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A New Approach to Estimate the In-mouth Release Characteristics of Odorants in Chewing Gum

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The objective of this study was to develop a new approach that can be used to estimate the in-mouth release characteristics of odorants in chewing gum. This technique, called the "Retronasal Flavor Impression Screening System (R-FISS)," is based on a modified dynamic headspace gas sampling technique. By application of the R-FISS technique, the in-mouth release characteristics of odorants during the chewing of gum was indicated by the peak area ratio, which was calculated by comparing the peak area for 10 min to the peak area for 1 min, of each odorant exhaled from the human nose. In addition, a good overall regression coefficient was found for the correlation between the peak area ratios of each odorant in the model chewing gum obtained by R-FISS and the retention indices on a polar stationary phase GC column (DB-Wax). Therefore, the in-mouth release of odorants in chewing gum seems to be capable of being predicted by their RIs on a polar stationary phase GC column (DB-Wax), and these results appear to suggest that two parameters (vapor pressure and hydrophobicity/hydrophilicity) are the key factors for determining the in-mouth release of odorants from chewing gum.

Keywords: flavor release, aroma release, flavor impression, chewing gum, retention index

Introduction

Food aroma (flavor) is one of the important factors influencing the quality of foodstuffs, and one of the major research areas in flavor science is the understanding of the characteristics of odorants from high-quality foods. Flavor assessment by aroma extract dilution analysis (AEDA) (Schieberle, 1995) is a technique that combines instrumental analysis by gas chromatography (GC) with sensory evaluation, which can be used to estimate the odor quality and the degree of contribution for the important characteristics of an odorant. Therefore, the AEDA is already widely utilized as a practical method to estimate the characteristics of the odorants in various foodstuffs.

On the other hand, for odorants in foods, the immediate aroma impression and duration of perception during food consumption are also important characteristics. Therefore, the characteristics of the in-mouth release of each odorant must be considered in order to understand in detail the characteristics of the odorants in foodstuffs. The perception of food aromas is the result of odorant/receptor interactions taking place in the odorant receptors on the olfactory epithelium, which is located in the human nasal cavity. The odorants reach the olfactory epithelium via the throat, allowing detection of the aroma of food in the mouth. To understand flavor perception during eating and drinking, it is important to know the composition and amount of the odorants that reach the olfactory epithelium (Taylor, 1996; Taylor and Linforth, 1997). However, it is difficult to analyze the odorants reaching the olfactory epithelium. Therefore, several analytical techniques to measure the exhaled odorants from the human nose have been developed, such as nosespace analysis, by measuring the APCI-MS (Linforth *et al.*, 1999; Brauss *et al.*, 1999; Taylor *et al.*, 2000; Hodgson *et al.*, 2005) or PTR-MS (Roberts *et al.*, 2003; Mestres *et al.*, 2005; Mestres *et al.*, 2006) and EXOM (Buettner and Schieberle, 2000). These analytical techniques are based on the experimental results of Taylor *et al.*, which show a better correlation between the intensity of the flavor perceptions during eating/drinking and the quantity of odorants exhaled through the nostril via the nasal cavity than the amount of odorants included in the foods themselves (Taylor and Linforth, 1997; Linforth *et*

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al., 1999; Hollowood *et al.*, 2000). However, it seems that application of these techniques to real foodstuffs was quite difficult because of the low concentrations of the exhaled odorants in addition to the presence of many kinds of included odorants in the real foodstuffs. Therefore, application of these techniques tended to be limited to model experiments.

In general, for the quality of the chewing gum, aroma is a factor more important than for other foodstuffs, since excellent in-mouth release of the aroma of chewing gum is required. The immediate aroma impression and the duration of perception of odorants during chewing in addition to excellent odor quality and the appropriate intensity are important to the perception of chewing gum quality. Therefore, to understand the characteristics of the in-mouth release of odorants during the chewing of gum and to utilize this knowledge is extremely important for the production of high-quality chewing gum products. However, the characteristics of in-mouth release, which are related to the immediate impression and persistence of the aroma, of the odorants in the chewing gum are still unclear. Therefore, the aim of the following investigation was first, to develop a technique that estimates the in-mouth release characteristics of odorants from chewing gum, and second, to investigate the possibility of predicting their behavior during the chewing of gum.

Materials and Methods

Materials Commercial and model chewing gums were tested. The commercial chewing gum was purchased from a local market. The model chewing gum was prepared by kneading a mixture of about 60 odorants (see Table 2; final concentrations of the odorants were each ca. 30 ppm dissolved in glyceryl triacetate) or a single odorant (final concentration of ethyl propionate or menthyl acetate was 400 ppm dissolved in glyceryl triacetate), powdered sugar (64 g), and 85%-sugar syrup (13 g), with a gum base (23 g).

Determination of in-mouth odorant release via the nasal cavity Assessors placed the chewing gum samples (samples of commercial chewing gum weighed 2.5 g and those of model chewing gum weighed 2 g) into their mouths and chewed at the rate of 100 chews per minute using a metronome. To trap the odorants, the exhaled air was passed through a glass nosepiece fitted to the nose of each assessor (Fig. 1). During this experiment (1 min or 10 min), the exhaled air from the nostril was passed through a small glass column (6 cm × 5 mm i.d.) filled with tenax TA (100 mg, 80/100 mesh, GL Science, Tokyo, Japan), which had been heated at 220°C prior to the analysis. The end of the glass column was connected to a pump by a silicon rubber tube, and during trapping of the exhaled air from the nose, a suction of approximately 1 L/min was applied to the system. This sampling system allowed the assessors to exhale normally without the need to press the exhaled air through the Tenax-column. After trapping, the water was removed from the Tenax TA with dry nitrogen (30 min, 100 mL/min). Three replicates of each experiment were performed by each assessor. These experiments were carried out at room temperature (25 ± 2°C).

Gas chromatography-mass spectrometry (GC-MS) Thermal desorption of the trapped odorants on the Tenax TA was performed using a TDU thermal desorption system (Gerstel GmbH, Mulheim an der Ruhr, Germany) in combination with the ATEX option of an MPS2 auto-sampler (Gerstel GmbH, Mulheim an der Ruhr, Germany) and CIS-4 injector (Gerstel GmbH, Mulheim an der Ruhr, Germany) for cryofocusing the analytes prior to transfer onto the analytical column. The following sampling parameters were used: Thermal desorption was performed by programming the TDU from 20°C to 220°C (held for 3 min) at the rate of 12°C/sec and using the split mode (the split ratio was 1:30). Cryofocusing was performed with liquid nitrogen at -150°C. Injection was performed with a ramp of 12°C/sec from -150°C to 220°C (held for 3 min), and the split ratio was 1:5. The odorants were analyzed by an Agilent 6890 N gas chromatograph coupled to an Agilent 5975 B series mass spectrometer (Agilent Technologies, Palo Alto, CA, USA). The column was a 30 m × 0.25 mm i.d. DB-Wax fused silica capillary (J & W Scientific, Folsom, CA, USA) with a film thickness of 0.25 μm. The column temperature was programmed from 30°C (held for 3 min) to 210°C at the rate of 5°C/min. The flow rate of the helium carrier gas was 1 mL/min. The mass spectrometer was used with an ionization voltage of 70 eV (EI) and ion

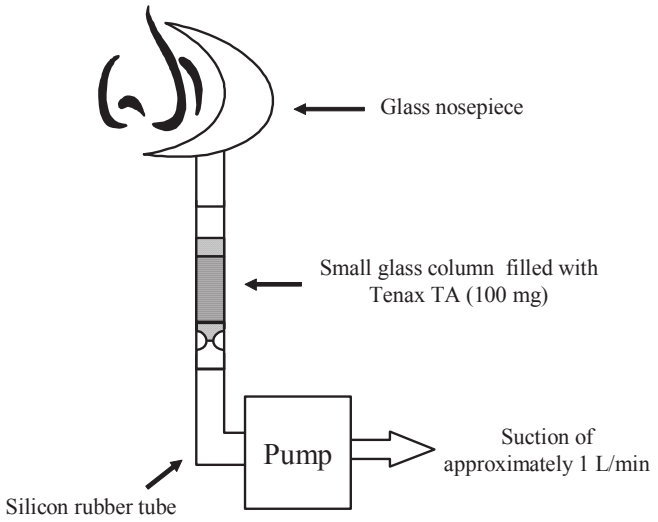

Fig. 1. Schematic diagram of the device for trapping exhaled odorants from the human nose.

source temperature of 150°C. The peak area ratio of each exhaled odorant from the human nose was calculated by comparing the peak area for 10 min to the peak area for 1 min. For the overlapping odorants on the GC chromatogram using the DB-Wax stationary phase column, each peak area ratio was determined by comparing the peak area of the extracted ions for 10 min to that for 1 min. The Kovats GC retention indices (RI) of the odorants were calculated from the retention time of *n*-alkanes.

Sensory analysis A sensory assessor panel consisting of five judges (two female and three male) was trained for time-intensity (TI) analysis of the flavor sensation from the chewing gum. They were asked to rate the perceived flavor intensity with time while chewing the gums (100 chews/min). The intensity of the flavor sensation was scored on a scale of 1 (weak)–10 (strong). Ten results per sample were obtained from the five assessors (each assessor tested each model chewing gum twice, and the model chewing gum contained a single odorant). The scores for each time were normalized as a percentage of the maximum score per sample, and then the ten normalized scores were averaged.

Results and Discussion

Estimation of the in-mouth release characteristics of odorants in chewing gum Measuring the in-mouth release of odorants from the chewing gum was expected to be quite difficult because the exhaled air from the human nose includes many kinds of odorants and their amounts are extremely low. Therefore, to analyze the exhaled in-mouth odorants from the human nose, the odorants need to be concentrated, followed by separation by GC for application using real foods such as chewing gum. A technique that is coupled with the trapping of odorants in the air on adsorbents such as Tenax, and a GC-MS equipped with a thermal-desorption and cryo-focusing injection system, have been used for the dynamic headspace gas sampling technique. The major advantage of this technique is to be able to improve the detection limit of low-amount odorants and to achieve the separation of a mixture consisting of a great number of odorants by one measurement. By application of this analytical technique for the in-mouth odorants in commercial chewing gum, a good separation of the exhaled odorants over a wide range of concentrations could be achieved by optimizing the TDU-CIS-4 injection system and GC conditions (Fig. 2).

The reproducibility of this analytical technique regarding the major peaks of commercial chewing gum was examined by six assessors (Table 1). The peak areas of the major in-mouth odorants, which were sampled for 10 min from the start of chewing, by each assessor indicated a good reproducibility within a 20% relative standard deviation (RSD (%) = SD × 100/mean) for the tested odorant. However, reproducibility of the average peak areas among the six assessors was not recognized (51 < RSD (%) < 99). On the other hand, the RSD values (%) of the peak area ratios, which were calculated by comparing the peak area for 10 min to the peak area for 1 min, were more significantly reduced than the average peak areas of each exhaled odorant among the six

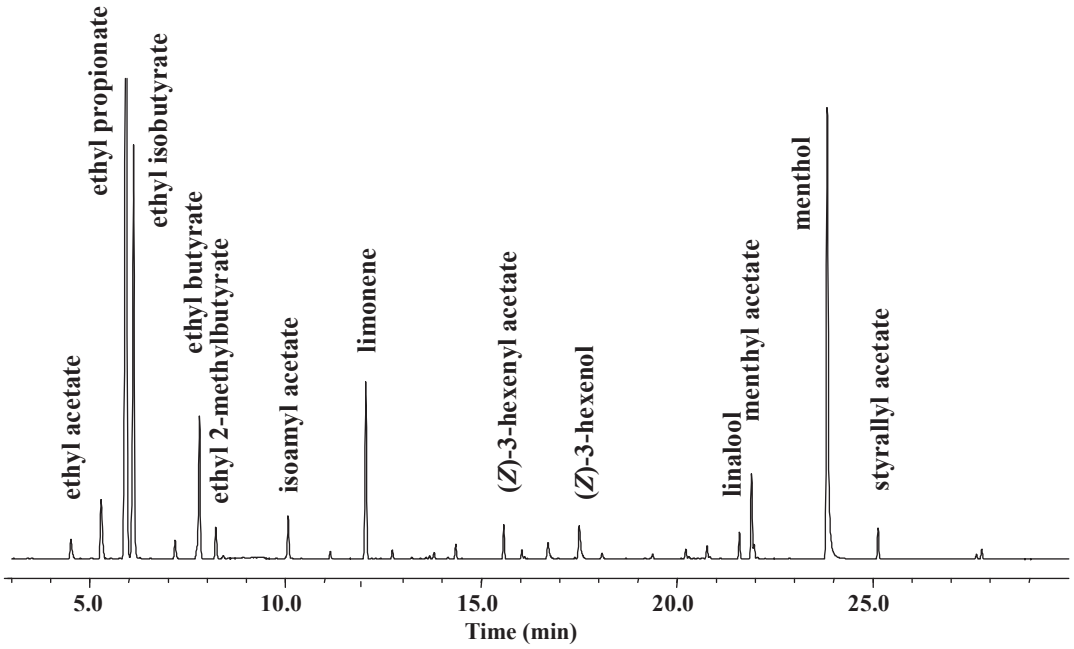

Fig. 2. Typical gas chromatogram of the exhaled in-mouth odorants from the human nose (commercial chewing gum).

Table 1. Comparison of the RSDs (%) of the peak area and peak area ratio of the major exhaled odorants in the commercial chewing gum.

		RSD (%)[a]		
		Peak Area[b]		Peak Area Ratio[c]
No.	Compound	Same Assessor[d]	Between Different Assessors	Between Different Assessors
1	ethyl acetate	17	51	23
2	ethyl propionate	8	84	19
3	ethyl isobutyrate	8	85	15
4	ethyl butyrate	9	92	30
5	ethyl 2-methylbutyrate	8	88	15
6	isoamyl acetate	9	78	24
7	limonene	9	89	24
8	(*Z*)-3-hexenyl acetate	7	93	24
9	(*Z*)-3-hexenol	12	99	32
10	linalool	12	82	18
11	menthyl acetate	10	91	28
12	menthol	6	67	22
13	styrallyl acetate	18	91	25

[a] Each RSD (%) was calculated based on the value from six assessors. [b] Peak area was the mean value of the triplicate results for 10 min.

[c] Peak area ratio of each compound was calculated by comparing the peak area for 10 min to the peak area for 1 min (10 min/1 min).

[d] RSD (%) was the mean value from six assessors.

assessors. There was little individual difference in the peak area ratio regardless of the significant individual difference in the amount of exhaled odorants from the human nose. These findings suggested that it is possible to estimate the in-mouth release characteristics of odorants in chewing gum by comparing the peak area ratios because the peak area ratio was considered to indicate the in-mouth release kinetics of each odorant. In preliminary experiments with this sampling system, the peak area of each exhaled odorant from the human nose increased with the extension of time from the start of chewing (the range from 1 min to 10 min). Therefore, to indicate more definitely the difference of the peak area ratio of each odorant, the sampling times (1 min and 10 min) to calculate the peak area ratios were chosen. By comparison of the peak area ratios of each tested odorant, it was demonstrated that each odorant had a specific value of the peak area ratio (Fig. 3). Therefore, the magnitude of the peak area ratio was assumed to reveal the in-mouth release characteristics of odorants in the chewing gum, since the peak area ratios of each odorant seem to be the kinetics corresponding to the odorant release from the chewing gum during chewing. It was expected from this result that ethyl propionate (low peak area ratio) and menthyl acetate (high peak area ratio) have an immediate aroma impression and longer duration of perception, respectively.

To verify the relationship of the difference in the peak area ratios and the in-mouth release kinetics of each odorant in chewing gum, a comparison of the in-mouth release of ethyl propionate and menthyl acetate from the model chewing gum was made by sensory evaluation using the time-intensity measurement. The time-intensity curves produced by the normalizing data of the tested odorants showed similar patterns among assessors despite the individual differences in the perceived flavor intensities. Fig. 4 shows the change in the flavor intensities of ethyl propionate and menthyl acetate of the model chewing gum. The results indicate that the time-intensity curves of both odorants were quite different, and it can be observed that the flavor intensity of ethyl propionate was lowered in a short time even though that of menthyl acetate lasted for a long time. These results indicated that the peak area ratios obtained by this analytical technique concept, called the "Retronasal Flavor Impression Screening System (R-FISS)," agreed well with the results of the sensory time-intensity measurement, and the system can be used to estimate the in-mouth release characteristics of the odorants in chewing gum.

Prediction of the in-mouth release characteristic of odorants in chewing gum For volatile compounds that were drunk as an aqueous solution, it is known that the hydrophobicity/hydrophilicity (Log *P*) and vapor pressure (Log *pL*) of each compound are the most important factors in their persistence in the human breath (the in-mouth release characteristics of the volatile compounds) (Linforth and Taylor, 2000). In addition, it has also been suggested that the polarity/boiling point interaction of the compound plays a role in the rate and time of release from mint-flavored sweets (Ingham *et al.*, 1995). On the other hand, the vapor pressure (boiling point) and hydrophobicity/hydrophilicity (polarity)

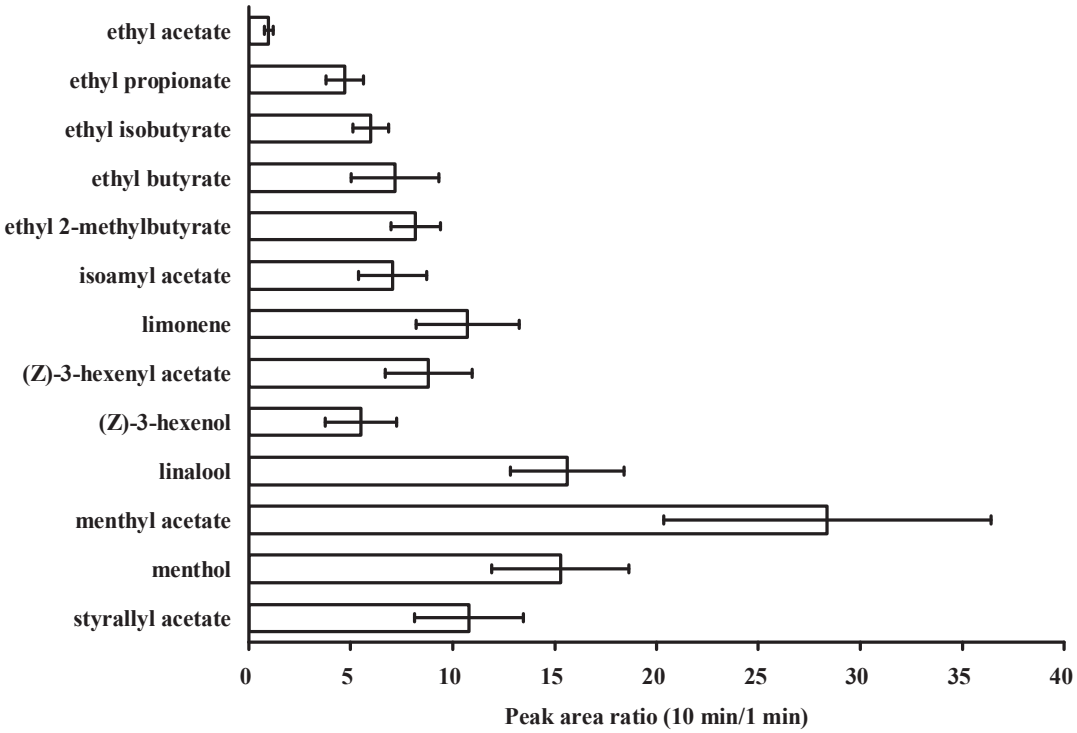

Fig. 3. Average peak area ratios, which were calculated by comparing the peak area for 10 min to the peak area for 1 min, of the exhaled odorants in commercial chewing gum. Each average was the mean value of triplicate results from six assessors.

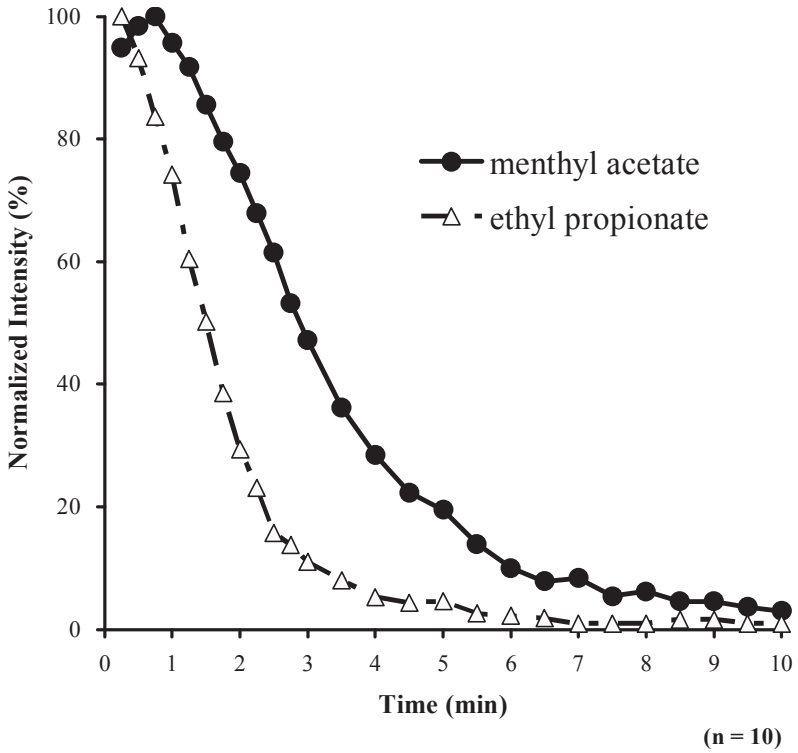

Fig. 4. Simultaneous time-intensity curves of ethyl propionate and menthyl acetate in the model chewing gum. Ten results per sample were obtained from five assessors (two replicates of each chewing gum per assessor), and the scores of each perceived intensity value have been normalized for easy comparison.

Table 2. Average peak area ratios[a] of the tested odorants in the model chewing gum.

No.	RI DB-Wax	RI DB-1	ΔI[c]	Compound	Extracted ion[b] (m/z)	Peak area ratio (10 min/1min)	No.	RI DB-Wax	RI DB-1	ΔI	Compound	Extracted ion[b] (m/z)	Peak area ratio (10 min/1min)
1	890	593	297	ethyl acetate		1.4	30	1321	1079	242	propyl hexanoate	117	7.7
2	894	714	180	acetaldehyde dea		2.4	31	1316	888	428	2-heptanol		8.1
3	927	673	254	methyl isobutyrate		2.7	32	1356	1133	223	isobutyl hexanoate		8.1
4	956	690	266	ethyl propionate		3.6	33	1337	846	491	hexanol		6.4
5	965	735	230	ethyl isobutyrate		4.4	34	1371	1057	314	allyl hexanoate		8.0
6	990	661	329	2-pentanone		2.8	35	1385	836	549	(*Z*)-3-hexenol	67	6.1
7	962	850	112	isobutanal dea		3.4	36	1412	1074	338	methylthio hexanoate		7.8
8	971	705	266	methyl butyrate		3.6	37	1401	854	547	(*E*)-2-hexenol	82	6.2
9	1036	774	262	ethyl butyrate		4.9	38	1416	1177	239	butyl hexanoate	117	8.1
10	1058	760	298	3-hexanone		5.1	39	1437	1228	209	hexyl isovalerate		8.9
11	1055	837	218	ethyl 2-methylbutyrate		5.3	40	1453	1079	374	(*Z*)-6-nonenal	122	9.7
12	1074	758	316	butyl acetate		5.2	41	1448	972	476	1-octen-3-ol	57	10.3
13	1091	774	317	hexanal		4.8	42	1455	957	498	heptanol	70	7.6
14	1111	852	259	isoamyl acetate		5.8	43	1501	990	511	(*E*,*E*)-2,4-heptadienal	81	7.1
15	1136	875	261	ethyl valerate		6.0	44	1510	1089	421	2-nonanol		14.2
16	1149	934	215	butyl isobutyrate		6.6	45	1541	1280	261	ethyl nonanoate		9.1
17	1195	1010	185	1,4-cineole		5.5	46	1550	1084	466	linalool		15.4
18	1191	866	325	2-heptanone	114	6.1	47	1555	1052	503	octanol	84	13.1
19	1195	877	318	heptanal	96	5.8	48	1588	1110	478	fenchyl alcohol		11.7
20	1215	867	348	methylthio butyrate		5.7	49	1612	1371	241	hexyl hexanoate	117	9.4
21	1224	1027	197	1,8-cineole		4.5	50	1611	1373	238	octyl butyrate	89	9.5
22	1234	825	409	(*E*)-2-hexenal	98	5.2	51	1645	1171	474	menthol		10.6
23	1219	979	240	butyl butyrate	89	7.1	52	1724	1009	715	4-hexanolide	85	11.2
24	1241	871	370	(*Z*)-4-heptenal		5.1	53	1706	1183	523	α-terpineol		15.3
25	1264	928	336	ethyl amyl ketone		7.9	54	1842	1373	469	β-damascenone	121	12.3
26	1275	991	284	hexyl acetate		8.2	55	1854	1408	446	α-ionone	121	16.5
27	1294	959	335	2-octanone		8.3	56	1937	1215	722	4-octanolide	85	13.8
28	1299	980	319	octanal		6.3	57	1928	1104	824	2-phenylethyl alcohol	91	12.7
29	1320	983	337	(*Z*)-3-hexenyl acetate	82	7.7							

[a] Each average was the mean value of triplicate results from one assessor.
[b] Each peak area ratio of the overlapped odorants on the gas chromatogram was determined by comparing the peak area of the extracted ions for 10 min to that for 1 min.
[c] ΔI is the difference of the RI of the polar and apolar columns.

of the compounds are also generally major factors affecting their retention in gas chromatography (Masada and Kojima, 1983; Kawai, 1987). For instance, the major factors of retention of compounds on apolar or polar stationary phase GC columns, such as the DB-1 or DB-Wax, depend on the vapor pressure or the polarity/vapor pressure interaction of the compounds, respectively. Based on these factors, it can be presumed that the peak area ratios, corresponding to the in-mouth characteristics of the odorants obtained by R-FISS, have a close relation to the retention time or retention index in the gas chromatography analysis; in addition, these values can be expected to be used for the prediction of the in-mouth release characteristics of the odorants in chewing gum.

In order to confirm the relationship of the peak area ratio and retention index (RI) of each odorant, the peak area ratios of the odorants, which had various functional groups (such as ethers, ketones, aldehydes, esters, and alcohols), were determined by R-FISS using the model chewing gum. Table 2 shows the RIs on the polar and apolar GC columns, showing ΔI (ΔI is the difference in the RI of the polar and apolar columns, and these values correspond to the polarity of each odorant) and the peak area ratios of 57 odorants, which gave comparatively good reproducibilities. Each RI can correspond to the vapor pressure, polarity, and polarity/vapor pressure interactions of the compounds. First, the RIs on the apolar GC column and ΔI relations with the peak area ratios of the 57 odorants are shown in Fig. 5. The tested odorants of the model chewing gum were recognized to have a weak correlation with both the peak area ratios and the RI on the apolar GC column ($R^2 = 0.6282$) and ΔI ($R^2 = 0.3521$). These data indicated that the in-mouth release of odorants in the chewing gum was related to the vapor pressure and polarity of each odorant, as was the case of the volatile compounds that were drunk as an aqueous solution, and each coefficient of correlation suggested that the vapor pressure of each odorant was a more significant factor than the polarity. Furthermore, the RIs on the polar GC column (DB-Wax), which were affected by the polarity/vapor pressure interaction of the compounds for the retention of the compounds on GC, and the relation with the peak area ratios of the 57 odorants are shown in Fig. 6, and a good overall regression coefficient was found for the correlation between the peak area ratios of each tested odorant and the retention indices on a polar stationary phase GC column (DB-Wax). The coefficient of cor-

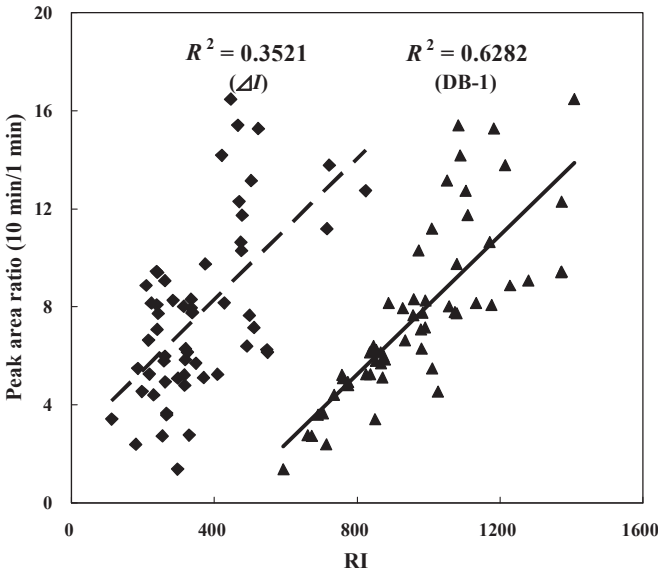

Fig. 5. Relationship between the peak area ratio of odorants in the model chewing gum and its retention index (RI) on the apolar stationary phase GC column (DB-1) and ΔI (ΔI is the difference in the RI of the polar and apolar columns). Each value is the mean of three replicates by one assessor.

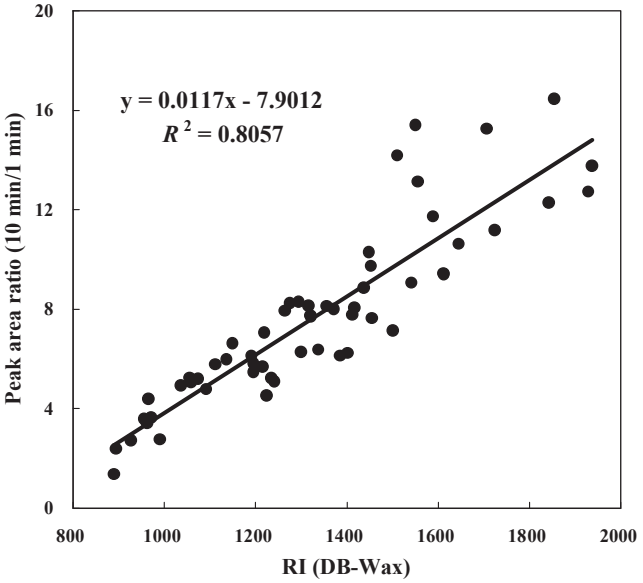

Fig. 6. Relationship between the peak area ratio of odorants in the model chewing gum and its retention index (RI) on the polar stationary phase GC column (DB-Wax). Each value is the mean of three replicates by one assessor.

relation (R^2) for the tested odorants was 0.8; therefore, it can be used to predict the in-mouth release of various odorants in chewing gum using the RI of the polar stationary phase GC column (DB-Wax).

Conclusions

On the basis of these results, it is suggested that the in-mouth release of odorants in chewing gum can be estimated by the "Retronasal Flavor Impression Screening System" (R-FISS) approach, which compared the peak area ratios (10 min/1 min) of each exhaled odorant from the human nose via the nasal cavity, and it was found possible to predict the in-mouth release of odorants in chewing gum from their RIs on the polar stationary phase GC column (DB-Wax). The R-FISS and RI approaches are relatively simple methodologies that provide a useful approach to clarify the characteristics of the in-mouth release of odorants in chewing gum. However, it is well known that the food matrix composition has important effects on the release rate of aroma compounds (Taylor, 2002); for instance, fat content is important in the flavor perception of products (Brauss *et al.*, 1999; Odake *et al.*, 2006). There are many types of chewing gums (sugar or sugarless, stick or tablet, etc.), and it has been reported that the sucrose concentration and their form had effects on the perceived flavor intensity of chewing gum (Davidson *et al.*, 1999). In addition, the functional groups of the tested odorants in the present paper were limited. Therefore, further investigations will be necessary to provide detailed information about the in-mouth release characteristics of the odorants in chewing gum, such as the relationship between the chewing gum matrix and aroma release.

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