Association between olfactory performance of individuals and their ability to smell odor-active compounds in complex chemical mixtures during the gas-chromatography-olfactometry (GC-O) analysis.

Giorgia Sollai[1], Iole Tomassini Barbarossa[1], Paolo Usai[2], Thomas Hummel[3], Roberto Crnjar[1]

[1] Department of Biomedical Sciences, Sect. of Physiology, University of Cagliari, Italy

[2] Department of Medical Sciences and Public Health, University of Cagliari, Italy; Presidio Policlinico of Monserrato, Cagliari, Italy.

[3] Smell and Taste Clinic, Department of Otorhinolaryngology, TU Dresden, Dresden, Germany

Corresponding author: Giorgia Sollai, Department of Biomedical Sciences, Section of Physiology, University of Cagliari, SP 8 Km 0.700, 09042 Monserrato (CA), Italy. E-mail: gsollai@unica.it; Phone: +39 070 6754160; Fax: +39 070 6754191

Abstract

Humans can accurately discern thousands of odorants, although there is a considerable inter-individual variability. Individuals can be classified as normosmic, hyposmic or anosmic, depending on their olfactory sensitivity or blindness. In this research we studied the olfactory sensitivity to banana head-space as a complex odor mixture in a group of 53 subjects classified for their olfactory status, by means of the "Sniffin' Sticks" extended test. Using the coupled Mass Spectrometry-Gas Chromatography-Olfactometric Detection Port (MS-GC-ODP3) technique, the single components of the banana flavour mixture were separated, identified and verbally evaluated by each subject. For each compound both the "odor type" and "odor descriptor" from data in the literature were reported, so that we could identify molecules that were defined as smelling of banana. The results show that the threshold olfactory score is linearly correlated with the number of total molecules and with the number of molecules smelling of banana. The intensity of the aroma of banana during the sniffing of pen #5 in the identification test is positively correlated with the number of molecules smelling of banana, the hedonic odor valence reported, but not with the mean intensity reported for molecules smelling of banana. Instead, the intensity for pen #5 was correlated with the intensity for isoamyl acetate odor, which is the molecule with the highest number of detections. In conclusion, our findings confirm the potentials of the GC-O methodological approach to identify odor-active compounds and show that human perception of single compounds is strongly conditioned by the olfactory status of the subject.

Keywords: physiological variations of olfactory performance; VARU intensity and hedonic valence; pleasantness; isoamyl acetate; Sniffin' Sticks test; olfaction.

1. Introduction

The sense of smell enables animals, from insects to humans, to detect and discriminate the odors in the environment where they live [1-2]. The ability to encode the intensity and quality of these odors allows animals to locate food sources, to identify mates and to avoid predators, permitting survival and reproduction [3-6]. In humans the olfactory function strongly influences the quality of life, playing a major role in the feeding behavior, in the ability to detect odors signaling dangers (e.g.: gas, smoke, spoiled food), in social communication (reproductive behavior, mother-child recognition), and in personal hygiene [7-8].

Most of the odors of food and drinks are mixtures of molecules; this means that some odors could be masked by others and therefore not be perceived, thus preventing access to the information that they carry. It has been reported that the odor of the most intense compound prevails in the mixture [9-10]. Many studies have shown that humans can reliably identify up to three components in mixtures of odorants [11-15], and that sommeliers, who are experts in olfactory identification, are not able to recognize more than 4 molecules within a mixture [16].

Recently, several studies were aimed at identifying single odor-active molecules within a mixture and the characteristics of their perception by humans, in order to improve the quality of food and perfumes, to make them more pleasant and desirable to consumers [17-18]. In fact, a very important part of food flavour is its aroma, which is the response of the olfactory epithelium to volatiles entering the nasal cavity [19]. In general, the aroma of a food consists of many odor-active volatile compounds, only a few of which are sensorially relevant. The objective of those studies was to identify odor-active compounds and to find a relationship between their qualitative and quantitative characteristics with human perception [17]. One of the major problems in the study of a food aroma is the identification of the compounds that really contribute to it [20-21].

The Gas Chromatography-olfactometry (GC-O) is an instrumental technique that uses human assessors to detect and evaluate volatiles eluting from a GC separation [22]. By means of this technique it is possible to evaluate the odor contribution of a single chemical component to the overall aroma; in fact, GC-O is a combination of sensory and instrumental analysis, allowing simultaneous chromatographic separation and odor evaluation by a human assessor [20,23]. This method of investigation has been widely used to provide useful information on which odor-active compounds are the major contributors to food aroma [20,24-25]. However, when doing this kind of investigation, one must keep in mind that there is a considerable inter-individual variability [26-27]. Individuals can be classified as normosmic, hyposmic or anosmic depending on whether they exhibit normal, reduced or absent ability to detect odors; anosmia can be general or specific [8,28].

On the basis of these considerations, the main goal of this study was to assess whether a relationship exists between the individual ability to detect the molecules isolated from a mixture and the subject's olfactory status.

To this end we chose to study the flavour of banana as an example of a complex chemical mixture epitomized in food industry by one specific molecule (isoamyl acetate also known as banana oil; hereafter IAA) [20,29-33]. So far, no study has focused on the relationship between human odor perception of odor-active compounds and general olfactory function. By means of the coupled Mass Spectrometry-Gas Chromatography-Olfactometry (MS-GC-O) technique, the single components of the banana aroma were separated, identified and verbally evaluated by each subject. All molecules were classified on the basis of their "odor type" and "odor descriptors", allowing us to identify the odor-active molecules described as smelling of banana. The individual ability to detect the number of molecules was correlated to the olfactory status of each subject previously classified as normosmic, hyposmic or functionally anosmic (hereafter referred to as "anosmia") by means of the "Sniffin' Sticks" extended test. Furthermore, we looked for a correlation between the intensity of the item #5 of the Odor Identification Test (one of

the sub-test of the Sniffin' Sticks extended test) containing the banana aroma and the number of molecules detected by each subjects, as well as its relationship with the intensity of the odor-active compounds. Finally, we investigated the presence of a correlation between the pleasantness attributed to pen #5 and its intensity.

2. Material and methods

2.1. Subjects

The study was done on fifty-three Caucasian healthy non-smoking volunteers (41 females and 12 males), aged 19-53 years, recruited in Cagliari (Sardinia, Italy). All subjects were asked to be perfume-free and with at least 2 hours of fasting prior to testing. Before any tests, they were informed of the experimental procedures and were asked to sign an informed consent. The study, approved by the local Ethical Committee, was conducted in accordance with the Declaration of Helsinki of 1975.

2.2. Olfactory sensitivity screening

Sniffin' Sticks Extended Test (SSET; Burghart Instruments, Wedel, Germany), based on odor-containing felt-tip pens and consisting of Threshold (T), Discrimination (D) and Identification (I) test, was used to evaluate the orthonasal olfactory function of each subject [34]. Odor presentation was conducted according to Hummel et al [35]. Using a single-staircase, 3-alternative forced choice paradigm, the olfactory threshold was assessed. The subject had to identify the pen containing the odorant among three: two pens contained the solvent and the third one the odorant (n-butanol). 16 triplets of pens containing increasing concentrations of n-butanol, were available to the experimenter. If the subject correctly identified twice in a row the pen containing the odorant, a reversal of the staircase was validated. The olfactory threshold is given by means of the dilution steps of the average of the last 4 reversals out of 7 (score assigned was between 1 and 16). 16 triplets of pens were also used to evaluate the olfactory

discrimination. For each triplet, the subject had to identify the target pen, which is the one containing the odor different from that of the other two pens. The number of correct responses represents the score obtained (from 0 to 16). The olfactory identification performance was assigned by means of 16 pens containing common odors. For each pen and by using a four-alternative forced choice, the subject had to identify the presented odor. The score obtained was the number of correct identifications.

The subjects were classified as normosmic, hyposmic or anosmic for their overall olfactory performance or their olfactory threshold, discrimination and identification performance, on the basis of the TDI or T, D, I score respectively obtained. The classification of the subjects was made by taking into account the reference values reported in previous studies [35].

Finally, for each odor smelled during the identification test, the subject marked on a visual analogue scale the hedonic valence and intensity. The relative scaling for "unpleasantness/pleasantness" was from -10 to +10 visual analogue rating units (VARUs), and for "low/high intensity" from 0 to +20 VARUs [36].

2.3. Dynamic headspace sampling

Volatile compounds were collected using the dynamic headspace method as described by Rizzolo et al [37] and Nuzzi et al [23]. In details, about 200 g of cut banana pulp were placed in an airtight 0.5-L glass vessel, flow-through system fitted to a Porapak Q (150/75mg, 50/80; Supelco) in a glass adsorption tube (5 mm Ø) inserted into the collection port on top of the vessel. Volatiles were collected at room temperature by flushing the system for 3 h with purified air at 30 L/h (550 ml/min). Trapped volatiles were eluted from the Porapak Q tube with 1.5 ml of 1-hexane, providing a solution that contained the isolated volatile compounds. Samples were then stored at -20 °C until used.

2.4. Mass Spectrometry - Gas Chromatography – Olfactometry (MS-GC-O) analysis

The analyses were performed in an Agilent 6890N gas chromatograph (Santa Clara, CA, USA) simultaneously coupled to a Gerstel ODP3 temperature programmable sniffing port (Mülheim an der Ruhr, Germany) with an olfactometry detection system and an Agilent model 5973 series mass spectrometer. The carrier gas was helium at constant flow of 1.2 mL/min. Injection volume was 1 µl and, at the output of the chromatographic column, the flow was splitted 1:1 between the ODP sniffing port and the MS detector.

The chromatographic column was a 30 m HP-INNOWax column, 0.25 mm internal diameter x 0.50 µm film thickness (Agilent 19091N-233; Agilent technologies, USA). The temperature of the injector and the MS interface temperature were set at 250 and 260 °C respectively. The oven temperature was maintained at 40 °C (0.2 min), 40°C/min to 90°C (0.50 min), 2°C/min to 150°C, 30°C/min to 230°C (12 min). The injector mode was splitless; the temperatures for the ion source and the quadrupole mass filter were 230°C and 150°C, respectively. Chromatograms were recorded by monitoring the total ion current in a 40-550 mass range. The transfer line to the GC-ODP3 sniffing port was held at 220°C.

The volatiles were identified by comparing the mass spectrum found in the MS Standard Library NIST2014 (US National Institute of Standards and Technology; Gaithersburg, MD, USA), and isomyl acetate was confirmed by analysing a reference compound (Sigma-Aldric, Milan, Italy). Information about the natural presence of odorants, “odor type” (i.e. fruity, floral, green, etc.) and “odor descriptors” (i.e. banana, apple, rose, wood, etc.) were obtained from the Good Scents Company Information System (www.thegoodscentscompany.com), according to Gonzales-Kristeller et al [38].

Prior to testing each panelist was characterized for his/her olfactory status (normosmic, hyposmic, anosmic) by means of the "Sniffin' Sticks" extended test, as previously described.

For the GC-O analyses, participants were asked to evaluate both the intensity and the duration of the compound while eluting [23,39]. The subject had a voice recording and digital signaling system

connected to the PC (GERSTEL ODP recorder 3 for Windows 7). The signaling system is characterized by the presence of 4 keys that represent a 4-point intensity scale: 1 = weak odor, 2 = distinct odor, 3 = intense odor, 4 = very intense odor. Whenever an odor was perceived, the subject pressed one of the keys of the signaling system and could express his/her subjective evaluation of the aroma intensity (based on which button was pressed), the duration of the stimulus, the degree pleasantness/unpleasantness and the description of the odor-active volatile compound. The PC automatically recorded the retention time and sniffing time of odor-active compound individually and, in this way, the olfactograms obtained overlap with the chromatograms (Fig. 1). The samples were presented completely blindly in order to avoid psychological conditioning.

2.5. Statistical analysis

The Pearson correlation test was used to evaluate the relationship between: a) the total number of molecules (from now on, total-molecules) or the number of molecules smelling of banana (from now on, banana-molecules) perceived by each subject and the T score; b) the intensity for the pen of the identification test containing the banana aroma (from now on, pen #5) and the number of total-molecules, the number of banana-molecules, the main intensity of banana-molecules and the intensity of IAA also known as banana oil, commonly added to foods and drinks to give the banana aroma); c) the hedonic valence attributed to pen #5 and its intensity.

Statistical analyses were performed using GraphPad Prism 6 (GraphPad Software, San Diego, CA, USA). P values < 0.05 were considered to be significant.

Post-hoc comparisons, subsequent to one-way ANOVA, were conducted with the Fisher's least significant difference (LSD) test. Statistical analyses were performed using STATISTICA for WINDOWS (version 7.0; StatSoft Inc, Tulsa, OK, USA). P values < 0.05 were considered to be significant.

3. Results

Table 1 shows that a total of 43 molecules were obtained from the extract of a banana sample using the dynamic headspace method. We classified the molecules by means of the information available from "The Good Scent Company System" on the organoleptic properties (odor type and odor descriptors) of each compound. On the basis of the odor type information we found 23 fruity molecules, 4 green, 3 floral, 2 herbal, 1 brown, 1 fatty, 1 spicy and 1 terpenic molecule. Instead, based on odor descriptors, 14 molecules were described as smelling of banana, 13 of which are of the fruity odor type, while 1 is of the herbal odor type. For 7 molecules we did not find any information regarding either the type or the odor descriptor: in particular, 3 of them were not identified, while the other 4 are not present in the Good Scent Company System. Table 2 shows that 33 of the 43 molecules found in the extract were odor-active and they were divided as follows: 19 fruity, 3 floral, 2 herbal, 2 green, 1 brown, 1 fatty and 1 spicy. Finally, only 12 of the 14 molecules described as smelling of banana were odor-active; in fact, none of the subjects smelled the odors of “acetic acid, hexyl ester” (fruity) and “butanoic acid, 1-methyloctyl ester” (fruity), reported as n. 17 and n. 20 respectively in Table 1. Among the odor-active molecules the detection frequency was highest (N = 36) for isoamyl acetate (1-butanol, 3-methyl, acetate), high for 2-pentanol, acetate (N = 28) and propanoic acid, 2-methyl, 3-methylbutyl ester (N = 23), intermediate for butanoic acid 3-methyl, 3-methylbutyl ester (N = 17) and isopentyl hexanoate (N = 17), and gradually lower for all others. As reported in Table 2, the subjects described the odor of the molecules they perceived during the GC-O experiment as fruity or floral, and in particular they described 8 of the 12 odor-active banana-molecules as actually smelling of banana.

Examples of the chromatogram and related aromagram obtained in response to volatiles from banana extract eluting from chromatographic separation during the GC-O experiment by a normosmic and an hyposmic subject, are shown in Fig. 1. In order to assess if the ability to smell volatiles by each subject

was correlated with his/her olfactory performance, we tested for a correlation between the number of odor-active compounds and the T score of each subject, by means of the Pearson correlation test. In detail, correlation analyses shown in figures 2 indicated that the T score was positively correlated with the number of total-molecules (Pearson $r = 0.18$, $p = 0.001$) and of banana-molecules smelled by each subject (Pearson $r = 0.53$, $p < 0.0001$).

The Pearson correlation test also revealed that the intensity of pen #5 reported by each subject was positively correlated with both the number of banana-molecules smelled by each subject and the intensity of IAA (Pearson $r > 0.29$, $p < 0.05$), but not with the number of total-molecules smelled by each subject (Pearson $r = 0.18$, $p = 0.10$) and the mean intensity reported for banana-molecules (Pearson $r = 0.14$, $p = 0.31$) (Fig. 3). Besides, the Pearson correlation test has also revealed a positive correlation between the hedonic valence and the intensity value that each subject attributed to pen #5 (Pearson $r = 0.42$, $p = 0.002$) (Fig. 4). Finally, we found that the mean value of the hedonic valence perceived for pen #5 increased significantly ($p = 0.022$; Fisher's LSD test subsequent to one-way ANOVA) from 2.33 ± 0.97 to 6.53 ± 0.62 depending on whether the participants smelled the IAA odor as pleasant or unpleasant, respectively.

4. Discussion

The olfactory system has a wide sensitivity and discriminatory power, even if the exact number and order of magnitude of olfactory stimuli that humans can detect is still unknown [40]. Indeed, due to a combination of environmental, genetic and cultural factors [41-46], humans present a considerable inter-individual variability [47]. Besides, the ability to discriminate different compounds depends on whether they are single or in a mixture. In fact, it is known that humans have an extraordinary ability to discriminate one mixture from another, but at the same time have difficulty in identifying more than 3 or 4 compounds within a mixture [11-12,14,48]. The odors that surround us rarely consist of a single molecule, but more often are mixtures of many volatiles. By means of the Gas Chromatography-

Olfactometry (GC-O) technique it is possible to perform a sensory analysis of single volatile compounds, separated by the GC, using the human nose as a sensor. Since it is not known whether the human nose, as a sensor, presents inter-individual variability, the main scope of this study was to evaluate the presence of a correlation between the ability to smell single molecules as they are eluted from the gas chromatograph and the olfactory performance of subjects. In particular, the results we obtained show that the threshold olfactory performance of the subjects was positively correlated with the number of total-molecules and banana-molecules being odor-active compounds for each of participants. In order to establish the number of compounds that are odor-active, we used the frequency detection method [25], by which the number of individuals who smell an odor is counted [49]. The detection frequency method has been reported as being the simplest because it does not require the use of qualified assessors and the results obtained reflect the inter-individual differences [50-51]. By means of the detection frequency method, we also found that only 12 of the 14 banana-molecules were odor-active compounds for subjects who participated in this study. In addition, during the GC-O experiment, they provided an odor description based only on their experience and olfactory memory. Subjects defined 8 of the 12 odor-active banana-molecules as actually smelling of banana. Probably not all banana-molecules were identified as such, because the participants were not informed of the mixture injected into the GC column. This means that they did not have a mental representation of the odor. In fact, the visual representation has been shown to exert a great influence on the formation of the quality odor percept [52].

The second aim of the study was to evaluate whether the intensity of the banana aroma sniffed from pen #5 in the identification sub-test was correlated with the number of total-molecules and/or banana-molecules smelled by each subject, and whether a correlation existed with the intensity reported for the banana-molecules. The results show a positive correlation with the number of banana-molecules smelled, but not with the number of total-molecules, and even more interestingly, the intensity of pen #5 is not correlated with the average intensity of banana-molecules, but only with that of IAA. This result

252 reinforces the theory according to which compounds smelled most frequently are also those that have the
253 greatest influence in determining the odor of a given aroma [51]. In fact, IAA is the odor-active
254 compound that obtained the highest number of detections, having been smelled by 36 subjects out of 53.
255 Even though the aroma of banana is represented by a very complex mixture of esters, IAA among them,
256 commonly known as “banana oil”, it has been reported as a key component of the banana fruity odor and
257 is used as an additive to give the aroma of banana to foods and beverages [20,29-33,53].
258 Humans perceive the odors in the environment where they live in a rather variable way and this variability
259 applies not just to the overall olfactory performance, but also to the ability to perceive specific odors and
260 the way in which they are perceived [26,54]. In fact, the perceived quality and pleasantness reported for
261 some odors differ greatly among individuals [27,55]. Since IAA was the most relevant compound in the
262 banana aroma, we evaluated whether the pleasantness reported for pen #5 depends on that reported for
263 IAA. The results showed a significant effect of the pleasantness reported for IAA on the pleasantness
264 reported for pen #5: in fact, the subjects who defined IAA as pleasant, attributed a greater pleasure also
265 to the aroma of banana.
266 Finally, we found that the pleasantness and intensity reported for the aroma of banana during the sniffing
267 of pen #5 are positively correlated. Similar results were found in a previous study about the relationship
268 between the pleasantness and the intensity reported for 10 diverse chemical stimuli, with the IAA
269 molecule among them [56].
270 In conclusion, our findings, while confirming the potentials of the GC-O methodological approach to
271 identify odor-active compounds deriving from food and to study the human perception of them, highlight
272 the fact that human perception of single compounds is strongly conditioned by the olfactory status of the
273 subject, thus suggesting the presence of inter-individual variations in the perception of compounds that
274 are odor-active. The results also clearly show that olfactory function was positively associated with the
275 number of perceived individual odorous compounds. Furthermore, the results of this work could be of

great interest to the food or perfume industry, which continually seeks to identify odor-active compounds to make their products more pleasant and desirable to consumers [17-18].

Conflict of interests: All authors declare no conflict of interest.

Funding: This work was supported by a grant from the University of Cagliari (Fondo Integrativo per la Ricerca, FIR 2017-2018) and a grant from the Deutsche Forschungsgemeinschaft to TH (DFG HU411/18-1).

Acknowledgments

The Authors thank the volunteers without whose contribution this study would not have been possible.

References

[1] Sollai G, Solari P, Masala C, Crnjar R, Liscia A. Effects of avermectins on olfactory responses of Culicoides imicola (Diptera: Ceratopogonidae). J Med Entomol (2007) 44:656-659. DOI: 10.1603/0022-2585(2007)44[656:eoaoor]2.0.co;2.

[2] Li H, Wang P, Zhang L, Xu X, Cao Z, Zhang L. Expressions of olfactory proteins in locust olfactory organs and a palp odorant receptor involved in plant aldehydes detection. Front Physiol (2018) 9: 663. doi: 10.3389/fphys.2018.00663.

[3] Solari P, Crnjar R, Frongia A, Sollai G, Secci F, Spiga M, Masala C, Liscia A. Oxaspiropentane derivatives as effective sex pheromone analogues in the gypsy moth: electrophysiological and behavioral evidence. Chem Senses (2007) 32:755-763. doi:10.1093/chemse/bjm043.

[4] Su CY, Menuz K, Carlson JR. Olfactory perception: receptors, cells, and circuits. Cell (2009) 139:45-59. doi: 10.1016/j.cell.2009.09.015.

[5] Sollai G, Solari P, Loy F, Masala C, Crnjar R, Liscia A. Morpho-functional identification of abdominal olfactory receptors in the midge Culicoides imicola. J Comp Physiol A (2010) 196:817-824. DOI 10.1007/s00359-010-0561-1.

[6] Sollai G, Solari P, Crnjar R. Olfactory sensitivity to major, intermediate and trace components of sex pheromone in Ceratitis capitata is related to mating and circadian rhythm. J Insect Physiol (2018) 110:23-33. doi.org/10.1016/j.jinsphys.2018.08.007.

[7] Hummel T, Nordin S. Olfactory disorders and their consequences for quality of life. Acta Otolaryngol (2005) 125(2):116-21.

[8] Croy I, Nordin S, Hummel T. Olfactory Disorders and Quality of Life-An Updated Review. Chem Senses (2014) 39:185-194. doi: 10.1093/chemse/bjt072.

[9] Ferreira V. Revisiting psychophysical work on the quantitative and qualitative odour properties of simple odour mixtures: a flavour chemistry view. Part 1: intensity and detectability. A review. Flavour Frag J (2012a) 27:124–140. doi.org/10.1002/ffj.2090.

[10] Ferreira V. Revisiting psychophysical work on the quantitative and qualitative odour properties of simple odour mixtures: a flavour chemistry view. Part 2: qualitative aspects. A review. Flavour Frag J (2012b) 27:201–215. doi.org/10.1002/ffj.2091.

[11] Laing DG, Francis GW. The capacity of humans to identify odors in mixtures. Physiol Behav (1989) 46:809–814.

[12] Laing DG, Glenmarec A. Selective attention and the perceptual analysis of odour mixtures. Physiol Behav (1992) 52:1047-1053.

[13] Laing DG, Link C, Jinks A, Hutchinson I. The limited capacity of humans to identify the components of taste mixtures and taste-odor mixtures. Perception (2002) 31:617-635. DOI: 10.1068/p3205.

[14] Livermore BA, Laing DG. The influence of training and experience on the perception of multi-component mixtures. J Exp Psychol Hum Percept Perform (1996) 22:267-277.

[15] Jinks A, Laing DG. A limit in the processing of components in odour mixtures. Perception (1999) 28:395-404. DOI: 10.1068/p2898.

[16] Poupon D, Fernandez P, Boisvert SA, Migneault-Bouchard C, Frasnelli J. Can the identification of odorants within a mixture be trained? Chem Senses (2018) 43:721-726. DOI: 10.1093/chemse/bjy060.

[17] Brattoli M, Cisternino E, De Gennaro G, Giungato P, Mazzone A, Palmisani J, Tutino M. Gas chromatography analysis with olfactometric detection (GC-O): an innovative approach for chemical characterizatio of odor active volatile organic compounds (VOCs) emitted from a consumer product. Chem Eng Trans (2014) 40:121-126. DOI: 10.3303/CET1440021.

[18] Schilling B, Kaiser R, Natsch A, Gautschi M. Investigation of odors in the fragrance industry. Chemoecol (2010) 20:135-147. DOI : 10.1007/s00049-009-0035-5.

[19] Baigrie B (2003) Taints and off-flavours in food. Edition Brian Baigrie. Cambridge (UK): Woodhead Publishing Limited.

[20] Jordán MJ, Tandon K, Shaw PE, Goodner KL. Aromatic Profile of Aqueous Banana Essence and Banana Fruit by Gas Chromatography-Mass Spectrometry (GC-MS) and Gas Chromatography-Olfactometry (GC-O). J Agric Food Chem (2001) 49:4813-4817. DOI: 10.1021/jf010471k.

[21] Blank I, Devaud S, Fay LB, Cerny C, Steiner M, Zurbriggen B (2001) Odor-active compounds of dry-cured meat: Italian-type salami and Parma ham. In: Takeoka GR, Günter M, Engel KH, editors. Aroma Active Compounds in Foods. Vol. 794. Washington: ACS Symposium Series. 9-20.

[22] Delahunty CM, Eyres G, Dufour JP. Gas-chormatography-olfactormetry. J Sep Sci (2006) 29:2107-2125. DOI 10.1002/jssc.200500509.

[23] Nuzzi M, Lo Scalzo R, Testoni A, Rizzolo A. Evaluation of fruit aroma quality: comparison between gas chromatography-olfactormetry (GC-O) and odour activity value (OAV) aroma patterns of strawberries. Food Anal Methods (2008) 1:270-282. DOI 10.1007/s12161-008-9039-y.

[24] Mayol AR, Acree TE (2001) Advances in gas chromatographyolfactometry. In: Schieberle P, Buettner A, Acree TE, editors. Gas Chromatography-Olfactometry. The State of the Art. Washington DC: Leland, J.V. American Chemistry Society. 1-10.

[25] Ruth SM. Methods for gas chromatography–olfactometry: A review. Biomol Eng (2001) 17:121-128.

[26] Amoore JE. Specific anosmia: a clue to the olfactory code. Nature (1967) 214:1095–1098.

[27] O'Connell RJ, Stevens DA, Akers RP, Coppola DM, Grant AJ. Individual-differences in the quantitative and qualitative responses of human subjects to various odors. Chem Senses (1989) 14:293–302. doi.org/10.1093/chemse/14.2.293.

[28] Feldmesser E, Bercovich D, Avidan N, Halbertal S, Haim L, Gross-Isseroff R, Goshen S, Lancet D. Mutations in Olfactory Signal Transduction Genes Are Not a Major Cause of Human Congenital General Anosmia. Chem Senses (2007) 32(1):21-30. doi:10.1093/chemse/bjl032.

[29] Aurore G, Ginies C, Ganou-Parfait B, Renard CMGC, Fahrasmane L. Comparative study of free and glycoconjugated volatile compounds of three banana cultivars from French West Indies: Cavendish, Frayssinette and Plantain. Food Chem (2011) 129:28-34. doi: 10.1016/j.foodchem.2011.01.104.

[30] Eisenmenger MJ, Reyes-De-Corcuera JI. Enhanced synthesis of isoamyl acetate using an ionic liquid–alcohol biphasic system at high hydrostatic pressure. J Mol Catal B-Enzym (2010) 67:36-40. doi: 10.1016/j.molcatb.2010.07.002.

[31] Romero MD, Calvo L, Alba C, Daneshfar A, Ghaziaskar HS. Enzymatic synthesis of isoamyl acetate with immobilized Candida Antarctica lipase in n-hexane. Enzyme Microb Tech (2005) 37:42-48. doi: 10.1016/j.enzmictec.2004.12.033.

[32] Wolfson A, Saidkarimov D, Dlugy C, Tavor D. Green synthesis of isoamyl acetate in glycerol triacetate. Green Chem Let Rev (2009) 2(2):107-110. DOI: 10.1080/17518250903170850.

[33] Zhu X, Li Q, Li J, Luo J, Chen W, Li X. Comparative study of volatile compounds in the fruit of two banana cultivars at different ripening stages. Molecules (2018) 23:2456. doi: 10.3390/molecules23102456.

[34] Hummel T, Sekinger B, Wolf SR, Pauli E, Kobal G. "Sniffin' sticks": olfactory performance assessed by the combined testing of odor identification, odor discrimination and olfactory threshold. Chem Senses (1997) 22:39-52.

[35] Hummel T, Kobal G, Gudziol H, Mackay-Sim A. Normative data for the "Sniffin' Sticks" including tests of odor identification, odor discrimination, and olfactory thresholds: an upgrade based on a group of more than 3,000 subjects. Eur Arch Otorhinolaryngol (2007) 264:237-243. doi: 10.1007/s00405-006-0173-0.

[36] Fischer M, Zopf Y, Elm C, Pechmann G, Hahn EG, Schwab D, Kornhuber J, Thuerauf NJ. Subjective and objective olfactory abnormalities in Crohn's disease. Chem Senses (2014) 39:529-538. doi: 10.1093/chemse/bju022.

[37] Rizzolo A, Polesello A, Polesello S. Use of headspace capillary GC to study the development of volatile compounds in fresh fruit. HRC-J High Res Chrom (1992) 1992:472-477. doi.org/10.1002/jhrc.1240150713.

[38] Gonzalez-Kristeller DC, doNascimento JBP, Galante PAF, Malnic B. Identification of agonists for a group of human odorant receptors. Front Pharmacol (2015) 6:35. doi: 10.3389/fphar.2015.00035.

[39] Van Ruth SM, O'Connor CH. Evaluation of three gas chromatography-olfactometry methods: comparison of odour intensity-concentration relationships of eight volatile compounds with sensory headspace data. Food Chem (2001) 74(3):341-347. DOI: 10.1016/S0308-8146(01)00142-X.

[40] Gerkin RC, Castro JB. The number of olfactory stimuli that humans can discriminate is still unknown. Elife (2015) 4: e08127. DOI: 10.7554/eLife.08127.

[41] Calderón-Garcidueñas L, Franco-Lira M, Henríquez-Roldán C, Osnaya N, González-Maciel A, Reynoso-Robles R, Villarreal-Calderon R, Herritt L, Brooks D, Keefe S, Palacios-Moreno J, Villarreal-Calderon R, Torres-Jordan R, Medina-Cortina H, Delgado-Chavez R, Aiello-Mora M, Moronpot RR, Doty RL. Urban air pollution: influences on olfactory function and pathology in exposed children and young adults. Exp Toxicol Pathol (2010) 62:91-102. doi: 10.1016/j.etp.2009.02.117.

[42] Hasin-Brumshtein Y, Lancet D, Olender T. Human olfaction: from genomic variation to phenotypic diversity, Cell 25 (2009) 178-184. doi: 10.1016/j.tig.2009.02.002.

[43] Menashe I., Abaffy T., Hasin Y., Goshen S., Yahalom V., Luetje C.W., Lancet D. Genetic elucidation of human hyperosmia to isovaleric acid. Plos Biology (2007) 11, e284. https://doi.org/10.1371/journal.pbio.0050284.

[44] Seo HS, Guarneros M, Hudson R, Distel H, Min BC, Kang JK, Croy I, Vodicka J, Hummel T. Attitudes toward olfaction: a cross regional study. Chem Senses (2010) 36:177-87. doi: 10.1093/chemse/bjq112.

[45] Silva-Teixeira C.S, Cerqueira N.M.F.S.A., Silva Ferreira A.C. Unravelling the olfactory sense: from gene to odor perception. Chem. Senses 41 (2016) 105-121. doi: 10.1093/chemse/bjv075.

[46] Sollai G, Melis M, Magri S, Usai P, Hummel T, Tomassini Barbarossa I, Crnjar R. Association between the rs2590498 polymorphism of Odorant Binding Protein (OBPIIa) gene and olfactory performance in healthy subjects. Behav Brain Res (2019) 372:112030. doi.org/10.1016/j.bbr.2019.112030.

[47] Hummel T, Whitcroft KL, Andrews P, Altundag A, Cinghi C, Costanzo RM, Damm M, Frasnelli J, Gudziol H, Gupta N, Haehne A, Holbrook E, Hong SC, Hornung D, Huttenbrink KB, Kamel R, Kobayashi M, Konstantinidis I, Landis BN, Leopold DA, Macchi A, Miwa T, Moesges R, Mullol J, Mueller CA, Ottaviano G, Passali GC, Philpott C, Pinto JM, Ramakrishnan VJ, Rombaux P, Roth Y, Schlosser RA, Shu B, Soler G, Stjarne P, Stuck BA, Vodicka J, Welge-Luessen A. Position paper on olfactory dysfunction. Rhinol. (2017) S54:1-30. doi: 10.1007/s00415-008-0807-9.

[48] Livermore A, Laing DG. The influence of odor type on the discrimination and identification of odorants in multicomponent odor mixtures. Physiol Behav (1998) 65(2):311–320.

[49] Fur Y, Mercurio V, Moio L, Blanquet J, Meunier JM. A new approach to examine the relationships between sensory and gas chromatography–olfactometry data using generalized procrustes analysis applied to six French Chardonnay wines. J Agr and Food Chem (2003) 51:443-452. DOI: 10.1021/jf0205458.

[50] Dussort P, Deprete N, Bou-Maroun E, Fant C, Guichard E, Brunerie P, Le Fur Y, Le Quere J-L. An original approach for gas chromatography-olfactometry detection frequency analysis: application to gin. Food Res Int (2012) 49:253-262. doi: 10.1016/j.foodres.2012.07.011.

[51] Plutowska B, Wardencki W. Application of gas chromatography – olfactometry (GC – O) in analysis and quality assessment of alcoholic beverages - A review. Food Chem (2008) 107:449-463. DOI: 10.1016/j.foodchem.2007.08.058.

[52] Jadauji JB, Djordjevic J, Lundström JN, Pack CC. Modulation of olfactory perception by visual cortex stimulation. J Neurosci (2012) 32(9):3095–3100. doi: 10.1523/JNEUROSCI.6022-11.2012.

[53] Ishii-Foret A, Roudnitzky N, Beno N, Bensafi M, Hummel T, Rouby C, Thomas-Danguin T. 2008. Synergy and masking in odor mixtures: an electrophysiological study of orthonasal vs. retronasal perception. Chem Senses (2008) 33:553-561. doi:10.1093/chemse/bjn022.

[54] Keller A, Hempstead M, Gomez IA, Gilbert AN, Vosshall LB. An olfactory demography of a diverse metropolitan population. BMC Neurosci (2012) 13:122. doi: 10.1186/1471-2202-13-122.

[55] Stevens DA, O'Connell RJ. Semantic-free scaling of odor quality. Physiol Behav (1996) 60:211-215.

[56] Doty RL. An examination of relationships between the pleasantness intensity and concentration of 10 odorous stimuli. Percept Psychophys (1975) 17(5):492-496.

[57] Bugaud C, Alter P. Volatile and non-volatile compounds as odour and aroma predictors in dessert banana (Musa spp.). Postharvest Biol Technol (2016) 112:14-23. DOI: 10.1016/j.postharvbio.2015.10.003.

[58] Pino J, Febles Y. Odour-active compounds in banana fruit cv. Giant Cavendish. Food Chem (2013) 141:795-801. doi: 10.1016/j.foodchem.2013.03.064.

Figure legends

Figure 1. Examples of the chromatogram and related aromagram obtained in response to volatiles from banana extract eluting from chromatographic separation during the GC-O experiment by a normosmic (A) and an hyposmic (B) subject.

Figure 2. Correlation analysis between the T score and the number of total molecules smelled by each subject (A; total-molecules) and the number of molecules described in the literature as smelling of banana (B; banana-molecules).

Figure 3. Correlation analysis between the intensity of pen #5 and the number of total molecules smelled by each subject (A; total-molecules), the number of molecules described in the literature as smelling of banana (B; banana-molecules), the mean of the intensity reported for the molecules described as smelling of banana (C), the intensity from 0 to +4 reported for isoamyl acetate (D; IAA intensity). VARUs = Visual Analogue Rating Units from 0 to +20 for low/high intensity for pen #5.

Figure 4. Correlation analysis between the hedonic valence and the intensity reported by each subject for pen #5 of the identification test. VARUs = Visual Analogue Rating Units; from 0 to +20 for low/high intensity and from -10 to +10 for “unpleasantness/pleasantness” of pen #5.

Table 1. Headspace volatile compounds detected in freshly cut banana fruit

N.	Compound	RT[a]	Odor type[b]	Odor descriptors[b]
1	Isobutyl acetate*	6.59	Fruity	Sweet, banana, tropical
2	Butanoic acid, ethyl ester*	6.98	Fruity	Juicy fruit, pineapple, banana, cognac
3	2-Pentanol, acetate*	7.61	Herbal	Banana, green, orange juicy
4	Propanoic acid, 2-methyl-, 2-methylpropyl ester*	7.94	Fruity	Pineapple, grape skin, tropical, banana
5	Isoamyl acetate*	8.71	Fruity	Sweet, banana, fruity
6	Propanoic acid, 2-methyl-, butyl ester*	9.31	Fruity	Sweet, tuti frutti, melon, banana, citrus
7	Butanoic acid, 2-methylpropyl ester*	9.66	Fruity	Sweet, pineapple, rum, apple, berry
8	2-Hexanol, acetate	9.83	Fruity	Apple, pear, sour
9	Butanoic acid, 2-methyl-, 2-methylpropyl ester*	10.12	Fruity	Sweet, fruity
10	Butanoic acid, 3-methylbutyl ester*	10.62	Fruity	Green, apricot, pear, banana
11	Butanoic acid, 1-methylbutyl ester*	11.24	Fruity	Sweet, banana, pineapple, cherry, tropical
12	Butanoic acid, butyl ester*	11.50	Fruity	Fruity, banana, pineapple, sweet
13	Butyl 2-methylbutanoate	11.94	Green	Fruity, Cocoa
14	Pentanoic acid, 2-pentyl ester	12.16	Fruity	Ripe, fruity, apple
15	2-Heptanol, acetate*	12.95	Brown	Fenugreek, fruity, fatty, green
16	Propanoic acid, 2-methyl-, 3-methylbutyl ester*	13.31	Fruity	Waxy, apricot, pineapple, green, banana
17	Acetic acid, hexyl ester*	13.47	Fruity	Green, apple, banana, sweet
18	p-Cymene*	13.85	Terpenic	Citrus, terpenic, woody, spicy
19	Butanoic acid, 3-methyl-, 3-methylbutyl ester*	14.24	Fruity	Sweet, green, ripe, apple, tropical
20	Butanoic acid, 1-methyloctyl ester*	14.56	Fruity	Sweet, banana, apricot
21	4-Hexen-1-ol, acetate*	14.83	-	Found in banana fruit
22	Acetic acid, 1,4-dimethylpent-4-enyl ester*	15.01	-	No information
23	2-Hexen-1-ol, acetate, (Z)-*	15.95	-	No information
24	4-Hexen-1-ol, (4E)-, acetate*	16.48	-	Found in banana fruit
25	Hexanoic acid, 2-methylpropyl ester*	16.69	Fruity	Sweet, pineapple, green apple, peach
26	Butanoic acid, 2-methyl-, 3-methylbutyl ester*	17.06	Fruity	Sweet, citrus, cherry, blueberry, apple
27	Butanoic acid, 1-methylhexyl ester*	18.74	Fruity	Green, vegetable, cheesy, walnut
28	Unknown	19.41	-	No information
29	Butanoic acid, hexyl ester*	19.73	Green	Sweet, fruity, apple, waxy, soapy
30	Butanoic acid, 3-methyl-, hexyl ester*	21.12	Fruity	Sweet, apple, unripe, strawberry
31	Isopentyl hexanoate*	21.90	Fruity	Banana, apple, pineapple, green
32	Acetic acid, octyl ester*	22.81	Floral	Waxy, mushroom, green, fruity, apple
33	Butanoic acid,4-hexen-1-yl ester*	23.66	Green	Fresh, green apple, fruity
34	3-Octen-1-ol, acetate, (Z)-*	24.77	Herbal	Bergamot, woody, grapefruit, rose, apple
35	Unknown	25.31	-	No information
36	Cyclohexaneethanol, acetate	25.74	Floral	Mint, rose, raspberry, green
37	Unknown	28.02	-	No information
38	Propanoic acid, 2-methyl-, 1-methylbutyl ester*	30.88	-	Found in banana fruit
39	Propanoic acid, 2-methyl-, 2-methylbutyl ester	32.38	Fruity	Fruity, ethereal, tropical, banana
40	5-Octen-1-ol, (Z)-*	33.16	Green	Fresh, watermelon, melon
41	3-Decen-2-ol, (Z)-	33.94	Fatty	Green, fruity, apple, earthy, jasmine
42	Linolenic acid	35.34	Floral	Sweet, fresh, gentle, soft, mild
43	Elemicin*	44.23	Spicy	Spicy, flower

[a] RT = retention time in I-Wax column.

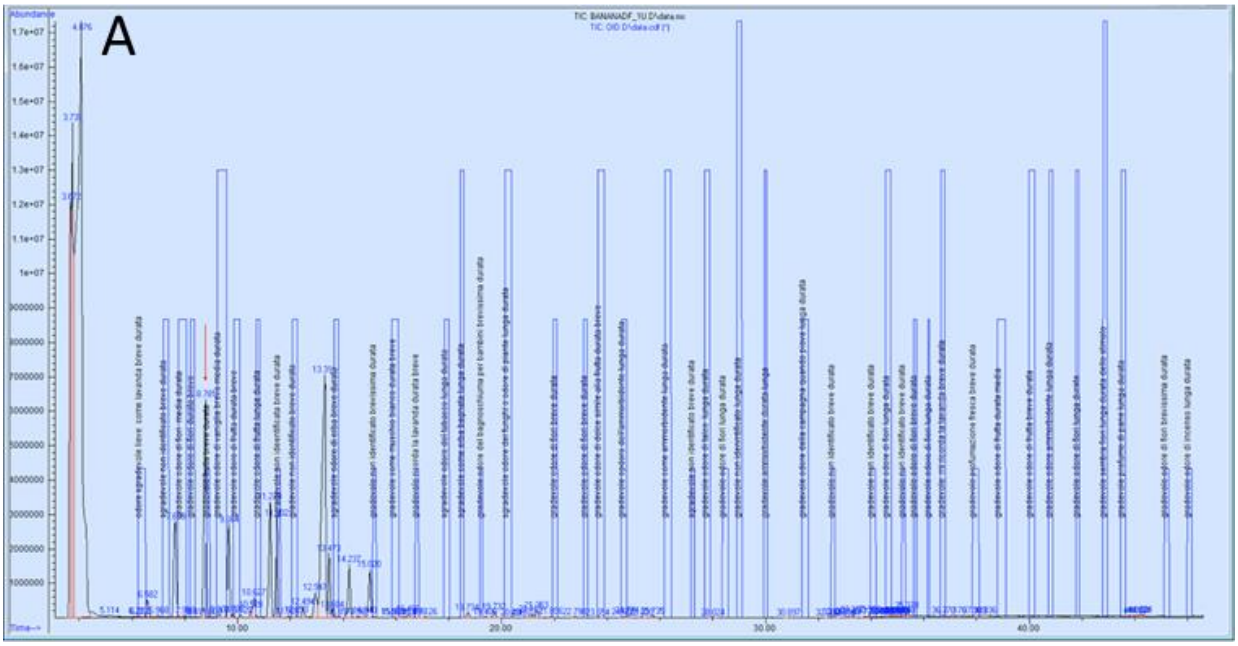
[b] Odor type and odor descriptors from the Good Scent Company Information System (www.thegoodscentscompany.com).

Asterisks indicate the molecules that have also been found in other banana extracts. References: [20,29,33,57-58]

Table 2. GC-O analysis: odor-active compounds and odor descriptions by subjects.

N.	Odor-active compound	Odor description	df
1	Isobutyl acetate	Sweet, fruity, caramel, banana	6
2	Butanoic acid, ethyl ester	Sweet, fruity, wine, banana, strawberry, orange	15
3	2-Pentanol, acetate	Fruity, floral, banana, strawberry, chewing-gum, red wine	28
4	Propanoic acid, 2-methyl-, 2-methylpropyl ester	Floral, sweet	5
5	Isoamyl acetate	Banana, fruity, sweet, sugar, strawberry, orange, vanilla	36
6	Propanoic acid, 2-methyl-, butyl ester	Sweet, floral, vanilla, fruity	5
7	Butanoic acid, 2-methylpropyl ester	Fruity, sweet, alcohol, rose	7
10	Butanoic acid, 3-methylbutyl ester	Banana, fruity, sweet	6
11	Butanoic acid, 1-methylbutyl ester	Fruity, sweet, vanilla	4
12	Butanoic acid, butyl ester	Fruity, sweet, banana	10
14	Pentanoic acid, 2-pentyl ester	Sweet, fruity	4
15	2-Heptanol, acetate	Fruity	3
16	Propanoic acid, 2-methyl-, 3-methylbutyl ester	Fruity, sweet, banana, wood, strawberry, blueberry	23
19	Butanoic acid, 3-methyl-, 3-methylbutyl ester	Sweet, herbal, blueberry	17
22	Acetic acid, 1,4-dimethylpent-4-enyl ester	Sweet, floral, white musk	8
25	Hexanoic acid, 2-methylpropyl ester	Floral, lemon	7
26	Butanoic acid, 2-methyl-, 3-methylbutyl ester	Sweet, herbal, fruity	4
27	Butanoic acid, 1-methylhexyl ester	Floral, sweet, cheesy, spicy, musty, fruity	14
28	unknown	Garlic	2
29	Butanoic acid, hexyl ester	Sweet, floral, soapy, mushroom	11
30	Butanoic acid, 3-methyl-, hexyl ester	Sweet, floral, rose	13
31	Isopentyl hexanoate	Banana, green, floral	17
32	Acetic acid, octyl ester	Banana, floral, vanilla	10
33	Butanoic acid,4-hexen-1-yl ester	Fruity, sweet	2
34	3-Octen-1-ol, acetate, (Z)-	Sweet, floral, strawberry, rose	11
36	Cyclohexaneethanol, acetate	Mint, rose, floral, food, orange	11
37	unknown	Floral, strawberry, fruity, sweet	6
38	Propanoic acid, 2-methyl-, 1-methylbutyl ester	Floral, wet earth	13
39	Propanoic acid, 2-methyl-, 2-methylbutyl ester	Sweet, banana	4
40	5-Octen-1-ol, (Z)-	Fresh, floral, orange	4
41	3-Decen-2-ol, (Z)-	Floral, fruity	5
42	Linolenic acid	Floral, fresh, citrus fruits	5
43	Elemicin	Floral, wood	11

Odor-active compounds: list of compounds eluted by the GC during GC-O experiments that were smelled by at least one of the subjects who participated in the study.
Odor description indicates the specific description that each subject gave of the odor smelled during the GC-O experiment.
df = detection frequency (number of subjects who smelled the compound).



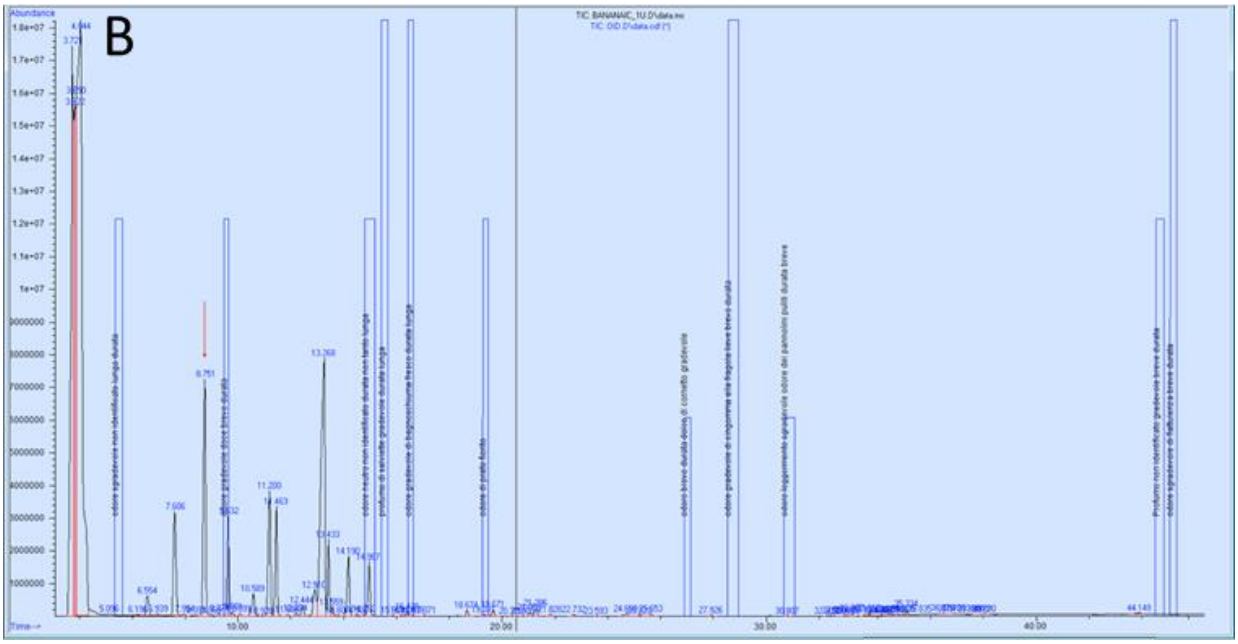


Figure 1

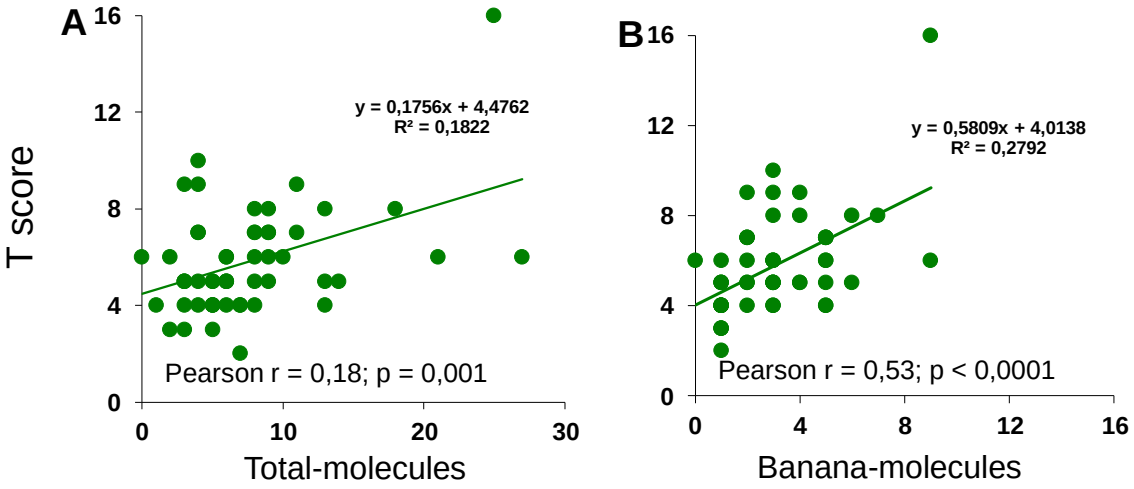

A
B
T score
y = 0,1756x + 4,4762
R² = 0,1822
Pearson r = 0,18; p = 0,001
Total-molecules
y = 0,5809x + 4,0138
R² = 0,2792
Pearson r = 0,53; p < 0,0001
Banana-molecules
0
4
8
12
16
20
30

Figure 2

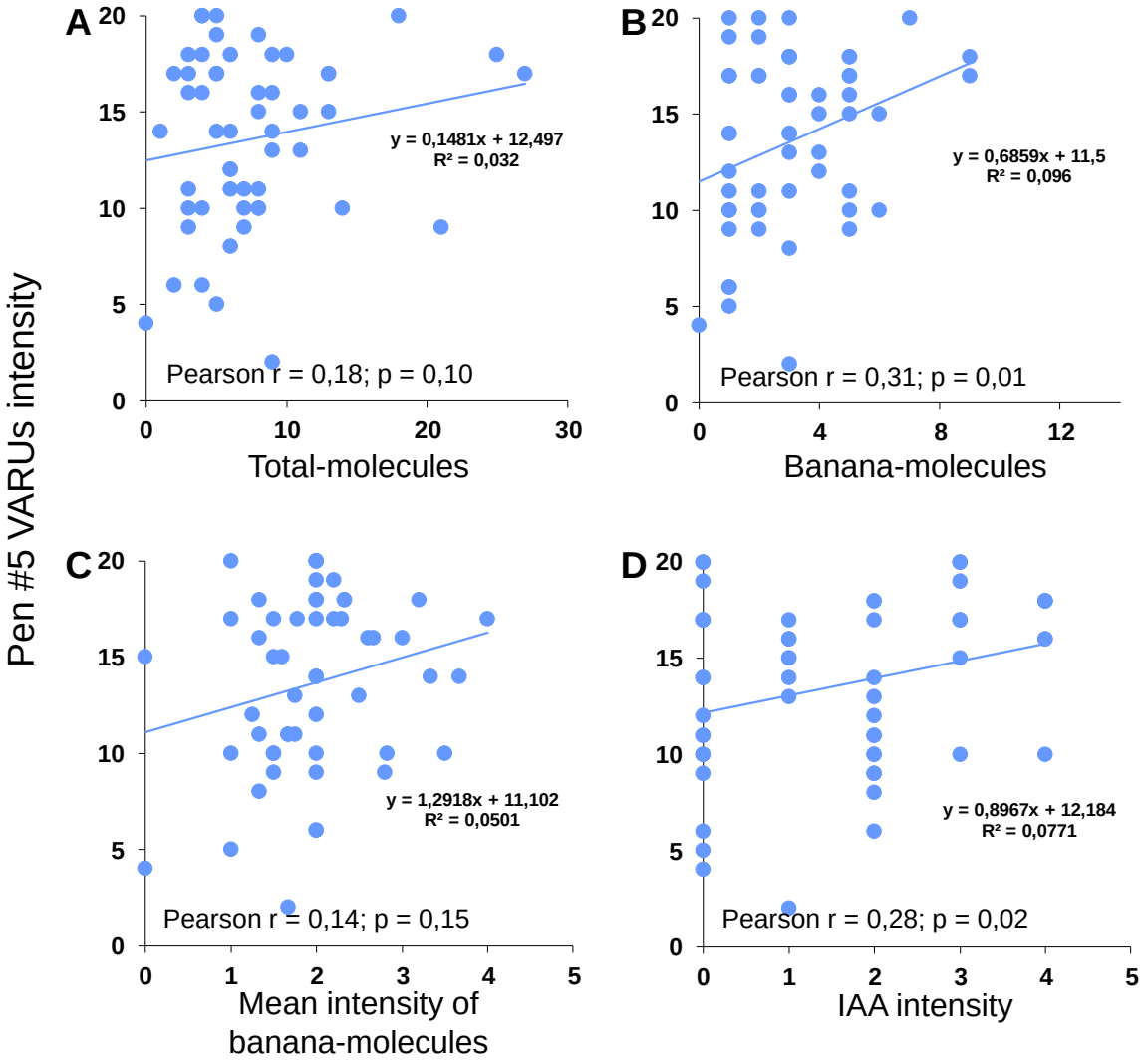

A
y = 0,1481x + 12,497
R² = 0,032
Pearson r = 0,18; p = 0,10
Total-molecules
B
y = 0,6859x + 11,5
R² = 0,096
Pearson r = 0,31; p = 0,01
Banana-molecules
C
y = 1,2918x + 11,102
R² = 0,0501
Pearson r = 0,14; p = 0,15
Mean intensity of banana-molecules
D
y = 0,8967x + 12,184
R² = 0,0771
Pearson r = 0,28; p = 0,02
IAA intensity
Pen #5 VARUs intensity

Figure 3

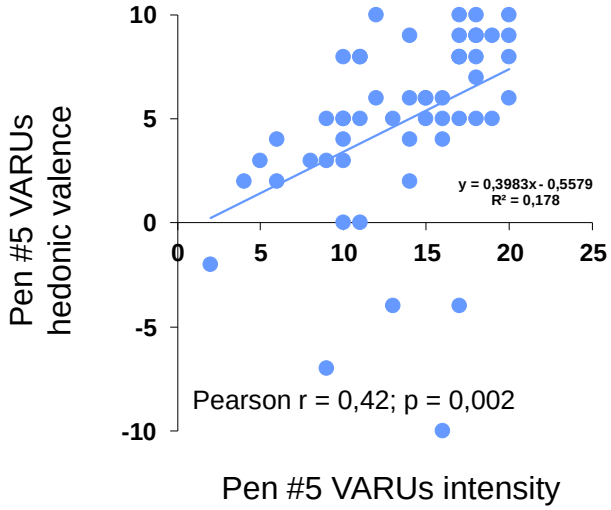

Pen #5 VARUs hedonic valence
10
5
0
-5
-10
0
5
10
15
20
25
y = 0,3983x - 0,5579
R² = 0,178
Pearson r = 0,42; p = 0,002
Pen #5 VARUs intensity

Figure 4