Perceived Intensity and Pleasantness of Coffee Aroma: Role of Odor-Active Molecules, Sex and Olfactory Function

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Posted Date: 21 November 2025

doi: 10.20944/preprints202511.1610.v1

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 doi:10.20944/preprints202511.1610.v1

Article

Perceived Intensity and Pleasantness of Coffee Aroma: Role of Odor-Active Molecules, Sex and Olfactory Function

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Abstract

Coffee is the most popular non-alcoholic beverage in the world, and its consumption has increased over the last decades. Recent studies have identified the social and environmental factors that determine whether an individual is a coffee drinker or non-drinker. Knowing the key aroma compounds of coffee and identifying inter-individual differences in the number and type of odor-active compounds could be important to understand what guides consumers towards the choice of drinking or not drinking coffee. In this study, using the coupled Gas Chromatography-Olfactometry technique, the components of the headspace of roasted coffee beans were separated and evaluated by volunteers. Each participant had to identify and provide a personal evaluation of the pleasantness and intensity perceived for each odor molecule. The results show that individuals with normosmia perceive single molecules with a greater intensity than those with hyposmia, and that females report perceiving the odor of single molecules with a higher intensity than males. The reported pleasantness for the coffee aroma is determined by the hedonic valence attributed to each molecule in terms of pleasantness/unpleasantness. These results could be of great interest to the coffee industry, providing useful information for the development of new blends.

Keywords: smell; coffee beans; gender; Sniffin' Sticks test; olfactory status; individual variability; gas chromatography-olfactometry

1. Introduction

The main functions of the olfactory system are oriented towards the identification of danger signals, social communication and eating behavior [1,2]. Regarding the role of smell in eating behavior, individuals use it to choose foods, and this is reflected in the composition and size of the meal. Through the orthonasal perception of given odors, the appetite for foods that contain those odors is stimulated, while retronasal stimulation decreases the appetite not only for these foods, but also for others [3–6]. The impairment of the sense of smell leads individuals to change their food choices: these people prefer foods with a high energy content (such as sugars and fats) because they are more gratifying than healthier foods such as fruits and vegetables. Besides, individuals tend to add salt and spices to enhance the flavor of what they eat, thus increasing its gratifying power, which is instead reduced by a lower olfactory sensitivity [7–14]. Most food and drink odors are mixtures of molecules, which means that some odors may be masked by others and therefore not perceived, thus preventing access to the information they contain. It has been reported that the smell of the strongest compound(s) prevails in the mixture and determines its overall odor [15,16]. Masking odors perceived as pleasant could make a food smell unpleasant, while the inability to perceive unpleasant odors could make it more pleasant and acceptable to individuals.

Coffee is not only the most popular non-alcoholic beverage in the world, but its consumption has increased over the last 20 years, in accordance with its social significance and its functional effects [17–19]. From a social point of view, coffee consumption is seen as a moment of break from work

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activities, of socialization between individuals and is an integral part of the lifestyle of individuals [18,20–24]. From a functional point of view, coffee can prevent chronic diseases such as tumors, prevent or alleviate neurodegenerative diseases such as Parkinson's and Alzheimer's, prevent cardiovascular disorders and alleviate hypertension, present antiadipogenic, antidiabetic and neuroprotective effects [19,25–30].

Recent studies have focused on understanding the factors that drive individuals to be coffee drinkers or non-drinkers. Quality, flavor and ethics were the main drivers of consumption and, with the new century, the act of drinking a coffee beverage has evolved. It includes several mixed factors, such as pleasure, experience, lifestyle, and social status [18,24,31]. Sensations of energy, gratification and pleasantness are among the reasons that drive individuals to consume coffee, while taste and fear of the impact of coffee on health are among the reasons that make an individual a non-consumer [18]. Coffee is appreciated and loved mainly for its unique sensory properties, which include an intense and full-bodied aroma, a bitter/acidic taste and a pleasant aftertaste [20,32,33].

Using the gas chromatography technique it is possible to separate the molecules that make up the complex odor of a food and by coupling it with an olfactometer (an olfactory evaluation device that uses the human nose as a detector), it is possible to evaluate the contribution of the odor of each single component to the overall aroma, that is, the subjective response of how each single molecule is perceived by individuals [34–36]. Recently, several studies have been aimed at identifying single active odor molecules, defined as “odor-active” compounds, within a mixture and the characteristics of their human perception, in order to improve the quality of odorous products, such as food and beverages, through the identification of natural and/or synthetic products that make them more pleasant and desirable for consumers [34,37–39]. Knowing the key aroma compounds of coffee, identifying inter-individual differences in the number and type of odor-active compounds could be important to understand what guides to drink or not drink coffee.

Given the increase in coffee consumption over the past 20 years, the social and functional significance that this beverage has in people’s lives, and considering that most studies on the olfactory properties of coffee have evaluated the blend’s complex aroma and how its odor can influence individuals’ attention span and appetite, our laboratory has been enrolling an ever-increasing and diverse number of people to understand how the perception of active compounds in the odor can influence the perception of the blend and, consequently, their choice and acceptance as beverage. Aim of this study was to investigate, in detail, the intensity and pleasantness with which odor-active compounds were perceived and to highlight any differences between males and females and between normosmic and hyposmic individuals. Our goal was to reduce the information gap on the factors underlying individual variability in coffee perception, both as a complex aroma and as the odor-active compounds that compose it.

2. Results

Table 1 shows the volatiles smelled by at least two participants, the odor description that was reported and the number of individuals who perceived each odor-active compound as it was eluted from the chromatography column, separately for males and females. Participants perceived 25 of the 48 odor-active molecules as coffee-smelling, even though only 21 of them are actually reported in the literature as having a coffee odor. However, of the 25 molecules described as having a coffee odor, only 19 are described as such in the literature; in fact, the molecules 2,3-pentanedione and pyrazine, 2-acetyl-6-methyl (indicated in the table as number 31 and 43, respectively), although described in the literature as having a coffee odor, were not described as such by the panel members. This means that 6 of the molecules described as coffee by the participants do not match the odor description reported in the literature.

 doi:10.20944/preprints202511.1610.v1

Table 1. GC-O analysis: odor-active molecules and odor descriptions by participants.

N.	Odor-active molecule	Odor description	Df (M-F)
1	Octane, 3,5-dimethyl-	Woody, burnt, unknown	3-3
2	Oxalic acid, isobutyl nonyl ester	Burnt, unknown	1-2
3	Toluene	Coffee, smoked, solvent, roasted, fruit	9-21
4	β-Pinene	Sweet, floral, vanilla, herbs, incense, sulfur, pungent	8-10
5	p-Xylene	Vanilla, medicinal, floral, gas, pungent, plastic	7-8
6	Oxalic acid, isobutyl pentyl ester	Floral, fruity, vanilla, sweet	3-5
7	Pyridine*	Coffee, smoked, roasted, cheese, plastic, woody	3-18
8	D-Limonene*	Sweet, sour, citrus, caramellic	10-2
9	Furan,2-pentyl-*	Smoked, sweet, solvent, plastic, cocoa, herbs	10-6
10	Pyrazine, methyl-*	Coffee, nutty, roasted, smoke, floral, caramellic, alcohol	7-7
11	Acetoin	Coffee, sweet, roasted, parfum, fruit, woody, caramellic	15-18
12	2-Propanone, 1-hydroxy-	Sweet, pungent, fish, solvent, wet, medicinal, feet, burnt	25-13
13	Pyrazine, 2,5-dimethyl-*	Coffee, citrus, medicinal, sweet, cocoa, burnt, shoes	13-15
14	Pyrazine, ethyl-*	Coffee, nutty, egg, sweet, vinegar, pungent, shoes	4-5
15	Pyrazine, 2,3-dimethyl-*	Coffee, burnt, caramellic, fruity, citrus	5-4
16	DL-2,3-Butanediol*	Sweet, caramellic, rose, wet, smoke	4-4
17	Vinyl butyrate	Floral, parfum, bitter, solvent, pungent, fresh, plastic	5-9
18	Hex-4-yn-3-one, 2,2-dimethyl-	Sweet, solvent, acidic, pungent	3-4
19	Pyrazine, 2-ethyl-6-methyl-*	Coffee, sweet, smoked, medicinal, solvent, parfum, roasted, ammonia, balsamic, fruit	31-29
20	Pyrazine, 2-ethyl-3-methyl-*	Coffee, cocoa, solvent, bitter, nutty, roasted, burnt, musty, medicinal, solvent, herbs	31-33
21	Pyrazine, 2-(n-propyl)-*	Green, musty, woody, earthy, wet, herbs, floral, fruit	28-19
22	Pyrazine, 2,6-diethyl-*	Coffee, roasted, earthy, musty, burnt, mushrooms, vegetable, tobacco	32-32
23	Pyrazine, 3-ethyl-2,5-dimethyl-*	Coffee, nutty, roasted, floral, bitter, woody, solvent, wet	24-24
24	2-Propanone, 1-(acetyloxy)-	Pungent, parfum, musty, saltiness	3-6
25	Pyrazine, 2-ethyl-3,5-dimethyl-*	Coffee, musty, roasted, wet, cocoa, herbs	22-22
26	Furfural*	Coffee, sweet, solvent, floral, pungent, cocoa, wet	11-25
27	Pyrazine, tetramethyl-	Coffee, roasted, burnt, vanilla, parfum, solvent. bitter	17-14
28	Pyrazine, 3,5-diethyl-2-methyl-*	Floral, musty, wet, solvent, fresh	29-16
29	Pyrazine, 2-ethenyl-5-methyl-	Coffee, nutty, bitter, plastic, musty, earthy, wet	11-22
30	Furan, 2-acetyl-*	Coffee, parfum	2-3
31	2,3-Pentanedione*	Floral, herbs, earthy, sweat, musk, cheese, pungent, citrus, musty, burnt, stinky feet, legumes, woody	31-33
32	2-Furanmethanol, acetate*	Coffee, roasted, fruit, earthy, herbs, woody, bitter, fish Pungent, sour, bitter, wet grass, plastic, herbs, spicy	16-29
33	Pyrazine, 2-methyl-6-(2-propenyl)-	Coffee, sweet, floral, lavender	3-11
34	2-Cyclopenten-1-one, 2,3-dimethyl-	Coffee, roasted, solvent, rotten, musty, herbs, wet earth	2-3
35	Acetic acid, diethyl-*	Sweet, cocoa, herbs, nutty	18-26
36	Pentanoic acid, 4-oxo-, methyl ester	Coffee, sweet, parfum, fruity, solvent	4-5
37	2-Furancarboxaldehyde, 5-methyl-*	Coffee, pungent, floral, musty, herb, sweet, burnt, rubber	4-7
38	2-Furanmethanol, propanoate*	Coffee, nutty, popcorn, roasted, fish, sour, biscuit, smoke	15-29
39	Furan, 2,2'-methylenebis-*	Coffee, smoke, popcorn, nutty, roasted, cheese, sweet	22-40
40	2-Furanmethanol*	Cheese, smoke, stinky feet, acidic, fruity, putrid	14-24
41	Butanoic acid, 3-methyl-*	Roasted, biscuit, saltiness, nutty, plastic	25-20
42	Furan, 2-(2-furanylmethyl)-5-methyl-*	Putrid, musty, cheese, medicinal	4-5
43	Pyrazine, 2-acetyl-6-methyl	Shoes, wet, sweat, pungent, legumes, plastic, cheese	6-15
44	4(H)-Pyridine, N-acetyl-*	Sweat, acidic	10-9
45	Octaethylene glycol monododecyl ether	Cheese, musty, putrid, plastic, chicken, shoes, burnt	2-2

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46	2-Hexadecanol	Coffee, solvent, cheese, musty, smoke, feet, caramellic	31-28
47	N-Furfurylpyrrole*	Coffee, roasted, almond, sweet, burnt, parfum, fresh, cocoa	29-27
48	2-Acetylpyrrole*		16-26

Odor-active molecules: list of molecules eluted by the chromatographic column during GC-O experiments and smelled by participants. Odor description: specific description that each participant attributed to each odor smelled during the GC-O experiment. df = detection frequency (number of subjects who smelled the compound, separately for males and females). Volatile compounds described in the literature as molecules smelling of coffee are listed in red printter.

Fisher's exact test highlighted a different distribution between males and females in the perception of some molecules. In detail, the data reported in Table 2 show that a significantly greater number of females perceived the coffee-molecules of toluene ($\chi^2 = 6.989$, $p = 0.008$), pyridine ($\chi^2 = 13.767$, $p < 0.001$), furfural ($\chi^2 = 8.883$, $p = 0.003$), pyrazyne, 2-ethenyl-5-methyl- ($\chi^2 = 5.579$, $p = 0.018$), 2-furanmethanol acetate ($\chi^2 = 7.345$, $p = 0.007$), 2-furanmethanol propanoate ($\chi^2 = 8.566$, $p = 0.003$), furan,2,2-methylenebis- ($\chi^2 = 17.782$, $p < 0.001$), 2-furanmethanol ($\chi^2 = 4.344$, $p = 0.037$), pyrazine 2-acethyl-6-methyl ($\chi^2 = 4.846$, $p = 0.028$), and total-molecules of pyrazyne, 2-methyl-6-(2-propenyl)- ($\chi^2 = 5.251$, $p = 0.022$) and 2-acetylpyrrole ($\chi^2 = 4.254$, $p = 0.039$). Instead, significantly more males perceived total-molecules as D-limonene ($\chi^2 = 6.432$, $p = 0.011$), 2-propanone, 1-hydroxy- ($\chi^2 = 7.375$, $p = 0.007$), pyrazine, 2(n-propyl)- ($\chi^2 = 4.344$, $p = 0.037$) and pyrazine, 3,5-diethyl-2-methyl ($\chi^2 = 8.645$, $p = 0.003$). No coffee odorant molecule was perceived by significantly more males.

Table 2. Different distribution between Males (M) and Females (F) in their ability to perceive some molecules from the headspace of roasted coffee beans.

Molecule	Perception ability	M n	F n	*p*-Value
Toluene	Yes	9	21	0.008
	No	33	22	
Pyridine	Yes	3	18	< 0.001
	No	39	25	
D-limonene	Yes	10	2	0.011
	No	32	41	
2-Propanone, 1-hydroxy-	Yes	25	13	0.007
	No	17	30	
Pyrazine, 2-(n-propyl)-	Yes	28	19	0.037
	No	14	24	
Furfural	Yes	11	25	0.003
	No	31	18	
Pyrazine, 3,5-diethyl-2-methyl-	Yes	29	16	0.003
	No	13	27	
Pyrazyne, 2-ethenyl-5-methyl-	Yes	11	22	0.018
	No	31	21	
2-Furanmethanol, acetate	Yes	16	29	0.007
	No	26	14	
Pyrazine, 2-methyl-6-(2-propenyl)-	Yes	3	11	0.022
	No	39	32	
2-Furanmethanol, propanoate	Yes	15	29	0.003
	No	27	14	
Furan, 2,2-methylenebis-	Yes	22	40	<0.001
	No	20	3	
2-Furanmethanol	Yes	14	24	0.037
	No	28	19	
Pyrazine, 2-acetyl-6-methyl	Yes	6	15	0.028

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	No	36	28	
2-Acetylpyrrole	Yes	16	26	0.039
	No	26	17	

p-Value derived from Fisher's Exact Test. Females (n = 43), Males (n = 42). Volatile compounds described as molecules smelling of coffee are listed in red print.

Figure 1 shows the relationship between the perceived intensity of the coffee mixture (contained in pen #10 and rated by each participant during the identification test) and the intensity with which the single molecules were perceived during the GC-O tests. Considering all participants together (Figure 1A), we found a positive relationship with both total molecules (Spearman $r = 0.58$, $p < 0.0001$) and coffee molecules (Pearson $r = 0.65$, $p < 0.0001$). When participants were divided by sex, r values became ≥ 0.35 ($p \leq 0.023$; Pearson's Correlation test) and ≥ 0.65 (total molecules: $p < 0.0001$, Pearson's Correlation test; coffee molecules: $p < 0.0001$, Spearman's Correlation test), for males (Figure 1B) and females (Figure 1C) respectively.

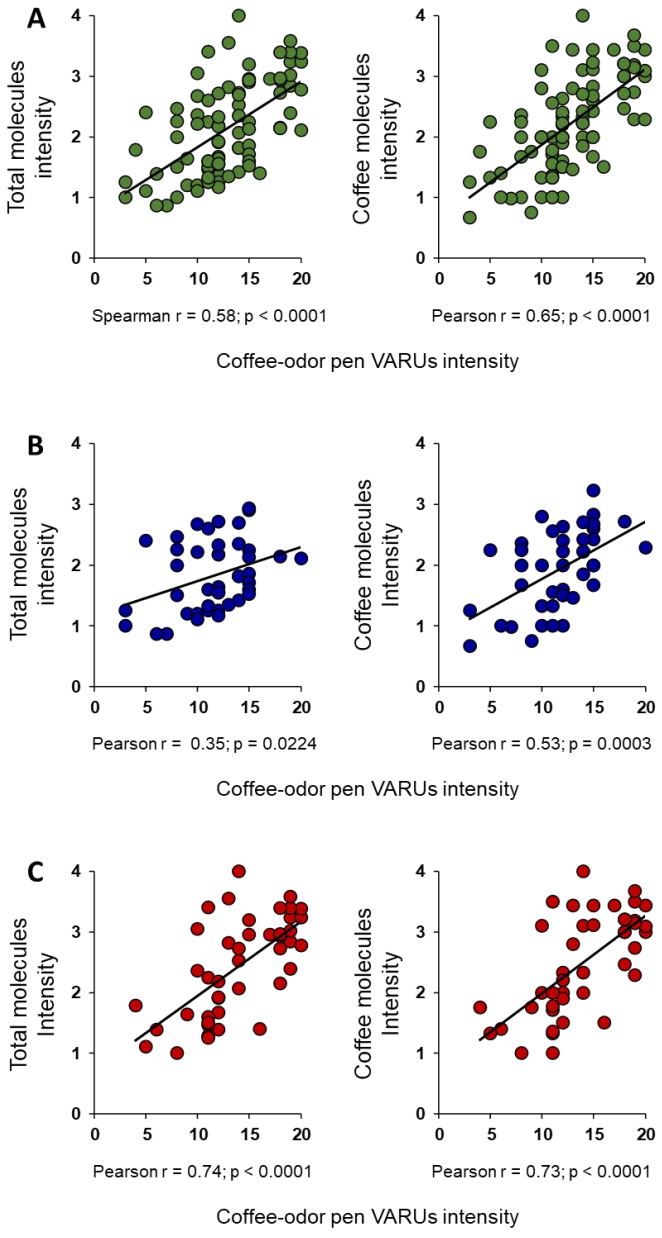

Figure 1. Relationship between perceived intensity for single molecules and complex mixture. Pearson's or Spearman's correlation analysis between the perceived intensity for each odor-active molecules and that for

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the complex mixture by each participant, considering them all together (A) and separately in males (B) and females (C).

Figure 2 shows the mean value ± SE of the intensity with which both total and coffee molecules were perceived while eluting from the chromatographic column, in relation to both olfactory status and sex. In particular, post-hoc analyses following a two-way ANOVA (total-molecules: $F(1,81) = 9.14$, $p = 0.003$; coffee-molecules: ($F(1,81) = 3.81$, $p = 0.055$), showed that normosmic males and females reported perceiving molecules with greater intensity than hyposmic individuals, both for total-molecules ($p < 0.001$; Fisher's LSD test subsequent to two-way ANOVA) and for coffee-smelling molecules ($p < 0.001$; Fisher's LSD test subsequent to two-way ANOVA). Normosmic females perceived the molecules (both total and coffee) with a significantly greater intensity than normosmic males ($p < 0.001$; Fisher's LSD test subsequent to two-way ANOVA). Besides, limited to coffee odorant molecules, we also found significant differences among individuals with hyposmia, with females reporting a greater intensity than males ($p = 0.047$; Fisher's LSD test subsequent to two-way ANOVA).

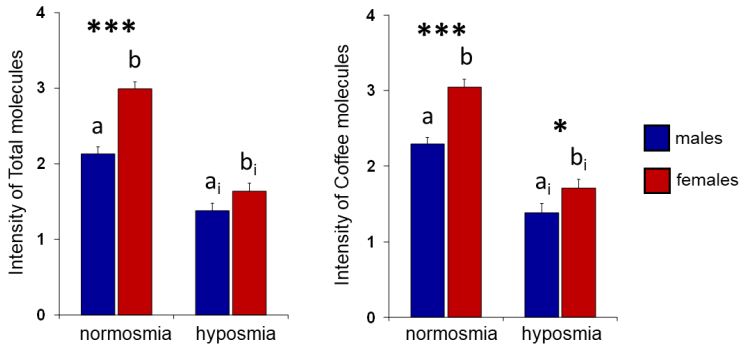

Figure 2. Relationship between TDI olfactory score and ability to perceive single molecules. Mean values ± SE of the intensity referred by males and females for the total- and coffee-molecules during the GC-O experiments, according to their TDI olfactory status. Different letters indicate significant differences between normosmic or hyposmic individuals, within the same sex (males: a-a_i; females: b-b_i; $p < 0.001$; Fisher's LSD test subsequent to two-way ANOVA). * Indicates significant differences between males and females within the same TDI olfactory status (*** $p < 0.001$; * $p < 0.05$; Fisher's LSD test subsequent to two-way ANOVA).

The correlation test revealed a positive relationship between the hedonic valence attributed to the coffee-odor pen and the number of both total and coffee-odorant molecules perceived as pleasant, both when participants were considered all together (Spearman $r \geq 0.44$, $p < 0.0001$; Figure 3A), and separately in males (Total-molecules: Pearson $r = 0.38$, $p = 0.013$; Coffee-molecules: Spearman $r = 0.35$, $p = 0.022$; Figure 3B) and females (Total-molecules: Spearman $r = 0.60$, $p < 0.0001$; Coffee-molecules: Pearson $r = 0.52$, $p = 0.0003$; Figure 3C). Instead, a negative correlation was found between the hedonic valence for the coffee-odor pen and the number of total molecules and coffee molecules defined as unpleasant by the participants, both when participants were considered all together (Spearman $r \leq -0.46$; $p < 0.001$; Figure 4A), and separately as males (Spearman $r \leq -0.68$; $p < 0.0001$; Figure 4B), and females (Total-molecules: Pearson $r = -0.34$, $p = 0.025$; Coffee-molecules: Spearman $r = -0.36$, $p = 0.019$; Figure 4C).

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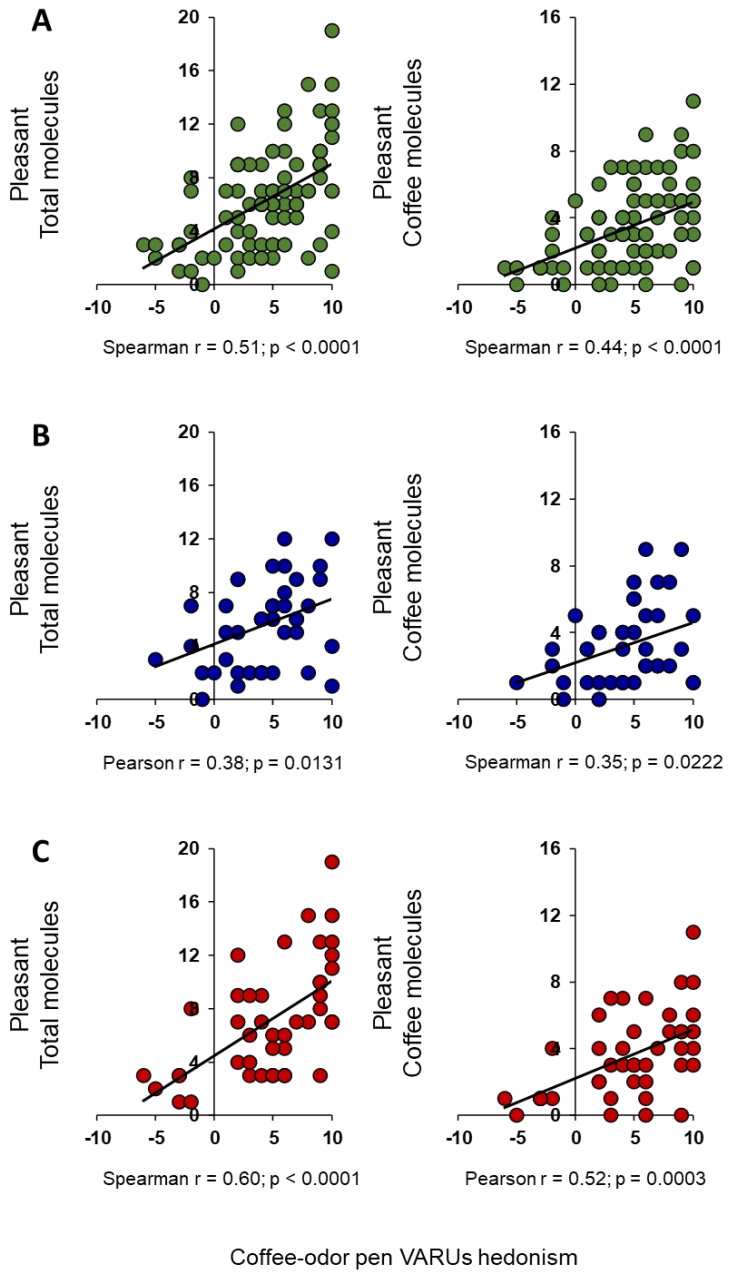

Figure 3. Relationship between single molecules pleasant and hedonic valence of coffee aroma. Pearson's or Spearman's correlation analysis between the reported hedonic valence for the coffee-odor pen and the number of both total and coffee molecules perceived as pleasant by each participant, considering them all together (A) and separately in males (B) and females (C).

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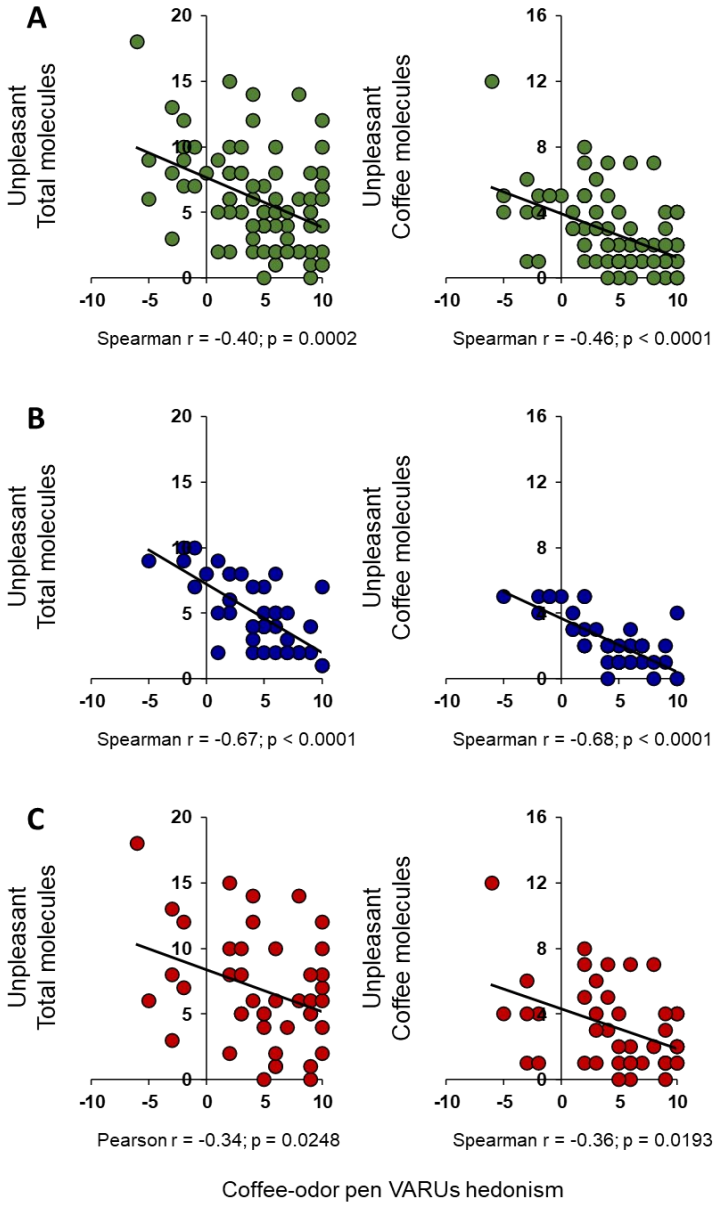

Figure 4. Relationship between single molecules unpleasant and hedonic valence of coffee aroma. Pearson's or Spearman's correlation analysis between the reported hedonic valence for the coffee-odor pen and the number of both total and coffee molecules perceived as unpleasant by each participant, considering them all together (A) and separately in males (B) and females (C).

3. Discussion

One of the three main functions of the olfactory system is to provide information about foods and drinks, helping to determine food choices and eating behavior of individuals [1,14,40]. Foods and drinks are characterized by a complex aroma, given by the mixture of the odors of the individual molecules that compose it. Within the mixture, not all molecules have the same importance from a sensorial point of view: in fact, it has been previously shown that the number of molecules perceived is directly correlated with the intensity reported for the mixture and that the most sensorially active molecules, i.e. those perceived by the greatest number of participants, are those that contribute most to the aroma of the mixture [15,16,34,36,39,41–43]. This means that the number and type of molecules perceived are the basis of the intensity and pleasantness with which each of us perceives a complex odor. This aspect is very important because if an odor as a whole is unpleasant, it is likely to make a food or drink less appetizing, while if the perceived odor is overall pleasant, that food or drink will

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be more appetizing. If the pleasant and appetizing foods preferred by individuals also have a high energy content, their consumption can affect body weight and consequently the health of the individuals themselves. Likewise, if the intensity with which a food is perceived is low, sensorial satiety will be reached late and this, as is known, affects the start, duration and end of a meal [3–6,10,44–48]. The results of this study show that participants correctly identified as coffee 19 of the 21 odor-active molecules described in the literature as coffee odorants (i.e., perceived by at least two participants during the gas chromatography-olfactometry experiments): in particular, 8 molecules were perceived and correctly identified by at least 50% of the participants; of the remaining 13 molecules, 5 were perceived and correctly identified by at least 30% of the participants and only 6 by less than 20% of the participants. This result is very important because, as already mentioned, the most sensorially active molecules are also those that have the greatest influence within the mixture in determining the odor of the mixture itself, thus facilitating the participants' ability to correctly identify the coffee-pen odor. Conversely, participants who did not correctly identify the coffee-pen odor, perceived a maximum of 3 coffee-molecules out of the 8 most sensorially active. Based on this result, we can hypothesize that the number of coffee-odor molecules perceived is likely insufficient to guide the individual in correctly identifying the coffee odor.

The second objective was to evaluate the role of the perceived intensity for the single molecules and to study the differences related to both olfactory function and the sex of the participants. In fact, it is known that due to factors that are genetic [49–55], environmental [56–59], physiological [60–66] and pathological [48,67–82] the olfactory function of individuals can vary from normosmia (normal function), to hyposmia (reduced or compromised function) or anosmia (function totally or specifically absent), both with respect to complex stimuli and single molecules [39,83–88]. An interesting aspect that we analyzed was to understand whether there is a relationship between the perception of the mixture and that of the single molecules, and whether this is in some way linked to the olfactory function and to the sex of the individuals. The results we obtained show a direct relationship between the intensity with which single molecules are perceived and the intensity reported for the mixture, both when individuals are considered all together and when they are divided into males and females. In particular, our data highlight two important aspects: on the one hand, they show that normosmic individuals perceive the odor-active molecules, both total and coffee-smelling, with a significantly higher intensity than individuals with hyposmia; on the other hand, females with normosmia report perceiving odor-active molecules, both total and coffee, with a significantly higher intensity than males and, limited to coffee-molecules, even between females and males with hyposmia. Overall, our findings highlight that females outperform males also in specific olfactory abilities (such as number and intensity in the perception of single molecules), confirming previous studies suggesting that, due to the effect of social, cognitive, neuroendocrine and genetic factors, females show better olfactory performance than males [51,63,89–95].

We previously found that normosmic females perceived a greater number of molecules than males and therefore reported perceiving the odor of the coffee pen with greater intensity [96]. The results of this study show that the ability to perceive 15 of the 48 odor-active molecules, differs between males and females. For 11 of them, females perceived the odor more than males; in particular, we found that 9 of the 11 are coffee-molecules. Instead, the four molecules for which the number of detections was greater for males than for females were all of the total-molecules type. Females not only perceived the odor-active molecules with a significantly higher intensity than males, but also a greater number of the main coffee odorant molecules, suggesting that females likely have a more precise sensory representation than males. Overall, these results could explain why females perceive the coffee-odor pen more intensely than males: 1) females perceive a greater number of odor-active molecules in the odor of coffee. This is important, considering that there is a direct relationship between the intensity for the coffee-odor pen and the number of molecules perceived; 2) females perceive the odor-active molecules more intensely. Therefore, females appear to perform better than males, not only because they perceive a greater number of molecules, but also more

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intensely. As a future perspective, it would be interesting to evaluate the factors (e.g., environmental and genetic) underlying these differences.

During the GC-O experiments, participants could also express a personal evaluation of the hedonic valence attributed to the odor they perceived. Specifically, they were asked to report whether the odor was pleasant or unpleasant. Therefore, as a final goal, we assessed whether the reported pleasantness/unpleasantness for the coffee-odor pen was related to the number of odor-active molecules defined as pleasant or unpleasant. Our results show that there is a positive correlation between the number of pleasant molecules and the perceived pleasantness for the coffee-odor pen. On the contrary, a negative correlation was found between the pleasantness for the coffee-odor pen and the number of molecules defined as unpleasant. The results are similar for males and females and for both types of odor-active molecules (both total and coffee). These findings are consistent with a previous study, which found that the reported pleasantness for the complex banana odor was directly related to that for the odor of isoamyl acetate, used in the food industry to impart the banana flavor to foods and drinks and identified as the most sensorially active within the mixture [39]. This aspect is very important because it provides a possible explanation of why the same complex odor is perceived as pleasant by some individuals and unpleasant by others. Just as intensity is related to the number of odor-active molecules, pleasantness is related to how odor-active molecules are perceived. Therefore, an individual who perceives the pleasant odor of coffee, perceives most of the odor-active molecules as pleasant. On the contrary, those who perceive the unpleasant odor of coffee, perceive the sensorially active molecules within the blend as unpleasant. Since it has been reported in the literature that sensory properties are among the factors that contribute to making an individual a coffee drinker or a non-drinker, these aspects are of particular importance [18].

4. Materials and Methods

4.1. Subjects

Volunteers (43 F, 42 M, age 18-56 years, BMI 18.5-24.99 Kg/m^2) were recruited through a public call at the University of Cagliari. On the day of the experiment, each volunteer was required to be perfume-free and to have fasted for at least an hour and a half before starting the olfactory tests. Each participant was asked to sign an informed consent and was read the experimental protocol approved by the local ethics committee (Prot. PG/2021/14278, 22.09.2021). The following inclusion criteria were applied in the selection of volunteers: healthy individuals, with a good sense of smell (subjectively assessed), non-smokers, with a COVID-19 infection lasting longer than 12 months. On the contrary, individuals reporting chronic pathologies such as inflammatory/autoimmune, neurodegenerative, tumoral, metabolic, cognitive, cardiovascular and respiratory diseases were excluded [48,68,69,71,76,77,97–103].

4.2. Olfactory Sensitivity Screening

The olfactory function of the participants was assessed by the TDI olfactory score (rating from 0 to 48), obtained by adding the scores of the Th-test (Threshold test; rating score from 0 to 16), the Dis-test (Discrimination test; rating 0 to 16) and the Id-test scores (Identification test; rating from 0 to 16). These represent the 3 tests that make up the Sniffin' Sticks battery (Burghart Instruments, Wedel, Germany), used internationally for olfactory screening [104]. More details on the delivery procedure for the felt-tip pens containing the odors can be obtained at the following link: https://www.uniklinikum-dresden.de/de/das-klinikum/kliniken-polikliniken-institute/hno/forschung/interdisziplinaeres-zentrum-fuer-riechen-und schmecken/neuigkeiten/downloads.

Based on the TDI olfactory score obtained, each participant was classified as normosmic or hyposmic, also taking into account sex and age group, according to Hummel et al 2007 [105].

During the Id-test, each participant was asked to rate on a scale, the "Visual Analogue Rating Units" (VARUs), the intensity (score 0-20) and the perceived pleasantness (negative score from -1 to

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-10; positive score from 1 to 10) for the complex aroma of the coffee contained in the pen #10 (coffee-odor pen) [39,106].

4.3. Dynamic Headspace Sampling

The dynamic headspace method was used to extract volatile compounds from roasted coffee beans [38,107]. The headspace method allows to obtain an extract, whose composition in terms of volatiles, is directly related to the quality of the aroma assessed by the user [108].

Furthermore, the extracts thus obtained can be used both for analysis with a mass spectrometer coupled to a gas chromatograph (MS-GC analysis) and for sensory evaluation by a human assessor (GC-O analysis) [38].

A 0.5 L airtight glass tank with a flow-through mechanism was filled with approximately 100 g of roasted coffee beans (Crnjar et al 2023). The volatile-impregnated air was then directed toward a glass tube (5 mm Ø) containing a Porapak Q filter (150/75 mg, 50/80; Supelco) placed in the vessel's top collection port. The volatiles were recovered at room temperature after the system was flushed with purified air for three hours at a rate of 30 L/h (500 ml/min). A solution comprising the separated volatiles was obtained by releasing the trapped volatiles from the Porapak Q tube using 1.5 ml of 1-hexane. The samples were then stored at -20 °C until use. Three GC runs were carried out 24 hours after sample preparation to confirm the efficacy of the extracted material and the repeatability of the chromatogram. The validity of the headspace was demonstrated by the fact that the chemical profile was the same as in the previous study [37,96] and comparable to other studies [109–117].

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

To perform GC-O tests, we injected a volume of 1 µL of coffee extract into the HP-INNOWax column (30 m × 0.25 mm × 0.50 µm; Agilent 19091N-233; Agilent technologies, Santa Clara, CA, USA) of the gas chromatograph (GC; Agilent 6890N). This volume was split 1:1 between the olfactometry detection port (Gerstel ODP3; Mülheim an der Ruhr, Germany) and the mass spectrometer (MS) detector (Agilent 5973; Santa Clara, CA, USA) coupled to the GC [39]. The carrier gas (1.2 mL/min) was Helium. For the GC runs, we used the same protocol reported in previous studies [37,39]. Volatiles present in our coffee extract were identified by means of the mass spectrum found in the MS Standard Library NIST2014 (US National Institute of Standards and Technology; Gaithersburg, MD, USA).

Each time that a volatile was smelled, the participant recorded on a computer his/her individual rating of the perceived odor-active molecule: intensity, duration, hedonic value and identification, via a digital recording and reporting system (GERSTEL ODP 3 for Windows 7) [38,118]. The identification reported by the participants was compared with the organoleptic information regarding the odor descriptors available at the Good Scents Company Information System (www.thegoodscentscompany.com) [37,119]. Participants recorded their comments by pressing one of the 4 buttons present in the reporting system. Each button represents a different value (scale 1-4) of perceived intensity: 1 = weak odor, 2 = distinct odor, 3 = intense odor, 4 = very intense smell. By automatically recording the retention and sniffing periods of each odor-active molecule, olfactograms were superimposed on chromatograms. Samples were presented blindly to avoid pre-conditioning.

The method we chose to assess the ability to perceive single molecules during GC-O experiments by each participant is the detection frequency method, as it is representative of inter-individual variability and does not require expert raters [34,120–123].

4.5. Statistical Analysis

Fisher's Exact Test was used to analyze differences between males and females in their ability to perceive some odor-active molecules.

Correlation analyses were used to assess the relationship between: a) the intensity reported for the coffee-odor pen and that for each odor-active molecule (both total and coffee-smelling) perceived

 doi:10.20944/preprints202511.1610.v1

by each participant, also considering males and females separately; b) the hedonism value reported for the coffee-odor pen and that for each odor-active molecule (both total and coffee-smelling) perceived by each participant, also considering males and females separately. Pearson's correlation or Spearman's correlation test was used if the normality assumption was met or not, respectively. Statistical analyses were performed using GraphPad Prism 8.1 (GraphPad Software, San Diego, CA, USA). A statistically significant correlation was defined as a p-value < 0.05.

Two-way ANOVA was used to test for a significant interaction between TDI olfactory status × sex on the perceived intensity for the odor-active molecules, for both total- and coffee-molecules.

Post-hoc comparisons were conducted using 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 significant.

5. Conclusions

In conclusion, the perception that each individual has of a complex odor is different and subjective. Quantitatively, this appears to depend both on the number of sensorially active molecules in the mixture and on the average intensity with which these molecules are perceived. Qualitatively, it seems to be determined by the hedonic valence attributed to each molecule in terms of pleasantness/unpleasantness. Furthermore, these factors seem to be closely related to the olfactory function and sex of the individual. In particular, females perform better than males both in quantitative (perceived intensity) and qualitative (type of smelled molecules) terms. On this basis, it becomes interesting to evaluate other factors involved in an individual's ability to perceive single molecules: genetic factors, such as polymorphisms of genes involved in the olfactory function of individuals, but also their age and eating habits.

Studying the factors underlying inter-individual differences and the organoleptic properties of the coffee blends which influence not only perceived pleasantness, but also intensity, can be of great interest in understanding why an individual is a coffee consumer or non-consumer. It has recently been suggested that coffee consumers prefer novel blends, and this information could help the coffee industry develop and market new products. For the coffee industry, sex differences are important because knowing that a blend composed of certain molecules can be more or less intense and pleasant for females than for males allows them to more accurately choose the beverage with which they want to serve it. For coffee consumers, this information can be useful in choosing the right blend. Furthermore, knowing that not only the chemical composition and sex of individuals, but also their olfactory function can influence the perception of a blend is important for consumer choice. We believe that the coffee industry, by understanding the key molecules that influence the olfactory perception of coffee aroma, and how these vary based on the sex and individual olfactory function, could market blends diversified by sex and olfactory performance. For example, a blend that is too intense might be unpleasant for a female individual with normal olfactory function, while it might be acceptable for a male consumer with reduced olfactory function.

Author Contributions: Conceptualization, R.C. and G.S.; methodology, D.D. and G.S.; formal analysis, D.D. and G.S.; resources, P.S., R.C. and G.S.; data curation, D.D. and G.S.; writing—original draft preparation, G.S.; writing—review and editing, D.D., P.S., R.C. and G.S.; supervision, G.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethical Committee of the University Hospital of Cagliari (Prot. NP/2020/3883 del 30 September 2020).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

 doi:10.20944/preprints202511.1610.v1

Data Availability Statement: The original contributions presented in the study are included in the article further inquiries can be directed to the corresponding author.

Acknowledgments: The authors thank the volunteers; without their contribution, this study would.

not have been possible. The authors have reviewed and edited the output and take full responsibility for the content of this publication.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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