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The Human Nose as a Chemical Sensor in the Perception of Coffee Aroma: Individual Variability

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Abstract: The flavor of foods and beverages is generally composed of a mixture of volatile compounds, however not all the molecules that form an aroma are sensorially relevant. The odor-active compounds present in a mixture are different for each subject, both in quantitative and qualitative terms. This means that the ability of the human nose to act as a chemical sensor varies among individuals. In this study, we used the headspace of roasted coffee beans as a complex olfactory stimulus and, by means of the coupled Gas Chromatography-Olfactometry (GC-O) technique, the single components of coffee flavor were separated. Each subject, previously classified for his/her olfactory status (normosmic, hyposmic or anosmic) by means of the Sniffin' Sticks battery (composed of Threshold, Discrimination and Identification subtests), had to identify and evaluate each smelled molecule. The results show that the individual ability to detect individual compounds during the GC-O experiments and the odor intensity reported during the sniffing of pen #10 (the pen of the identification test) containing coffee aroma were related to TDI olfactory status (based on the score obtained from the sum composed of Threshold, Discrimination and Identification scores). We also found that the number of total molecules and of molecules smelling of coffee is linearly related to the TDI olfactory score. Finally, the odor intensity reported when sniffing pen #10 containing coffee aroma is positively correlated with the number of molecules detected and the average intensity reported. In conclusion, our findings show that the human perception of both individual compounds and complex odors is strongly conditioned by the olfactory function of subjects.

Keywords: inter-individual physiological variations; olfactory function; VARU intensity; Sniffin' Sticks test; olfaction

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1. Introduction

All living organisms are able to sense the chemicals present in the environment where they live to obtain useful information on the availability of energy-rich food sources, potential mating partners and on the presence of predators [1–8]. All odorous molecules, whether natural or synthetic and whether perceived as pleasant or unpleasant, are highly volatile and can activate the olfactory receptors present in the human nose or in the olfactory organs of all animals [8–14]. In particular, in humans the perception of odors has been observed to affect the quality of life, exerting a relevant influence on eating habits of individuals and consequently on their body weight, on the ability to perceive molecules that signal the presence of dangers (e.g., toxic and/or harmful gases, smoke and spoiled food) and in interpersonal relationships [15–20].

Most odors found in nature, such as those of food and drink, are rarely formed by individual compounds; more commonly they are complex mixtures, composed of multiple molecules, only some of which are sensorially relevant [21–23]. An important challenge is therefore represented by the understanding of which molecules within a mixture are perceived, and thus constitute the odor-active compounds, and which remain irrelevant from a sensory point of view. This problem can be solved using the gas chromatography-olfactometry (GC-O) technique which simultaneously uses the chromatographic column to

separate the individual compounds within a mixture and the human nose as a chemical sensor capable of perceiving and evaluating the volatiles coming out of the olfactometric port [24–28]. In addition to understanding olfaction at a physiological level, many attempts have been recently made to detect odorants and volatile organic compounds by means of artificial olfactory sensor technology, which was widely introduced in many fields, such as environmental monitoring, detection of food conditions and clinical diagnostics [29–31]. Semiconductor metal oxides such as SnO_2 and TiO_2, for instance, are largely employed as gas sensors for a number of favorable functional properties like good stability and sensitivity combined with low cost [32,33].

An important aspect that should not be underestimated is that the ability to perceive individual compounds is directly correlated with the olfactory function of individuals who, as known, present a great variability [34–36]. In fact, as a result of physiological, genetic, behavioral and environmental factors, humans can be classified as normosmic (normal olfactory function), hyposmic (reduced olfactory function) or functionally anosmic (olfactory blindness, which can be general or specific) [16,37–50]. It is known that the human olfactory system has a high potential in terms of discrimination and sensitivity even if, at present, the number of stimuli that humans are able to perceive has not yet been quantified [51]. Furthermore, the information available on the potential of the human nose as a chemical sensor in relation to the olfactory function of individuals is still lacking, in terms of odor-active compounds, in the intensity at which they are perceived and in our ability to identify and/or associate them with the aroma of the mixture to which they belong [26,36,52].

Based on these considerations, this study had three different but interconnected objectives, with the aim of better understanding the relationship between the olfactory function of individuals, in terms of normosmia or hyposmia, and the ability of the human nose to act as a chemical sensor of individual molecules belonging to a complex mixture, both in quantitative (number of molecules perceived) and qualitative (intensity of perception) terms. The first objective regards the effect of the olfactory function of individuals on their ability to smell the individual compounds of the complex aroma of coffee as they are eluted from the chromatographic column and the correlation between the number of odor-active compounds and the TDI olfactory score obtained by each subject. With the second objective we evaluated whether the intensity reported by subjects for the odor of coffee contained in the pen #10 of the identification test (one of the subtests of the Sniffin' Sticks battery) was correlated with the number of odor-active compounds and with the average intensity with which they were perceived. Finally, we investigated the effect of olfactory status (normosmic vs. hyposmic) on perceived intensity for pen #10 and its correlation with the TDI olfactory score obtained by each participant.

2. Materials and Methods

2.1. Subjects

Thirty-eight Caucasian healthy non-smoking volunteers (24 females, 14 males, 28.8 ± 1.61 y), recruited in Cagliari (Sardinia, Italy), took part in this study. To estimate the ability to detect the odor-active compounds, during the GC-O analysis we used the detection frequency method, which does not require the presence of qualified evaluators and the results it produces represent the inter-individual variability [24,28,53–55].

All participants fasted for at least 90 min prior to testing and were free of perfume. Before starting the olfactory tests, the experimental protocol approved by the local Ethical Committee was read to them and they were asked to sign an informed consent (Prot. PG/2018/22 of 2 January 2018).

2.2. Olfactory Sensitivity Screening

The olfactory function of each individual was evaluated by means of the TDI olfactory score given by the sum of the scores obtained with the tests of Threshold (T-test), Discrimination (D-test) and Identification (I-test), which represent the sub-

tests of the Sniffin' Sticks test (Burghart Instruments, Wedel, Germany), based on odor-containing felt-tip pens [56]. According to the reference values reported by Hummel et al. [57], based on the total TDI olfactory score obtained, and on sex and age, each participant was classified as normosmic, hyposmic or functionally anosmic. For each olfactory test the score is between 0 and 16. For the T-test, the score is given by the average of the last 4 reversals out of 7, while for the D-test and I-test the score is given by the number of correct discriminations and identifications (for details visit: https://www.uniklinikum-dresden.de/de/das-klinikum/kliniken-polikliniken-institute/hno/forschung/interdisziplinaeres-zentrum-fuer-riechen-und-schmecken/neuigkeiten/downloads).

For the coffee-odor of pen #10 presented during the I-test, the subjects must also give a personal evaluation of the perceived intensity, marking a sign on the "Visual Analogue Rating Units" (VARUs) scale, ranging from 0 to 20 VARUs [58].

2.3. Dynamic Headspace Sampling

The dynamic headspace method, as described by Rizzolo et al. [59] and Nuzzi et al. [27], was used to collect the volatile compounds. In terms of volatiles, the dynamic headspace method is considered the most appropriate for obtaining an extract whose composition is closely linked to the quality of the scent as assessed by the consumer [60]. In addition, it has the ability to acquire extracts for GC-MS and sensory assessment via GC-O analysis by a human assessor [27].

In detail, approximately 100 g of roasted coffee beans were placed in a 0.5 L airtight glass vessel, with a flow-through system fitted to a Porapak Q (150/75 mg, 50/80; Supelco; St. Louis, MO, USA) in a glass adsorption tube (5 mm Ø) inserted into the collection port at the top of the vessel. By flushing the system with purified air for three hours at a rate of 30 L/h (500 mL/min), volatiles were recovered at room temperature. Using 1.5 mL of 1-hexane, trapped volatiles were released from the Porapak Q tube, resulting in a solution containing the isolated volatile chemicals. Samples were then stored at −20 °C until used. By performing three GC runs 24 h after sample preparation and before they were used for GC-O experiments, the effectiveness of the extraction and the reproducibility of the chromatogram were confirmed. Besides, to verify that the sample was not altered, before each section of the GC-O experiment, a GC-run without any sniffing session was made. The fact that the volatile chemical profile observed in this study is remarkably comparable to the headspace volatile profiles published in the literature data provides evidence of its validity [61–69].

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

An Agilent 6890N gas chromatograph (GC; Agilent technologies; Santa Clara, CA, USA) was simultaneously connected with an Agilent model 5973 series mass spectrometer (MS) and with an olfactometry detection system (Gerstel ODP3; Mülheim an der Ruhr, Germany) to perform the analyses. A constant flow of 1.2 mL/min of He was used as carrier gas. The flow was split 1:1 between the olfactometry and the MS detector at the outlet of the chromatographic column and the injection volume was 1 µL [36].

The chromatographic column was a 30 m HP-INNOWax, 0.25 mm internal diameter × 0.50 µm film thickness (Agilent 19091N-233; Agilent technologies, Santa Clara, CA, USA). The temperature of the injector and the MS interface temperature were set at 250 °C 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 [36].

To identify the volatiles, we used the mass spectrum found in the MS Standard Library NIST2014 (US National Institute of Standards and Technology; Gaithersburg,

MD, USA). In accordance with Gonzales-Kristeller et al. [70], the Good Scents Company Information System (www.thegoodscentscompany.com; 20 January 2023) was used to obtain information regarding odorant natural occurrence, "odor type" (i.e., roasted, floral, woody, etc.) and "odor descriptors" (i.e., coffee, fruit, cheese, wood, etc.).

The "Sniffin' Sticks" test, as previously described, was used to characterize the olfactory function of each panelist, prior to testing. Participants were asked to assess the volatile strength and duration during elution for the GC-O analyses [27,71] by using a PC-connected audio recorder and digital signaling system (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. The participant pressed one of the signaling system keys whenever he/she detected an odor to express his/her subjective assessments of the aroma intensity (based on which button was pressed), the stimulus duration, the degree of pleasantness or unpleasantness and the description of the odor-active compound. The chromatograms were overlaid with the obtained olfactograms after the PC automatically recorded the retention time and sniffing time of each odor-active compound. The samples were presented completely blind to avoid psychological conditioning.

2.5. Statistical Analysis

The Pearson correlation test was used to evaluate the relationship between: (a) the total number of odor-active compounds (hereafter, total-compounds) or the number of odor-active compounds smelling of coffee (hereafter, coffee-compounds) smelled by each subject and his/her TDI olfactory score; (b) the intensity perceived by each subject for the pen of the identification test containing the coffee aroma (hereafter, the coffee-odor pen) and his/her TDI olfactory score, (c) the number of total- and coffee-molecules smelled and the intensity perceived for the coffee-odor pen by each subject, (d) the reported average intensity for total- and coffee-molecules and the perceived intensity for the coffee-odor pen by each subject. The correlation coefficient "r" was considered to measure the strength of the linear relationship or straight-line between two variables: $r < 0.3$ means lower correlation, $0.3 < r < 0.7$ means medium correlation, $r > 0.7$ means higher correlation [72,73]. Statistical analyses were performed using GraphPad Prism 6 (GraphPad Software, San Diego, CA, USA). A statistically significant correlation was defined with a p-value < 0.05.

One-way ANOVA was used to analyze the effect of the TDI olfactory status of the subject on: (a) his/her ability to smell individual compounds during the GC-O analysis and (b) the intensity referred for the coffee-odor pen. Post-hoc comparisons, following one-way ANOVA, were conducted with the Fisher's test of least significant difference (LSD). Statistical analyses were performed using STATISTICA for WINDOWS (version 7.0; StatSoft Inc., Tulsa, OK, USA). p values < 0.05 were considered significant.

3. Results

3.1. Volatile Compounds of Coffee Aroma

The 50 volatile compounds obtained from the extract of the roasted coffee beans by means of the dynamic headspace method are listed in Table 1. Using the information from "The Good Scent Company System" on the organoleptic properties, we classified each volatile for its odor type and odor descriptors. On the basis of the odor type information, we found nine nutty compounds, five fruity, two buttery, two bready, two fishy and one for each of the following odor type: coffee, chocolate, sweet, potato, creamy, caramellic, cheesy, roasted, winey, citrus, green, vegetable, waxy, musty, balsamic, aromatic, phenolic, acidic and terpenic. Based on odor descriptors, 21 compounds were identified as smelling of coffee, eight of which are of the nutty odor type, three are of the fruity odor type, two are of the bready odor type, one is of the phenolic, fishy, coffee, balsamic buttery, caramellic, roasted or vegetable odor type. In the Good Scent Company System, we did not find any information regarding odor type for 11 compounds (no. 1, 2, 7, 18, 33, 35, 36, 38, 44 and 47 in Table 1) and for 7 regarding odor descriptor (no. 1, 6, 33, 35, 36, 44 and 47 in Table 1).

Table 1. Volatile compounds found in the headspace of roasted coffee beans.

N.	Compound	RT [a]	Odor Type [b]	Odor Descriptors [b]
1	Octane, 3,5-dimethyl-	8.10	-	-
2	Oxalic acid, isobutyl nonyl ester	8.33	-	Bland, mild, caramellic
3	Toluene	8.54	Phenolic	Solventy, woody, roasted, coffee
4	β-Pinene	10.20	Terpenic	Sweet, fresh, pine, woody, hay, green
5	Ethylbenzene	10.69	-	Petroleum-like odor
6	p-Xylene	10.90	Aromatic	-
7	Oxalic acid, isobutyl pentyl ester	11.85	-	Bland, mild, caramellic
8	Pyridine *	12.55	Fishy	Sour, sickening, putrid, coffee
9	D-Limonene *	12.81	Citrus	Citrus, orange, fresh, sweet
10	Furan,2-pentyl- *	13.30	Fruity	Fruity, green, earthy, beany, vegetable, metallic
11	Pyrazine, methyl- *	15.65	Nutty	Coffee, cocoa, roasted, chocolate, peanut, green, nutty brown, musty, earthy
12	Acetoin	16.50	Buttery	Sweet, creamy, green, butter, dairy, milk, fatty, buttery, creamy, sour, fatty, vanilla
13	2-Propanone, 1-hydroxy-	17.28	Sweet	Pungent, sweet, caramellic, ethereal
14	Pyrazine, 2,5-dimethyl- *	18.11	Chocolate	Cocoa, roasted nutty, beefy roasted, beefy, woody, grassy, medicinal
15	Pyrazine, ethyl- *	18.67	Nutty	Peanut, butter, musty, woody, roasted, cocoa, coffee
16	Pyrazine, 2,3-dimethyl- *	19.30	Nutty	Musty, nut skin, cocoa, powdery, caramellic, roasted, potato, coffee, peanut, butter,
17	DL-2,3-Butanediol *	19.81	Creamy	Fruity, creamy, buttery
18	Vinyl butyrate	20.02	-	Organic solvent
19	Hex-4-yn-3-one, 2,2-dimethyl-	20.67	Winey	Chemical, winey, fruity, fatty, terpenic, cauliflower
20	Pyrazine, 2-ethyl-6-methyl- *	21.08	Potato	Roasted, potato
21	Pyrazine, 2-ethyl-3-methyl- *	22.09	Nutty	Nutty, peanut, musty, corn, raw, coffee, bready
22	Pyrazine, 2-(n-propyl)- *	22.89	Green	Green, vegetable, nutty
23	Pyrazine, 2,6-diethyl- *	23.59	Nutty	Nutty, potato, cocoa, roasted, coffee
24	Pyrazine, 3-ethyl-2,5-dimethyl- *	24.08	Nutty	Potato, cocoa, roasted, nutty, coffee
25	2-Propanone, 1-(acetyloxy)-	24.70	Fruity	Fruity, buttery dairy, nutty
26	Pyrazine, 2-ethyl-3,5-dimethyl- *	25.01	Nutty	Peanut, caramellic, coffee, cocoa
27	Furfural *	25.27	Bready	Sweet, brown, woody, caramellic, bread baked, coffee, almond
28	Pyrazine, tetramethyl-	25.71	Nutty	Nutty, musty, chocolate, coffee, cocoa, brown, lard, burnt, dry, vanilla
29	Pyrazine, 3,5-diethyl-2-methyl- *	26.66	Nutty	Nutty, meaty, vegetable
30	Pyrazine, 2-ethenyl-5-methyl-	27.10	Coffee	Coffee
31	Furan, 2-acetyl- *	27.67	Balsamic	Sweet, balsamic, almond, cocoa, coffee, caramellic, nutty, brown, toasted, milky, lactonic

Table 1. *Cont.*

N.	Compound	RT [a]	Odor Type [b]	Odor Descriptors [b]
32	2,3-Pentanedione *	28.21	Buttery	Pungent, sweet, buttery, creamy, nutty, caramellic, cheesy, coffee
33	2-Butanone, 1-(acetyloxy)-	28.52	-	-
34	2-Furanmethanol, acetate *	28.81	Fruity	Coffee, sweet, fruity, banana, horseradish, roasted, cocoa
35	Pyrazine, 2-methyl-6-(2-propenyl)-	29.37	-	-
36	2-Cyclopenten-1-one, 2,3-dimethyl-	30.12	-	-
37	Acetic acid, diethyl- *	30.56	Acidic	Acidic, fruity, whiskey, dry berry, dairy, tropical
38	Pentanoic acid, 4-oxo-, methyl ester	31.05	-	Caramellic, flavouring agent
39	2-Furancarboxaldehyde, 5-methyl- *	31.54	Caramellic	Sweet, caramellic, bready, brown, coffee, spicy, maple
40	2-Furanmethanol, propanoate *	32.16	Fruity	Sweet, fruity, green, banana, oily, coffee, spicy
41	Furan, 2,2′-methylenebis- *	32.81	Fruity	Rich, roasted, coffee
42	2-Furanmethanol *	34.17	Bready	Sulfurous, estery, chemical, musty, sweet, brown, caramellic, bready, coffee, alcoholic
43	Butanoic acid, 3-methyl- *	34.35	Cheesy	Cheesy, dairy, acidic, sour, pungent, ripe, fatty, fruity, stinky feet, sweaty, tropical
44	Furan, 2-(2-furanylmethyl)-5-methyl- *	34.68	-	-
45	Pyrazine, 2-acetyl-6-methyl	35.33	Roasted	Roasted, coffee, cocoa, popcorn
46	4(H)-Pyridine, N-acetyl- *	35.76	Fishy	Sour, fishy, putrid, ammoniacal
47	Octaethylene glycol monododecyl ether	36.21	-	-
48	2-Hexadecanol	36.38	Waxy	Waxy, clean, greasy, floral, oily
49	N-Furfurylpyrrole *	37.95	Vegetable	Plastic, green, waxy, fruity, coffee, vegetable
50	2-Acetylpyrrole *	40.68	Musty	Musty, nut, skin, cherry, maraschino, cherry, bready, coumarinic, licorice, walnut

[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 coffee extracts [56–64].

Table 2 shows that 47 compounds found in the extract were odor-active for at least two of the participants; in fact, the "ethylbenzene" and "furan, 2-(2-furanylmethyl)-5-methyl" (indicated with no. 5 and 44 in Table 1) was found to be active for just one individual; while, the "2-Butanone, 1-(acetyloxy)-" (indicated with no. 33 in Table 1) it was not perceived by any of the participants. Furthermore, the panelists described 21 of the odor-active compounds as smelling of coffee, even though the odor descriptors reported in Table 1 define only 17 of them as actually having a coffee odor.

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

N.	Odor-Active Compound	Odor Description	df
1	Octane, 3,5-dimethyl-	Woody, unknown	2
2	Oxalic acid, isobutyl nonyl ester	Burnt, unknown	2
3	Toluene	Coffee, smoked, solvent, roasted	8
4	β-Pinene	Sweet, floral, vanilla	8
5	Ethylbenzene	Petrol	1
6	p-Xylene	Vanilla, medicinal, floral	5
7	Oxalic acid, isobutyl pentyl ester	Floral, fruity, vanilla	6
8	Pyridine *	Coffee, smoked, roasted, cheese	3
9	D-Limonene *	Sweet, sour, citrus	6
10	Furan,2-pentyl- *	Smoked	2
11	Pyrazine, methyl- *	Coffee, nutty, roasted, smoke	3
12	Acetoin	Coffee, sweet, roasted, parfum	10
13	2-Propanone, 1-hydroxy-	Sweet, pungent, fish, solvent, wet	10
14	Pyrazine, 2,5-dimethyl- *	Coffee, citrus, medicinal, sweet, cocoa	7
15	Pyrazine, ethyl- *	Coffee, nutty, egg	3
16	Pyrazine, 2,3-dimethyl- *	Coffee, burnt, caramellic, fruity	5
17	DL-2,3-Butanediol *	Sweet, caramellic, rose, wet	5
18	Vinyl butyrate	Floral, parfum, bitter, solvent, pungent	7
19	Hex-4-yn-3-one, 2,2-dimethyl-	Sweet, solvent	4
20	Pyrazine, 2-ethyl-6-methyl- *	Coffee, sweet, smoked, medicinal, solvent, parfum, roasted	19
21	Pyrazine, 2-ethyl-3-methyl- *	Coffee, cocoa, solvent, bitter, nutty, roasted	25
22	Pyrazine, 2-(n-propyl)- *	Green, musty, woody, earthy, wet, herbs, floral	22
23	Pyrazine, 2,6-diethyl- *	Coffee, roasted, earthy, musty, burnt, mushrooms	25
24	Pyrazine, 3-ethyl-2,5-dimethyl- *	Coffee, nutty, roasted, floral, bitter	20
25	2-Propanone, 1-(acetyloxy)-	Pungent, parfum	6
26	Pyrazine, 2-ethyl-3,5-dimethyl- *	Coffee, musty, roasted, wet	21
27	Furfural *	Coffee, sweet, solvent, floral, pungent	13
28	Pyrazine, tetramethyl-	Coffee, roasted, burnt, vanilla	13
29	Pyrazine, 3,5-diethyl-2-methyl- *	Floral, musty, wet, solvent, fresh	15
30	Pyrazine, 2-ethenyl-5-methyl-	Coffee, nutty, bitter, plastic	14
31	Furan, 2-acetyl- *	Parfum	2
32	2,3-Pentanedione *	Floral, herbs, earthy, sweat, musk, cheese, pungent	24
34	2-Furanmethanol, acetate *	Roasted, fruit, earthy, herb, woody, coffee	21
35	Pyrazine, 2-methyl-6-(2-propenyl)-	Pungent, sour, bitter	6
36	2-Cyclopenten-1-one, 2,3-dimethyl-	Sweet, floral, lavender	4
37	Acetic acid, diethyl- *	Roasted, solvent, rotten, musty, herbs, wet earth	22
38	Pentanoic acid, 4-oxo-, methyl ester	Sweet	4
39	2-Furancarboxaldehyde, 5-methyl- *	Coffee, sweet, parfum	4
40	2-Furanmethanol, propanoate *	Coffee, pungent, floral, musty, herb, sweet, burnt	14
41	Furan, 2,2′-methylenebis- *	Coffee, nutty, popcorn, roasted, fish, sour	21
42	2-Furanmethanol *	Coffee, smoke, popcorn, nutty, roasted	24
43	Butanoic acid, 3-methyl- *	Cheese, smoke, stinky feet, acidic, fruity, putrid	16
44	Furan, 2-(2-furanylmethyl)-5-methyl- *	Unknown	1
45	Pyrazine, 2-acetyl-6-methyl	Putrid, musty, cheese	6
46	4(H)-Pyridine, N-acetyl- *	Shoes, wet, sweat	7
47	Octaethylene glycol monododecyl ether	Sweat, acidic	4
48	2-Hexadecanol	Cheese, musty, putrid, plastic	29
49	N-Furfurylpyrrole *	Solvent, cheese, musty	15
50	2-Acetylpyrrole *	Coffee, roasted, almond, sweet, burnt, parfum, fresh	29

Odor-active compounds: list of compounds eluted from the chromatography column and smelled by at least one participant. Odor description: personal description given by each subject for the odor smelled. df = detection frequency (number of participants who smelled the compound). Asterisks indicate the molecules that have also been found in other coffee extracts [56–64].

3.2. Olfactory Function and Odor-Active Compounds

One-way ANOVA showed that the number of molecules perceived for both total and smelling of coffee is significantly higher for normosmic individuals than for hyposmic ones (total-molecules: F (1,36) = 16.19, $p = 0.0003$; coffee-molecules: F (1,36) = 24.25, $p < 0.0001$) (Figure 1).

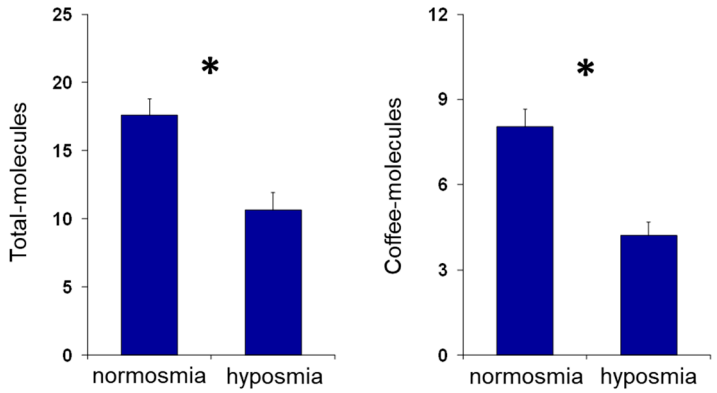

Figure 1. Mean (±SEM) values on the number of total- and coffee-molecules smelled during the GC-O experiments by each subject, according to their TDI olfactory status. Asterisk indicates significant differences between individuals with normosmia or hyposmia ($p < 0.0005$; Fisher's LSD test subsequent to one-way ANOVA).

The Pearson correlation test was used to investigate for a correlation between the number of odor-active compounds and the TDI olfactory score reached by each subject. In detail, the results shown in Figure 2 indicated that TDI olfactory score was positively correlated with both the number of total-molecules (Pearson r = 0.56, $p = 0.0002$) and of coffee molecules smelled by each subject (Pearson r = 0.55, $p = 0.0004$).

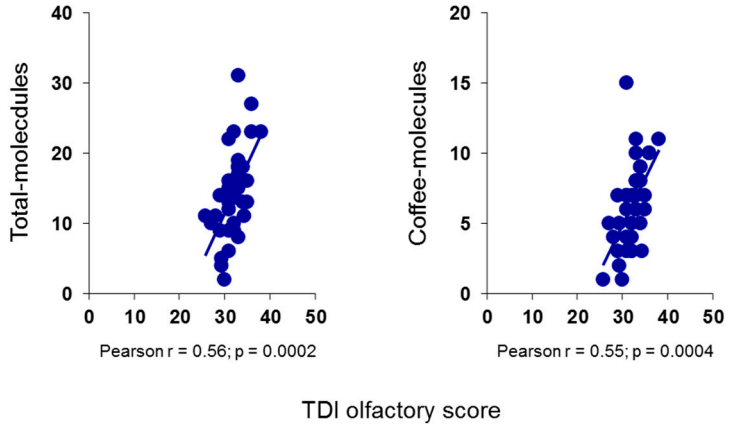

Figure 2. Correlation analysis between the number of total- and coffee-molecules smelled by each subject and his/her TDI olfactory score.

Besides, a positive correlation was found between the intensity value that each subject attributed to the coffee-odor pen and the number of odor-active compounds (total-molecules: Pearson r = 0.67, $p < 0.0001$; coffee-molecules: Pearson r = 0.65, $p < 0.0001$) (Figure 3) and the average intensity referred for total- and coffee-molecules (total-molecules: Pearson r = 0.56, $p = 0.0003$; coffee-molecules: Pearson r = 0.62, $p < 0.0001$) (Figure 4).

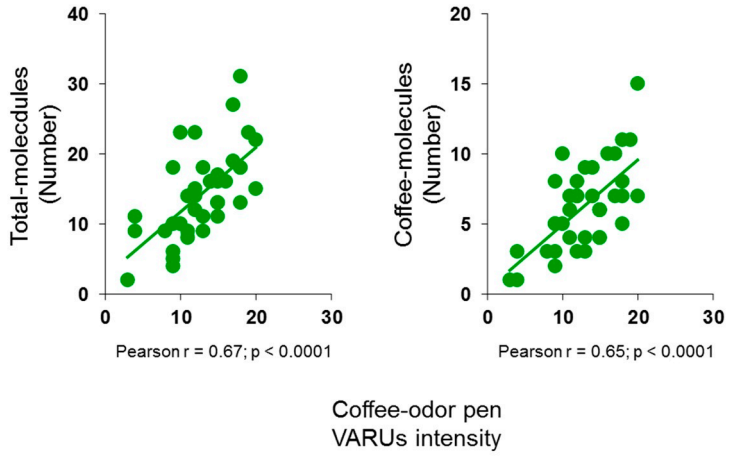

Figure 3. Correlation analysis between the number of total- and coffee-molecules smelled, and the intensity perceived for the coffee-odor pen by each subject.

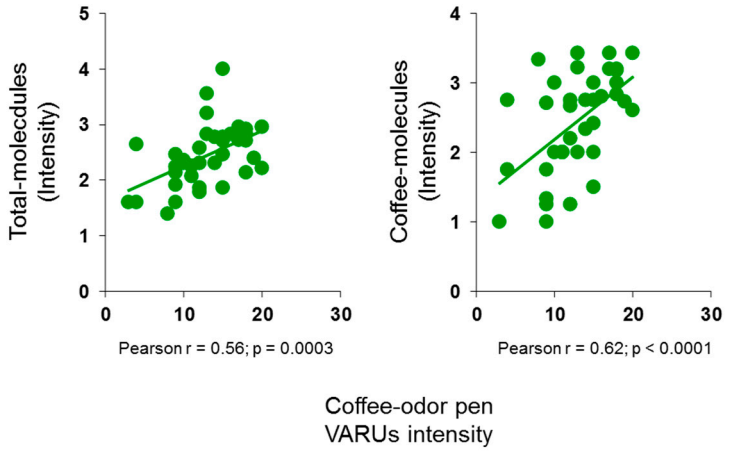

Figure 4. Correlation analysis between the average intensity referred for total- and coffee-molecules and the intensity perceived for the coffee-odor pen by each subject.

The mean values ± SEM of the intensity perceived for the coffee-odor pen by panelists classified by their TDI olfactory status are shown in Figure 5. One-way ANOVA revealed that the intensity perceived by normosmic individuals was significantly higher than that of hyposmic individuals (F (1,36) = 11.63, p = 0.0016).

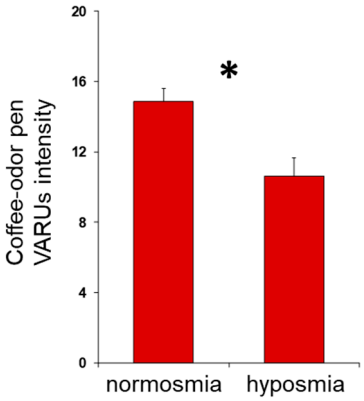

Figure 5. Mean (±SEM) values of the intensity perceived for the coffee-odor pen by subjects, according to their TDI olfactory status. Asterisk indicates significant differences between individuals with normosmia or hyposmia (p < 0.005; Fisher's LSD test subsequent to one-way ANOVA).

The Pearson correlation test also revealed that the intensity of the coffee-odor pen reported by each subject was positively correlated with his/her TDI olfactory score (Pearson r = 0.49, $p = 0.0016$) (Figure 6).

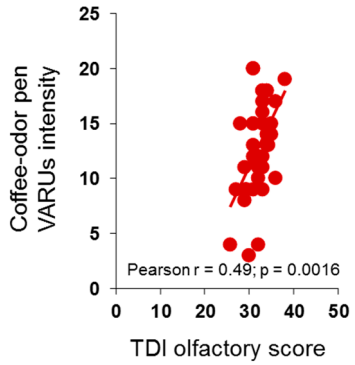

Figure 6. Correlation analysis between the intensity perceived for the coffee-odor pen by each subject and his/her TDI olfactory score.

4. Discussion

The main objective of this study was to evaluate the role of the human nose as a chemical sensor, particularly, in relation to its ability to perceive the single odorous molecules that make up a complex odor. It is known that the human nose has a vast sensitivity and a high discriminative power, but the number of odorants it can perceive is still unknown [51], especially when considering that among humans there is a great inter-individual variability due to multiple factors: physiological, genetic, environmental, cultural and behavioral [18,20,37,38,40,42–50]. Most of the odors that surround us, and in particular those of food and drink, are composed by a mixture of volatile compounds that can be separated by means of a chromatographic column and used as single olfactory stimuli [24,25,53,71,74–77].

In this study, by means of the coupled Gas Chromatography-Olfactometry (GC-O) technique, the single components of the coffee aroma were separated, identified and verbally evaluated by each subject, using their own nose as a chemical sensor. The number of odor-active compounds (i.e., the number of sensory active molecules for the subject during the GC-O experiment) was evaluated by means of the frequency detection method [36,54,71], which has the advantage of not requiring qualified participants and of highlighting inter-individual variability [28,54]. The results show that all compounds eluted from the chromatographic column were perceived by the participants and that 17 of the 21 compounds commonly defined as smelling of coffee, were described as having coffee odor. This is remarkable considering that the participants were unaware of the mixture injected into the chromatographic column, so they did not have a mental representation of the odor, known to exert a great influence on the formation of the perceived odor quality [78].

Since little is known about the ability of the human nose to perceive individual compounds as they elute from the chromatography column, we evaluated the effect of subjects' olfactory function on their ability to smell the individual molecules that make up the complex odor of coffee. Our results show that the number of odor-active compounds smelled by each individual depends on his/her olfactory status. In fact, for individuals classified as normosmic the number of odor-active compounds was significantly higher than that of those classified as functionally hyposmic. The TDI olfactory status represents the general olfactory status of the individual attributed on the basis of the score obtained from the sum of the olfactory threshold, discrimination and identification scores. This means that a condition of hyposmia can be determined by reduced ability in all three olfactory performances, or in two of them or only in one. We believe that the number of odor-active compounds for hyposmic individuals is lower than for normosmic ones, due to a reduced ability to perceive and discriminate odors. The olfactory threshold represents

the minimum concentration that an odor must have in order to be perceived: hyposmic individuals could present an increased olfactory threshold (i.e., to perceive odors, they must have a higher concentration than that required by normosmic individuals) and consequently they may not perceive those odors that are eluted at subthreshold concentrations for them. Discrimination represents the ability to recognize different odors: hyposmic subjects show difficulty in recognizing different odors that are similar, and this could reduce the number of single compounds that they smell during GC-O experiments. This is compatible with the fact that in our sample the general state of hyposmia is mainly determined by a low score obtained with the threshold and discrimination tests. In addition, correlation analyses showed that the number of odor-active compounds is directly correlated with the TDI olfactory score achieved by each individual. Furthermore, results are similar whether we consider the number of total perceived molecules or of molecules commonly defined as coffee odorants. Within each complex odor there are both molecules that even individually smell like the complex odor and molecules whose odor is completely different. This aspect is noteworthy if we consider that the more sensorially active molecules are also those that contribute more to the odor of the mixture [14,26,53]. Therefore, the individual perception of a complex odor is strongly conditioned by its intensity, number and type of individual compounds perceived, making the odor unique and characteristic for each person. This means that the idea that each individual has of a complex odor, formed by a set of many molecules, may be different from that of other individuals for whom the odor-active compounds are different both quantitatively and qualitatively. This also explains, at least in part, why the intensity with which an odor is perceived differs between individuals.

Based on these considerations, the second aim of our work was to evaluate whether a correlation exists between the perceived intensity of the coffee-odor pen and both the number of odor-active compounds and the intensity with which they are perceived. The results we obtained show that the reported intensity for the coffee-odor pen is positively correlated with the number of odor-active compounds, both total and coffee-smelling. Moreover, a positive correlation was also found for the reported intensity of individual compounds perceived during the GC-O experiments: the greater the intensity with which each individual perceives each molecule, the greater the intensity with which the mixture is perceived. These results are in agreement with the fact that in our sample the condition of hyposmia of individuals is mainly determined by a reduced ability of odor discrimination and an increased odor threshold. Therefore, on the one hand the sensorially active molecules are less numerous and on the other, the odor-active compounds are perceived with less intensity, making the perception of the complex coffee blend less intense.

Finally, given the correlation between the number of odor-active compounds and the TDI olfactory score and between the number of odor-active compounds and the intensity of the coffee-odor pen, the last objective of this study was to look for a correlation between the perceived intensity of the coffee-odor pen and the olfactory function of each individual. The results show that the values of the coffee-odor pen intensity and those of TDI are linearly correlated and that normosmic individuals report perceiving the odor of coffee more intensely than hyposmic ones. These findings are in accordance with a previous study in which a positive correlation was found between the subjects' olfactory function and their ability to detect individual compounds eluted from a chromatographic column and between the perceived intensity of the complex odor of banana and the number of odor-active compounds smelling of banana sniffed by each subject [36].

5. Conclusions

In conclusion, the results of this study show that the ability of the human nose as a chemical sensor is strongly conditioned by the individual olfactory function and that the intensity with which a complex odor is perceived depends on the number of odor-active compounds and on the intensity at which they are perceived. Furthermore, the knowledge of which compounds of a complex odor are odor-active could be of great interest not only for the food and perfume industry, but also for developing electronic noses capable of

identifying specific volatile molecules even in complex mixtures and/or reproducing the functional organization of the olfactory system [14,53].

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patient(s) to publish this paper" if applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to restrictions (e.g., privacy or ethical).

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