Perception of odour mixtures: The next challenge in flavour analysis

THIERRY THOMAS-DANGUIN[1], Charlotte Sinding[1], Sébastien Romagny[1], Maiken Thomsen[1], Elisabeth Guichard[1] and Gérard Coureaud[2]
[1] *Centre des Sciences du Goût et de l'Alimentation, AgroSup Dijon, CNRS, INRA, Université Bourgogne Franche-Comté, F-21000 Dijon, France*
[2] *Centre de Recherche en Neurosciences de Lyon, CNRS, INSERM, Université Lyon 1, F-69007 Lyon, France*

Abstract

The olfactory dimension of food flavour is critical to the food identity and typicality. Food odour and aroma result from the processing of complex mixtures of volatile compounds activating the sense of smell. The perceptual properties of odour mixtures have been explored from both the aroma analysis point of view and the psychophysical point of view, thus revealing perceptual effects such as masking, synergy, or perceptual blending. However, considering odorants separately, the classical aroma analysis approach misses the central role of perceptual integration in odour mixture processing. Therefore, the challenge of food flavour analysis is now to integrate the mechanisms of complex odorant mixtures perception. Here, we briefly review recombination strategies and tools that are already available to go one step forward and consider not only key-odorants but also key-associations involved in overall flavour perception.

Introduction

The olfactory dimension of food flavour is critical to the food identity and typicality. This has been nicely showed in a basic experiment by Mozell et al. [1], in which a group of subjects had to identify real food flavours. In order to minimize identification by nonchemical cues, 20 food samples were prepared to be presented as liquids of about the same apparent viscosity. Subjects were allowed to swirl the liquid around their mouth before being asked to identify the flavour by a food name (e.g. "chocolate, "coffee," "onion,"). All the samples were presented twice to each subject but following two experimental procedures. In a first condition, the olfactory dimension was removed since subjects were equipped with an air stream apparatus, connected to their nostrils, which blew odourless air in the direction opposite to the movement of volatile molecules from the mouth to the nose via the nasopharynx. In the second condition, without the air stream apparatus, the nose remained normally accessible to the molecules. When deprived of the olfactory and the nasal trigeminal inputs, subjects were poorly able to identify the samples and were even unable to identify the flavour of coffee or chocolate.

Food odour and aroma are both percepts, namely cerebral representations, constructed on the basis of the olfactory processing of complex mixtures of volatile compounds able to activate olfactory receptors [2]. Following the aroma analysis classical methodology, GC/MS-O (Gas Chromatography/Mass Spectrometry-Olfactometry) is used to separate and identify those odorants that contribute to the odour of a given food sample headspace extract. The analysis process requires around half an hour to detect and identify the main odorants of the mixture that constitutes the headspace [3]. In contrast, the human nose, when confronted to the same mixture of odorants, analyses simultaneously all the chemicals to provide a pattern that is integrated by the brain to produce, in less than one second, a mental representation of the food sample. The result will be the rapid categorisation and likely recognition of the odour as an odour object [4].

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Therefore, we always have to keep in mind that there is a critical difference between the chemical analysis strategy and the perceptual strategy when focusing on complex odour mixtures responsible for the flavour of food.

The perception of odour mixtures, the case of perceptual blending

The processes underlying the perception of complex mixture of odorants as patterns and the elaboration of odour object representation in the brain are based on odour coding and perceptual interactions that take place along the olfactory pathway [4]. The simultaneous interplay of several odorants with the olfactory system induces various interactions at all the levels of integration, from the very periphery where competition at the olfactory receptors level takes place [5,6] to high order integrative processes involving cognitive and top-down modulations [7]. From a theoretical point of view, it is possible to consider several cases of perceptual interactions in odour mixtures (Figure 1).

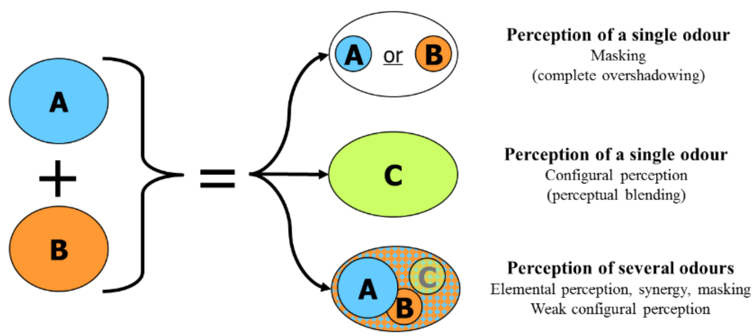

Figure 1: Theoretical outcomes on odour quality when two odorants are processed in mixture by the olfactory system. One odorant has an odour noted A and the other B, while odour C is specific to the mixture and results from configural processing of the so-called blending mixture (adapted from [4]).

In one case, the mixture carries a specific odour, which is not the superposition of the odorants' odour. Such a perceptual outcome is the result of the configural processing of certain mixtures called blending mixtures [8], which may be the chemical signature of odour objects [4]. Another processing strategy of odour mixture, namely elemental processing, leads to the recognition of the odorants' quality within the mixture (Figure 1). These two processing strategies, likely concurrent, can be influenced by individual-related factors such as physiological or cognitive state but also by the stimulus features, especially the odour quality of each of the odorants and their relative concentrations.

In a recent study, we investigated the configural and elemental perception of two 6-odorants mixtures in two mammal species, human adults and newborn rabbits, which have both assets with regard to the study of odour mixtures [9]. Using free-sorting tasks in humans, we evaluated the perception of a blending mixture (RC), which evoked the specific odour of Red Cordial and another mixture (RC^{mod}), made of the 6 same odorants but in different proportions, in comparison to the perception of the single odorants. In newborn rabbits, the perception of the same mixtures was assessed by measuring the orocephalic sucking response to the mixtures or their components after conditioning to one of these stimuli. The results revealed that the blending mixture (RC) was indeed configurally processed both in humans and rabbits. In contrast, the other mixture (RC^{mod}), containing the same odorants but in different concentration ratio, was elementally processed. These results demonstrate that configural perception is specific not only to the odorants included in a blending mixture but also to their respective proportion [10]. Interestingly, rabbit neonates also responded to each odorant after conditioning to the red cordial mixture, which demonstrated their ability to perceive elements in addition to the configuration in the mixture [11] and, in turn, supports the hypothesis that both elemental and configural processing are concurrent.

Key odorants and key associations in odour mixtures

Within the aroma chemical analysis framework, it is usually considered that if the omission of an odorant from a recombined mixture changes the overall perception, then this odorant is a key aroma compound [12]. However, key odorants may have a different status depending on whether the mixture has blending properties or not, which may also explain why key odorants reported in the literature for a lot of food sometimes carry an odour similar to the overall food odour and sometimes not. We tested this hypothesis through the study of the perceptual roles of the odorants that are included in mixtures elementally or configurally perceived. We examined, in humans, the perceptual impact of the nature and concentration ratio of the odorants included in two 6-components mixtures and their sub-mixtures containing 2 to 5 components [13]. The 6-odorants mixtures were RC and RC^{mod} used in the previous study [9]. Mixture processing was explored through a similarity rating task, in which 61 subjects rated the similarity of odour samples containing 1 to 6 components to either the RC or the RC^{mod} reference mixtures.

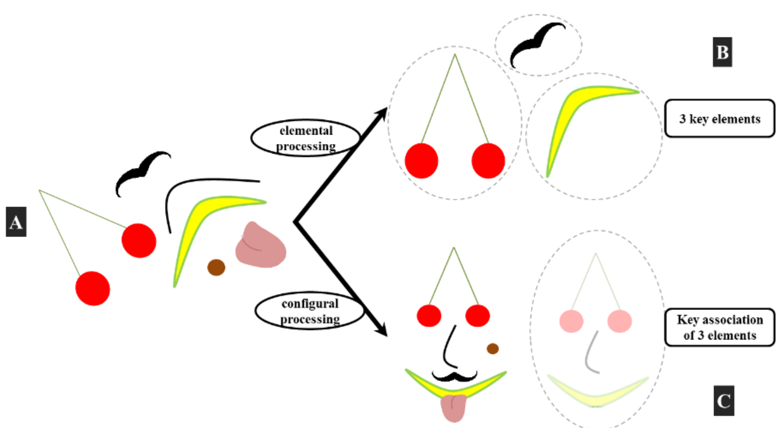

Figure 2: Illustration of the perception of key elements and key association in a 6-components mixture (A). Some of the elements carry a specific object identity (boomerang, bird and cherry); at a specific ratio (represented here by the spatial arrangement of the elements), their perception as individual elements can be still salient (B); they are *key elements*; the perception is *elemental*. In contrast, at another ratio (another spatial arrangement), the same elements may lead to a *key association* (C), in which the elements lose their object's identity but create another feature (a basic face); these elements are contributors to the *key association*. Adding other elements, which do not necessarily refer to specific objects (point, curved line), "polishes" the key association and provides an identity for the whole mixture; the perception is *configural*. (adapted from [13]).

The results highlighted that elemental perception depended primarily on the odour quality and concentration ratio of many of the mixed odorants, whereas configural perception depended on specific associations of odorants in strict concentration ratios. These findings led us to reconsider the impact of key elements in odour mixtures within the framework of a perceptual model, illustrated in Figure 2 owing to a visual analogy. In mixtures, some odorants may preserve their perceptual features such that the individual odour they carry as single molecules is still identifiable within the mixture. In that case, the mixture is elementally processed. It is perceived as a collection of a few individual odours carried by some of the odorants, which can be qualified as *key odorants*. Still, several odorants may lose some of their perceptual features [14] and create meaningful associations that strongly contribute to the mixture odour quality. These associations can be considered as *key associations*.

New developments in aroma analysis

If the aroma analysis methodology, relying on GC/MS-O, has been repeatedly shown to be efficient to identify impact odorants in complex food flavour, it appears that it can only point those molecules that are key odorants. Indeed, key associations can only be identified through the study of mixtures. Nevertheless, some odorants, likely contributors to key associations, may have been spotted during confirmatory recombination approaches, in which odorants, which odour is not similar to the odour of the overall food flavour, can appear as impacting compounds (e.g. [15,16]). The need for new tools to rapidly evaluate the perceptual importance of odorants in complex mixtures have led to the development of several methods based on dynamic reconstitution of mixtures online during GC-O analysis [17–20]. The Olfactoscan system couples two devices: a GC-O apparatus and a multi-channel dynamic dilution olfactometer [21]. The humidified air stream at the outlet of the olfactometer is connected to the GC-O sniffing port so that controlled mixtures of odorants, provided by the olfactometer, can be mixed with the odorants coming from the GC-O. Therefore, the olfactoscan system enables the screening of the olfactory active compounds delivered during a GC-O run, while mixed with a well-controlled background odour generated with the olfactometer.

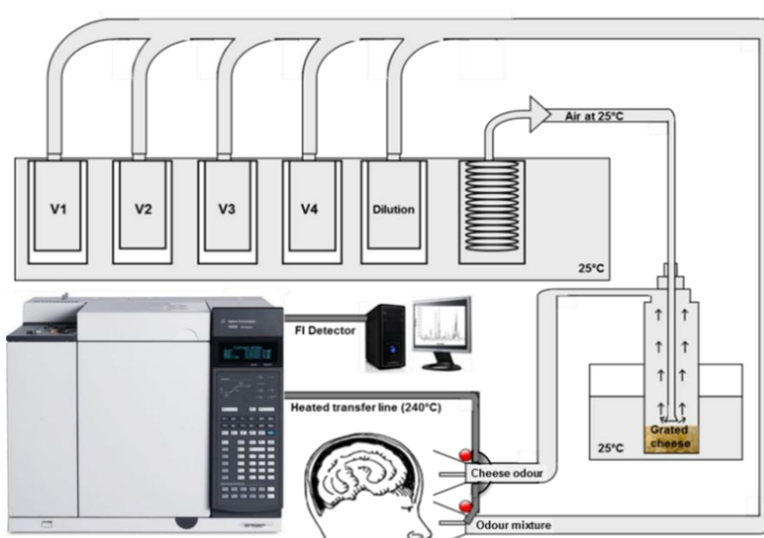

Figure 3: Olfactoscan setup including an olfactometer that delivered a precise mixture of odorants from the four vessels (V1, V2, V3, V4) to be combined with odorants eluted from a gas-chromatograph coupled to the outlet of the olfactometer; this formed the *Odour mixture* olfactory port. The olfactometer was also used to control the delivery of real cheese odour at the *Cheese odour* olfactory port, which served as the reference odour for the direct similarity rating task (adapted from [22]).

We used the Olfactoscan system to screen for specific associations of odorants responsible for the odour specificity of 3 non-processed semi-hard cheeses (setup presented on Figure 3) [22]. Eight odorants, identified as contributors to the basic odour of the cheeses, were dispatched into the four vessels of the olfactometer to form an optimal basic composition, specific to each of the three cheeses. Eight odorants, among which four were also present in mixture in one of the olfactometer vessels, were individually added to the basic composition owing to the GC-O system. All the combinations formed complex odour mixtures that were systematically compared to the real odour of each cheese, by 16 trained subjects through a direct similarity rating.

The results highlighted that the relative concentrations of the same few odorants in a mixture can be adjusted via a recombination approach to reach an optimum of similarity with the odours of different non-processed semi-hard cheeses. More precisely, when combined with acetic acid, butan-2,3-dione and methional, the odorant dimethyl trisulphide contributed to one cheese odour, whereas butanoic acid contributed to another cheese odour. Still, for the third cheese odour, the combination of dimethyl trisulphide, butanoic acid, 3-methylbutanoic acid and 3-methylbutan-1-ol is required.

Conclusion

Odour mixture processing, which constitutes the basic rule when perceiving the flavour of a food, induces several perceptual effects that contribute to the olfactory system striking efficiency in coding complex odour objects. The concept of configural-elemental dual olfactory processing has led to consider a new perspective in the identification of key components of odour sources, namely the importance of key odorants but also of key associations. In the framework of food flavour analysis, online recombination strategies and specifically relevant tools have been developed and are now available to go one step forward and take up the challenge of integrating odour mixture processing specificity into the aroma analysis path. Beyond the expected impact in terms of food flavour analysis, the study of odour mixtures is an original window allowing the investigation of olfaction-specific mechanisms certainly crucial to interpret -and provide an efficient representation of- our food and more broadly our environment.

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References

1. Mozell, M.M., Smith, B.P., Smith, P.E., Sullivan, R.L., and Swender, P. (1969). Arch. Otolaryngol. 90, 367–73.
2. Dunkel, A., Steinhaus, M., Kotthoff, M., Nowak, B., Krautwurst, D., Schieberle, P., and Hofmann, T. (2014). Angew. Chem. Int. Ed. Engl. 53, 7124–43.
3. d'Acampora Zellner, B., Dugo, P., Dugo, G., and Mondello, L. (2008). J. Chromatogr. A 1186, 123–143.
4. Thomas-Danguin, T., Sinding, C., Romagny, S., El Mountassir, F., Atanasova, B., Le Berre, E., Le Bon, A.-M., and Coureaud, G. (2014). Front. Psychol. 5, 504.
5. El Mountassir, F., Belloir, C., Briand, L., Thomas-Danguin, T., and Le Bon, A.-M. (2016). Flavour Fragr. J. 31, 400–407.
6. Chaput, M., El Mountassir, F., Atanasova, B., Thomas-Danguin, T., Le Bon, A.-M., Perrut, A., Ferry, B., and Duchamp-Viret, P. (2012). Eur. J. Neurosci. 35, 584–97.
7. Gottfried, J.A. (2010). Nat. Rev. Neurosci. 11, 628–41.
8. Sinding, C., Coureaud, G., Chabanet, C., Chambault, A., Béno, N., Dosne, T., Schaal, B., and Thomas-Danguin, T. (2014). Chapter 5 - Perceptual Interactions in Complex Odor Mixtures: The Blending Effect. In Flavour Science: Proceedings from XIII Weurman Flavour Research Symposium, V. Ferreira and R. B. T.-F. S. Lopez, eds. (San Diego: Academic Press), pp. 27–31.
9. Sinding, C., Thomas-Danguin, T., Chambault, A., Béno, N., Dosne, T., Chabanet, C., Schaal, B., and Coureaud, G. (2013) PLoS One 8, e53534.
10. Le Berre, E., Béno, N., Ishii, A., Chabanet, C., Etiévant, P., and Thomas-Danguin, T. (2008). Chem. Senses 33, 389–95.
11. Coureaud, G., Thomas-Danguin, T., Wilson, D.A., and Ferreira, G. (2014). Proc. R. Soc. B Biol. Sci. 281, 20133319–20133319.
12. Grosch, W. (2001). Senses 26, 533–545.
13. Romagny, S., Coureaud, G., and Thomas-Danguin, T. (2017). Flavour Fragr. J., 1–9.
14. Jinks, A., and Laing, D.G. (2001). Physiol. Behav. 72, 51–63.
15. Escudero, A., Gogorza, B., Melus, M.A., Ortin, N., Cacho, J., and Ferreira, V. (2004). J. Agric. Food Chem. 52, 3516–3524.
16. Paravisini, L., Septier, C., Moretton, C., Nigay, H., Arvisenet, G., Guichard, E., and Dacremont, C. (2014). Food Res. Int. 57, 79–88.
17. Hallier, A., Courcoux, P., Sérot, T., and Prost, C. (2004). J. Chromatogr. A 1056, 201–208.
18. Johnson, A.J., Hirson, G.D., and Ebeler, S.E. (2012). PLoS One 7, e42693.
19. Hattori, S., Takagaki, H., and Fujimori, T. (2005). Food Sci. Technol. Res. 11, 171–174.
20. Williams, R.C., Sartre, E., Parisot, F., Kurtz, A.J., and Acree, T.E. (2009). Chemosens. Percept. 2, 173–179.
21. Burseg, K., and de Jong, C. (2009). J. Agric. Food Chem. 57, 9086–9090.
22. Thomsen, M., Dosne, T., Beno, N., Chabanet, C., Guichard, E., and Thomas-Danguin, T. (2017). Flavour Fragr. J. 32, 196–206.