

Review

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[Jean-Luc Le Quéré](#)^{*} and Rachel Schoumacker

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Review

Dynamic Instrumental and Sensory Methods Used to Link Aroma Release and Aroma Perception: A Review

Jean-Luc Le Quéré * and Rachel Schoumacker

Centre des Sciences du Goût et de l'Alimentation (CSGA), CNRS, INRAE, Institut Agro, Université de Bourgogne, F-21000 Dijon, France; rachel.schoumacker@gmail.com

* Correspondence: jean-luc.le-quere@inrae.fr or jean-luc.lequere@wanadoo.fr

Abstract: Perception of flavor is a dynamic process during which the concentration of aroma molecules at the olfactory epithelium varies with time as they are released progressively from the food in the mouth during consumption. The release kinetics depends on the food matrix itself, but also on food oral processing, such as mastication behavior and food bolus formation with saliva, for which huge inter-individual variations exist due to physiological differences. Sensory methods, such as time-intensity (TI), or the more recent methods temporal dominance of sensations (TDS) and temporal check-all-that-apply (TCATA), are used to account for the dynamic and time-related aspects of flavor perception. Direct injection mass spectrometry (DIMS) techniques that measure in real-time aroma compounds directly in the nose (nosespace), aimed at obtaining data that reflect the pattern of aroma release in real-time during food consumption, supposed to be representative of perception, have been developed since 25 years. Examples obtained with MS operated in chemical ionization mode at atmospheric or sub-atmospheric pressure (atmospheric pressure chemical ionization APCI or proton transfer reaction PTR) will be given, with emphases on studies conducted with simultaneous dynamic sensory evaluation. Inter-individual variations in terms of aroma release and their relevance for understanding flavor perception will be discussed, as well as evidenced cross-modal interactions.

Keywords: in vivo; aroma release; aroma perception; dynamic methods; nosespace; DIMS; TI; TDS; TCATA; APCI-MS; PTR-MS

1. Introduction

Among the criteria that determine food choice by the consumer, sensory properties of foods are of prime importance. Amid these, flavor is an essential element in food perceived quality. Flavor is sensed by the integration of sensations in the brain, including possible cognitive interactions. Flavor perceptions occur in the mouth when foods are eaten and can be defined as taste and aroma, feeling of pain, heat and cold (chemesthesis or trigeminal sensitivity) and tactile sensation. However, sensory food qualities are generally experienced as unique perception commonly called 'taste'. This familiar 'taste' is a holistic perception of at least aroma and taste, generally called 'flavor perception' [1]. Aroma plays a major role in the overall flavor, as easily demonstrated by the difficulties encountered when trying to identify a particular flavor if the airflow through the nose is prevented. The molecular sensory science approach, so-called 'sensomics' [2], or equivalent procedures, has constituted a major breakthrough in identifying aroma-active compounds in food. Combining extraction of volatile organic compounds (VOCs), identification of odor-active compounds among them, and a validation step through recombination and/or omission protocols, this approach constitutes the state-of-the-art for identifying aroma-active components in food [2].

However, relating aroma compounds composition to aroma perception by humans is not straightforward. Poor correlations are often found between all the aroma compounds identified in a food and the sensory perception by a consumer eating this food. Monitoring specific odor-active compounds with their known individual aroma quality does not necessarily indicate their actual contribution to the overall flavor. In fact, it is still not completely understood how the various

components, their interactions, and their interactions with physiology combine to produce a sensory impression [3,4]. Moreover, a significant correlation between analytical and sensory data does not necessarily imply a causal relationship between the two [5]. Food aroma compounds elicit a transduction cascade after interacting primarily in a combinatorial code with ca. 400 olfactory receptors (OR) in humans. A few hundreds of foodborne volatiles lead to a multitude of odor and aroma perceptual qualities [6]. Moreover, in a sensory evaluation of foodstuffs, flavor is evaluated in a complex mixture context where interactions with the food matrix occur [7], and where masking or enhancing effects may influence the overall sensory perception [8,9].

Perception of flavor is a dynamic process [10], changing in both short and long term and time dimension is de facto implicit in perceptual experience [8]. During food consumption, aroma compounds are released progressively from the food into the air in the mouth and are delivered through swallowing and breathing to the olfactory epithelium in the upper part of the nose via the nasopharynx in what is referred to as the retronasal route [3]. Therefore, the concentration of retronasal aroma compounds at the olfactory receptors varies continuously with time, from the beginning of perception until fading, towards persistence and after-smell. Their release kinetics depends on the food matrix itself [7,11], but also on in-mouth physiological mechanisms, sometimes referred to as food oral processing, producing food breakdown [12,13]. Thus, salivation, mastication and tongue movement conduct to bolus formation with incorporated saliva and subsequent swallowing [12], mechanisms for which huge inter-individual variations exist due to physiological and behavioral differences [14–18]. Moreover, release gradients occur due to aroma compounds adsorption on mucosa, inducing delayed releases partly explaining persistence and after-taste [3].

As temporal dimension is, therefore, of prime importance for any instrumental development aimed at studying in vivo aroma release, direct methods of analysis capable of continuously monitoring volatile compounds in the breath were an obvious option. Direct injection mass spectrometry (DIMS) techniques add this time dimension for the analysis of VOCs [19]. Thus, soft chemical ionization techniques using proton transfer such as atmospheric pressure chemical ionization (APCI), proton transfer reaction (PTR) or selected ion flow tube (SIFT) coupled to mass spectrometry (MS) are able to monitor in real time aroma compounds present in the exhaled breath directly in the nose [20]. Referred to as nosespace analyses [21], continuous breath-by-breath analyses [22] aimed at obtaining data that are supposed to reflect the pattern of aroma release in real time during food consumption, which is supposed to explicate perception [23]. These DIMS techniques accommodate the necessary instrumental constraints in terms of speed and response (linearity and limit of detection) compatible with real-time in vivo analysis of VOCs present in the breath of humans [20,24].

To account for dynamic and time-related aspects of aroma perception, specific time-dependent sensory methods have been developed [25]. Obviously, well-established static sensory measures such as descriptive analyses are not suitable. These measures made at a single time-point do not capture the full temporal sensory experience and such evaluations are an integration over time of the whole perception experience [26]. Very early, the time-intensity method (TI) that allows following the intensity evolution of a sensory attribute was available [27]. Since TI focuses on a single attribute only, it is far from assessing the multi-component profile of aroma release and capturing the multidimensionality of perception. Therefore, other quantitative and qualitative temporal methods able to measure several attributes simultaneously have been proposed. Among quantitative-based ones, dual-attribute TI (DATI, [28]), modified TI [29] or multi-attribute TI (MATI, [30]) must be cited. Amongst qualitative-based ones, temporal dominance of sensations (TDS) that selects amid a predefined list of descriptors which one is dominant at each time of consumption [31], and temporal check-all-that-apply (TCATA) that enables to select several pertinent descriptors at each time-point of consumption [32], are the most popular.

Combining dynamic instrumental and sensory methodologies in order to try to understand better aroma perception through the prism of aroma release seems desirable. Inter-individual variability in aroma release and perception, on the dependence of physiological and behavioral factors, is well documented, but intra-personal variations should also be taken into account. In time-

dependent perceptive tasks, since continuous evaluation is highly attention demanding, individuals may vary in their sensory acuity and present fatigue detrimental to their capacity to stay focused on a particular task and the time elapsed between stimulation and response [8]. Therefore, important intra-individual variations are inherent to temporal sensory evaluation, and may be related to circadian rhythm [8], mood, and physiological aspects like preprandial or postprandial status. Efficient training and planning tests at the same time in the day for each replicate session can reduce these variations in a certain extent [8]. However, aroma release experiments are also subject to intra-personal variations. Thus, although a strict breathing and sampling protocol was applied, significant intra-individual variabilities between replicates were effective in the nosespace analysis of flavored liquid samples, even if the five replicates were measured in the same session with sufficient recovery time between them [18]. The variation was proved to be due to human release behavior, despite the strict evaluation protocol used; moreover, the variation was found volatile compound-dependent, larger variation being obtained for compounds with higher air-water partition coefficient [18]. Implication of intra-personal variations in aroma release experiments could be an important issue for replicate measurements, particularly when replicates are being measured in different sessions, at different moments. Aroma release and perception appear as multidimensional phenomena, with variations highly dependent on humans, with time-related aspects of inter- and intra-individual variability. As such, in order to understand better the mechanisms that link *in vivo* aroma release and dynamic sensory perception during food consumption, one should rely on a protocol where both instrumental and sensory methods are conducted simultaneously. Therefore, real-time simultaneous data capture seems preferable for measuring changes over time in order to avoid, or at least reduce, inherent variability that should increase significantly if non-concurrent assessments are being used.

The aim of the present review is to present the temporal sensory methods available to analyze dynamically aroma perception, the *in vivo* dynamic instrumental methods used to analyze aroma release, and to emphasize studies that conducted aroma release and sensory evaluation simultaneously. Challenges related to various sources of variability such as interactions with the food matrix, food oral processing and sensory cross-modal interactions are also discussed. Some perspectives for future research are also presented.

2. Dynamic Sensory Methods to Analyze Aroma Perception

To account for the dynamic character of flavor perception, temporal sensory methodologies that allow following the evolution of perceived sensations with time has been developed. The area of temporal methods has grown to such an extent that books (e.g. Hort et al., 2017 [25]) and reviews (e.g. Visalli and Galmarini, 2022 [33]) dedicated to the topic of time-dependent measures of perception in sensory evaluation have been published recently.

Historically, the first method proposed to follow the intensity evolution of a sensory attribute (e.g. one aroma descriptor) in the time-course of food consumption has been the time-intensity (TI) evaluation. Initially manually handled, data recording and treatment benefited early from computerized systems [27]. The TI evaluation had been considered as an established sensory methodology for some 40 years as early as in the beginning of the 1990s [34,35]. However, TI focuses only, although continuously and quantitatively, on a single sensory attribute. As a food consumption experience is rarely monodimensional, panelists must complete as many analyses as number of sensory attributes to be evaluated. Quickly the methodology can become lengthy and costly. Moreover, continuous TI, sometimes referred to as CTI [36], requires extensive training of the panelists who tend to reproduce a stereotypical response. It is also subject to the halo-dumping effect [8,37] when intensity evaluation of one descriptor can be biased by the concomitant perception and influence of other attributes, questioning the quantitative measure [36,37]. To tackle this problem, a method that allows evaluating the intensity of two attributes simultaneously has been proposed (dual-attribute TI or DATI, [28,38]). Although DATI tests require half the time to complete in comparison to successive single-attribute tests [28], it doesn't fully answer to the multidimensionality of flavor perception and needs careful panelists' training, but is particularly suited to study the relationships between two attributes (e.g. one taste and one aroma, [28]). The data parameters

generally used with TI curves are the maximum intensity (I_{max}), the time to reach I_{max} (T_{max}) and the area under the curve (AUC) that measures the overall perception.

To examine the halo-dumping effect, Clark and Lawless [37] developed a discontinuous TI methodology using specified discrete time-points to follow simultaneously two flavor attributes (i.e. sweetness and aroma). They found that odor-induced enhancement of sweetness was effectively lessened or eliminated [37]. Discrete (discontinuous) time-intensity (so-called DTI), used to evaluate perceived intensities at specified, distinct time-points [39], involves the rating of single or multiple sensory attributes at these discrete points in time [39]. DTI can measure temporal changes over longer periods and appears more flexible while allowing for more than one attribute to be assessed at once, lessening or eliminating attribute dumping effect. However, its discrete nature means that some information may be lost between time-points, data are often noisy, and the design of experimental protocols could be rather complex [40]. It does not appear as a method of choice for assessing changes over short time periods [40], but seems to suit better long evaluation periods such as persistence or after-taste phenomena. However, this was the basis of a so-called 'modified TI' method developed to evaluate the time-related intensities of various flavor descriptors of a model cheese consumed within one bite [29]. Schematically, the attributes were presented to each panelist alternatively and randomly during successive masticatory sequences of ca. 3 minutes. During these periods, which were divided in nine measuring time-points, the descriptors were randomly presented on a computer screen at each measuring time. The assessors had to evaluate their intensities at each time-point during a 3-seconds delay. Therefore, a complete evaluation of several descriptors necessitated several sequences for each panelist [29]. The method, later rationalized as the multi-attribute TI (MATI) method [30,39], although avoiding the attribute dumping effects, keeps the drawback of a lengthy and potentially costly method. In fact, an extended time duration is required to cycle through the attributes list repeatedly in order to capture a sufficient number of points to model the MATI curves [39]. Moreover, as papers using these techniques are particularly scarce, concern has been raised about the difficulty for participants to handle the procedure [35]. A derived method called alternate time-intensity (ATI) has been used recently to evaluate two sensory modalities, the salty taste and the specific aroma of flans [41], but it appears quite equivalent to the DATI, 'modified TI', or DTI methods described above.

TI was initially considered as a kind of temporal version of quantitative descriptive analysis (QDA), while allowing measuring only one attribute at a time [33]. However, descriptive analyses, with the evaluation of the intensity of several descriptors that gives rise to common sensory profiles of foodstuffs, are static methods. The descriptors intensities are integrated by the panelists at one time, which can be different for each panelist, all over a sensory session. Some discontinuous temporal versions of descriptive analyses that somewhat simplify the procedure [33] have been published, such as intensity variation descriptive methodology (IVDM, [42]), an early version of progressive profiling (PP, [43]). They enable the measure of attributes intensities within a single intake at uniform intervals steps or at specific moments and allow quantitative profiles of several attributes at different time to be obtained. Time scanning descriptive analysis (TSDA) was originally designed to tackle perception discrepancies caused by differences in evaluation temperatures between panelists evaluating hot beverages [44]. As such, as an alternative to QDA, TSDA accounts for the dynamics of sensory perception as a function of the temperature of the test sample at which it is evaluated [44]. Nevertheless, it could also be used as a convenient dynamic descriptive method as it introduces intensity scaling of specialty attributes at designed time blocks. Based on the same time-steps evaluation process, but for multiple intakes during consecutive consumption protocols, sequential profiling (SP) that extends progressive profiling to multi-bites (or sips) has been proposed [45]. Finally, a very demanding dynamic flavor profile method, combining the descriptive approach and the TI approach, has been described [9]. It consisted in recording the TI response of each descriptor previously determined by QDA; a 3D drawing of the data allowed to get a descriptive profile of a food at each consumption moment [9]. The quantitative methods described above require the generation of the attributes by the panel prior to the main analysis, which requires anterior descriptive tasks. They are all demanding methods that share the requirement of highly trained

assessors and the drawback of a quite lengthy (and costly) process, which almost preclude simultaneous nosespace analyses if several sensory attributes are in question.

In order to simplify the task, qualitative dynamic methods have developed recently. Temporal dominance of sensations (TDS, [31]) is a method that allows evaluating dynamically several sensory attributes (up to twelve in practice [46]) simultaneously during consumption. Methodologically, TDS lies between conventional static descriptive analysis of several descriptors and dynamical but monodimensional TI [35,46]. The method has developed considerably, including data treatment advances [47], and could be considered as the ideal means to dynamically follow the sensory perceptions associated with the release of aroma-active molecules. However, TDS introduced the concept of dominance, different from intensity. Practically, the panelists have to identify continuously during consumption the dominant sensation among a given list of attributes beforehand determined consensually. Therefore, a TDS analysis results in a sequence of dominant sensations measured by the dominance rate of the panel (Figure 1) during the evaluation period that can include a post-ingestion time to evaluate persistence [46].

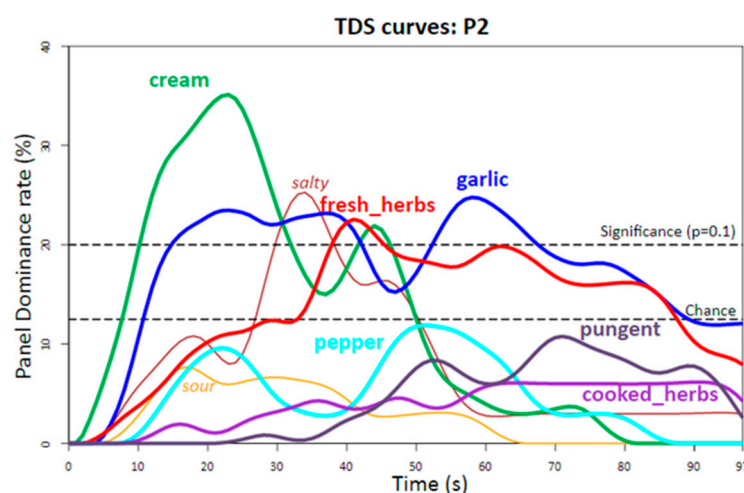


Figure 1. TDS curves obtained for a flavored ('garlic and herbs') fresh cheese (P2), evaluated by sixteen panelists using eight predefined attributes: garlic, cream, fresh herbs, cooked herbs, pungent, pepper, salty, and sour [48]. On these graphs, the "chance level" corresponds to the dominance rate that could be reached by chance for a given attribute. Its value, P_0 , is equal to $1/n$, n being the number of attributes. The "significance level" is the minimum value that must be reached for the dominance rate to be considered significantly higher than P_0 and is calculated from the confidence interval of a binomial proportion based on a normal approximation [31]. Only TDS curves above the significance level are considered significantly dominant.

However, the dominance notion is a complex mental construct that includes more than one single aspect of sensory perception [49]. With TDS only the most salient sensations at a given time are dynamically assessed reliably. Therefore, some descriptors, although being important to explain particular perceived sensations whose intensities could be possibly evaluated in a descriptive analysis, may appear as not noticeable because never significantly dominant (e.g. pepper, pungent and cooked herbs aromas in Figure 1).

As an alternative to TDS, the temporal check-all-that-apply method (TCATA, [32]) was developed. TCATA integrates the temporal monitoring of all applicable attributes, chosen among a predefined given list, and transforms the static CATA (check-all-that-apply) method [50] in a dynamic one. Method and data treatment developments have made TCATA as operational as TDS [51,52]. Allowing a dynamic monitoring of several attributes (and not only the dominant ones), TCATA seems attractive (Figure 2) to be hyphenated to aroma release analyses.

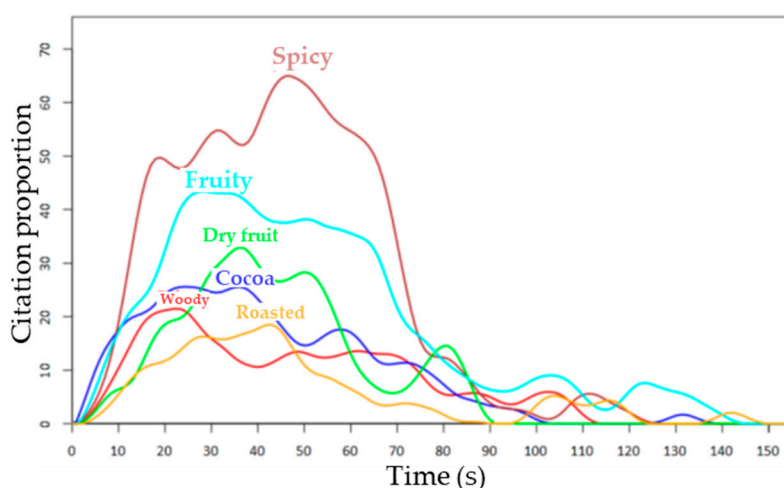


Figure 2. TCATA curves obtained for a dark chocolate evaluated by sixteen panelists using six predefined aroma descriptors [53]. Results are expressed as panel citation proportion.

However, as for TDS, some drawbacks soon appear in practice: a limited number of attributes in the predefined list and difficulties to handle the method for the panelists (they have to check descriptors when they are perceived and uncheck them when they are no longer apparent), although a familiarization step seems to afford a certain comfort improvement [51]. Moreover, for both methods, data treatment average results on the panel, leading to the loss of individual information.

The two methods have been compared very early [54] and both were found equally adequate to discriminate food samples [54]. TCATA was found slightly more efficient giving rise to more detailed dynamic sensory profiles [54,55]. However, this alleged preponderance could depend on the type of food samples, on panels performance (use of familiarized, trained or naïve panels), or on the lack of specific criteria necessary to compare the two temporal methods [47]. DTS suffers from dithering and dumping effects, which can be important when two or more sensory modalities are being evaluated in the same sequence (e.g. aroma and/or taste and/or texture). Then, only a few attributes are available for each modality and the panelists can be indecisive when choosing modality and descriptor at the same time [49]. To alleviate this drawback, TDS by modality (M-TDS) that differentiates TDS sequences for each sensory modality was proposed [56,57], with, however, the inherent disadvantage for the panelists to perform as many evaluation sessions as modalities to be evaluated. The three methods (TDS, TCATA and M-TDS) have been compared in a study aimed at characterizing semi-solid foods of yogurt type [58]. They were found equally able to discriminate the samples, with some advantages for M-TDS and TCATA for the dynamic multimodal sensory description of the products. TCATA afforded additional information on potential interactions between modalities or/and descriptors. The three methods seem adequate for the discrimination of a set of samples, but they have never been compared in combination of aroma release analyses in order to explain perception. Moreover, this only comparative study has been conducted using a single intensively trained panel in a predetermined evaluation order (TDS, TCATA and then M-TDS), and no definitive conclusion on the respective advantages and drawbacks of the three methods could be inferred [58]. The use of trained panels or naïve consumers for these qualitative temporal methods is also debated [58]. In a recent study on chocolate-hazelnut spreads consumed without or with a carrier food by a consumer panel (n=72), TCATA better discriminated between spreads while TDS revealed clearer temporality of sensations [59].

In the method presented above, the studied time-lapse concerns mostly the time it takes a subject to evaluate continuously one intake (sip or bite) of a food product from the moment of food intake in the mouth until a few moments after swallowing. However, food and beverage consumption generally needs multi-intake, and the temporality of full portion evaluation (bite after bite, or sip after sip) has been rarely studied [33]. Thus, with the objective of measuring the order in which key attributes appear over a complete eating experience (including each mouthful until aftertaste),

temporal order of sensations (TOS) was proposed [60]. Within a predefined list of attributes, the subjects were asked to indicate for each bite/sip and for aftertaste, in order which three, for instance, they perceived first [40,60]. The results are then presented according to the proportion of each attribute emerging first at each evaluation time. Another method, so-called 'pick 3 and rank' (P3R, [61] cited by [62]) was proposed to measure the temporality between several bites of a full portion. P3R consisted in retrospectively (i.e. not in real time) picking then ranking the 3 most important descriptors perceived during a bite [62]. Based on TOS and P3R, a new retrospective temporal method so-called attack-evolution-finish (AEF) inspired from the sequence often used by wine professionals [62] has been recently proposed [62]. It consists in retrospectively selecting the most important descriptor during each of the 3 tasting periods [62], this selection being related to the concept of dominance rated in TDS [62]. Compared in a study on dark chocolates, it was concluded that AEF and TDS produced very similar results in terms of product discrimination [62]. In order to avoid the potential bias induced by presenting a limited number of predefined descriptors to the panelists, it has been proposed recently to use free-comment with AEF (FC-AEF, [63]). FC-AEF allows the subjects to evaluate their temporal perception using their own words instead of a limited predefined list of attributes. Used with a panel of 63 consumers evaluating five dark chocolates, thus avoiding the necessary training of assessors, it was claimed that FC-AEF allowed providing temporal discrimination and characterization of the products [63].

The newest methods escaped from the real-time simultaneous tasting-evaluating paradigm as they proposed a retrospective evaluation of several attributes at discrete moments of consumption immediately after tasting [33]. Advantages and disadvantages of concurrent versus retrospective sensory data collection are discussed in detail in the recent papers that proposed the retrospective measures [62–64]. While losing temporal resolution in real-time, it is claimed that retrospective methods do not require training nor familiarization and, therefore, can be implemented without difficulty with naive consumers contrarily to other temporal methods [64]. They seem adequate for discriminating food samples. However, they still need to be confronted with dynamic instrumental aroma release methods in order to evaluate their ability to strengthen the release-perception relationship issues.

3. Dynamic Instrumental Methods to Analyze In Vivo Aroma Release

Aroma compounds directly measured in the expired air from the nose (so-called nosespace) during food consumption are supposed to reflect aroma release in real time. They are supposed to be representative of the molecules that interact with the olfactive receptors (the active odorants) via the retronasal route, and hence to cause aroma perception.

Techniques for measuring aroma released in expired air from the human nose have been developed during the last three decades. Significant robust results were obtained for sampling aroma using a collection of expired air samples at discrete time-points during consumption on adsorbents of Tenax® type [65,66] or on solid phase microextraction (SPME) fibers [67,68]. After thermal desorption of the adsorbent and analysis by gas chromatography coupled to mass spectrometry (GC-MS) of each desorbed sample, it was possible to construct aroma release curves, although obtained discontinuously (e.g. Linforth et al., 1996 [69] and Pionnier et al., 2004 [67]). Moreover, this discontinuous sampling presents a low sensitivity due to the limited adsorption time available for each time-point. Therefore, this method is essentially used to study highly flavored model foods. Real-time continuous in vivo aroma analysis has been obtained using atmospheric or sub-atmospheric pressure ionization MS [70], often referred to as direct-injection MS (DIMS, [19]). Aroma release curves are thus obtained in real-time in a continuous way as air from the nose is sampled directly into a mass spectrometer through a heated interface, making real-time breath-by-breath analysis routinely possible. These techniques operate in soft chemical ionization (CI) mode [24], generally by proton transfer from the reactant hydronium ion H_3O^+ . Most of the volatile compounds have higher proton affinities (PA) than water ($\text{PA}_{\text{H}_2\text{O}} = 691 \text{ kJ/mol}$) and they ionize by proton transfer from H_3O^+ giving rise essentially to protonated molecular ions MH^+ and a few fragments [70] that are accelerated into a mass spectrometer. Advantageously, common constituents of air have PAs lower

than the PA of water, and are not ionized. Among the DIMS techniques, those which are based on proton transfer CI such as atmospheric pressure chemical ionization (APCI-MS), proton transfer reaction (PTR-MS) and more recently selected ion flow tube (SIFT-MS) appear especially well suited to explore the dynamic process of aroma release [19,20,70] and they have been used quite extensively for the last twenty years. Secondary electrospray ionization (SESI-MS) has demonstrated some potentialities in the domain of real-time breath analysis (e.g. Berchtold et al., 2014 [71], Gaugg et al., 2016 [72], Weber et al., 2023 [73]) and should also contribute to real-time aroma release studies in the near future. Noteworthy, the technique has recently proven its utility in VOCs fingerprinting [74]. These DIMS techniques accommodate the necessary instrumental constraints in terms of fragmentation, speed and response (linearity and limit of detection) compatible with *in vivo* analysis of volatiles present in the breath of human subjects [75]. From the early beginning, many examples of nosespace analyses and their fundamental advances may be found in dedicated or specialized treatises [24,75–79].

The first significant robust results were obtained using APCI-MS with an ion source optimized for the detection of volatile substances hyphenated to a quadrupole mass analyzer [69,80]. This so-called MSNose™ interface [81] has been used in numerous aroma release studies that can be found in dedicated reviews [22,23,79,82]. Aroma release curves also reflect the respiratory cycle of individuals (signal increase on exhalation, signal decrease on inhalation), allowing the measure of respiratory frequencies. From the start, inter-individual differences in aroma release kinetics, linked to oral physiology variability, were evidenced [80] and compound-dependent temporal release delays, linked to in-mouth enzymatic reactions, disclosed [69]. Optimized APCI sources have also been interfaced with ion-trap mass spectrometers [75,83–86] or triple-quadrupoles [87], providing sensitivity, selectivity and structural capability benefits of tandem MS (MS/MS). Instruments sensitivity is an important issue, as only a minor part of the food aroma components is actually able to reach the nasal cavity [3]. A large part of the odorants is simply swallowed with the food and adsorbed via the gastro-intestinal tract [88]. Moreover, in the mouth odorants partition between food media and saliva or air, adsorb on mucosa and are diluted in the breath during transport to the nose. All these compound-dependent events limit their availability for perception [12]. Thus, retronasal concentration has been reported to reach only 0.1 to 10 % of the concentration in the food measured by static headspace, and to be 10 to 100-fold lower than in mouth [89]. APCI instruments hyphenated to quadrupole mass analyzers present some limitations. To reach sufficient time-resolution and sensitivity, necessary for *in vivo* aroma release studies, they need to be run in the multiple-ion detection mode (or selected ions monitoring) with dwell times that limit the practical measurable number of targeted ions (i.e. individual VOCs) to 5-10. This limitation is also true for PTR-MS and SIFT-MS instruments run with quadrupole analyzers. Contrarily, ion-trap instruments are fast analyzers that allow full scan to be performed, compatible with the necessary time-resolution of *in vivo* aroma release, with comparable sensitivity to that of multiple-ion monitoring of quadrupole instruments. Therefore, ion-trap mass analyzers allow untargeted analyses. Numerous examples of *in vivo* nosespace studies using APCI-MS may be found in dedicated publications (e.g. [19,24,75,79,82]). The PTR-MS technique [90–92] has been also largely used contemporarily to APCI for real-time breath and nosespace analyses [18,93,94]. Contrarily to APCI where reagent ions are produced in the vicinity of the ionization region in the source, in PTR the generation of the reactant H_3O^+ ion in a specially designed source is spatially and temporally separated from the proton transfer reaction that occurs in a dedicated reaction chamber, the drift-tube [20]. Therefore, a better control of the ionization process is achieved, and individual optimization and quantitation are made accessible [20,95]. The same applies to the SIFT-MS technique [96] where the reaction chamber is a flow tube, with the additional advantage of thermal energy ionization conditions that allow studies on reaction rate coefficients between reagent ions and neutral analytes [20]. However, the necessary addition of a carrier gas in the flow tube produces a dilution of the breath samples, thus limiting *de facto* the sensitivity of the technique. For a long time used in breath research [97,98], SIFT-MS has naturally proven its utility in nosespace analyses [99–103].

A major breakthrough has been achieved when a time-of-flight (ToF) mass analyzer has been hyphenated to PTR instruments [104,105]. As already outlined, quadrupole mass analyzers (QMA) are limited to analyses targeted on a few analytes whose molecular ions or fragment ions are obtained at nominal masses. ToF affords higher mass resolving power, allowing isobaric compounds to be distinguished, speed of analysis in full scan mode, and a better sensitivity on the whole mass range. These features afford tremendous benefits with full scan compatible with breath-by-breath time-resolution and sensitive untargeted analyses. While nosespace analyses conducted using QMA generally address highly flavored food models or foodstuffs reinforced with a few aroma molecules, ToF instruments may address real food issues [106]. Moreover, tentative identifications are made possible with theoretical assignment of sum formulae to each detected ion thanks to the exact mass measurement allowed by the mass accuracy of the ToF analyzer. However, a sum formula may correspond to one or more compounds (isomers) and it remains advisable to ascertain aroma composition by GC-MS to provide confirmatory data on compound identities [20]. Numerous examples of nosespace analysis using the PTR-ToF-MS technique can be found in recent publications [24,77,107]. Noteworthy, both SIFT-MS and PTR-MS ion sources have the possibility to produce other reactant ions such as O_2^+ and NO^+ that ionize neutral molecules via charge transfer or hydride abstraction. Intrinsic feature of SIFT-MS in the 'selected ion' process (negative reagent ions O^- , O_2^- , OH^- , NO_2^- may also be selected), it necessitates a switchable reagent ion (SRI) option for PTR-MS [108]. The ammonium ion NH_4^+ may also be used as a protonating agent using both instruments [109,110]. Although all these reagent ions can be useful in specific applications (e.g. OH^- for detecting molecules bearing labile protons or NH_4^+ for detecting amines and labile oxygenated compounds), they still need to demonstrate their usefulness in routine aroma release analyses.

As for the data treatment used in TI, the data treatment parameters generally retained for the release curves are the signal maximum intensity (I_{max}), the time to reach this maximum intensity (T_{max}), and the area under the curve (AUC) that measures the total amount of the released compound. The curves may be smoothed to attenuate the up and down signal variations due to breathing (signal up on exhaling, signal down on inhaling), and thus determine more easily the I_{max} and T_{max} parameters (Figure 3).

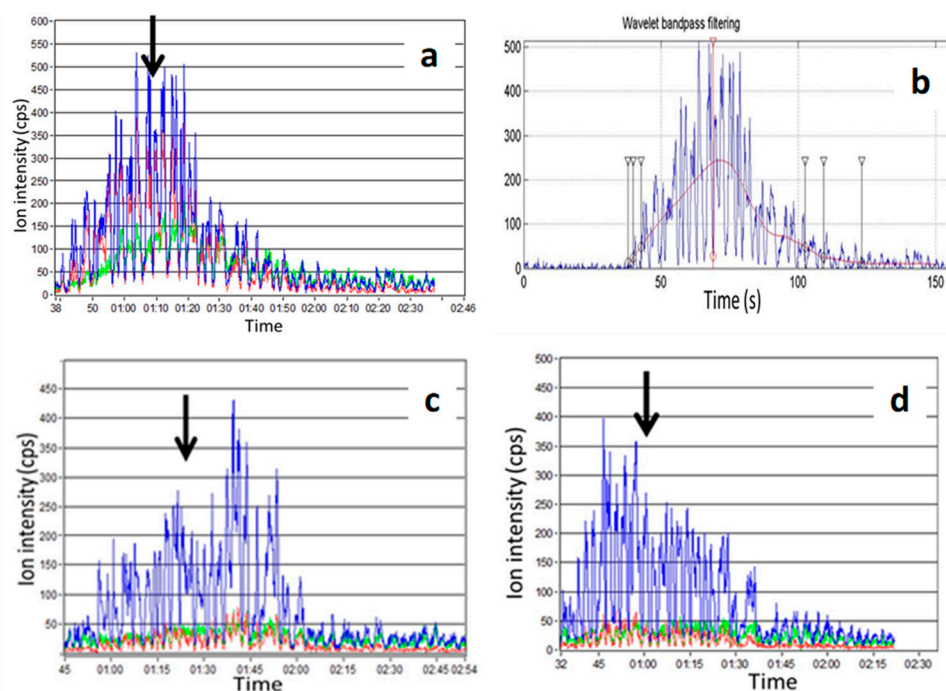


Figure 3. Examples of aroma release curves obtained for a flavored (“garlic and herbs”) fresh cheese evaluated by sixteen panelists using a PTR-ToF-MS (in blue m/z 73.067 butan-2-one, in red m/z 87.044 diacetyl, in green m/z 89.060 butyric acid/acetoin) [48]. Vertical arrows on a), c) and d) stand for swallowing time. The release curves illustrate inter- and intra-individual variability: a) stands for one panelist while c) and d) are replicates of another panelist. Vignette b displays an example of signal smoothing (red trace is the smoothed signal of m/z 73.067 of a) using wavelet bandpass filtering [111].

4. Aroma Release and Sensory Evaluation Conducted Simultaneously

Many attempts to relate *in vivo* dynamic aroma release to perception relied on static sensory methodologies, such as descriptive analysis. Both analyses were conducted at different moments and not simultaneously for practical impossibility. Sometimes conducted using the same panel for both analyses, a majority of studies employed a different panel for sensory evaluation, often with more panelists for more robust sensory results. Static sensory methods, which result in an integration by the panelists of each sensory modality, are not able to assess time-related aspects of perception. Moreover, the panelists meeting together at a different moment, individual aspects apprehended by nosespace analyses, compulsory conducted at individual level, are lost. In efforts to establish the link between release and perception, not taking into account those inter-individual variations may result in possible bias, and static sensory methods are essentially good at differentiating products. Nevertheless, some significant relationships have been established on the effect of fat, thickener or sugar on aroma release and perception in a descriptive approach. For instance, a descriptive profile was used in a study on the effect of thickener type and fat concentration in citrus emulsions [112] and a protocol based on descriptive sensory analysis was proposed to study flavor release and perception in cheese [113].

Observed discrepancies between release and perception were hypothesized to be due to interactions not taken into account by static methods. Dynamic sensory methods (essentially TI) have also been used in parallel while independently of the release studies, either by the same panel or by two different panels. While, as previously stated, inter- and intra-individual variations may be responsible of some bias, significant results have been obtained. Thus, for instance, multi-attribute TI allowed establishing some relationships between sensory attributes and released flavor compounds in a model cheese [29], and discontinuous TI was used to decipher the respective role of aroma release, salivary composition and oral processing parameters in model cheese aroma perception [114].

So, different strategies have been adopted to investigate non-concurrently the dynamic aspects of aroma release and aroma perception during food consumption. Conducted essentially on model foods using static or dynamic sensory methodologies by single or different panels, observed discrepancies in the results have been often linked to the complexity of the involved phenomena, including matrix effects, cross-modal interactions and inter-individual variations. Therefore, despite interesting information have been obtained in parallel studies, as already outlined, to clearly establish the link between *in vivo* aroma release in the nosespace and aroma perception, and avoid bias as much as possible, it seems advisable to conduct the two complementary analyses simultaneously, with the additional advantage of a single panel appropriateness.

Due essentially to limited sensitivity inherent to APCI- or PTR- quadrupole instruments for *in vivo* studies, real food systems were rarely investigated initially. Thus, the first studies conducted simultaneously dealt with highly flavored model food or flavored-reinforced real food (Table 1), while staying sensorially acceptable for the panelists.

Using a gelatin gel flavored with increasing concentration of carvone [115] or a solid food aromatized with a rosemary flavor [5], a perfect correlation between aroma release and simultaneous perception was found (Table 1). In the carvone experiment, the aroma quantity delivered in the nosespace was proportional to the carvone concentration in the product, although absolute measured quantity varied greatly between individuals, and the perceived mint intensity was correlated to the aroma concentration [115]. These simple models seem to attest a clear link between release and perception. The time of maximum perceived intensity (T_{max} of TI experiment) occurred before the

time of maximum aroma release (T_{max} of nosespace experiment), and this was attributed to adaptation [116] to the stimulus [115]. Huge inter-individual variations were noticed, but the speed of eating seemed to influence the level of adaptation to the stimulus, the slower the eating event, the greater the adaptation [115], confirming previous results obtained by Linforth et al. with more complex models (Table 1, [117]).

Aroma molecules are generally hydrophobic entities, and, therefore, food fat content is an important factor that affects aroma release and perception. The link was investigated in flavored yogurts varying in fat content [118]. Nosespace measurements revealed faster and more intense aroma delivery, but with less persistence, in low fat yogurts (Table 1). Sensory data revealed also faster and more intense aroma perception in low fat samples, while no significant differences were found between samples for perceived persistence [118]. Similar results were obtained with flavored milks varying in fat content (Table 1, [119]) and with flavored liquid emulsions differing in fat level (Table 1, [120]). An adjustment of aroma content in low and high fat milks in order to deliver the same aroma quantity resulted in isointense aroma perception [119]. In these examples, the effect of fat on in vivo release globally conformed to the theory when significant decreases in both in-nose volatile release and corresponding perceived aroma intensities were found on increasing fat content. However, contrasted results were observed when considering the respective hydrophobicity of the aroma molecules (Table 1, [118]), which was later confirmed by an experiment conducted non-concurrently on flavored milk with varying fat content [121]. In flavored emulsions, the fat content also produced a significant effect on pre- and post-swallowing events [120]. With a rigorous breath and consumption protocol, it was found that on increasing the fat content, the ratio of volatiles released in the post-swallow phase increased significantly compared to the pre-swallow phase. The authors hypothesized that these data could contribute to the understanding why low-fat and high-fat foods are perceived differently [120]. These results globally confirmed others that were obtained in non-concurrent analytical/sensory experiments (e.g. [122]). However, changing fat content in food may affect other parameters such as structure and texture, with for instance differences in particle size and viscosity, which could also affect aroma release [118]. These structure and concomitant texture issues have been the subject of several studies on the impact of various thickeners on aroma release and perception (Table 1).

When the only studied parameter was the thickener (e.g. gelatin or whey protein) concentration, in mono-flavored model gels it was clearly established that texture determines aroma perception rather than in-nose aroma concentration, evidencing texture-aroma cross-modal interactions (Table 1, [17,123,124]). Moreover, perception seemed correlated to the different rates of aroma release in differently textured gels [123]. Quick release during the chewing phase was noticed for soft gels while aroma delivery from hard gels increased slowly, reaching maximum intensity during swallowing [17]. However, for soft gels individual release curves presented two different patterns. Some panelists released aroma during the chewing phase while others released aroma essentially on swallowing. A proposed explanation was that the latter kept their velum (soft palate) unconsciously closed during jaw movement, while the former opened their velum allowing aroma transfer to the nose, already changing their chewing behavior according to that of a more rigid system [17]. Directly related to aroma delivery, aroma perception showed high temporal resolution largely modulated by individual physiological differences that were not fully mirrored by the TI sensory methodology [17].

TDS was applied to differently textured gelified candies (Table 1) flavored with three aroma molecules imparting 'green', 'strawberry' and 'butter' olfactory notes to the candies (namely Z-hex-3-en-1-ol, ethyl hexanoate and diacetyl, respectively). TDS sensory data indicated a linear increase of the global duration of dominance in hard candies [125]. An imposed in-mouth melting protocol revealed interesting features. Temporal sensory profiles were found dependent on candies texture. Thus, the 'strawberry' attribute was dominant in the liquid gel (0% gelatin). In the 2%-gelatin gel, the 'green' attribute dominated before the 'strawberry' descriptor became significantly dominant. For the 5%- and 15%-gelatin candies, 'butter' was dominant in the initial melting time before 'strawberry' became dominant in perfect coincidence with swallowing [125]. Temporal relations with corresponding released aroma molecules in the nosespace were evidenced. When the panelists

chewed the candies freely, out of the melting protocol, quicker (T_{max}) and higher amount (I_{max}) of aroma release was obtained, but only the 'strawberry' attribute was judged as dominant around a shortened swallowing time. In this case, temporal relationships between release and perception were more difficult to establish. The use of an imposed melting protocol, involving a longer residence time of the product in mouth, probably allowed easier relationships to be evidenced. However, all the conclusions were mainly based on a descriptive analysis of instrumental and sensory data averaged on the panel where the variety and the complexity of the phenomena could not be fully apprehended [125].

When a mechanical treatment was coupled to varying protein content in flavored stirred yogurts in order to obtain varying yogurt viscosity, the different viscosities influenced aroma release and perception (Table 1, [126]). However, the phenomena were more influenced by the applied mechanical treatment than by protein content, revealing the importance of viscosity in the release and perception relationships [126]. Finally, when differing sugar level was combined to various thickener contents in flavored model custards, perceptual sweetness-aroma cross-modal interactions were mainly evidenced, whatever the texture of the desserts (Table 1, [127]).

One of the first clear demonstration of a sweetness-aroma cross-modal interaction was obtained using mint-flavored chewing gums in a combined nosespace-TI experiment (Table 1, [128]). If menthone release and mint perception concomitantly increased quickly, mint perception rapidly decreased while the nosespace menthone content remained almost constant. It was found that mint perception followed the decrease of the sucrose level measured in the saliva of the panelists, hence sweetness perception. Thus, a taste/aroma interaction between sweetness and the congruent mint aroma was evidenced [128]. However, although the panel was rigorously trained for aroma TI assessments, one cannot exclude that the assessors might have confused loss of sweetness with mint flavor [128]. Alternatively, the panelists could have become adapted to menthone with time and this adaptation [116] period could have coincided with the sucrose release time [128]. To limit adaptation, the food matrix must have a shorter residence time in mouth. This is clearly the case for beverages. The effects of sugar and CO_2 levels were investigated in mint-flavored (menthol, menthone and (Z)-hex-3-en-1-ol) carbonated beverages in the context of reducing sugar levels in such beverages, which represents a challenge for the industries of soft drinks (Table 1, [129]). While increasing CO_2 level resulted in increased aroma release and perception, regardless of sucrose content, increasing sugar concentration only tended (not significantly) to induce higher aroma release in the nasal cavity, with, however, no effect on perception for carbonated beverages [129]. Perceptual sweetness-aroma interactions between congruent flavors (mint and sweet) were thus evidenced in the absence of CO_2 . Similarly, Lethuaut et al. found that a higher level of sucrose in their flavored gels increased aroma perception without a significant effect on aroma release (Table 1, [127]). The presence of CO_2 in the carbonated beverages could have induced a trigeminal perception that could mask aroma perception in a perceptual trigeminal-aroma interaction, or have increased complexity involving difficulties to assess the carbonated products [129].

Real foods have also been investigated with simultaneous instrumental/sensory protocols. Thus, three commercial soft cheeses were submitted to nosespace (APCI-MS) and dynamic sensory (TI on the three predefined 'sulfury', 'buttery' and 'mushroom' attributes, evaluated in separate sessions) analyses (Table 1, [130]). This study revealed the sensitivity and technical limits of APCI-QMA instruments for studying real foods. While 19 compounds were identified as major odor-active volatiles of the cheeses by GC-O, only six protonated molecular ions were detected in the nosespace of 15 panelists, namely m/z 63 (dimethylsulfide), m/z 91 (S-methylthioacetate), m/z 94 (dimethyldisulfide), m/z 87 (3-methylbutanal/diacetyl), m/z 115 (heptan-2-one) and m/z 143 (nonan-2-one). Temporal correlations have been established only between the 'sulfury' note and the three sulfur compounds. Poor correlations displayed for the other attributes were mainly due to insufficient sensitivity of the APCI-MS for some key-odorants (octan-3-one and oct-1-en-3-ol, responsible for the 'mushroom' note, were not detected in the nosespace) and unachieved separation of isobaric compounds (diacetyl, responsible for the 'buttery' note, found in low concentration, and 3-methylbutanal, present in much higher concentration) [130].

Two protocols (spitting-out and swallowing) were compared for the evaluation of aroma release and perception of an alcoholic beverage (a commercial flavored vodka) using TDS as the sensory methodology (Table 1, [131]). Significant differences in both release and perception were observed when comparing the two protocols. The more usual swallowing one produced successive dominances that are more complex but decreased the panel dominance rates of the significant attributes. Ethanol perception was also important when the beverage was swallowed. Aroma release data accounted only partly for the perception differences. Nevertheless, despite the lack of specific key odorants of the predefined sensory attributes, some temporal parameters of release data (mean values on the panel) could be related to the time at which dominance appeared and to the dominance duration of some attributes. However, only limited conclusions could be inferred due to the variety and complexity of the involved mechanisms [131]. On the one hand, all aroma compounds fragmented to a large extent under the chosen PTR-MS operating conditions and most of the fragments were common to several molecules; targeted analysis imposed by the quadrupole mass analyzer allowed monitoring a limited number of ions representing several volatiles. On the other hand, as already outlined, TDS revealed only the significant dominant sensations, at panel level, and mean values of the release parameters were used for an only descriptive linking of analytical and sensory data [131].

As outlined above, the launch of the PTR-ToF-MS technology changed the game for real food systems. In a study on espresso coffees, a roasting effect was evidenced by both TDS and nosespace data obtained concurrently (Table 1, [132]). Thus, a change in aroma perception was observed when roasting increased with a switch of dominance from 'roasted' to 'burnt'. At the same time, more volatiles in higher concentrations were released in the nosespace with increasing roasting degree [132]. Moreover, TDS revealed differences in aroma dominances between samples at the middle/end of perception time, while the release of potent odorants displayed different behaviors. Thus, untargeted volatile tracers of the 'burnt' sensory attribute could be tentatively identified as *N*-heterocycles such as substituted methyl-pyrroles and pyridine [132]. Sugar addition did not modify the nosespace composition, but completely altered coffee perception. As expected, the 'sweetness' attribute became dominant and an increase of the perceived aromatic complexity with time was evidenced, with 'caramel' and 'hazelnut' gradually dominating the 'roasted' and 'burnt' notes. Congruency between sweet taste and several coffee aromas thus provoked a perceptive taste-aroma cross-modal interaction that could be temporally monitored by both dynamic methods [132]. Furthermore, a cluster analysis conducted on the nosespace data allowed characterizing two different temporal behaviors for aroma release, making possible the identification of potential markers of the temporal dominances revealed in TDS [132].

Relationships between release and perception have been obtained yet essentially thanks to temporal links obtained through descriptive analyses of the two types of data averaged at panel level. However, intra- and inter-individual behavioral variations in both release and perception have been evidenced, which complicates interpretation and prohibits a complete understanding. Taking into account those differences in data treatment could improve our knowledge of such mechanisms, recognized as particularly complex. In an attempt to correlate better the two types of data, it was proposed to use an index calculated at individual level for each panelist and replicate in a study on commercial flavored ("garlic and herbs") fresh cheeses that paired nosespace PTR-ToF-MS and TDS (Table 1, [48]). This index was defined as the abundance of each compound detected in the nosespace of each panelist while a given attribute was dominant; it was called AWD for abundance while dominance. As this index was computed at individual level, statistical treatments were made possible and correspondence analysis (CA) of the resulting AWD contingency tables was suggested to highlight the potential relationships between the aroma compounds released in the nosespace and the sensory attributes [48]. Among all the ions detected in the nosespace using PTR-ToF-MS, sixteen aroma compounds characterized by the experimental exact mass of diagnostic ions postulated to be their protonated molecular ions MH^+ , whose identities have been confirmed by independent GC-MS analyses of the products, were selected. They were characteristic of dairy aroma with additional sulfur compounds probably coming from garlic and terpenes probably coming from herbs. Their

MH⁺ masses ranged from m/z 61.028 (acetic acid) to m/z 179.002 (diallyltrisulfide). Meanwhile, eight predefined sensory attributes were followed using TDS: 'garlic', 'cream', 'fresh herbs', 'cooked herbs', 'pungent', 'pepper', 'salty', and 'sour'. Averaged on the entire panel, the release curves showed a clear temporal effect with significant longer T_{max} for some molecules. Thus, diacetyl (m/z 87.044) appeared early followed by terpenes (m/z 137.132) and sulfur compounds (like diallyldisulfide at m/z 147.030). These could correspond to the successive panel dominance rates of the 'cream', 'garlic' and 'fresh herbs' attributes that reached significance [48]. Other molecules were released later, such as 3-vinyl-1,2-dithi-4-ene (m/z 145.017) whose T_{max} was delayed by more than 30 s compared to diacetyl T_{max}. The latter, together with butyric acid (m/z 89.060), also lasted longer in the breath and both could correspond to the long-lasting 'pungent' sensation significantly dominant at the end of the evaluation sequence. CA of the contingency tables containing AWD means scores for each product revealed significant correspondences between attributes and ions. Thus, the 'cream' attribute was clearly associated to diacetyl and butan-2-one. The 'pungent' and 'pepper' characters were associated to carboxylic acids but also, in a lesser extent, to the sulfur compounds 3-vinyl-1,2-dithi-4-ene and diallyltrisulfide. The 'garlic' sensation was clearly associated to a mixture of sulfur compounds, and the herbaceous-related attributes seemed associated to a mixture of odorants. These encouraging results confirmed the tendencies found in the simple descriptive associations based on temporal considerations, while extending the possible correspondences between attributes and ions. However, the authors concluded on the necessity of developing analyses that are more sophisticated to assess statistically the significance of the relationships between multiple key-aroma compounds released in the nospace and the temporal perception of real foods [48].

Nevertheless, the method was used to investigate the relationships between sensory attributes and released aromas in eight dark chocolates differing in characterized sensory properties (Table 1, [133–135]). In the nospace analyses conducted with a PTR-ToF-MS, 35 ions tentatively identified to the molecular MH⁺ ions of aroma compounds were significantly detected. They were characteristic of chocolate aroma with notably pyrazines, Strecker aldehydes, furanones and terpenes. Their masses ranged from m/z 45.033 (acetaldehyde) to m/z 173.150 (ethyl octanoate). Meanwhile, using TDS eleven predefined sensory attributes were evaluated among which nine were aroma descriptors (e.g. 'fruity', 'milky-buttery', 'roasted nuts', ...). Despite huge inter-individual variability in terms of release behaviors and dominance perceptions, the averaged release curves displayed some temporal effects with significant longer T_{max} for some molecules. These could be hardly related to the successive panel dominance rates that reached significance. However, CA of the AWD scores contingency tables of each chocolate revealed some correspondences between attributes and ions. Thus, for one chocolate the 'milky-buttery' note was clearly associated to butan-2-one (m/z 73.065) and the 'roasted nuts' attribute was associated to di- and tri-substituted pyrazines (m/z 123.089 and m/z 137.106, respectively, Figure 4, [133–135]).

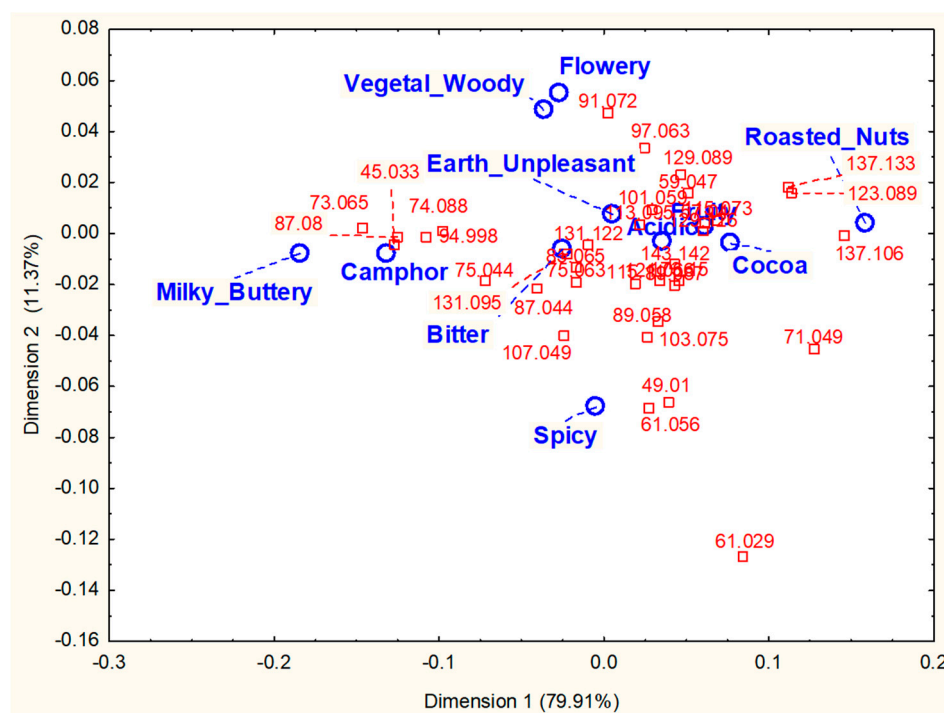


Figure 4. Correspondence Analysis of the AWD scores obtained by a panel of 12 assessors (3 replicates) for aroma release (PTR-ToF-MS, 35 ions) and aroma perception (TDS, 11 predefined descriptors) of a dark chocolate [134].

However, the main information inferred from the CA maps was that dominant sensations were essentially due to a particular mixture of odorants released at a certain time, typical of each product. In fact, the combinatorial code of aromas that could explain most of the dominant sensations depended on the product. The proportion of the different odorants in the mixtures perceived by a panelist over time during consumption appeared more important than specific odorants that exceptionally could explain specific sensations [134,135]. In another study on three dark chocolates differing in sensory properties, sensory evaluation was conducted with both TDS and TCATA procedures, while the nosespace of 16 assessors was concurrently measured in duplicates using PTR-ToF-MS (Table 1, [53]). The release of 19 discriminant aroma compounds whose MH^+ masses ranged from m/z 49.011 (methanethiol) to m/z 143.145 (nonanal) was followed and 6 predefined sensory attributes ('fruity', 'dry fruit', 'roasted', 'woody', 'cocoa', 'spicy') were evaluated as dominant sensations (TDS) or citable attributes (TCATA). The three chocolate samples were clearly distinguished at the nosespace and sensory levels, whatever the temporal sensory method used [53]. CA of AWD indices calculated for each sensory method allowed to draw the same conclusions as previously stated. The links between ions and dominant sensations (TDS) or cited attributes (TCATA) displayed various combinatorial codes of aromas that could explain the different perceptions, allowing samples differentiation [53]. However, a multiblock analysis of the paired aroma release and sensory temporal data revealed that the chocolates discrimination obtained with TDS was more similar to the discrimination displayed in nosespace than the TCATA one [136]. In this case, TDS appeared to reflect better the nosespace data [136].

Two recent studies using TI as the sensory methodology afforded interesting results. Thus, discontinuous TI evaluation of mint aroma and sweetness of chewing gums was conducted with concurrent PTR-ToF-MS in-nose release monitoring of VOCs linked to mint aroma: monoterpenes ($C_{10}H_{16}H^+$) at m/z 137.133, menthol ($C_{10}H_{19}^+$, dehydrated) at m/z 139.148, menthofuran ($C_{10}H_{14}OH^+$) at m/z 151.112 and menthone/1,8-cineole ($C_{10}H_{18}OH^+$) at m/z 155.144 (Table 1, [137]). Significant differences for aroma release were found between Chinese and European panelists, this ethnicity effect being also significant for mint aroma and sweetness perception. Contrastingly, no gender effect was evidenced [137]. Moreover, it was found that measured physiological parameters (oral cavity

volume, salivary flow, breath acetone concentration, and fungiform papillae density on the tongue) did not explain the relationships between aroma release in the nosespace and associated perception [137]. In a different register, condiments like spreads, mayonnaises or vinaigrettes are generally consumed with solid carrier foods like bread or vegetables, making composite foods. In these composite foods, interactions between condiment and carrier food are susceptible to affect aroma release and perception during consumption. Lemon flavored mayonnaises were evaluated in the absence or the presence of solid carrier foods (bread or potatoes) using TI for the lemon flavor and PTR-ToF-MS for the in-nose release of corresponding added aroma molecules, citral and limonene (Table 1, [138]). When mayonnaises were evaluated alone, a perfect correspondence between aroma release and perception was observed, modulated by mayonnaises viscosity (Table 1, [138]). On addition of carriers (bread or potatoes) in-nose aroma (citral and limonene) release increased while perceived lemon aroma intensity decreased [138]. The increasing aroma release in the nosespace could be explained by varying masticatory behavior and/or a surface area increase induced by the presence of the carrier food. The concomitant perception decrease clearly highlighted a cross-modal texture-aroma interaction [138]. Demonstration that not only physicochemical characteristics of foods but also cross-modal interactions play a role in flavor perception of composite foods was further confirmed in a study on chocolate-hazelnut spreads consumed with or without carrier (bread, wafer) foods (Table 1, [139]). If fat and sugar content of the spreads had only a limited effect on in vivo aroma release and perception, in contrast, addition of carriers strongly affected aroma release for all target molecules and perception of the predefined corresponding attributes measured using TCATA. The addition of carriers to spreads resulted in an increasing aroma release in the nosespace (duration and intensity) and a decreasing aroma perception [139]. Thus, carrier food addition modulates aroma perception of composite foods by cross-modal texture–aroma interactions.

Three additional recent investigations are worthwhile to be cited. Addition of oenological tannins to wines is supposed to provide wine stability, protection from oxidation and sensory persistence. However, their incidence in red wines is controversial. In a study pairing PTR-ToF-MS nosespace and TDS sensory evaluation of Pinot Noir red wine, it was found that oxidation resulted in decreasing dominance and persistence of the 'fruity' attribute while the dominance of the 'maderized' and 'prune' descriptors increased. Concurrently, a decrease of the fruity ester ethyl decanoate and an increase of oxidative Strecker aldehydes were noticed in the nosespace [140]. Addition of ellagitannins, but not proanthocyanidins, protected the wine from oxidation, preserving perception of fruitiness and preventing the increase of 'maderized' notes. Moreover, ellagitannins increased the aroma persistence in the non-oxidized wine [140]. The impact of capsaicin (a chemesthesis agent responsible for spiciness) on aroma release and simultaneous perception was investigated using a flavored (nutty note) solution of 3-methylbutanal (Table 1, [141]). It was found that capsaicin had no significant impact on the in-nose aroma release concentration, while the presence of capsaicin significantly enhanced aroma perception by 45% during 60s observation. The capsaicin-enhanced aroma perception found in this study revealed a perceptive cross-modal (chemesthesis-aroma) interaction, although a halo-dumping effect could not be completely ruled out [141]. So, to consolidate the knowledge on capsaicin's effect on aroma perception, further studies conducted with fully trained participants on more congruent flavor (such as savory) should be envisaged [141]. Finally, in the worldwide context of salt reduction in food products for a more healthy diet, two promising strategies based on odor-induced saltiness enhancement and heterogeneous distribution of flavor compounds were tested in four-layer cream-based hot flans (Table 1, [41]). Salt and an aroma mixture were added homogeneously, or heterogeneously, to the four-layer snacks according to an experimental design giving seven variants. Globally, the sensory results on aroma perception suggested that a homogeneous distribution of salt induced higher aroma intensity perception regardless of the added aroma distribution. Concurrently, the measured in-nose aroma levels revealed a higher release for the products with a homogeneous salt distribution compared to the products with a heterogeneous one [41]. Regardless of the added aroma distribution, products with heterogeneous salt distribution were perceived as significantly saltier than products with homogeneous one. The products containing salt in the only outer layer were perceived as the

saltiest. This salty taste enhancement could be due to the initial strong dominance of the salty sensation at the very beginning of the eating process as measured using TDS [41]. Thus, a heterogeneous distribution of salt could constitute an interesting strategy to enhance saltiness in reduced-salt food. Besides, aroma release was not affected by aroma compound distribution but only by salt distribution in the food product, probably revealing a salting-out effect. The involved mechanisms based on a combination of physicochemical and perceptual effects need further investigations to be fully understood [41].

Table 1. Dynamic nosespace and sensory analyses conducted simultaneously.

Food Product	Type of Variation Studied	Number of Panelists (Replicates)	Sensory Method	Instrumental Method	Relevant Findings	Reference
Carvone flavored gelatin gels	Carvone concentration	14	TI	APCI	Linear relationship between stimulus and perception. Effect of speed of eating on adaptation to the stimulus	Hollowood et al., 2000 [115]
Rosemary flavored solid food	Flavoring time in process	6	TI	APCI	Correlation between aroma release and simultaneous perception of rosemary flavor	Cook et al., 2005 [5]
Flavored yogurt	Fat content	10 (5)	Modified DTI (questionnaire)	APCI	Quicker and greater aroma release in low fat yogurts, but with less persistence. Lipophilic compounds more affected by fat for I _{max} , but not for T _{max} or persistence. Significant sensory differences (intensity and timing) evidenced. Differences in particle size and viscosity might also affect aroma release	Brauss et al., 1999 [118]
Flavored milk	Fat content	98 (4)	Paired test *	APCI	Good correlation between aroma delivery and perception, with higher intensity in low fat milk	Shojaei et al., 2006 [119]
Flavored liquid emulsions	Fat content	6 (3)	TI	PTR	Significant effect of fat on release and perception, and on pre- and post-swallow events	Frank et al., 2011 [120]
Flavored model gels	Gelatin concentration	11	TI	APCI	Decreased aroma perception on increasing gelatin concentration with no significant differences in aroma release (texture-aroma cross modal interaction). Correlation of sensory data with the different rates of aroma release in the different gels	Baek et al., 1999 [123]
Flavored whey protein gels	Whey protein content	10 (3)	TI	APCI	Texture of gels determines perception of aroma intensity rather than in-nose aroma concentration. Texture-aroma cross-modal interactions evidenced	Weel et al., 2002 [124]
Flavored model gel	Whey protein content	7 (3)	TI	PTR	Correlation between individual-specific consumption patterns and respective sensory perception. Correlation between gel texture and release patterns and corresponding aroma perception	Mestres et al., 2006 [17]

Flavored stirred yogurts	Viscosity (protein content and mechanical treatment)	8 (4)	3-points DTI **	APCI	Complex viscosity of yogurts influenced in-nose release and perception. Aroma release and perception stronger in low-viscosity yogurts than in high-viscosity ones. Aroma release and perception more influenced by mechanical treatment than by protein composition	Saint-Eve et al., 2006 [126]
Flavored candies	Gelatin concentration; Melting or chewing protocols	12 (4)	TDS	PTR	Highest aroma release (Imax) obtained with low gelatin content. Aroma release determined by interaction between product properties and oral behavior. Relations between dynamics of release and perception established for temporal parameters	Délérès et al., 2011 [125]
Flavored model gels	Thickener type and level, sugar and flavor level	6	TI	APCI	Significant correlation between stimulus and perception, depending on gel strength. Effect of speed of eating on adaptation to the stimulus	Linforth et al., 1999 [117]
Flavored model custards	Thickener and sugar level	7 (6)	TI	APCI	Perceptual sweetness-aroma interactions, whatever the texture of the desserts	Lethuaut et al., 2004 [127]
Mint flavored commercial chewing gum	Gum type	11 (3)	TI	APCI	Decreasing perception of mint flavor followed sucrose release rather than menthone release. Sweetness-aroma cross-modal interaction evidenced	Davidson et al., 1999 [128]
Mint-flavored carbonated beverages	CO ₂ and sugar level	4 (8)	3-points DTI **	PTR	CO ₂ increased aroma release and perception, regardless of sugar content. Perceptual sweetness-aroma interactions evidenced. Impact of sugar content on aroma release but not on perception for carbonated beverages	Saint-Eve et al., 2009 [129]
Soft cheeses	Cheese type	15 (3)	TI	APCI	Correlation between temporal sulfury notes and main sulfur compounds temporal release. Sensitivity and technical limitations of APCI evidenced for highlighting other relationships	Salles et al., 2003 [130]
Alcoholic beverages	Spitting-out or Swallowing	10 (4)	TDS	PTR	On swallowing, aroma release data partly accounted for the observed differences in perception	Délérès et al., 2011 [131]
Espresso coffee	Roasting degree and sugar level	18 (3)	TDS	PTR-ToF	Significant effect of roasting on release and perception. Sweet taste-smell perceptual interaction	Charles et al., 2015 [132]
Commercial flavored ('garlic and herbs')	Brand	16 (2)	TDS	PTR-ToF	Significant relationships between dominant sensations and released aromas, highlighted in correspondence analyses (CA) of	Schlich et al., 2015 [48]

fresh cheeses					Abundance While Dominant (AWD) indices ***	
Dark chocolate	Categorized sensory properties	12 (3)	TDS	PTR-ToF	Sensory categories confirmed by TDS. Significant relationships between dominant sensations and released aromas, revealed by CA of AWD indices ***	Deuscher et al, 2019 [133–135]
Dark chocolate	Sensory properties	16 (2)	TDS and TCATA	PTR-ToF	Samples differentiation confirmed by sensory (both TDS and TCATA) and aroma release. Relationships between dominant sensations (TDS) or cited attributes (TCATA) and released aromas, revealed by CA of AWD indices ***	Le Quéré et al., 2021 [53]
Commercial mint chewing-gums	Ethnicity, gender and physiology	29 (3)	DTI	PTR-ToF	Effect of ethnicity on correlated aroma release and perception, not explained by physiological parameters. No gender effect.	Pedrotti et al., 2019 [137]
Composite food (lemon flavored mayonnaise on carrier foods)	Fat content and viscosity level; Carrier food	14 (3)	TI	PTR-ToF	Increasing mayonnaise viscosity resulted in lower aroma release and perception. Addition of carriers increased in-nose aroma release while decreasing perceived aroma intensity. Carrier addition modulates aroma perception of composite foods by cross-modal texture–aroma interactions	Van Eck et al., 2021 [138]
Red wine	Oenological tannins; Wine oxidation	17 (2)	TDS	PTR-ToF	Addition of ellagitannin extract in wine impacts the dynamic of sensations of oxidized wine, the length of aroma release in mouth, and preserves fruitiness under oxidative conditions	Pittari et al., 2022 [140]
Composite food (flavored chocolate-hazelnut spreads on carrier foods)	Fat and sugar content; Carrier food	8 (3)	TCATA	PTR-ToF	Carriers attributes perceived at beginning of consumption, while spreads attributes perceived after swallowing. Limited effect of fat and sugar content on aroma release and perception. Addition of carriers increased aroma release (duration and intensity) and decreased perception. Cross-modal texture–aroma interactions evidenced	Gonzalez-Estanol et al., 2023 [139]
Flavored solutions	Capsaicin present or not	15 (3)	Sequential profiling	APCI	No significant impact of capsaicin on aroma release, but aroma perception significantly higher. Capsaicin enhanced saliva flow	Yang et al, 2021 [141]
Flavored four-layer hot flans	Odor-induced saltiness enhancement; heterogeneous distribution of	15 (3)	ATI****	PTR-ToF	Increased aroma release and perception in products salted homogeneously. Increased saltiness in heterogeneously salted products regardless of aroma distribution	Emorine et al., 2021 [41]

flavor
compounds

* paired comparison test: is a significant perceivable difference in flavor intensity existing between samples? Not a dynamic method, but conducted simultaneously with the nosespace analyses of 98 individuals. ** 3-points DTI: discrete time-intensity of overall olfactory intensity measured at three consumption times, i.e. intake, swallowing and 60 s after intake (persistence). *** AWD: calculated index of abundance of each volatile compound during a given attribute is dominant [48]. See text for a complete description. **** ATI : alternate TI, equivalent to DTI evaluating only two attributes (salty taste and one specific aroma).

Dynamic instrumental and sensory analyses conducted simultaneously revealed several relationships between aroma release and aroma perception, and these relationships were modulated by food structure and texture, perceptual cross-modal interactions and, last but not least, inter-individual variability in release and perception behaviors. These aspects are discussed below. However, these analyses conducted concurrently present the main drawback of being realized during individual sessions only, due to instrumental constraints. Typically conducted with a dozen of panelists, and with the necessary replicates, data acquisition on several food variants could be rather lengthy. Moreover, the sensory and instrumental data used for establishing the relationships are generally treated as averaged data over the whole panel, which partially prevents from taking into account the true temporality of the phenomena and the inter-individual variability. This is a challenge for the future.

5. Aroma Release and Aroma Perception: Is the Link So Close?

As outlined above, relating in vivo aroma release and aroma perception is generally not straightforward. A simple relationship between aroma release and corresponding perception is rarely observed, essentially in very simple systems (e.g. aromatized solutions or gels) far from ecologically-relevant foodstuffs, unfamiliar and likely unpleasant. As soon as food systems are made more complex to resemble real foods, or when real foods are considered, interactions of aroma with the food ingredients within the food matrix occur, which results in variable consequences on aroma release and perception [7,142]. Moreover, a given sensory attribute may result from the combined action of several aroma compounds, and a particular ion detected using DIMS techniques may correspond to several distinct molecules. Furthermore, perceptual interactions between odorants add another sensory dimension, as confirmed in recent studies on wine [143,144]. Also linked to more complex systems, perceptive cross-modal interactions between aroma and other sensory modalities (texture, taste, chemesthesis or trigeminal sensations, although rarely investigated for the latter) have been evidenced. Finally, all the effects on perception are complicated by interactions with human oral physiology and food oral processing behavior that result in huge inter-individual variability in aroma release and perception [3].

5.1. The Food Matrix

Food systems comprise various ingredients (carbohydrates, lipids and proteins) that influence their structure and texture. Aroma compounds interact with these major food ingredients through different mechanisms such as phase partitioning, binding or altered diffusion and these interactions may affect aroma compounds volatility and hence their availability in the retronasal pathway for perception [142]. Any formulation modification, using e.g. fat replacers, thickeners or sweeteners, is thus susceptible to modify aroma release and perception by changing the nature of the interactions involved [7].

Proteins interact with aroma compounds through reversible (mainly hydrophobic interactions and hydrogen bonds) or irreversible (covalent) binding. The reversible binding strength is dependent of aroma compounds hydrophobicity, hence inducing variable effects on release and perception [7], exemplified in several results presented above (Table 1). Furthermore, if aldehydes are known to irreversibly bind to proteins through covalent binding, a recent study has shown that aroma

compounds of various chemical classes may covalently bond to proteins during thermal processing through Schiff base, Michael addition and disulfide linkages [145].

The addition of carbohydrates clearly modifies the structure of the matrix, affording viscosity changes that modulate texture. Molecular interactions between polysaccharides and aroma compounds may also occur. However, these nonspecific molecular interactions have a moderate impact on aroma release compared to the effects caused by the changes in viscosity [7]. Moreover, the respective characteristics of both the carbohydrates and the aroma compounds (volatility, polarity and hydrophobicity) induce various retention and release effects that do not allow drawing a clear picture of carbohydrate-aroma interactions. However, carbohydrate concentration plays a major role and aroma perception was shown to decrease rapidly when this concentration was higher than a critical concentration, c^* , which is the point at which the viscosity of the system abruptly increases. It corresponds to the transition from a solution where macromolecules can move freely to a more organized gel. The reduced diffusion of aroma compounds in harder gels produces a decreasing release in the mouth, hence a decrease in perception [146]. Generally, an increase in viscosity seems to result in a decrease in the intensity of aroma perception, but not necessary in a decreasing aroma release. Thus, in gelified systems such as yogurts, Saint-Eve et al. [126] showed that for the same protein concentration, a decrease in viscosity induced by the application of a mechanical treatment resulted in an increase in aroma release and the intensity of aroma perception (Table 1). In model dairy desserts textured with carrageenan variants and sweetened with sucrose, Lethuaut et al. [127] showed that changes in sweetness and texture induced changes in aroma perception while aroma release remained largely unaffected, and highlighted perceptual sweetness-aroma interactions (Table 1). A perceptual interaction between odor and oral texture was also evidenced when a cream odor was presented ortho- or retronasally to assessors while milk-like foods with different viscosities were simultaneously present in the mouth [147]. Thus, the perceived aroma intensity decreased with increasing viscosity of the liquid, irrespective of whether or not the odor was presented ortho- or retronasally at a constant concentration. Remarkably, the odor stimulus also increased the perceived intensities of thickness and creaminess of the fluid in the mouth, but only when being presented in the retronasal mode that is as if the odor would have originated from the liquid [147]. Sucrose itself affects aroma release and both an increase and a decrease of release following the addition of sucrose to water were noticed. Increase in sucrose concentration caused an increased release of the more volatile compounds and a decreased release of the less volatile ones. However, no significant differences in sensory perception were evidenced [7]. An increase in the mole fractions of the highly volatile compounds in the liquid phase on addition of sucrose could explain the increased aroma release [7]. An increased release of more hydrophilic compounds can also be explained by stronger competition with sucrose for water molecules [146]. The decrease observed for the less volatile compounds was attributed to an increase in viscosity, which may affect the diffusion of aroma molecules [7].

Lipids influence the flavor of foods through various effects on multimodal aspects of perception (mouth-feeling, taste, and aroma). For aroma compounds, lipids influence their perception by direct impacts on food texture and aroma release [142]. Lipids induce phase partitioning and aroma compounds are distributed between fat and the aqueous phase, following partition governed by hydrophobicity [7,142]. For most aroma compounds that are essentially lipophilic, the effect of fat on their release is greater than that of other ingredients. Thus, small changes in fat content have significant effects on the volatility of aroma compounds, hence their perception (Table 1). However, these effects are largely dependent on the compounds hydrophobicity and polarity, the vapor pressure of the more polar compounds decreasing only slightly [7]. Thus, for a mixture of aroma compounds exhibiting different polarities, the relative proportions of their release significantly change on changing the fat ratio, inducing changes in overall aroma perception [7]. Furthermore, to explain aroma perception in lipid-containing food, it is also necessary to consider the release rate of aroma compounds that influences significantly their odor thresholds, influences that, once more, are modulated by their hydrophobicity and polarity [7,142]. Moreover, the nature of fat affects aroma release [142,148] and the presence of emulsifying proteins at the interface of oil in water emulsions

affects the hydrophobicity-dependent release of aroma compounds through the additional protein-aroma interactions described above [7,142]. Emulsion properties such as droplet size and distribution, microstructure and viscosity also affects aroma release and perception [142].

Among other food ingredients, phenolic compounds may be involved in interactions with aroma compounds that may affect aroma release and perception. This effect is particularly relevant for wine and olive oil where the polyphenols content is significant. However, the real effect of these macromolecules on aroma release and perception should be considered through the prism of their associations with proteins [7,142]. Salt (NaCl) is another ubiquitous food ingredient important for food palatability, but also for technological issues (inhibition of pathogen microorganisms' growth, texture improvement, involvement in enzymatic and biochemical reactions during food maturation). Salting-out effect that results in increasing the release of volatile compounds is dependent on the chemical nature of the volatiles, which may result in modulations of aroma perception [7]. Moreover, in association with lipids, salt modifies in-mouth aroma release and perception [111,149]. Furthermore, health concerns lead to reducing the sodium content in foodstuffs and to developing low-salt foods. One of the main consequences of decreasing salt content is the modification of the sensory characteristics of the product through a perceptive aroma-saltiness interaction [150].

For interested readers, detailed consequences of food ingredients interactions with aroma compounds on release and perception may be found in dedicated treatises (e.g. [151–153]) and specialized reviews (e.g. [7,142,146,154]).

5.2. Cross-Modal Interactions

Once halo-dumping effects have been ruled out, with adequate training of the panelists for instance, perceptive cross-modal interactions have been evidenced, as exemplified in several studies described above (Table 1). Several perceptive aroma-texture interactions were evidenced as a consequence of altering food viscosity by modifying the content or the nature of thickeners. Moreover, these interactions should always be considered for composite foods when the use of a carrier food systematically modifies the aroma perception of the spreads. Cross-modal aroma-taste interactions were also evidenced essentially with several examples of sweetness-aroma interaction that can be found in Table 1. For a long time it has been demonstrated that an odor can enhance taste perception, and conversely, that a taste can increase an odor intensity [155]. It has been also established that such perceptual interactions occur when odor and taste are congruent [150]. Thus, sucrose, but not salt and citric acid, significantly increased the perceived intensity of sweet-related aromas vanillin, citral, and furaneol [156]. Concomitantly, the salt-related sardine aroma induced an increased saltiness perception in low-salt solutions, while a sweet-related carrot aroma did not [157]. This phenomenon known as odor-induced taste enhancement (OITE) is considered to be an efficient strategy to compensate for sugar reduction [158] and salt reduction [159] in food while maintaining acceptability for the consumers. Noteworthy, these cross-modal interactions seem processed in high-level integratory areas of the brain with potential top-down effects on primary sensory regions [1].

5.3. Inter-Individual Variability

For a long time, huge inter-individual variability in aroma release and perception has been evidenced [3], which requires a significant number of panelists and replicates to infer relevant results, often only at panel level, however. The properties of the food matrix, which undergoes physicochemical interactions with aroma compounds as described above, and oral physiological characteristics and their interactions during food oral processing are the main drivers that produce inter-individual variability. It is out of the scope of the present review to exhaustively detail all the implications of these complex relationships on aroma release and perception, and the interested reader is invited to refer to dedicated reviews (e.g. [3,12,23,160]) and treatises (e.g. [152,153]). Only the most salient causes of variation will be described briefly.

Obviously, the first cause of variation in odor perception comes from the human genome. Polymorphism in OR genes results in OR genotypes and olfactory phenotypes that alter odor perception in terms of both intensity and pleasantness [161]. Whether the existence of these

phenotypes could explain specific anosmia and hyposmia, or not, is still debated [161]. Following food intake and before being transferred to the breath, aroma compounds are released in saliva. Saliva variations in both flow and volume contribute to varying aroma release in the oral cavity during food oral processing. Moreover, saliva composition in proteins and enzymes plays a major role. Thus, a low saliva flow and a low α -amylase content were found responsible for a higher aroma release [162]. Variations in the salivary proteome modulate sensory perception and in-mouth enzymes originating from the oral microbiote, saliva or epithelial cells are able to metabolize aroma compounds in the time-course of food consumption (e.g. [163–165]). Mouth coating of residual food that sticks to the oral surface after food ingestion, dependent on matrix composition, and selective adsorption of aroma compounds to the oral and pharyngeal mucosa modify aroma compounds immediate availability and participate to ‘after-smell’ perception [3] or persistence (e.g. [160,164]). With solid food, salivation and mastication combine to form a food bolus ready to be swallowed. The aroma compounds transported from the saliva to the air phase in the mouth are transferred to the upper airways essentially on swallowing. This so-called ‘swallow-breath’ comes with an aroma pulse [166] that generally presents the highest aroma release signal, whatever the type of food [167]. Swallowing requires forcing the bolus or the liquid beverage into the pharynx by a tongue movement while the velum retracts and elevates, preventing food material from being swallowed the wrong way [12]. Nevertheless, as already outlined above [17], some individuals release aroma during the chewing phase, opening their velum and allowing important aroma transfer to the nose on each masticatory pulse before swallowing [146]. This source of variation in aroma release is even complicated by the observation of groups of people releasing either a high amount (high releaser group HRG) or a very low quantity (low releaser group LRG) of aroma [168] whatever the food product, confirming previous observation [169]. HRG subjects were better discriminated from LRG ones by a higher chewing activity (number of chewing cycles, chewing duration, maximal amplitude and total muscle work), confirming previous results (e.g. [15]), higher mouth coating, and higher velum opening frequency [168]. A multiblock partial least square regression conducted on the different physiological and behavioral oral processing datasets (masticatory behavior, bolus rheology, saliva composition and flux, mouth coating and bolus moistening) confirmed the importance of masticatory behavior on aroma release [162]. Although breath flow was not measured, and despite some contradictory results [160], the influence of breathing flow on aroma release could also account for the difference between the two groups. Thus, during wine drinking, higher releasers were characterized by higher volume of air breathed out [170], indicating that breathing capacity could also be important in the aroma release process [160], confirming earlier results (e.g. [67]).

Finally, as another source of variability, recent studies highlight the importance of perireceptor molecular events in the modulation of aroma perception [165]. Thus, metabolism of odorant molecules by odorant metabolizing enzymes was found effective in human nasal cavity affecting odorant perception [171] and *in vivo* odorant competitive metabolism was demonstrated to be involved in human olfactory process, influencing intensity and quality of aroma perception [172].

6. Concluding Remarks and Future Trends

Aroma perception is a complicated phenomenon that results of a dynamic interplay between aroma release from a food matrix, where interactions of aroma compounds with food ingredients modulate the release, human physiology and food oral processing behavior, and perceptive cross-modal interactions. However, the resulting aroma release does not always explain sensory perception, due to other physiological mechanisms at the peripheral and central levels in the brain. Moreover, aroma perception contributes to a unitary flavor perception that arises from the central integration of multiple sensory inputs, among which aroma and taste are of prime importance. Thus, odor/taste integration that constructs the first level of flavor perception depends on neural processes that occur in chemosensory regions of the brain [173].

As aroma release and aroma perception are dynamic processes, the potential link between release and perception can only be understood if instrumental methods that apprehend the time dimension of aroma release are paired to dynamic sensory methods, preferably using a concurrent

protocol. Mass spectrometry methods based on DIMS techniques are satisfactory, but only the most sensitive instruments allow untargeted analyses of real foods. Currently developed on the PTR-ToF-MS technology, a gain in sensitivity is desirable to be able to follow key odorants that are often found in trace amount and are simply not detected in vivo using the current instruments. SESI-MS has to prove its usefulness in the near future, while the less expensive ion mobility spectrometry (IMS) technique has recently demonstrated some potentialities [174]. Available temporal sensory methods should also be ameliorated, particularly to take into account better the temporality of the phenomena and inter-individual variability. A method such as temporal order of sensations that apprehend the most salient perception moments could be an interesting alternative to the other temporal sensory methods used so far. Efforts on combined sensory/instrumental data treatments should also be undertaken, beyond the current models based on the AWD index [48] or multiblock analyses [136].

Finally, the valuable results reviewed above have allowed finding several relationships between aroma release and aroma perception, often highlighting perceptive cross-modal interactions. However, a perfect understanding of all the relationships that link food oral processing, aroma release and aroma perception is still incomplete because the complexity of the phenomena. Particularly, as inter-individual physiological variability leads to differences in aroma release, which affects aroma perception, future research should take into account these variations. Moreover, most of the results have been obtained using a single bite/sip food intake of model or simple foodstuffs. Nevertheless, heterogeneous foods or composite foods modify oral processing behavior, sensory perception and food intake [175], and recent results obtained for aroma release and perception of composite foods have highlighted aroma-texture perceptive interactions [138,139]. Therefore, it should be interesting to consider food oral processing and related aroma release and perception during more natural food consumption behavior, investigating multi-bites/sips food intake conditions, or even a whole meal. Available dynamic instrumental and sensory methods have the potentiality to tackle this new paradigm.

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