The Effects of Mixing Stage and Fermentation Time on the Quantity of Flavor Compounds and Sensory Intensity of Flavor in White Bread

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Received July 11, 2008; Accepted November 27, 2008

Flavor compounds in white bread have been analyzed by a static-headspace-type GC/MS and sensory evaluation for clarifying the effects of mixing stage and fermentation time on the intensity of flavor compounds as assessed in the crumb and sensory scores of flavor in white bread. Principal component analysis (PCA) and analysis of variance (ANOVA) were applied to the peak area of total ion chromatogram as well as sensory scores of 8 sensory descriptors for flavor intensity. Although the results of instrumental analyses showed the superiority of fermentation time over mixing energy as the governing factor of white bread flavor, the results of statistical analyses for the scores of sensory evaluation showed the superiority of mixing energy over fermentation time. The difference in the governing factor was probably due to overestimation for the effect of alcohols on the flavor quality of white bread, especially ethanol derived from the fermentation process.

Keywords: flavor compounds, fermentation time, GC/MS, mixing stage, principal component analysis, sensory evaluation, static headspace method, white bread

Introduction

The bread-making process can be broadly classified into three sub-processes: mixing, fermentation, and baking. The mixing and fermentation processes play an important role in determining the qualities of white bread. Numerous studies have been carried out on the aroma and flavor of white bread-complex flavor compounds derived from raw materials and the fermentation and baking processes (Baltes *et al.*, 1994; Chang *et al.*, 1995; Collyer, 1964; Grosch *et al.*, 1997; Heidelberg *et al.*, 1996; Henry *et al.*, 1996; Hwang *et al.*, 1994; Maga, 1974; Mulders, 1973; Rothe *et al.*, 1983; Schieberle *et al.*, 1989; Schieberle *et al.*, 1991a; Seits *et al.*, 1998). Flavor compounds produced by microorganisms such as bread yeast during the fermentation process markedly af-

fect the flavor compounds produced during the subsequent baking process as the most important factor of the characteristic flavor of white bread (Watanabe, 1997). Previous studies have revealed the effects of alcohols, esters, and amino acids produced during the fermentation process on bread flavor (Frasse *et al.*, 1992; Hironaka, 1985; Parnell, 1998; Schieberle *et al.*, 1991b; Tanaka, 1987; Watanabe, 1997).

Recent studies on bread flavor identified 100 flavor compounds in the crumb of white bread by GC/MS analysis using a dynamic headspace method (Chang *et al.*, 1995; Seits *et al.*, 1998). The dynamic headspace method includes a purge-and-trap method and a solid-phase microextraction (SPME) method. In the purge-and-trap method, targeted compounds are purged from a sample under an inert gas atmosphere to be absorbed in a Tenax trapping tube packed with porous polymer. In the SPME method, purged compounds are absorbed in a solid-phase fiber bonded with fine needles. Concentrated

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flavor compounds trapped by these methods are thermally desorbed before measurement by gas chromatograph (GC) or gas chromatograph and mass spectrometer (GC/MS).

The dynamic headspace method, however, detects the ratio of each flavor compound concentrated in specified absorption media, which means the difference in abundance ratio between flavor compounds as measured by instrumental analyses and sensory characteristics perceived by human beings when eating white bread. In contrast, the static headspace method has the advantage of enabling direct measurement of the ratio of each flavor compound contained in the gaseous phase within a vial.

According to a food *kansei* model proposed by Ikeda *et al.* (2004), the eating behavior of human beings; that is, the process of eating and evaluating foods over the short term, can be determined only by the routes of perception and cognition. The purposes of the model are 1) to evaluate quantitatively the process of emerging pleasantness and affective change in an individual person during the short period of eating behavior, and then 2) to use these quantitative data to optimize product design and the manufacturing process (Ueda *et al.*, 2008). Consequently, the model intends to correlate physicochemical properties with sensory characteristics of the targeted foods. However, available literature is limited on the correlation between the quantities of flavor compounds detected by GC/MS and sensory characteristics of the flavor contained in white bread.

The objectives of this work are 1) to measure the flavor compounds contained in the crumb of white bread samples under the conditions of different mixing stage and fermentation time using a static-headspace-type GC/MS, and then 2) to perform sensory evaluation on flavor intensity of the samples, and finally 3) to clarify the effects of mixing stages and fermentation time on the flavor compounds in the crumb and sensory characteristics of white bread.

Materials and Methods

Samples White bread samples were prepared using the no-punch straight-dough method, as shown in Table 1 (Takeya, 1981). All samples were mixed by a mixer (DTM-50; SK mixer Co., Ltd.) so that the dough temperature reached about 27°C after the mixing process. Next, primary fermentation was performed in a fermenting vessel (Took Final Proofer; Tokura Shoji Co., Inc.) controlled at an internal temperature of 27°C in the vessel and 75% relative humidity before the samples were divided evenly, weighed, and rolled for a 20-minute interval at room temperature. Then molded samples were further fermented (secondary fermentation) under the conditions of an internal temperature of 38°C in the vessel and 85% relative humidity. Fermented samples

were baked at an internal oven temperature of 210°C (Took Oven; Tokura Shoji Co., Inc.) for 38 minutes. Baked white bread samples were cooled to room temperature and then sliced into 18 mm thicknesses. The combinations of four mixing stages and four primary fermentation times provided 16 white bread samples (Table 2). All these processes were performed by well-trained professionals in the field of bread-making.

Identification of flavor compounds by GC/MS Instruments for GC/MS analyses were a static headspace (Tekmar 7000 Headspace Autosampler; Tekmar Company Inc.), a gas chromatograph (Model 6890; Agilent Technologies Inc.) with a column (DB-5; J&W Scientific Inc.) of 30 m in length, 0.25 mm in inside diameter, and 1.0 µm in membrane thickness, and a mass spectrometer (Automass SUN-200S; JEOL Ltd.)

Table 1. Ingredients of white bread samples.

Ingredients	(%)
Wheat Flour (Camera: Nisshin Flour Milling Inc.)	100.0
Yeast (Regular: Oriental Yeast., Ltd.)	2.0
Yeastfood (Oriental Yeast., Ltd.)	0.1
Sugar	6.0
Salt	2.0
Defatted skim milk	2.0
Shortning	5.0
Water	70.0

Table 2. IMixing and fermentation conditions for white bread samples.

Sample No.	Mixing stage	Fermentation time (min)
1	Pick-up ^a	15
2	Pick-up ^a	60
3	Pick-up ^a	120
4	Pick-up ^a	300
5	Clean-up ^b	15
6	Clean-up ^b	60
7	Clean-up ^b	120
8	Clean-up ^b	300
9	Final ^c	15
10	Final ^c	60
11	Final ^c	120
12	Final ^c	300
13	Over-mixing ^d	15
14	Over-mixing ^d	60
15	Over-mixing ^d	120
16	Over-mixing ^d	300

a: 100rpm for 3 min

b: 100rpm for 3 min following 200 rpm for 8 min

c: 100rpm for 3 min following 200 rpm for 10 min and 285 rpm for 3 min

d: 100rpm for 3 min following 200 rpm for 10 min and 285 rpm for 8 min

with a mass spectral search program (Version 2.0; National Institute of Standards & Technology) for library search and identification and software (EZChrom Elite; GL Sciences Inc.) for the quantification of identified total ion peak areas.

For the static headspace, 3 grams of samples in the center portion of the crumb were encapsulated in a glass vial container (22 mL). The analytical conditions of the headspace were 3 gram sample weight, 80°C sample temperature, 160°C injection temperature, 36-second duration of injection, 80°C needle temperature, and 160°C transferring temperature. High-purity helium carrier gas (1.2 mL/min) was employed for gas chromatography. The column was held at 50°C for 3 min, temperature programmed to 260°C at a heating rate of 4°C/min, and then held at 260°C for 15 min. The analytical conditions of the mass spectrometer were 250°C interface temperature, 160°C transferring temperature, and 230°C ion source temperature. The ionization energy of the mass spectrometer was 70 eV, and a scan cycle time of 0.5 ms (30-300 m/z) was used.

Sensory evaluation Sensory evaluation was performed by 23 expert sensory assessors employed in Nisshin Seihun, Co. Inc., on the samples shown in Table 2. Test samples of white bread were cooled to room temperature for 120 min after baking, and then sliced into 18 mm thicknesses, after which the slices were individually sealed in polyethylene plastic zipper bags. The samples were evaluated on the day after baking.

A 9-point category scale (1: very weak; 5: very strong) was used at 0.5 point intervals, and the standard sample (Sample No.11) was rated as 3 for each of the following 8 descriptors on the intensity of flavor: flavor intensity, sweet smell, alcoholic smell, milk-like smell, powder-like smell, acid smell, fruity smell, and fragrant smell.

Statistical analyses Analysis of variance (ANOVA) and principal component analysis (PCA) were applied to the total ion peak areas of flavor compounds identified by GC/MS as well as sensory scores of eight descriptors on flavor intensity. These statistical analyses were performed using the JMP 6.0.3.J software (SAS Institute, Japan).

Results and Discussion

Analyses of flavor compounds by GC/MS A static-headspace-type GC/MS produces a total ion chromatogram (TIC) for flavor compounds contained in the samples (Fig. 1). Such chromatograms for all the samples detected and identified 64 flavor compounds. Retention time and TIC peak area for each flavor compound are shown in Table 3. For all the samples, over 80% of the whole flavor quantity was ethanol (No. 2), which is the main flavor compound produced by bread yeast and naturally resulted in the largest quantity among all flavor compounds contained in the crumb of the samples.

The percentages of flavor compounds other than ethanol in Sample No. 11 were 62.8% for alcohol group, 11.8% for aldehyde group, 6.8% for carboxylic acid group, 5.1% for

Fig. 1. Typical total ion chromatogram of volatile components from white bread sample No.11 using static HS-GC/MS.

Table 3. Total ion peak area of flavor compounds in white bread samples.

No.	Retention time (min)	Compounds	Total ion peak area ($\times 10^4$)							
			Sample No.							
			1	2	3	4	5	6	7	8
1	5.24	Acetaldehyde	27,503	19,983	28,942	26,449	26,928	30,047	29,949	25,845
2	6.00	Ethanol	1,302,520	1,380,610	1,616,570	2,446,280	1,421,250	1,667,730	1,958,620	2,633,520
3	6.47	Acetone	14,663	10,863	7,451	3,816	13,319	11,457	8,846	3,976
4	6.75	Furan	864	410	1,169	745	548	869	1,602	524
5	6.98	Propanoic acid	526	482	1,259	872	708	706	1,077	568
6	7.26	Dimethylsulfide	916	873	1,044	1,234	1,015	579	814	665
7	8.02	1-Propanol	24,209	34,875	38,821	62,078	25,122	33,233	43,748	62,207
8	8.63	2,3-Butanedione	8,388	8,707	9,896	9,108	8,782	9,272	10,948	9,353
9	8.93	2-Butanone	2,808	2,780	2,578	2,234	2,768	2,847	2,628	2,183
10	9.64	Ethylacetate	3,684	3,597	4,394	8,114	3,605	4,200	4,852	8,919
11	10.13	1-Propanol, 2-methyl-	63,246	98,550	93,810	125,936	77,067	86,403	82,861	98,995
12	10.24	Acetic acid	15,452	8,178	9,813	18,511	14,212	14,451	25,249	27,919
13	10.50	(Z)-2-Butenal	4,053	3,760	4,533	7,104	3,857	4,694	5,213	7,210
14	11.02	Butanal, 3-methyl-	7,810	6,460	6,512	8,724	7,084	6,869	8,163	8,143
15	11.14	2-Butanone, 3-methyl-	972	1,034	1,538	1,840	983	1,170	1,627	1,954
16	11.43	Butanal, 2-methyl-	3,881	3,699	4,042	4,329	3,316	3,469	4,392	4,554
17	11.69	Benzene	414	411	667	596	427	364	687	454
18	12.11	(E)-2-Penten-1-ol	429	588	505	479	547	604	537	472
19	12.38	2,3-Pentanedione	722	950	1,257	1,537	744	876	1,335	1,379
20	12.59	Pentanal	1,903	2,021	2,042	2,325	2,106	2,379	2,325	2,134
21	12.86	2-Butanone, 3-hydroxy-	5,875	6,142	6,547	4,700	7,028	7,959	7,771	2,944
22	13.21	Furan, 2-ethyl-	388	526	336	362	418	507	481	500
23	13.37	Propanoic acid, 2-methyl-	125	159	149	193	107	121	142	177
24	13.60	Heptane	276	299	305	318	107	289	214	382
25	14.39	1-Butanol, 3-methyl-	79,487	98,992	104,717	162,093	78,863	92,897	107,509	165,756
26	14.58	1-Butanol, 2-methyl-	41,769	62,117	61,277	97,891	42,229	53,699	62,970	102,430
27	14.82	Propanoic acid, ethylester	13,705	16,247	18,484	29,876	15,138	17,911	20,594	32,820
28	15.07	Disulfide, dimethyl	902	913	803	847	959	936	839	549
29	15.78	1-Pentanol	1,026	968	1,160	1,200	1,149	1,115	1,079	1,030
30	16.34	Toluene	510	537	574	677	442	617	481	494
31	17.18	Hexanal	6,980	7,750	6,933	7,054	7,588	8,795	8,685	7,541
32	18.22	Octane	722	937	682	714	735	898	680	1,094
33	18.43	Furfural	351	355	398	362	263	350	397	427
34	18.63	Butanoic acid, 3-methyl-	1,427	1,542	1,779	2,645	1,816	1,915	2,047	2,467
35	19.14	Butanoic acid, 2-methyl-	788	854	1,181	2,418	925	1,045	1,299	2,363
36	20.56	1-Hexanol	3,666	3,799	3,219	3,697	3,684	3,967	3,157	3,672
37	20.83	Ethylbenzene	303	333	278	367	289	308	243	286
38	20.95	1-Butanol, 3-methyl-, acetate	341	415	369	1,065	304	388	342	1,159
39	21.22	Xylene	361	412	319	442	325	369	311	414
40	21.51	2-Heptanone	257	287	284	381	293	304	342	330
41	22.03	Heptanal	531	573	498	521	674	701	653	593
42	22.09	Styrene	314	362	309	392	292	307	300	309
43	22.36	Benzene, dimethyl-	142	193	115	173	130	116	116	160
44	24.50	(E)-2-Heptenal	294	337	306	374	349	349	339	420
45	24.75	Benzaldehyde	1,059	1,016	1,363	1,058	1,047	1,190	1,349	922
46	25.29	1-Heptanol	148	177	158	204	165	195	158	284
47	25.45	Pyridine, 3-methyl-	136	166	212	275	165	195	158	284
48	25.53	Benzene, 1-ethyl-2-methyl-	133	171	153	218	183	174	128	197
49	25.72	Trisulfide, dimethyl	1,059	1,016	1,363	1,058	1,047	1,190	1,349	922
50	25.84	1,3-Octadiene	247	252	261	271	278	300	283	271
51	25.88	Benzene, 1,2,4-trimethyl-	189	217	186	265	216	244	159	209
52	26.19	2-Octanone	116	133	132	214	131	164	113	162
53	26.56	Hexanoic acid, ethylester	195	270	341	985	221	279	349	1,088
54	26.64	Furan, 2-pentyl-	826	869	844	788	981	1,044	877	875
55	27.11	Benzene, 1,3,5-trimethyl-	191	208	189	163	201	212	165	174
56	27.75	Benzene, 1,3-dichloro-	308	338	305	330	263	264	325	402
57	28.33	Benzeneacetaldehyde	161	181	226	284	171	224	272	249
58	28.93	Limonene	475	512	395	516	712	787	679	690
59	29.09	(E)-2-Octenal	200	196	192	283	264	252	245	270
60	31.26	Nonanal	871	1,153	957	1,285	2,210	2,126	1,971	1,962
61	31.60	Phenethyl alcohol	1,373	1,466	3,159	7,802	3,071	3,093	4,697	8,380
62	33.47	(E)-2-Nonenal	200	223	236	297	331	353	327	330
63	34.96	Octanoic acid, ethylester	313	584	1,090	4,030	427	704	1,186	4,267
64	42.44	Decanoic acid, ethylester	154	149	269	1,096	127	197	283	1,115

ketone group, 2.4% for diketone group, 1.2% for ester group, 0.9% for alkenal group, 0.8% for aromatics group, 0.4% for sulfuric compound group, and 0.4% for furanic compound group (Table 4). The alcohol group other than ethanol included much 1-propanol (No. 7), 2-methyl-1-propanol (No. 11), 3-methyl-1-butanol (No. 25), and 2-methyl-1-butanol

(No. 26).

Comparison of the relative ratios of compound groups in the samples (Sample No. 9 and Sample No. 12) to the reference sample (Sample No. 11) revealed that the groups of alcohols, esters, and aromatics increased in relative ratio with increasing fermentation time. On the contrary, those of

Table 3. (Continued).

No.	Retention time (min)	Compounds	Total ion peak area ($\times 10^4$)							
			Sample No.							
			9	10	11	12	13	14	15	16
1	5.24	Acetaldehyde	26,567	26,323	29,714	25,437	24,590	25,437	30,764	26,206
2	6.00	Ethanol	1,489,580	1,524,030	1,750,890	2,367,060	1,259,000	1,729,020	2,283,750	2,867,410
3	6.47	Acetone	11,843	11,033	11,238	4,500	13,771	12,970	11,339	5,328
4	6.75	Furan	231	320	805	798	694	318	807	742
5	6.98	Propanoic acid	586	922	490	930	615	536	545	652
6	7.26	Dimethylsulfide	752	814	609	1,238	863	939	533	692
7	8.02	1-Propanol	26,438	29,742	35,494	57,789	22,945	30,104	40,151	60,007
8	8.63	2,3-Butanedione	8,340	8,489	9,852	8,886	6,984	8,864	11,542	9,542
9	8.93	2-Butanone	2,558	2,556	2,303	1,713	2,462	2,572	2,839	2,812
10	9.64	Ethylacetate	3,333	3,668	4,098	6,632	3,430	3,483	4,891	8,008
11	10.13	1-Propanol, 2-methyl-	71,171	80,343	85,384	123,272	75,180	64,318	62,586	85,197
12	10.24	Acetic acid	21,395	13,456	12,702	9,448	12,661	27,330	21,228	28,021
13	10.50	(Z)-2-Butenal	3,908	3,969	3,783	5,846	3,620	3,871	4,581	7,122
14	11.02	Butanal, 3-methyl-	6,749	7,500	7,189	8,454	6,408	7,084	9,119	10,084
15	11.14	2-Butanone, 3-methyl-	1,130	935	1,135	1,909	829	1,191	1,413	2,012
16	11.43	Butanal, 2-methyl-	4,333	3,811	3,905	4,336	3,212	3,498	4,414	5,394
17	11.69	Benzene	661	433	429	358	366	478	515	806
18	12.11	(E)-2-Penten-1-ol	474	416	398	430	568	584	665	640
19	12.38	2,3-Pentanedione	699	887	1,018	1,343	572	952	1,405	1,710
20	12.59	Pentanal	2,202	2,154	2,085	2,267	1,949	2,287	2,622	2,553
21	12.86	2-Butanone, 3-hydroxy-	5,848	6,666	6,474	3,634	5,973	6,826	8,057	2,903
22	13.21	Furan, 2-ethyl-	497	346	396	294	423	548	548	500
23	13.37	Propanoic acid, 2-methyl-	101	114	91	125	97	146	112	157
24	13.60	Heptane	379	236	302	337	181	226	362	382
25	14.39	1-Butanol, 3-methyl-	80,663	80,654	93,488	151,158	73,972	81,004	101,340	158,434
26	14.58	1-Butanol, 2-methyl-	41,095	40,437	50,686	90,224	36,264	39,237	56,205	99,290
27	14.82	Propanoic acid, ethylester	14,421	14,244	16,229	28,168	14,081	16,146	19,699	32,229
28	15.07	Disulfide, dimethyl	1,033	836	671	848	934	861	880	837
29	15.78	1-Pentanol	1,133	971	943	1,261	1,200	1,214	1,251	1,293
30	16.34	Toluene	635	529	316	726	452	420	452	575
31	17.18	Hexanal	8,045	7,746	7,870	7,630	8,215	8,784	10,014	8,838
32	18.22	Octane	929	735	641	818	1,043	715	682	1,296
33	18.43	Furfural	404	391	306	418	333	342	388	500
34	18.63	Butanoic acid, 3-methyl-	1,665	1,460	1,631	2,438	1,687	2,076	2,046	2,775
35	19.14	Butanoic acid, 2-methyl-	987	793	959	2,279	925	1,148	1,227	2,443
36	20.56	1-Hexanol	4,006	2,867	2,624	3,480	4,172	3,123	3,189	4,220
37	20.83	Ethylbenzene	292	218	207	382	334	272	251	364
38	20.95	1-Butanol, 3-methyl-, acetate	296	240	262	832	366	321	333	1,001
39	21.22	Xylene	319	264	257	438	370	324	322	420
40	21.51	2-Heptanone	327	274	280	318	343	327	365	426
41	22.03	Heptanal	712	651	584	669	824	818	761	748
42	22.09	Styrene	325	270	280	388	308	310	281	341
43	22.36	Benzene, dimethyl-	136	136	118	201	111	130	120	166
44	24.50	(E)-2-Heptenal	312	290	249	336	352	340	342	408
45	24.75	Benzaldehyde	1,018	1,066	1,290	877	1,085	1,273	1,443	1,009
46	25.29	1-Heptanol	214	179	182	214	199	206	204	241
47	25.45	Pyridine, 3-methyl-	153	114	143	237	178	188	171	210
48	25.53	Benzene, 1-ethyl-2-methyl-	121	130	103	196	164	135	176	184
49	25.72	Trisulfide, dimethyl	1,066	1,169	1,198	1,352	1,091	1,354	1,360	1,225
50	25.84	1,3-Octadiene	240	220	218	238	252	231	258	293
51	25.88	Benzene, 1,2,4-trimethyl-	169	208	158	192	179	165	185	255
52	26.19	2-Octanone	110	111	99	152	157	113	133	153
53	26.56	Hexanoic acid, ethylester	167	162	193	785	205	182	259	906
54	26.64	Furan, 2-pentyl-	943	907	756	804	1,193	1,042	1,056	1,039
55	27.11	Benzene, 1,3,5-trimethyl-	168	154	123	187	186	188	174	243
56	27.75	Benzene, 1,3-dichloro-	160	195	163	466	277	261	238	339
57	28.33	Benzeneacetaldehyde	155	155	172	233	192	167	227	267
58	28.93	Limonene	299	226	232	344	443	443	407	505
59	29.09	(E)-2-Octenal	196	193	203	228	270	287	266	287
60	31.26	Nonanal	884	886	633	1,281	2,153	2,041	1,785	1,841
61	31.60	Phenethyl alcohol	1,046	1,146	2,267	7,344	2,491	2,744	4,105	8,231
62	33.47	(E)-2-Nonenal	268	237	199	276	413	383	351	381
63	34.96	Octanoic acid, ethylester	394	501	804	3,854	325	515	951	3,993
64	42.44	Decanoic acid, ethylester	131	138	188	1,047	142	150	229	1,061

aldehydes, carboxylic acid, ketones and diketones decreased with increasing fermentation time. No significant changes in relative ratios were found in the groups of alkenals, sulfuric compounds and furanic compounds. In addition, the peak areas of total ion chromatogram for seven compound groups (alcohols, carboxylic acid, esters, alkenals, aromatics, sul-

furic compounds, and furanic compounds) increased with fermentation time.

Statistical analyses of TIC peak area The results of principal component analysis and analysis of variance revealed that flavor compounds were sufficiently summarized by four main principal components, and that 37 flavor com-

Table 4. Total ion peak area of compound groups in white bread samples.

Compound Group	Sample No. 9 Total ion peak area (%)		Sample No. 11 Total ion peak area (%)		Sample No. 12 Total ion peak area (%)	
Alcohol*	225,543	57.8	270,908	62.8	434,528	68.2
Aldehyde	48,779	12.5	50,683	11.8	49,405	7.8
Carboxylic acid	38,478	9.9	39,489	6.8	42,368	6.6
Ketone	22,037	5.6	22,127	5.1	13,100	2.1
Diketone	9,039	2.3	10,368	2.4	10,229	1.6
Ester	4,154	1.1	5,384	1.2	12,365	1.9
Alkenal	3,900	1.0	3,780	0.9	5,850	0.9
Aromatics	2,064	0.5	3,557	0.8	8,221	1.3
Sulfuric compound	1,818	0.5	1,809	0.4	2,592	0.4
Furanic compound	1,668	0.4	1,867	0.4	2,020	0.3
Others	32,690	8.4	21,358	7.4	56,442	8.9
Total	390,170	100.0	431,330	100.0	637,120	100.0

* Ethanol is not included.

pounds were significant in fermentation time, and 6 flavor compounds were significant in mixing energy at the level of 5 percent or less (Table 5). The first PC (PC1) was mainly affected by fermentation time, and its contribution ratio was 40.4%. In contrast, PC2 was affected by mixing energy and its contribution ratio was 12.6%. The contribution ratios of PC3 and PC4 were 9.8% and 8.1%, respectively, making the cumulative contribution ratio from PC1 to PC4 70.8%.

PC1 comprised 30 flavor compounds, which belonged to the groups alcohols, esters, aldehydes, carboxylic acid, aromatics, furanic compounds, and cyclic nitrogenous compounds. All compounds in PC1 were significant in fermentation time at the level of 5 percent or less. Similarly, PC2 comprised 9 flavor compounds belonging to the groups alcohols with carbon number of 4 to 8, aldehydes, ketones, furanic compounds, alkenals, and alkenols. Among these, 6 flavor compounds were significant in mixing energy at the level of 5 percent or less. PC3 comprised 7 flavor compounds belonging to the groups aromatics, sulfuric compounds, and aldehydes. Among them, 4 flavor compounds were significant in fermentation time at the level of 5 percent or less. The results of these statistical analyses indicate that baking process affected all PCs, especially PC2, and PC1 comprised the compounds derived from fermentation.

Flavor compounds significant in fermentation time at the level of 5 percent or less were sufficiently summarized by principal component analysis (Table 6). The contribution ratio of PC1 was 30.7%, that of PC2 was 24.2%, and that of PC3 was 16.2%, making the cumulative contribution ratio from PC1 to PC3 71.1%. Flavor compounds derived from the baking process and raw materials markedly affected five main PCs as well. PC1 comprised flavor compounds whose groups were alcohols and esters derived from the fermenta-

tion process; and aldehydes, ketones, and furanic compounds derived from the baking process. PC2 comprised compounds in the groups of esters and aromatics derived from the fermentation process; aromatics, alkenals, and nitrogenous compounds derived from the baking process; and aromatics derived from raw materials. PC3 comprised alcohols derived from the fermentation process, sulfuric compounds derived from the baking process, and aromatics derived from raw materials.

Sensory evaluation Applying principal component analysis and analysis of variance to the scores of sensory evaluation of flavor revealed that 8 sensory descriptors for flavor intensity were sufficiently summarized by three main principal components, and PC1 and PC2 were mainly affected by mixing energy and fermentation time, respectively (Table 7). The contribution ratios of PC1, PC2, and PC3 were 27.2%, 26.2% and 15.5%, respectively, and the cumulative contribution ratio was 68.8%. The results indicate that both mixing energy and fermentation time equally affect the sensory characteristics for flavor intensity of white bread.

The sensory descriptors for the smells of sweetness, milk, fruit, and fragrance were summarized in PC1. In general, the smell of milk, significant in mixing energy at the level of 5 percent, is due to 2,3-butanedione as well as 3-hydroxy-2-butanone, which are typically derived from the baking process and are known as the flavor compounds of butter smell. Similarly, the fragrant smell, significant in mixing energy ($p < 0.001$), is due to the flavor compounds produced by the amino-carbonyl reaction and strecker degradation during the baking process, such as pyrazines, pyrrols, pyridines, and thiazoles. The smell of fruit, significant in fermentation time ($p < 0.05$), is often called the flavor like that of quality sake brewed from rice, and its typical flavor compound is ethyl

Table 5. Factor loadings of flavor compounds in white bread samples.

No.	Compounds	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	ANOVA
1	Acetaldehyde			-0.55								
2	Ethanol	0.93										F***
3	Acetone	-0.93										F***
4	Furan						0.68					
5	Propanoic acid						0.80					
6	Dimethylsulfide			0.64								F***
7	1-Propanol	0.98										
8	2,3-Butanedione					0.81						
9	2-Butanone				0.55							F*
10	Ethylacetate	0.98										F***
11	1-Propanol, 2-methyl-	0.66										F**
12	Acetic acid							0.49				
13	(Z)-2-Butenal	0.96										F***
14	Butanal, 3-methyl-	0.72										F**
15	2-Butanone, 3-methyl-	0.94										F***
16	Butanal, 2-methyl-	0.73										F***
17	Benzene							0.88				
18	(E)-2-Penten-1-ol		0.68									
19	2,3-Pentanedione	0.85										F***
20	Pentanal					0.54						
21	2-Butanone, 3-hydroxy-	-0.74										F***
22	Furan, 2-ethyl-						-0.57					
23	Propanoic acid, 2-methyl-	0.67										F**
24	Heptane								0.65			F*
25	1-Butanol, 3-methyl-	0.98										F***
26	1-Butanol, 2-methyl-	0.96										F***
27	Propanoic acid, ethylester	0.98										F***
28	Disulfide, dimethyl	-0.64										F*
29	1-Pentanol		0.71									M*
30	Toluene			0.73								
31	Hexanal		0.75									M**
32	Octane					-0.61						
33	Furfural	0.59										F*
34	Butanoic acid, 3-methyl-	0.89										F***
35	Butanoic acid, 2-methyl-	0.97										F***
36	1-Hexanol					-0.53						
37	Ethylbenzene			0.79								
38	1-Butanol, 3-methyl-, acetate	0.93										F***
39	Xylene			0.61								F*
40	2-Heptanone		0.63									F*
41	Heptanal		0.93									M***
42	Styrene			0.81								F*
43	Benzene, dimethyl-			0.64								F*
44	(E)-2-Heptenal	0.64										F*
45	Benzaldehyde					0.73						
46	1-Heptanol	0.69										F**
47	Pyridine, 3-methyl-	0.84										F***
48	Benzene, 1-ethyl-2-methyl-	0.62										F*
49	Trisulfide, dimethyl					0.69						
50	1,3-Octadiene				0.84							
51	Benzene, 1,2,4-trimethyl-									0.58		
52	2-Octanone	0.60										F*
53	Hexanoic acid, ethylester	0.97										F***
54	Furan, 2-pentyl-		0.87									M**
55	Benzene, 1,3,5-trimethyl-				0.63							
56	Benzene, 1,3-dichloro-	0.65										F**
57	Benzeneacetaldehyde	0.79										F***
58	Limonene				0.88							
59	(E)-2-Octenal		0.73									
60	Nonanal		0.76									
61	Phenethyl alcohol	0.96										F***
62	(E)-2-Nonenal		0.93									M**
63	Octanoic acid, ethylester	0.98										F***
64	Decanoic acid, ethylester	0.96										F***
Variance		25.9	8.0	6.3	5.2	4.7	3.1	2.9	2.4		1.4	
Contribution rate (%)		40.4	12.6	9.8	8.1	7.3	4.8	4.6	3.7		2.2	
Cumulative contribution rate (%)		40.4	52.9	62.8	70.8	78.2	83.0	87.5	91.3		93.5	

F= Fermentation time; M=Mixing energy; *P < 0.05; **P < 0.01; ***P < 0.001

Table 6. Factor loadings of flavor compounds significant at the five percent level among different samples in fermentation time.

No.	Compounds	PC1	PC2	PC3	PC4	PC5
2	Ethanol	0.79				
3	Acetone	-0.64				
6	Dimethylsulfide			0.84		
9	2-Butanone				0.69	
10	Ethylacetate	0.63				
11	1-Propanol, 2-methyl-			0.74		
13	(Z)-2-Butenal	0.67				
14	Butanal, 3-methyl-	0.83				
15	2-Butanone, 3-methyl-	0.78				
16	Butanal, 2-methyl-	0.94				
19	2,3-Pentanedione	0.87				
21	2-Butanone, 3-hydroxy-					-0.62
23	Propanoic acid, 2-methyl-		0.50			
24	Heptane	0.64				
25	1-Butanol, 3-methyl-	0.65				
26	1-Butanol, 2-methyl-	0.65				
27	Propanoic acid, ethylester	0.66				
28	Disulfide, dimethyl				0.88	
33	Furfural	0.80				
34	Butanoic acid, 3-methyl-		0.64			
35	Butanoic acid, 2-methyl-	0.63				
38	1-Butanol, 3-methyl-, acetate		0.61			
39	Xylene		0.65			
40	2-Heptanone	0.63				
42	Styrene			0.86		
43	Benzene, dimethyl-			0.77		
44	(E)-2-Heptenal		0.88			
46	1-Heptanol					0.69
47	Pyridine, 3-methyl-		0.67			
48	Benzene, 1-ethyl-2-methyl-		0.81			
52	2-Octanone		0.83			
53	Hexanoic acid, ethylester		0.57			
56	Benzene, 1,3-dichloro-			0.61		
57	Benzeneacetaldehyde	0.66				
61	Phenethyl alcohol					
63	Octanoic acid, ethylester	0.60				
64	Decanoic acid, ethylester	0.57				
Variance		11.4	8.9	6.0	4.3	2.7
Contribution rate (%)		30.7	24.2	16.2	11.5	7.2
Cumulative contribution rate (%)		30.7	54.9	71.1	82.6	89.7

F= Fermentation time; M=Mixing energy; *P < 0.05; **P < 0.01; ***P < 0.001

Table 7. Factor loadings for sensory descriptors of white bread flavor.

Descriptors		PC1	PC2	PC3	ANOVA
'Fureba no tsuyosa'	(Flavor intensity)		0.75		<i>F</i> ***
'Amaikaori'	(Sweet smell)	0.86			
'Arukuru'	(Alcohol smell)		0.83		<i>F</i> ***
'Milk-shuu'	(Smell of milk)	0.84			<i>M</i> *
'Konapposa'	(Powder-like smell)			0.92	<i>F</i> ***
'Sanshuu'	(Acid smell)		0.80		
'Kajitsu no kaori'	(Fruity smell)	0.43			<i>F</i> *
'Koubashii'	(Fragrant)	0.67			<i>M</i> ***
Variance		2.2	2.1	2.1	
Contribution rate (%)		27.2	26.2	15.5	
Cumulative contribution rate (%)		27.2	53.3	68.8	

F= Fermentation time; M=Mixing energy; *P < 0.05; **P < 0.01; ***P < 0.001

acetate.

The sensory descriptors for the smells of alcohol and acid were summarized in PC2. Both descriptors were significant in fermentation time ($p < 0.001$). Alcohol smell is due to organic acids produced by lactic acid bacteria and other flavor compounds produced by degradation reaction during the baking process.

PC3 included the sensory descriptor 'powder-like,' which was significant in fermentation time ($p < 0.001$). The powder-like smell was considered to be derived mainly from flour serving as the main ingredient, and therefore is sensed more when either fermentation time or baking time is insufficient.

The PC scores of PC1 and PC2 for the sensory evaluation results of the samples are plotted in Fig. 2. Although the results of instrumental analyses showed the superiority of fermentation time over mixing energy as the governing factor of white bread flavor, sensory evaluation results showed the superiority of mixing energy over fermentation time, excepting samples for which fermentation time was 300 min.

Conclusions

A static headspace GC/MS detected and identified 64 flavor compounds for all samples. Since ethanol content was over 80% of the whole flavor quantity for all the samples, the percentages of flavor compounds other than ethanol were tabulated for each compound group for comparing the samples under the conditions of the same mixing stage as well as different fermentation times. As fermentation time increases, alcohols and aromatics increased and the groups of aldehydes and ketones decreased. The relative ratio of car-

boxylic acids was higher when fermentation time was shorter (15 min). When fermentation time is longer (300 min), the relative ratio of esters was higher and that of diketone group was lower. The results indicate that the difference in fermentation time changes the composition of flavor compounds in the gaseous phase, and then it affected the overall flavor in the crumb of white bread.

Analysis of variance revealed that 37 flavor compounds were significant in fermentation time, and 6 flavor compounds were significant in mixing energy, both at the level of 5 percent or less. The results of principal component analysis for all flavor compounds showed that PC1 was markedly affected by fermentation time and included 30 flavor compounds derived from the fermentation and baking processes. PC2 was significantly affected by mixing energy and included 8 flavor compounds derived from the baking process. Other PCs from PC3 to PC9 mainly comprised the flavor compounds derived from the baking process. In addition, applying PCA to the flavor compounds significant in fermentation time revealed that the PCs from PC1 to PC5 were affected not only by fermentation but also by baking, as well as by raw materials.

Applying principal component analysis and analysis of variance to the sensory evaluation results revealed that PC1 and PC2 were mainly affected by mixing energy and fermentation time, respectively. The cumulative contribution ratio from PC1 to PC3 was 68.8 %, and 8 sensory descriptors were sufficiently summarized. PC1 was correlated with the descriptors for the smells of sweetness, milk, fruit, and fragrance, while PC2 with those for the smells of alcohol and acid.

Fig. 2. PCA plot of first and second factors in sensory space of sensory evaluation data for sixteen white bread samples.

Acknowledgments Special thanks are due to Mr. Shinji Ishigami in the Headquarters of Research and Development, Nisshin Seihun, Co. Inc. and Mr. Masahiro Ando in the Food Business Department, Oriental Yeast, Co. Ltd. for their guidance and kind cooperation in conducting this research.

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