

# Application of Molecular Sensory Analysis in Determining Food Flavor: A Review

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## Abstract

Molecular sensory science represents a multidisciplinary and integrated methodology focused on exploring the sensory attributes of foods at a molecular level. Within this approach, gas chromatography coupled with olfactometry is employed to determine key odor-active compounds. Dilution analysis, detection frequency, and time-intensity methods are used to determine these aroma-active compounds. Odor Activity Values (OAVs) are also calculated to determine the individual contributions of each aroma component to the characteristic odor of food. Subsequently, aroma reconstitution, and omission tests are conducted to confirm the role of these key aroma compounds in shaping the overall flavor profile of the food. This review aims to outline the sequential stages within the application of molecular sensory science, serving as a recent and innovative model for verifying identified aroma-active compounds.

*Keywords: Aroma, aroma-active, gas chromatography-olfactometry, molecular sensory science, sensorics*

## 1. INTRODUCTION

In recent years, different technology models, called omic, have been developed and divided into groups such as metabolomics, proteomics, genomics, and transcriptomics (Capozzi and Bordoni, 2013). For food science, subgroups such as foodomics, sensomics, and flavoromics have been formed under the metabolomic group. The sensomics approaches in the field of food-related omic focus primarily on aroma-active compounds that indirectly affect consumer sensory perception of a specific food product. Sensomics, one of the omics technologies applied to food, aims to describe the sensory properties of foodstuffs at the molecular level (Vrzal and Olšovská, 2019). Sensomics, in other words molecular sensory science, is a multidisciplinary and integrated approach (Ling et al., 2023). It is performed when examining compounds related to sensory perception of food, not only to understand the function of aroma-active compounds in a particular food product, but also to identify a range of

molecules that can have an impact on sensory perception of food. The use of only instrumental methods in the evaluation of aroma compounds of a food can lead to a single conclusion and no decision can be drawn regarding the sensory properties of that food. Because other components in the food matrix can affect the sensory perception of food (Vrzal and Olšovská, 2019).

Apart from the color, taste, appearance and texture of a food, one of the key factors affecting a food's quality as well as consumer perception and preference is aroma. The distinctive scent of a food arises from a complex blend of volatile compounds (Baingana, 2024). The most common method used to identify the substances that give food its aroma is gas chromatography (GC) (Gou et al., 2021). Numerous aroma substances have been detected in various food products, and some of them are responsible for giving a particular food its distinctive aroma. These substances are known as

aroma-active compounds or key odorants (Gou et al., 2021; Cengiz et al., 2023). A pivotal component of aroma studies involves extracting and identifying the distinct aroma constituents present in a food product. The gas chromatography-olfactometry (GC-O) technique is a commonly employed method to identify these aroma compounds. (Mishra et al., 2019). Certain aroma compounds, despite their impactful influence on food flavor, may exist at relatively lower concentrations yet possess high odor intensities, making them undetectable by instrumental methods alone (Plutowska and Wardencki, 2008; Song and Liu, 2018). Additionally, during aroma extraction and GC analysis, some aroma compounds in some foods can change into other compounds due to their instability and reactivity (Molina-Calle et al., 2017). The GC-O method helps mitigate these challenges, allowing for the identification of aroma-active compounds even at lower concentrations. Consequently, it facilitates the exploration of the relationship between these significant aroma-active compounds and their sensory properties in food, a concept known as the sensomics approach. Molecular sensory science, also referred to as sensomics, is a method used to investigate both the quantitative and qualitative attributes of food odorants at the molecular level (Gou et al., 2021). This method is based on the combination of sensory analysis with instrumental methods such as gas chromatography-mass spectrometry (GC-MS), gas chromatography-olfactometry (GC-O) and gas chromatography-mass spectrometry olfactometry (GC-MS-O). The purpose of this article is to explain molecular sensory science which is a new approach model to clarify the steps of this model along with their importance and possible difficulties.

## 2. MOLECULAR SENSORY SCIENCE

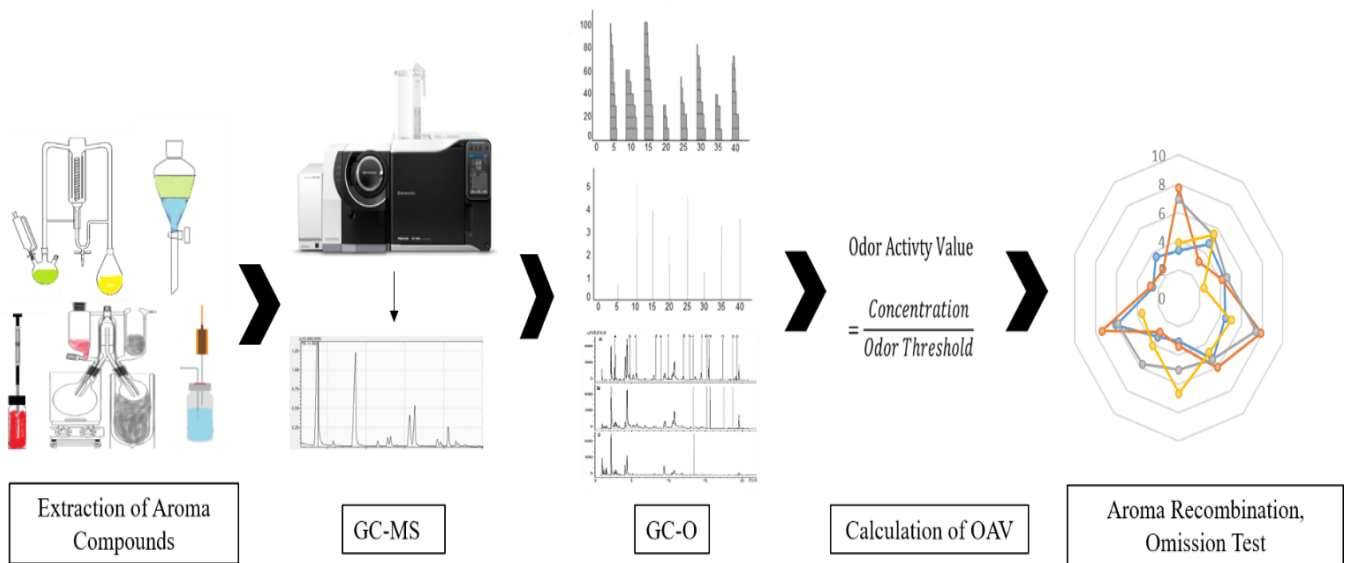
The aroma of food is formed by the combination of numerous complex volatiles. The unique aroma of food comes from a certain part of the volatile compounds and to accurately determine these essential aromas, a combination of analytical and

sensory research is necessary (Wagner et al., 2016). Human nasal cavity odor receptors exhibit notable variations in discrimination and sensitivity to volatile substances (Lu et al., 2017; Kilic-Buyukkurt et al., 2023). Indeed, it's essential to validate and confirm the accuracy of quantitative data acquired through GC with the assistance of sensory techniques. The approach of molecular sensory science, founded by the research teams of Schieberle and Hofmann, is employed precisely for this purpose to validate the components constituting food aromas (Steinhaus and Schieberle, 2007; Parker, 2015). In this approach, the essential steps include extraction, identification, quantification, and confirmation of aroma compounds contributing to odor. Within this methodology, the aroma compounds are initially extracted from the food using appropriate techniques, followed by their identification through instrumental methods like GC. Subsequently, the specific olfactometric techniques such as aroma extract dilution analysis (AEDA), detection frequency (DF), and direct intensity analysis (OSME) are used to determine the aroma-active compounds responsible for the distinctive odor of the food. Finally, aroma recombination and omission tests are applied with the molecular sensory science approach to verify the accuracy of the aroma-active compounds determined. A simple diagram illustrating the application of molecular sensory science is shown in Figure 1.

### 2.1. Aroma extraction

In the field of molecular sensory science, aroma compounds are extracted initially through multiple methods. The extraction of these compounds from the food matrix and non-volatile substances is a prerequisite before GC-MS-O analysis. Selecting an appropriate extraction method is crucial as it significantly impacts the accuracy and reliability of the foundational data utilized in subsequent stages of molecular sensory science (Kilic-Buyukkurt et al., 2023). A variety of techniques, such as steam distillation, simultaneous distillation-extraction (SDE), purge and trap extraction, solid-phase

Figure 1. Flowchart for applying molecular sensory science



microextraction (SPME), stir bar sorptive extraction (SBSE), liquid-liquid extraction (LLE), and solvent-assisted flavor evaporation (SAFE), are employed for this purpose (Delahunty et al., 2006). Steam distillation allows the separation of volatile components from plants, including essential oils and organic acids, as well as relatively volatile compounds insoluble in water. Nevertheless, this method can potentially lead to some loss of aroma due to the high-temperature application (Amanpour et al., 2019). In SDE, also known as the Lichens-Nicerson technique, involves both distillation and extraction occurring concurrently using a specially designed apparatus. During this process, water vapor aids in separating aroma compounds from the matrix, and subsequently, the organic phase, along with water, is condensed and collected. Further evaporation of more solvent results in a clear aromatic extract (Augusto et al., 2003; Guclu et al., 2020). Within the purge and trap system, also recognized as the dynamic headspace method, volatile compounds present in a food sample are captured using an inert gas like helium or nitrogen in an adsorptive trap. Subsequently, these trapped volatile compounds are once again separated—either by heating the trap

using a carrier gas or with the assistance of an appropriate solvent (Luque de Castro and Gámiz-Gracia, 2000; Sevindik et al., 2020). SPME is a quick and adaptable analytical method that entails adsorbing volatile compounds onto a coated fiber, followed by their release and separation within a GC column (Kilic-Buyukkurt, 2021). This technique is valued for its time-efficiency and eco-friendliness as it eliminates the need for solvents, but it has some limitations, such as the delicate nature of the extraction fibers, which demand careful handling (Xu et al., 2024). Similar to SPME, the SBSE method eliminates the necessity for solvents. This approach involves enriching the absorbent material by utilizing polydimethylsiloxane-coated stir bars, which are immersed in the aqueous sample and utilized for extraction while in a stirred state. (Zhao et al., 2021). Within the LLE method, aroma compounds in a sample are extracted utilizing an organic solvent with a similar polarity to the targeted compounds. Typically, volatile solvents such as benzene, dichloromethane, diethyl ether, and ethyl acetate are employed (Amanpour et al., 2019). The SAFE method involves the evaporation, extraction, and condensation of aroma compounds. This occurs

through evaporation at a lower temperature (< 50°C) and trapping at a very low temperature owing to a high vacuum ( $10^{-2}$  to  $10^{-3}$  Pa) facilitated by a specialized apparatus (Song and Liu, 2018).

## 2.2. Gas chromatography-mass spectrometry-olfactometry (GC-MS-O)

GC-MS, with its strong discrimination and identification capability, is a widely used technique for determining the aroma compounds of many foods. This technique is employed for both qualitative and quantitative analysis of aroma compounds (Verma and Srivastav, 2020). MS component within GC-MS serves as a detector proficient in identifying all ionizable compounds by capturing their mass spectra at distinct time intervals. In qualitative analysis, GC-MS matches volatile compounds with a standard spectrum library and/or utilizes internal/external standards (Gou et al., 2021).

Olfactometry is a valuable technique for detecting the aroma-active compounds of the samples (Wu et al., 2023). This technique involves an olfactometer, utilizing the human nose as a detector. GC-O or GC-MS-O represents a frequently employed combined method that integrates gas chromatography with olfactometry, enhancing the identification and understanding of aroma-active compounds (Mahmoud, et al., 2024). Especially within the context of the GC-MS-O setup, the olfactory (sniffing) port runs in parallel with either a mass spectrometer (MS) or flame ionization detection (FID). This design allows the aroma compounds within GC-MS-O extracts to be concurrently directed to both FID and MS, facilitating their identification while also enabling human olfactory detection through sniffing (Guclu et al., 2020). In olfactometry, when the concentration of a specific volatile compound exceeds the odor threshold, it generates interactions with human odor receptors, subsequently leading to the formation of corresponding aroma perceptions

in the human brain (Greger and Schieberle, 2007). Various techniques exist for gathering data on aroma-active compounds within GC-O, each grounded in distinct principles. These methods fall into three broad categories: dilution analysis, detection frequency, and time-intensity methods. (Li et al., 2020).

### 2.2.1. Dilution analysis

The dilution analysis method encompasses two techniques: Aroma Extract Dilution Analysis (AEDA) and Combined Hedonic Aroma Response Measurements (CharmAnalysis) (de-la-Fuente-Blanco and Ferreira, 2020). AEDA, a prominent technique within dilution analysis, involves a process developed by Schieberle and Grosch in 1987. It includes creating serial dilutions of the aroma extract at ratios like 1:2 or 1:3, followed by evaluation by a minimum of two experienced panelists. Each aroma compound is sniffed until no scent is detectable, noting the dilution factor (FD) at which the aroma becomes undetectable as specific to that compound (Grosch, 1993; Falcão et al., 2008). AEDA results are typically showcased by plotting the logarithm of FD (log FD) against the linear retention index or by listing the FD values of the odorant. Finally, at the conclusion of the analysis, the aroma compound with the highest FD is identified as the primary aroma-active compound contributing most significantly to the aroma profile (Gou et al., 2021). Regarding this issue, Sarhir et al. (2019) characterized the aroma-active compounds of the ayran with the GC-MS-O method. In the study, firstly aroma compounds were extracted by SAFE technique and then aroma-active compounds were determined by AEDA olfactometry method. A total of 14 aroma-active compounds were determined in the ayran, and 2-methylbutanal (FD: 512, fruity), ethyl lactate (FD: 512, whey/creamy), acetoin (FD: 256, buttery creamy), and butanoic acid (FD:256, cheesy-sweet) have been reported to be the strongest aroma-active compounds.

CharmAnalysis, an alternative approach to dilution analysis, was devised by Acree et al. in 1984. This method involves the random distribution of aroma extract dilutions for olfactometry, followed by subsequent sniffing procedures (de-la-Fuente-Blanco and Ferreira, 2020; Gou et al., 2021). Throughout this process, evaluators note the initial detection and fading times of each flavoring component, along with their intensities, utilizing the Charmware computer software. The collected intensity data for each dilution, in tandem with the corresponding peak areas, are then combined to generate a Charm chromatogram. The Charm value for the aroma-active compounds is calculated using the formula  $C=D^{n-1}$  where n represents the number of consistent odor responses noted by panelists, and D stands for the dilution level of the aromatic extract. (Blank, 1996; Brattoli et al., 2013).

#### 2.2.1. Detection frequency method

The detection frequency method (DFM) involves 6-10 untrained panelists engaging in sniffing sessions. The count of panelists identifying the same scent at matching retention times while smelling the extract is denoted as the detection frequency (Falcão et al., 2008). In this method, the intensity of an aromatic compound is determined by the number of individuals who perceive it (Botelho et al., 2007). Aroma-active compounds with higher detection frequencies are presumed to have a more pronounced presence, correlating with the intensity of the aromatic compound (Gou et al., 2021). In the resulting aromagram, the peak height represents the number of panelists detecting the aroma-active compound, quantified as the Nasal Impact Frequency (NIF). The area under the peak is referred to as the Nasal Impact Frequency Area (SNIF), depicting the surface area beneath the frequency curve (Falcão et al., 2008). In this approach, the panelists also measure the duration of the scent, and the SNIF is

determined by multiplying the detection frequency by the average duration of the scent duration (de-la-Fuente-Blanco and Ferreira, 2020). Pino and Trujillo, (2021) determined the aroma-active compounds of sour guava fruit, aroma compounds were extracted with headspace-solid-phase microextraction (HS-SPME) and then aroma-active compounds were determined by DFM. It was reported that a total of 26 aroma-active compounds emerged according to the results of the study, and all of them exhibited  $\geq 3$  frequency factors (maximum 9). It has been also stated that (*Z*)-3-hexenyl acetate (green, fruity), (*Z*)-3-hexenyl hexanoate (completely green), 3-methylbutyl butanoate (mature fruity), and hexyl acetate (fruity, floral), were the most important aroma-active compounds.

#### 2.2.3. Time-intensity method

In the time-intensity method (TIM), the sniffing process is carried out without the necessity of preparing aroma extract dilutions (Botelho et al., 2007). Throughout the sniffing process, a specialized 16-point electronic scale aids panelists in assessing the intensities of aroma compounds, along with their perceptions of the quality aspects and the moment of odor detection (Falcão et al., 2008; Gou et al., 2021). The analysis involves a team of four experienced panelists, each conducting four sniffs of every extract. Utilizing the aroma intensities and the times when the odor is perceived as reported by the panelists, an aromagram is generated (Botelho et al., 2007). Additionally, the Odor-Specific Magnitude Estimation (OSME) is a technique employed to measure the perceived intensity of an odor compound eluting from the GC-O. This involves the subject assigning an intensity rating to the compound's odor using a time-intensity device, resulting in the creation of an Osmegram. Furthermore, definitions of odor characteristics are also established (de-la-Fuente-Blanco and Ferreira, 2020). The aroma-active compounds of unifloral honey (*Croton heliotropiifolius*

Kunth) were evaluated using the OSME technique. In this study, a total of 17 aroma-active compounds were identified. Among these compounds, it was reported that the compounds contributing the most to the honey aroma were pentanoate acetate (ripe fruit, saffron, medicinal plant), and methyl eugenol (clove, tea). It was also noted that furanol, hotrienol, and benzaldehyde compounds contributed to the honey notes (Costa et al., 2019).

The posterior intensity method (PIM) is another technique used to assess aroma intensity in GC-O. This method involves determining the intensity of an odor compound within the GC effluent (Amanpour et al., 2019). The PIM closely resembles the OSME method, with the key distinction that the perceived intensity of each aroma compound is assessed using a memorized five-point intensity interval scale once a peak has emerged from the olfactory detection port. In a previous investigation pertaining to this topic, Botelho et al. (2007) employed and compared two GC-O methods (PIM and DFM) to analyze the aroma compounds present in three distinct clonal red wines. It was stated that a total of thirty-six aroma-active compounds were detected in both methods. The results of these two techniques were found to be a close relationship but, some compounds, such as 3-methyl butanoic acid (stinky, cheese-like), 2+3-methyl-1-butanol (pungent), 4-vinylguaicol (burnt and curry-like), and 2,5-dimethyl-4-hydroxy-3(2H)-furanone (burnt sugar, candy-like) exhibited the highest detection frequency according to DFM but not according to the PIM. Consequently, the authors concluded that the PIM proved to be a more dependable approach for measuring odors compared to the DFM.

### 2.3. Calculation of odor activity value (OAV)

Odor Activity Values (OAVs) serve to identify and assess the individual contributions of each aroma component to the characteristic odor of

food (Yang et al., 2019). Aroma compounds with higher OAVs predominantly contribute to the unique scent of the food (Grosch, 1993). Calculating the OAV entails dividing the quantity of an aroma constituent by its associated odor threshold value (Abe et al., 2020; Sarhir et al., 2021). This computation of OAVs for specific compounds aids in gauging their sensory significance within the flavor profile (Zhang et al., 2021). When an aroma compound's OAV exceeds 1.0, it signifies a noticeable impact on the overall flavor of the food. Moreover, the influence of an aroma substance on the food's overall flavor rises as its OAV increases (Parker, 2015; Gou et al., 2021).

### 2.4. Aroma recombination and omission test

The aroma reconstitution analysis is performed using the quantitative results determined in the study to confirm the accuracy of the quantitative data. In other words, in a recombination study using the actual concentration of aroma compounds in food, it is evaluated with the human nose whether these compounds can mimic odor responses as a result of mixing (Greger and Schieberle, 2007; Söllner and Schieberle, 2009). An aroma recombination is created by adding all components with an OAV value greater than 1.0 to a matrix (oil, water, alcohol, etc.) based on the quantitative results (Gou et al., 2021; Ayseli et al., 2021). In a previous study on this subject, key aroma compounds of pan-fried white mushrooms were characterized. After employing the AEDA olfactometric method, a total of 40 aroma-active compounds were identified, and it was stated that 4-hydroxy-2,5-dimethylfuran-3(2H)-one (caramel-like) had a high FD value of 8192. The study also reported that the aroma recombination, prepared using 13 aroma-active compounds with OAV greater than 1.0, closely resembled the aroma of the original sample (Grosshauser and Schieberle, 2013). The application of molecular sensory science approach involving AEDA was applied in

another study that examined the essential aroma-active compounds in raw licorice (*Glycyrrhiza glabra* L.). It was reported that fifty aroma-active compounds were identified in this study, with  $\gamma$ -nonalactone, 4-hydroxy-2,5-dimethylfuran-3(2H)-one, and 4-hydroxy-3-methoxybenzaldehyde showing the highest FD values of 1024. An aqueous reconstitution model was prepared by mixing 39 compounds with  $OAVs \geq 1$  at their actual concentrations. It was concluded that the recombinant exhibited an aroma profile highly similar to that of natural raw licorice, thereby accurately identifying and quantifying all significant aroma compounds (Wagner et al., 2016). In a study where the main aroma-active compounds in clear red raspberry juice were identified using molecular sensory science approaches, two different extractions (HS-SPME and SAFE) and olfactometric (DFM and AEDA) methods were employed. Samples extracted using HS-SPME were subjected to DFM analysis, while samples extracted using SAFE underwent AEDA. A total of 29 and 9 aroma-active compounds were identified in HS-SPME and SAFE extracts, respectively. Among these, hexanal, (*Z*)-3-hexenal, (*E*)-2-hexenal, and  $\beta$ -ionone compounds were detected in both extraction methods. It was reported that the three  $C_6$  aldehyde compounds exhibited the highest detection frequency, and  $\beta$ -ionone had the highest FD value. It was also reported that among the three different combinations prepared in aroma reconstitution models (model 1: based on DFM, AEDA, and OAV; model 2: based on DFM and OAV, and model 3: based on AEDA and OAV), model 1 which included all the main aroma-active compounds obtained from the HS-SPME and SAFE extract, was the closest and most similar to the original sample (Zhang et al., 2021).

Subsequent to the aroma recombination investigation, the omission test is employed to assess the impact of eliminating specific aroma components on the overall flavor (Parker, 2015). Ultimately, this process reveals the extent to

which the removal of individual compounds from the matrix contributes to the overall flavor of the food (Song and Liu, 2018). In a previous study, the aroma-active compounds of fresh apricot samples were determined using molecular sensory science. According to the AEDA results, a total of 26 aroma-active compounds were identified, with (*R*)- $\gamma$ -decalactone, (*E*)- $\beta$ -damascenone,  $\delta$ -decalactone, and (*R/S*)-linalool reported to have the highest FD values. Through the calculation of the OAV of volatile compounds, it was found that  $\beta$ -ionone, (*Z*)-1,5-octadien-3-one,  $\gamma$ -decalactone, (*E, Z*)-2,6-nonadienal, linalool, and acetaldehyde had OAV values exceeding 100 and were associated with apricot notes. Aroma recombination experiments were conducted with 18 of the identified aroma-active compounds with  $OAVs \geq 1$ , and the prepared recombination was found to be highly similar to apricot aroma. Finally, omission tests revealed that (*E, Z*)-2,6-nonadienal or (*Z*)-1,5-octadien-3-one compounds made significant contributions to the apricot aroma (Greger and Schieberle, 2007). In another study, the key odorants of rape honey were determined using molecular sensory science. After extracting honey samples using the SAFE method, aroma-active compounds were identified through the AEDA olfactometric method. A total of 28 aroma-active compounds were identified, with the compounds having the highest FD values being (*E*)- $\beta$ -damascenone (cooked apple-like, FD:2048), phenylacetic acid (honey-like, FD:128), 4-methoxybenzaldehyde (aniseed-like, FD:128), 3-phenylpropanoic acid (flowery, waxy, FD:128), and 2-methoxy-4-vinylphenol (clove-like, FD:128). Aroma reconstitution was prepared in a fructose-glucose matrix using all quantified aroma compounds, except for dimethyl trisulfide. It was reported that the recombinant was quite similar to the original honey. Omission analysis was performed by removing aroma compounds with  $OAV < 1$ , but only a small fraction of panelists could discern

the difference. Therefore, it was stated that these compounds did not significantly contribute to the overall honey aroma (Ruisinger and Schieberle, 2012).

### 3. CONCLUSION

In this review, the fundamental operational steps of molecular sensory science are outlined. Molecular sensory science focuses on identifying the compounds responsible for the distinctive aromas of food using GC and GC-O techniques, often in combination with odor activity values (OAV), aroma recombination analysis, and omission testing. Initially, aroma compounds are extracted from the food using suitable methods, followed by the identification and characterization of the most aroma-active compounds within the extract through chosen olfactometric methods. Subsequently, the OAVs of these aroma-active compounds are computed. Finally, aroma recombination and omission tests, based on the concentration of each aroma compound in the food, are devised and assessed through aroma profile analysis. Molecular sensory science finds application across a diverse range of food samples. Further

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studies utilizing this approach are necessary to more precisely determine the key odorants in the studied food.

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### Declaration of Interests

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### Author Contribution

Concept: O K-B

Design: O K-B

Data collecting: O K-B

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Critical review: O K-B



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