Real-time percept flavor balance derived from retronasal threshold and *in vivo* measurements of retronasal aroma release with PTR-MS

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Abstract

We devised to convert the contributions of retronasal aroma fluctuating with time during consumption into numerical values. Retronasally perceived aroma compounds were directly analyzed with proton transfer reaction mass spectrometry (PTR-MS) while subjects drank several samples of aqueous solutions of aroma compounds. The behavior of aroma compounds released from the nostrils was detected with breath-by-breath AUC (area under curve in a plot concentrations vs time) and was approximated as a power law function. Separately, subjects determined the flavor threshold for each aroma compound by drinking its aqueous solution. AUC of each compound at the 1st breath released from the nostrils was newly defined as retronasal threshold when subjects drunk an aqueous solution at its threshold concentration. Then, the value which was calculated from the AUC of retronasal aroma and retronasal threshold was defined as the contribution value of retronasal aroma (CRA). A contribution of an aroma compound at an arbitrary time after swallowing foods and drinks can be indicated with CRA. Consequently, our study enables the visualization of a perceived flavor balance and calculation of its time changes during consumption.

Introduction

Retronasal aroma is one of the most important factors for palatability of foods and drinks during consumption. It is different from orthonasal aroma inhaled from the nostrils. It is thought that nosespace analysis of aroma compounds released from the nostrils and olfactory sensation during ingestion of foods and drinks have a high correlation [1]. Aroma extract dilution analysis (AEDA) and odor activity value (OAV) are widely known as methods to determine the contribution value for aroma compounds in a food by sniffing via the orthonasal route; both are very useful to indicate the aroma contribution [2]. Even though several studies reported calculation methods of retronasal OAV by using odor thresholds determined retronasally [3, 4], they did not indicate time changes of the aroma contribution. An aroma contribution on AEDA or OAV is just derived from an aroma compound's concentration in a target sample so that it does not indicate flavor perception nor time changes of aroma during consumption. Real-time measurement of volatile compounds using atmospheric pressure chemical ionization mass spectrometry (APCI-MS) or PTR-MS has been possible since the 1990s, and has been used to measure aroma release from foods or *in vivo* aroma release from nostrils [5, 6]. Few reports, however, referred to time changes of the contribution value of retronasal aroma. In this study, we developed a method which shows time changes of aroma contribution during the ingestion of foods and drinks and predicts the time changes of the perceived flavor balance.

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Experimental

Sample preparation for nosespace analysis and odor profile creation

Nineteen aroma compounds were selected from aroma compounds which are known to be present in a coffee drink, and were separated into several groups in order to analyze them with PTR-MS. Each mixture of compounds was dissolved in water. The concentration of each compound was adjusted to be detected reliably with PTR-MS until the measurement was finished.

Separately, for odor profile verification, a coffee model flavor was prepared with the nineteen aroma compounds mentioned above at an appropriate composition ratio as coffee flavor. The model flavor was added to water purified by ion-exchange at 0.1% w/w, and it was used as a model coffee drink.

Nosespace analysis with PTR-MS

A commercial PTR-MS instrument (Ionicon Analytic GmbH, Innsbruck, Austria) was used for nosespace analysis. Two subjects sucked each aqueous aroma solution (10 ml) through a straw and swallowed at once. Then, expiration from each subject's nostrils was measured for one minute under controlling their breathing pattern (once per 3 sec). Each compound released from the nostrils was introduced with a flow of 100 sccm into the drift tube (2.0 mbar, 105 °C, 480 V drift voltage). The E/N ratio was 136 Td. The mass ion counts were normalized to H_3O^+ ion counts. The behavior of each retronasal aroma compound analyzed with PTR-MS was changed over to breath-by-breath AUC [7]. The behavior of AUC was approximated as a power law function [8]. The results of two subjects' measurement were averaged.

Determination of retronasal thresholds

Nineteen aroma compounds were dissolved in water separately, and were stepwise diluted with dilution factor 10 (0.1ppt to 1000ppm). Two assessors drank aqueous aroma solutions in ascending order of concentrations. The lowest concentration of each aqueous aroma solution that the assessors perceived on average was determined as flavor threshold of the aroma compound, respectively. When subjects drink an aqueous aroma solution, there is a proportional relationship between an aroma concentration in water and the aroma concentration released from a nostril [8]. AUC of each compound at the 1st breath released from nostrils was defined as retronasal threshold when subjects drink an aqueous aroma solution at its threshold concentration.

Calculation of the contribution value of retronasal aroma (CRA)

The behavior of AUC of each aroma compound is shown as a power law function by nosespase analysis with PTR-MS. In the case of any aroma concentration in water, a power law function of each compound can be used, because previous reports demonstrated an approximate linear relationship between each aroma concentration in water and breath concentration of each compound [8]. Therefore, AUC at arbitrary breath can be calculated in proportion to the aroma concentration in water. The contribution value of retronasal aroma (CRA) was calculated to divide AUC at arbitrary breath by retronasal threshold.

Sensory evaluation of odor intensity

Seven trained panelists assessed the odor intensity of aroma attributes according to seven descriptors (malty, butter, nutty, roast, green coffee bean, brown sugar, smoky/medicine) on a seven-point scale from 0 (not perceivable) to 6 (strongly perceivable) in order to visualize the sensory profile during the ingestion of the model

coffee drink. Each odor intensity was evaluated immediately after swallowing the drink. In addition, each odor intensity was evaluated after about 30 seconds after swallowing the drink.

Results and discussion

Prediction of CRAs

First of all, AUC of the 1st breath of each compound was calculated from each concentration of 19 aroma compounds in water during the ingestion of the model coffee drink. AUC of the 10^{th} breath of each compound was calculated using the individual power law function. The 1^{st} breath is assumed to be right after swallowing the drink and 10^{th} breath is assumed to be after about 30 seconds from swallowing the drink. Then, CRA of each aroma compound was calculated by dividing AUC of 1^{st} and 10^{th} breath, respectively by retronasal threshold, and the results showed that CRA of 11 compounds exceeded 1.0 at 1^{st} breath CRA (Table 1). CRA of other compounds was smaller than 1.0. In other words, this shows that we are not able to recognize these compounds at the concentration level in the model coffee drink, because their AUC of 1^{st} breath was considered smaller than retronasal threshold.

Table 1: Contribution values of retronasal aroma (CRA) of 11 compounds in model coffee drink

Aroma compound	*Aroma Attributes*	*CRA (1^{st} Breath)*	*CRA (10^{th} Breath)*
2-Methylpropanal	Malty	10	0.1
2-Methylbutanal	Malty	36	0.1
3-Methylbutanal	Malty	40	0.2
Diacetyl	Butter	10	1.6
2-Ethyl-3-methylpyrazine	Nutty	4	1.3
2-Ethyl-3,5-dimethylpyrazine	Nutty	20	6.7
Furfuryl mercaptan	Roast	50	0.1
Furfuryl alcohol	Roast	10	2.6
2-Methoxy-3-isobutyl pyrazine	green coffee bean	50	3.9
Methyl cyclopentenolone	brown sugar	20	6.8
Guaiacol	smoky, medicine	10	2.3

Comparison of the sensory profile and the predicted profile

In order to verify the advantage of our procedure, we compared the CRAs and the results obtained from sensory evaluation. It is difficult to linearly compare the CRAs of aroma compounds with the sensory evaluation because the CRAs were derived from quantitative values and it would be predominated by Fechner law when converted to a predicted flavor profile. So, CRAs of the same aroma attribute were first summed up. Next, the total CRA's value of each aroma attribute was taken as a logarithmic value. In addition, the calculated logarithmic values at 1^{st} breath were multiplied by 2.1 so that the "roast" values in Figure 1A overlapped. And the values at 10^{th} breath were multiplied by 3.7 so that the "brown sugar" values in Figure 1B overlapped. Therefore, the predicted profile and the sensory profile can be easily compared visually.

Figure 1A shows a comparison of the sensory intensity at right after swallowing and the predicted profile at the 1st breath. Profiles resulted in almost overlapping profiles. Figure 1B shows a comparison of the sensory intensity at after about 30 seconds and the predicted value at the 10th breath. Both of them changed over time from Figure 1A. The predicted profile deviated partly from the sensory profile recorded after about 30 seconds. However, we think that the profiles are still similar at several aroma attributes like nutty, green coffee bean, brown sugar, and smoky/medicine.

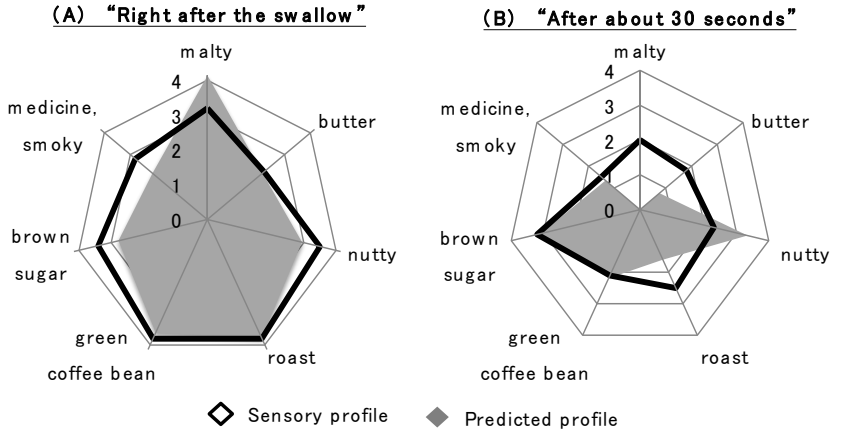

Figure 1: Comparison of sensory profile and predicted profile

Conclusion

We devised a method to predict aroma contributions and the time changes that we perceive after swallowing in the following sequence: *in vivo* measurements of retronasal aroma with PTR-MS, relationship between aroma concentration in water at swallowing and aroma concentration released from nostrils, then newly defined retronasal threshold derived from the relationship. The results of verification using the model coffee drink showed a good correlation between the predicted profile and the sensory evaluation. Therefore, it was suggested that this study is useful to indicate the real-time perceived flavor balance. On the other hand, there is an inevitable deviation between our prediction system and sensory evaluation because cross- or multi-modal sensory integration of olfaction and gustation has occurred during consumption [9]. Individual or genetic differences among subjects should be considered as well in future investigations.

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