 min. The run time was 52 min at a flow rate of 1.5 mL min^{-1}.

Program 2: The solvent composition and program were as previously described for chicken muscle (*19*). The run time was 30 min at a flow rate of 1.5 mL min^{-1}.

Analysis of Reducing Sugars. Nonphosphorylated reducing sugars were extracted from the sample and analyzed using the method previously described for chicken (*20*) with the following modifications. HPLC grade water (3 mL) was added to the yeast extract paste sample (ca. 6 g) before commencing the extraction to bring the moisture content of the sample to 75%. Following the four extractions and centrifugation steps (*20*), the supernatant was once more centrifuged at 800 g for 5 min to further remove any proteins. The final volume of the supernatant was ca. 45 mL. Any lipids present were removed by extraction with 160 mL of chloroform (Lab-Scan Analytical Sciences, Dublin, Ireland), and the aqueous phase containing the sugars was retained. The final volume of the extract was made up to 10 mL. After the removal of ionic compounds with resins and centrifugation (*20*), the supernatant (4.9 mL) was transferred into a 5 mL volumetric flask containing cellobiose (1.375 mg in 0.1 mL; Sigma-Aldrich) as an internal standard and mixed well to give the final extract. The HPLC apparatus and postcolumn derivatization used for analysis were the same as described for chicken (*20*), but the composition of the mobile phase mixture was as described previously (*21*). The flow rate of the mobile phase was also changed from 1.7 to 1.2 mL min^{-1} to facilitate the separation and complete elution of the analytes.

All of the samples were analyzed in duplicate. The peaks were identified based on the retention time of the respective standards and by spiking the sample with authentic standards. Blanks were analyzed by following the extraction procedure with 8 mL of HPLC grade water. For the recovery of sugars, the samples were spiked with fructose (0.871 mg), ribose (0.744 mg), glucose (0.307 mg), and xylose (0.226 mg) prior to extraction.

Informal Sensory Analysis. Eight panelists had been trained to develop their own vocabulary to describe the aroma attributes of the yeast extract samples and had defined and agreed on attributes for a separate study. In this short study, these panelists were asked to describe the aroma attributes of the three types of yeast extract paste samples, A (batch Y), B, and C. They were also asked to indicate any variations in the aroma intensity. The samples (10 mL) were presented as 0.5% solution (on dry weight basis; manufacturers' recommended concentration of yeast extract addition in foods) in 20 mL brown vials with caps, maintained at 60 °C using multiblock module heaters (Lab-line Instruments Inc., United Kingdom).

As part of another sensory analysis, 30 experienced but untrained panelists were asked to provide descriptions of the aroma of type A (batches X and Y) yeast extract paste samples along with an indication of aroma intensity. The samples were presented in the same manner as described above. The results from these two short sensory studies were collated by listing the most frequently used aroma descriptors in descending order of usage.

RESULTS

Table 1 compares the main odors in yeast extract pastes types A−C detected by GC-OA following extraction by SDE. Thirty-one odors are listed of which firm identities are suggested for 23 and tentative identities for a further four compounds. A full list of the volatile compounds identified is provided in the Supporting Information. An alternative extraction method, dynamic headspace extraction, yielded 31 odors for type A paste (**Table 2**). Eighteen odors and identifications were common between the two extraction methods. Two batches of this type of paste are compared. **Table 3** lists the terms used by the informal sensory panel to describe the overall odors of the diluted solutions of yeast extracts types A−C. **Table 4** lists the overall odor descriptors of the diluted solutions of type A, batches X and Y, paste samples.

Table 5 shows the results of the analysis of selected flavor precursors: sugars, ribonucleotides and related compounds, and thiamine. Significant differences were observed between the three types of pastes for all analytes except 5′-ATP.

DISCUSSION

The flavor of commercial yeast extract is formed partly due to biogeneration by the yeast and also, in large part, by the chemical reactions occurring during heat processing. Precursors present in the yeast extract are believed to react together to give the wide range of volatile compounds observed. Three commercial yeast extracts are compared in terms of the key odor compounds formed and the concentrations of selected precursors.

Key Odor Compounds in Three Types of Yeast Extract. The samples extracted by SDE suggested several odors that were important for all three types of yeast extract. These included the odors described as meaty, chicken (LRI 1310; 2-methyl-3-furanthiol); roasted, potato skin (LRI 1430; 2-furanmethanethiol); potato, roasted (LRIs 1455 and 1693; methional and unknown); honey, beer dregs (LRI 1821; *trans*-β-damascenone); and wood oil, frankfurter (LRI 1861; 2-methoxyphenol) (**Table**

Table 1. Main Odors Detected in the SDE Extract of Three Types of Yeast Extract Pastes

		mean odor score[c]									
		undiluted			diluted 1/10			sig. (P,		method of	odor of
LRI[a]	odor descriptor[b]	A	B	C	A	B	C	YE type)[d]	compound responsible[e]	identification[f]	ref compd[g]
1101	sulfur, metallic, green, savory	2.9	1.8	2.8	0.1	0.5	0.0	ns	2-methylthiophene	MS, LRI, O	sulfur, metallic
1232	savory, off, green	0.0	0.6	0.8	0.0	0.0	1.0	ns	2-pentylfuran	MS, LRI, O	green, sweet, fruity
1285	sweet, cabbage, metallic, sausage	0.0	2.3	1.3	0.0	1.0	0.5	<0.001	3-hydroxy-2-butanone	MS, LRI, O	sweet
									+ bis-(methylthio)methane	MS, LRI, O	sulfur, stale
1298	mushrooms, metallic	0.0	3.1	3.3	0.0	2.5	3.1	<0.001	1-octen-3-one	LRI, O	mushroom
1310	meaty, chicken	2.1	3.5	3.4	0.6	2.0	2.8	<0.001	2-methyl-3-furanthiol	MS, LRI, O	roasted, meaty, metallic
1335	cooked rice, popcorn	2.6	2.9	2.5	0.6	1.4	1.0	ns	*2-acetyl-1-pyrroline*	lri, o (*22*)	
1356	catty, metallic, roasted	0.8	0.8	2.2	0.4	0.0	0.0	ns	*2-methyl-3-methyl-thiofuran*	ms, lri, o (*40*)	
1373	sulfur, metallic, stale	0.4	3.3	2.1	0.0	1.1	0.9	<0.001	dimethyltrisulfide	MS, LRI, O	metallic, sulfur
1391	meaty, burning wood, green, alcoholic	0.0	1.4	2.2	0.0	0.0	1.0	0.008	2-nonanone	MS, LRI, O	green
									+ unknown		
1415	roasted, popcorn, meaty, pungent	3.1	2.5	2.3	0.0	0.0	0.6	ns	*2-methyl-5-isopropyl-pyrazine*	ms, lri	
1432	roasted, potato skin, sulfur	3.8	3.6	4.2	3.3	1.5	2.4	0.063	2-furanmethanethiol	MS, LRI, O	roasted, metallic, green
1455	potato, roasted, meat	3.1	3.8	3.8	1.5	2.3	2.5	0.014	methional	MS, LRI, O	potato, roasted
1468	plastic, rubber, metallic, potato	0.0	0.5	1.7	0.0	0.3	0.0	ns	2-furancarboxaldehyde	MS, LRI, O	potato, roasted, savory
									+ unknown		
1523	potato skins, pungent, savory	0.0	1.3	3.8	0.0	0.4	3.0	<0.001	2-methyltetrahydrothio-phen-3-one	MS, LRI, O	sulfur, roasted, metallic, oily
									+ unknown		
1570	roasted, curry leaves, burnt paper	1.8	1.0	2.6	0.5	0.0	0.3	ns	5-methyl-2-furancarbox-aldehyde	MS, LRI, O	dusty, burnt
									+ 1-methylthio-3-pentanone	MS, LRI, O	roasted
1586	roasted, burnt	0.0	1.9	0.6	0.0	0.0	0.0	<0.001	*cis-3,5-dimethyl-1,2,4-trithiolane*	MS, LRI	
1620	potato, savory, bitter	3.3	2.5	2.5	2.1	0.5	0.8	<0.001	unknown		
1644	floral, honey	3.0	3.0	1.3	0.3	0.0	0.0	ns	phenylacetaldehyde	MS, LRI, O	floral
1669	chicken, roasted, fatty, sulfur	0.8	2.3	2.8	0.8	1.8	2.4	<0.001	2-methyl-3-methyl-dithiofuran	MS, LRI, O	meaty, savory, roasted, metallic, pungent
1693	potato, roasted	2.9	2.8	4.0	1.5	1.4	1.3	ns	unknown		
1735	meaty, savory, burnt	0.0	0.9	1.3	0.0	1.0	0.5	0.018	dimethyltetrasulfide	LRI, O	metallic, off
1755	rice, mealy, burnt, pungent	0.0	0.6	1.2	0.0	0.0	0.4	ns	2-acetyl-2-thiazoline	LRI, O	cooked rice, popcorn
1782	stale, potato, burnt	1.8	0.6	1.1	0.0	0.0	0.0	ns	5-methyl-2-thiophene-carboxaldehyde	MS, LRI, O	stale, sweet fermented pickle
									+ unknown		
1795	savory, burnt, plastic	0.0	0.4	1.4	0.0	1.0	0.5	0.082	unknown		
1821	honey, beer dregs	2.9	2.6	3.2	1.3	1.3	2.4	ns	*trans-β*-damascenone	MS, LRI, O	honey, bitter, metallic
1861	wood oil, frankfurter	2.8	2.1	3.2	1.1	0.9	1.0	ns	2-methoxyphenol	MS, LRI, O	smoky, frankfurters
1932	honey, burning wood, metallic	0.0	0.8	0.7	0.0	0.6	0.5	0.09	2-phenylbut-2-enal	MS, LRI, O	honey, stale
1976	savory, pungent	1.5	2.3	1.8	0.0	0.6	0.8	ns	2-methyl-3-methyl-trithiofuran	MS, LRI, O	savory, sulfur, meaty
2022	burning wood, stock	0.5	0.5	1.0	0.0	0.6	0.8	ns	2-methylphenol	MS, LRI, O	smoky, frankfurters
2145	savory, roasted, burnt	0.4	0.8	0.9	0.4	1.0	0.8	ns	bis-(2-methyl-3-furyl) disulfide	MS, LRI, O	roasted, bitter
2394	burnt, honey, sulfur, rubber	0.0	0.3	1.3	0.0	0.0	1.3	<0.001	unknown		
	overall mean score	1.3	1.8	2.2	0.5	0.8	1.1				
	no. odors detected ≥0.05	19	31	31	14	21	25				

[a] Linear retention index (LRI) for the odors in CPWAX 52CB column. [b] Odor descriptors are those used by all assessors, with most frequently used terms cited first. [c] Mean odor scores on a scale of 1 (very weak) to 5 (very strong) for the two assessors common to all treatments, who assessed all samples in duplicate. Mean scores for all assessors showed a very similar pattern. [d] Significance of differences between the mean values for the three types of yeast extract paste are given as probability values for the two assessors common to all treatments. Values greater than $P < 0.10$ are shown as "ns". However, when statistical analysis was also conducted for all assessors, the same odors were found to be significantly different. [e] Compound responsible: Compound names in italics indicate that their identification as the odorant was tentative. [f] Method of identification as compound responsible for odor: MS, LRI, O: mass spectrum, linear retention index, description of odor of appropriate concentration of authentic compound agree with those detected in yeast extract by GC-O; ms, lri, and o: mass spectrum, linear retention index, and description of odor agree with literature data but should be regarded as tentative. Where a literature reference is not stated, the mass spectra agree with those in the Wiley mass spectral library computer software (6th edition, 1998, Hewlett-Packard, Manchester, United Kingdom). [g] Odor of reference compound: odor description of authentic reference compound determined by GC-O.

Table 2. Main Odor Responses and Odorants Detected in Yeast Extract Paste (Type A) Using Dynamic Headspace Method of Volatile Collection

LRI[a]	odor descriptor[b]	mean odor score[c] X	mean odor score[c] Y	sig. (YE batch)[d]	compound responsible[e]	method of identification[f]	odor of ref compd[g]
956	cheesy, stale, mushroom, chicory	2.5	1.3	ns	*2-ethylfuran*	MS, LRI, o (*25*)	
1049	fudge, caramel	0.0	1.5	ns	2,3-pentanedione	MS, LRI, O	sweet, sickly
1213	metallic, green, plastic, bitter, muggy	0.0	1.5	0.058	2-methylpyridine	LRI, O	metallic, green
1233	bitter, metallic, rancid, tea	0.0	1.0	ns	2-pentylfuran	MS, LRI, O	fruity, green
1297	mushrooms, earthy	2.1	1.8	0.008	1-octen-3-one	LRI, O	mushroom
1310	meaty, chicken, roasted, bitter, pungent	1.5	3.0	0.069	2-methyl-3-furanthiol	MS, LRI, O	roasted, meaty, metallic
1338	meaty, rice, popcorn, toast, savory	0.0	1.0	ns	*2-methyl-3-methyl-thiofuran*	ms, lri, o (*40*)	
1340	green, fatty, stale, mushroom, popcorn, old food	0.7	1.5	ns	*2-acetyl-1-pyrroline*	lri, o (*22*)	
1375	sulfur, metallic, geranium, pungent	3.4	3.1	ns	dimethyltrisulfide	MS, LRI, O	metallic, sulfur
1400	mushrooms, fresh, metallic, butter	1.3	1.6	ns	trimethylpyrazine + unknown	MS, LRI, O	metallic, hay
1437	fatty, butter fried, savory, burnt potato, stale	0.0	1.9	0.022	2-furanmethanethiol	MS, LRI, O	roasted, metallic, green
1443	burnt potato skin, fatty, gravy, savory, metallic	1.3	1.3	ns	2-ethyl-3,6-dimethyl-pyrazine	MS, LRI, O	burnt, roasted
1456	potatoes, dhal powder, meat, rice	3.1	3.0	ns	methional + 2-furancarbox-aldehyde + 2-ethyl-3,5-di-methylpyrazine	MS, LRI, O MS, LRI, O MS, LRI, O	potato, roasted potato, roasted, savory roasted, burnt
1485	potatoes, dhal powder, green, leafy	0.4	1.5	ns	2-furfurylmethylsulfide + a pyrazine	MS, LRI, O ms, lri	roasted, sulfur
1525	potato, savory, earthy, rancid, pungent	1.2	1.3	ns	2-methyltetrahydro-thiophen-3-one	MS, LRI, O	sulfur, roasted, metallic, oily
1605	potato, savory, slightly roasted, burnt	0.0	1.0	ns	*trans*-3,5-dimethyl-1,2-dithiolan-4-one	MS, LRI, O	potato, fatty
1642	floral, lavender	2.3	2.0	ns	phenylacetaldehyde	MS, LRI, O	floral
1648	metallic, geranium, fatty, pungent, meaty	1.6	2.0	ns	*3-methylthio-thiophene*	ms, lri	
1667	meaty, chicken, metallic, fatty, sulfur	2.8	2.8	ns	2-methyl-3-methyl-dithiofuran	MS, LRI, O	meaty, savory, roasted, metallic, pungent
1699	meaty, fatty, metallic, stale, geranium, savory	1.1	1.0	ns	unknown		
1748	sweet, fudge like	0.0	1.1	ns	unknown		
1756	metallic, geranium, pungent	0.0	1.5	0.058	unknown		
1769	chlorophenol, phenolic, disinfectant	0.0	1.5	ns	*2-acetylthiophene*	MS, LRI	
1810	musty, stale biscuits, stale cooking oil, oily, savory	0.0	1.3	ns	*trans,trans*-2,4-decadienal	LRI, O	stale, fatty, oily
1824	honey, sweet, bitter, chutney (pickled)	1.4	1.3	ns	*trans*-β-damascenone	LRI, O	honey, bitter, metallic
1861	frankfurters, bacon, smoky, stale	0.0	2.5	0.030	2-methoxyphenol	MS, LRI, O	smoky, frankfurters
1937	chicken, metallic, geranium, phenolic, smoked ham, meaty, spicy, plastic, stock	0.1	2.3	0.021	2-thiophenemethanol + benzothiazole	LRI, O MS, LRI, O	metallic, chicken rubber, metallic
1979	meaty, chicken, tomato, metallic, sulfur, bitter	0.0	2.8	0.022	2-methyl-3-methyl-trithiofuran	MS, LRI, O	savory, sulfur, meaty
2000	meaty, stale, burnt, geranium, metallic	1.9	0.8	ns	unknown		
2019	stale air, smoky, meaty, chicken, metallic	2.3	0.5	ns	2-methylphenol + unknown	LRI, O	smoky, frankfurters
2179	meaty, chicken, sulfur, burnt paper	0.0	1.5	ns	unknown		
	overall mean score	1.0	1.7				
	no. odors detected $\geq$0.05	18	31				

[a] Linear retention index (LRI) for the odors in CPWAX 52CB column. [b] Odor descriptors are those used by all assessors, with most frequently used terms cited first. [c] Mean odor scores on a scale of 1 (very weak) to 5 (very strong) for the four assessors who assessed all samples in duplicate. [d] Significance of differences between the two batches of yeast extract paste are given as probability values. Values greater than $P < 0.10$ are shown as "ns". [e] Compound responsible: compound names in italics indicate that their identification as the odorant was tentative. [f] Method of identification as compound responsible for odor: Because of insufficient sample quantity, GC-MS was not performed on the type A (batch X) samples. Therefore, identifications are based on detection of compounds in type A (batch Y) samples. MS, LRI, O: mass spectrum, linear retention index, and description of odor of appropriate concentration of authentic compound agree with those detected in yeast extract by GC-O; ms, lri, o: mass spectrum, linear retention index, and description of odor agree with literature data. Where a literature reference is not stated, the mass spectra agree with those in the Wiley mass spectral library computer software (6th ed., 1998, Hewlett-Packard, Manchester, United Kingdom). [g] Odor of reference compound: odor description of authentic reference compound determined by GC-O.

Table 3. Odor Descriptors for Three Types of Yeast Extract Paste Samples

sample	description and comments in descending order of frequency of use
type A (batch Y)	beef, root vegetables, yeast extract, soup, metallic, malty
type B	beef, yeast extract, burning methylated spirit/chemical, root vegetables
type C	meaty, roasted, sweet, root vegetables, roast beef, yeasty, metallic/sulfur
	overall odor intensity of type C > types A and B

Table 4. Odor Descriptors for Two Batches of Type A Yeast Extract Paste

sample	description and comments in descending order of frequency of use
type A (batch X)	meaty, beefy, sweet, chicken
type A (batch Y)	beefy, sweet, chemical, metallic, phenolic, yeasty, stronger, sharper
	overall odor intensity of batch Y > batch X

Table 5. Quantities of Selected Precursors Present in Samples of Yeast Extract (mg/100 g Dry Weight)

	paste A	paste B	paste C	sig[b]	SEM[c]	recovery (%)[d]
thiamine	2.58[a] b	3.00 b	1.12 a	***	0.16	102 ± 4
5′-IMP	93 a	88 a	496 b	***	4.63	98 ± 2
inosine	71 b	74 b	27 a	***	0.39	
5′-GMP	99 a	98 a	138 b	***	1.24	
guanosine	213 b	291 c	124 a	***	1.10	103 ± 3
5′-CMP	226 a	340 a	480 b	***	7.20	
5′-UMP	230 b	203 b	93 a	***	1.89	87 ± 2
5′-ATP	68	72	65	NS	5.63	100 ± 2
5′-ADP	436 a	585 b	501 a	**	9.71	66 ± 8
5′-AMP	150 b	138 b	80 a	***	1.28	
adenosine	469 b	625 c	321 a	***	6.32	
3′-AMP	344 b	361 b	206 a	**	7.69	
2′-AMP	1566 a	1870 b	1528 a	***	10.97	
3′-GMP	686 a	831 b	697 a	**	6.76	
ribose	11.08 b	6.04 a	13.17 b	*	1.02	72 ± 0.3
xylose	4.17 b	1.07 a	1.33 a	**	0.17	98 ± 1
fructose	10.92 a	16.45 ab	21.15 b	**	0.89	97 ± 2
glucose	1.92 a	1.62 a	2.40 b	**	0.06	87 ± 3

[a] Quantity of precursor is expressed as the mean of duplicate analysis for each sample; quantities are quoted without correction for recovery. [b] Degree of significance between the samples (analysis of variance, single factor): NS = no significant difference; * = $P \leq 0.05$; ** = $P \leq 0.01$; and *** = $P \leq 0.001$. [c] SEM = standard error of means. a–c, For each compound, values that do not share a common superscript are significantly different ($p < 0.05$) according to Fisher's LSD test. [d] Values are means ± standard deviations of duplicate analyses.

1). These compounds have already been reported as key odorants in one or more yeast extract compositions by Munch et al. (*9*, *13*). The nature of these odors has some similarities with the descriptors used by an informal sensory panel of experienced assessors asked to describe the three yeast extracts: beef, yeast extract, and root vegetable notes were common to all three types of pastes (**Table 3**).

Of particular interest were those odors that were more important in some yeast extracts than others as these may suggest why the odor quality can vary. Two of the pastes, types A and B, were from the same supplier, but type B paste had been subjected to additional heating and evaporation. This perhaps explains why type B paste had generally more intense odors than type A, as indicated by a higher mean odor score and a greater number of odors detected (**Table 1**). Type B paste had significantly ($P < 0.001$) more sweet, cabbage, metallic [LRI 1285; 3-hydroxybutanone and bis-(methylthio)methane]; mushrooms, metallic (LRI 1298; 1-octen-3-one); meaty, chicken (LRI 1310, 2-methyl-3-furanthiol); sulfur, metallic (LRI 1373; dimethyltrisulfide); roasted, burnt (LRI 1586; *cis*-3,5-dimethyl-1,2,4-trithiolane); and chicken, roasted (LRI 1669; 2-methyl-3-methyldithiofuran) odors than type A, in which these odors were very weak or not detected. Some of these additional odors may have contributed to increased yeast extract, chemical notes in the sensory description of this paste (**Table 3**). Although 1-octen-3-one was not detected by GC-MS, the LRI and odor description of the authentic standard matched well. It has a low odor threshold of 0.03−1.12 ng kg^{-1} (*22*). The additional heat treatment for type B paste may have influenced the greater odor intensity of these two compounds along with smaller increases in other odors. However, the large relative increase in odor intensities for these compounds may indicate another factor. The 1-octen-3-one, dimethyltrisulfide, and 2-methyl-3-methyldithiofuran all arise from oxidation reactions, the ketone arises from the oxidative breakdown of n-6 fatty acids, and dimethyltrisulfide and 2-methyl-3-methyldithiofuran arise from oxidative reactions with methanethiol. The presence of di- and trisulfides can be affected by the oxidation state of proteins or peptides present (*23*). All of those odors caused by compounds with di-, tri-, or tetrasulfide groups tended to be stronger in the type B paste than the type A. Perhaps type B paste, whether due to its composition or treatment, was more conducive to the oxidation reactions that form these compounds than type A.

While several odors were absent or low in type A paste, that described as potato, savory (LRI 1620; unknown) was significantly ($P < 0.001$) more intense than in type B paste. This may contribute to an increased prevalence of root vegetables in the sensory description.

Not surprisingly, type C paste, from a different manufacturer, showed even greater differences; odors of potato skins (LRI 1523; 2-methyltetrahydrothiophen-3-one and an unknown compound) and burnt, honey, sulfur, rubber (LRI 2394; unknown) were much stronger in this paste, perhaps explaining a greater prevalence of roasted odor in the sensory descriptions. Indeed, type C paste was also described as having a more intense odor overall than types A and B (**Table 3**), which corresponds to a greater mean odor score for the odors for type C paste (**Table 1**).

Among those compounds detected that did not evidently contribute to odor, type C paste contained many more thiazoles (Supporting Information). These were either not detected or detected at trace levels in types A and B paste samples. They included trimethylisothiazole (LRI 1394), 4-ethyl-2,5-dimethylthiazole (LRI 1398), a thiazole of MW 141 (LRI 1444) and MW 183 (LRI 1569), 2-methyl-4-(1-methylethyl)thiazole, and 2-(2-methylpropyl)-4,5-dimethylthiazole (LRI 1514). These differences between the three yeast extract pastes could arise either from differences in conditions of reaction or in available precursors. The pH of all three pastes was the same, but the reaction conditions would have differed, especially between type C and types A and B pastes, and considerable differences were observed in the precursors present (**Table 5**). Both sulfur-containing furans and thiazoles can be formed from the thermal degradation of thiamin and from other pathways such as reaction of reducing sugars with cysteine (*25*, *26*). Type C paste contained the least amount of thiamin and greater quantities of glucose, ribose, fructose, and certain ribonucleotides than the types A and B pastes (**Table 5**). Although these precursor

concentrations were measured after the thermal evaporation step to give the paste, this may indicate that the Maillard reaction rather than thiamin degradation is the source of these thiazoles. Similarly, type C paste gave more intense odors due to the compounds, 2-methyl-3-furanthiol and 2-methyl-3-methyldithiofuran, and had five times more 5′-IMP than types A and B. Inosine 5′-monophosphate undergoes thermal degradation to form ribose (*27*, *28*), and Mottram and Madruga (*29*) observed a 23-fold increase in 2-methyl-3-methyldithiofuran on addition of IMP to beef at 10 times its natural concentration. Further work on the relative roles of these precursors in the formation of flavor compounds will be reported separately.

In contrast to the above compounds, *cis*- and *trans*-3,5-dimethyl-1,2-dithiolan-4-one, reported previously in yeast extract (*8*), were among the dominant peaks of the chromatograms of both the types A and B paste samples. *cis*- and *trans*-3,(5 or 6)-Dimethyl-1,2-dithian-4-one were also detected only in samples A and B. This may suggest that a greater degree of sugar fragmentation had occurred in the types A and B pastes than the type C paste.

Some of the volatiles responsible for main odors in the yeast extracts studied (**Tables 1** and **2**) such as 2-methylthiophene, dimethyltrisulfide, 1-octen-3-one, 1-methylthio-3-pentanone, 5-methyl-2-furancarboxaldehyde, 2-methyl-3-methyldithiofuran, and 2-methyl-3-methyltrithiofuran have not been previously reported as key odorants in a pure yeast extract composition.

Precursors in Three Types of Yeast Extract. There is little information available in the literature regarding the presence and quantity of reducing monosaccharides in yeast extract. The concentrations of glucose and fructose reported in **Table 5** differ from those reported by Schieberle (*30*) in the low molecular weight fraction of disrupted baker's yeast cells (fructose, 3.3 mg/100 g yeast; and glucose, 126.5 mg/100 g yeast). The lower concentration of glucose in the samples in our study may be due to differences in the molasses used or the utilization of glucose in enzymatic reactions during the process of autolysis and in thermal reactions (such as Maillard reaction) during the thermal evaporation process.

All of the yeast extracts had a wide range and substantial concentrations of ribonucleotides and nucleosides. The balance of these compounds was similar but not identical for the two related pastes, A and B, and different in several respects for type C paste, which had higher quantities of 5′-IMP, 5′-GMP, and 5′-CMP and lower amounts of inosine and guanidine.

The quantities of ribonucleotides found in the samples studied (**Table 5**) were comparable with those cited in the literature (*31*, *32*). Fish (*31*) reported a wide variation in 5′-IMP content, detecting 1500 mg/100 g dry weight in one sample of yeast extract but none in another sample of yeast extract, using a similar method of analysis as used in this study. Of the total ribonucleotides, the 2′ and 3′ isomers constituted the major proportion, i.e., approximately 56% of the total ribonucleotides in type A paste, 61% in type B paste, and 51% in type C paste. This is in agreement with the trend observed by Iguchi (*32*). The amount of thiamin quantified in our samples is in the range that is expected for yeast extract (*33*−*35*).

Comparison of Methods of Extraction. SDE is efficient at extracting many of the less volatile flavor components of a food material, but because of the extra heat processing, it can miss some of the more volatile components and also introduce artifacts. Nevertheless, this method was deemed appropriate as yeast extract pastes themselves undergo further thermal treatment when used in processed foods. In order to complement these characteristics of SDE, one of the pastes studied, type A paste (batch Y), was also subjected to dynamic headspace analysis (**Table 2**). This method does not involve the same additional heating and is more effective at collecting the more volatile flavor components. Detection by both methods provides improved evidence of the odor impact of these compounds.

The main odors for both batches of type A paste detected by dynamic headspace analysis were meaty, chicken (LRIs 1310 and 1667; 2-methyl-3-furanthiol and 2-methyl-3-methyldithiofuran); potato, dhal powder, meat (LRI 1456; methional, furfural and 2-ethyl-3,5-dimethylpyrazine); sulfur, metallic (LRI 1375; dimethyltrisulfide); and floral, lavender (LRI 1642; phenylacetaldehyde), which contribute to the beef, yeast extract, and root vegetable descriptors given to their overall aroma. These odors agree with the main odors detected by SDE but, as expected, showed a different balance. Dynamic headspace concentration gave greater importance to the odor due to the volatile compound dimethyltrisufide while SDE indicated a greater role for the less volatile components such as *trans*-β-damascenone and 2-methoxyphenol. While these may have been affected by the additional heat from the SDE extraction, the observed results would be expected from the greater ability of SDE to extract less volatile compounds. On the other hand, the greater odor scores for 2-furanmethanethiol and two unknown compounds at 1620 and 1693 were probably due to this additional heating step.

There were other differences in the compounds detected by the two methods (Supporting Information), with some thiophenes, aliphatic sulfur compounds, and cyclic polysulfur compounds only detected in volatiles collected by dynamic headspace concentration and some of most compound classes and all alcohols, acids, oxazoles, pyrroles, and terpenes only detected by SDE (Supporting Information). These differences could be due to the different sample size, concentration methods, or selectivity of the methods or, of course, due to artifact formation. While the additional heating of the SDE samples is bound to have had an effect, it is noticeable that many of the thiols, sulfides, and di- or trisulfides, which are among the most labile of the volatile products, were detected by both methods.

Effect of Batch on Key Odor Compounds in Type A Paste. Comparison of two batches of type A yeast extract, to determine the influence of batch-to-batch variation, indicated that although there were similarities in the overall sensory odor notes such as beefy and sweet, considerable differences were observed between the two batches (**Table 4**). Batch Y was stronger in overall aroma, corresponding to an increase in mean odor score and numbers of odors detected (**Table 2**). Batch Y also possessed additional overall odor notes such as metallic, sharp, phenolic, and yeasty (**Table 4**). These additional notes may be caused by some odors detected by GC-OA (**Table 2**) that were significantly more intense in batch Y such as chicken, metallic (LRI 1937; 2-thiophenemethanol + benzothiazole); frankfurters, bacon, smoky (LRI 1861; 2-methoxyphenol); meaty, chicken, sulfur (LRI 1979; 2-methyl-3-methyltrithiofuran); and fatty, butter fried, savory (LRI 1437; 2-furanmethanethiol). Several metallic odors also tended ($P < 0.06$) to be more intense in batch Y (LRI 1213 and 1756; 2-methylpyridine and unknown).

Two of the odors increased in batch Y are caused by compounds (2-pentylfuran and 2-methyl-3-methyltrithiofuran), which require oxidative reactions for their formation, but further work would be required to determine whether the availability of pro-oxidants and antioxidants or times, temperatures, and other processing conditions are the cause for these interbatch variations. It has been shown that varying the temperature, moisture level, and time of processing causes variations in the

type of volatile compounds and their levels in a sample (*36*, *37*). Variations in the yeast cream may be caused by the variations in the yeast growth medium. In addition, the constituents of molasses, used as a growth substrate, can vary drastically from batch to batch and from season to season (*38*, *39*). These factors affect the consistency in the yeast quality and, hence, that of the extract produced from it.

In conclusion, the main odor volatiles in the different yeast extract pastes include 2-methyl-3-furanthiol, 2-methyl-3-methyldithiofuran, methional, 1-octen-3-one, and dimethyltrisulfide together with a number of pyrazines, thiophenes, and aliphatic compounds. Differences between the yeast extract pastes are due to differences in the balance of odor volatiles with the sample having higher intensity of beefy, roasted, and metallic aroma showing an increased intensity of odor caused by furanthiols and aliphatic sulfur compounds. Not only do pastes from different suppliers differ in terms of odor volatiles but so do different treatments and batches of yeast extract from one supplier. Differences are also observed in the concentrations of flavor precursors. This suggests that the normal variations in ingredients and processing in commercial yeast extract production can affect the sensory quality of the final product and raises questions about the role of precursors and conditions of reaction. Papers on the effect of processing conditions and role of precursors are in preparation.

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Supporting Information Available: Table of volatile compounds collected by simultaneous distillation and extraction from three types of yeast extract paste samples. This material is available free of charge via the Internet at http://pubs.acs.org.

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