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Review

# Gas chromatography–olfactometry in food flavour analysis

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**Abstract**

The application of gas chromatography–olfactometry (GC–O) in food flavour analysis represents to be a valuable technique to characterise odour-active, as well as character impact compounds, responsible for the characterizing odour of a food sample. The present article briefly reviews the use of GC–O in the flavour investigation of dairy products (milk and cheese), coffee, meat and fruits. Particular attention has been devoted to extraction techniques, GC–O hardware commonly utilised and olfactometric assessment methods, which can be applied to food analysis.


*Keywords:* Gas chromatography–olfactometry; GC–O; Food flavour analysis; Dairy products; Meat; Coffee; Fruits

## Contents



## 1. Introduction

The consumption of foods and beverages is tightly related to the stimulation of the human chemical senses, odour and taste. The flavour of food, along with its appearance and texture, is considered to be decisive for the consumer in the selection and ingestion of a particular food [1].

According to Laing and Jinks [2], food flavour is commonly defined as being the sensation arising from the integration or interplay of signals produced as a consequence of sensing smell, taste, and irritating stimuli from food or beverage. However, it is noteworthy that the term flavour is often inconsistently used; in reference to the above-mentioned biological receptor stimulation, or to the chemicals responsible for the process. In the present review, for the sake of readability, the terms flavour and odour will be used as synonyms for the overall sensory impression or the mixture of compounds responsible for the latter, while odourants and flavour compounds will be used to describe the single chemicals.

With respect to food aroma, the sensation of odour is triggered by highly complex mixtures of volatile molecules, mostly hydrophobic, and usually occurring in trace level concentrations (ppm or ppb). These volatiles interact with a G-protein-coupled odourant receptor (OR) of the olfactive epithelium located in the nasal cavity. Once the receptor is activated, a cascade of events

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is triggered to transform the chemical-structural information contained in the odourous stimulus into a membrane potential [3,4]. The latter is projected to the olfactory bulb, and then transported to higher regions of the brain [5] where the translation occurs.

The first comprehensive list of volatile molecules present in food matrices comprised a few hundred compounds. At the beginnings of the 1970s less than 1500 flavour chemicals had been identified in food products [6]. As well-known, nature herself produces most of the world's food flavours, many of which investigated following the advent of gas chromatography (GC) and mass spectrometry (MS), which marked a real turning point for flavour research. In the early stages of research in this field, attention was devoted to the development of methods in order to acquire deeper knowledge on the profiles of food volatiles; however, this analytical task was made troublesome due to the complexity of many real-world samples. Over the last decades, flavour research has benefited from the improvements in instrumental analytical chemistry, and, nowadays, the number of known flavours has increased more than four-fold, reaching over 7000 compounds [6], with around 300 identified in strawberry flavour and over 1000 only in coffee flavour.

In spite of the considerable instrumental advances made, the detection of new volatile flavours is turning out to be increasingly difficult. Extrapolating from the number of facultative aroma precursors, being these from confidential industrial results, as also from recently published achievements, it has been estimated that up to 10,000 volatiles may be present in food. Furthermore, a remarkable aspect is the considerable overlap of many volatiles in foods resulting in particular flavour profiles. Another intriguing aspect regards the isolation of volatile compounds; many studies in this field have been carried out without considering changes that may occur during the eating process, such as temperature increase, salivation and mastication. This acted as a stimulant towards the development of several systems which should simulate oral food processing, such as the artificial mouth [7,8] and artificial throat [9]. However, it is well-known that the combination of olfactometry and GC (GC–O) is an effective tool for the discrimination of relevant food flavour components.

In the past, a vast number of investigations have been carried out on the flavour of foods, and the introduction of GC–O was a breakthrough in analytical aroma research, enabling the differentiation of a multitude of volatiles in odour-active and non-odour-active, related to their existing concentrations in the matrix under investigation. Many extraction techniques were also developed, boosting the results attained. The continuous demand for new synthetic compounds reproducing the sensations elicited by natural flavours, triggered analytical investigations towards the attainment of information on scarcely known properties of well-known matrices. The present review provides an overview on the application of GC–O to extensively studied food matrices with the aim to demonstrate that even thoroughly investigated samples may continuously reveal new facets. For this reason, the present contribution will be focused on four key food matrices; dairy products (milk and cheese), coffee, meat and fruits. Extended sample preparation theory will not be dealt with in this article. As a prelude to food flavour analysis, some aspects regarding the assessment of food flavour and the relationship between odour-active compounds concentration and its exerted intensity will be briefly described.

## 2. Principles of gas chromatography–olfactometry

The human nose perception of volatile compounds, released from foods and fragrances, depends on the extension of the release from the matrix and the odour properties of the compounds. It is known that only a small portion of the large number of volatiles occurring in a fragrant matrix contributes to its overall perceived odour [10,11]. Further, these molecules do not contribute equally to the overall flavour profile of a sample, hence, a large GC peak area, generated by a chemical detector, does not necessarily correspond to high odour intensities, due to differences in intensity/concentration relationships. Consequently, the general interest of researchers was directed to the determination of the contribution of single constituents to the overall flavour of a product. In general, the sensory importance of an odour-active compound depends on its concentration in the matrix, and on its human nose limit of detection. Moreover, the unpredictable extent of interaction of flavour molecules with each other, and with other food constituents (lipids, protein, carbohydrates etc.) must also to be considered.

GC–O is the most appropriate analytical solution to such issues, as it enables the assessment of odour-active components in complex mixtures, through the specific correlation with the chromatographic peaks of interest; this is possible because the eluted substances are perceived simultaneously by two detectors, one of them being the human olfactory system. Consequently, GC–O provides not only an instrumental, but also a sensorial analysis. The latter is defined as the quantification of the human responses to the stimuli perceived by the senses of sight, smell, taste, touch and audition [12,13]. When coupled to analytical techniques, such as in GC–O, it becomes a precise, descriptive approach to characterise stimuli, evaluating and measuring impressions, as also an important process which enables the comprehension and quantification of a sensorial characteristic.

The description of a gas chromatograph modified for the sniffing of its effluent to determine volatile odour activity, was first published in 1964 by Fuller et al. [14]. The GC system was equipped with a non-destructive thermal conductivity detection (TCD) system with the outlet connected to a sniffing port (also called olfactometry port or transfer line). The latter was located inside a telephone booth, in order to isolate the evaluator from the potential influences of odourants present in the ambient. In 1971, a more sophisticated GC–O system was reported; humid air was added to the GC effluent, thus avoiding nasal mucosa dry-out [15]. Further improvements included the use of a Venturi tube, to maintain capillary column resolution and to deliver, ergonomically, the effluent to the evaluator. Over the following years, the sniffing ports began to incorporate design features and, nowadays, well-planned options are available on the market.

The introduction and diffusion of GC–O, proved to be vital for the development in the research field of odour-active compounds, providing valuable information on the chromatogram locations on which to focus attention and resources. GC–O is

a unique analytical technique which associates the resolution power of capillary GC with the selectivity and sensitivity of the human nose.

### 2.1. Sample preparation for GC–O analysis

Some considerations have to be made on a rather laborious, but significant step of food flavour analysis by means of GC, namely sample preparation. A food flavour profile is closely related to the isolation procedure, which should yield a product which is representative of the sample; therefore, the choice of an appropriate sample preparation method becomes crucial. According to the properties of a foodstuff, the preparation may include mincing, homogenisation, centrifugation, steam distillation (SD), solvent extraction (SE), fractionation of solvent extracts, simultaneous distillation–extraction (SDE), supercritical fluid extraction (SFE), pressurised-fluid extraction, Soxhlet extraction, solvent assisted flavour evaporation (SAFE), microwave-assisted hydrodistillation (MAHD), direct thermal desorption (DTD), headspace (HS) techniques, cryofocussing, solid-phase microextraction (SPME), matrix solid-phase dispersion (MSPD) and/or methylation, among others.

Commonly, distillation and SE methods are considered to yield the near complete flavour of food extracts, which is not always of relevance for the determination of a characteristic odour profile. In order to obtain more representative samples, SDE is widely applied; providing elegant and rapid extractions, through which the recovered isolates, after being concentrated, are ready to be injected into the GC system. However, the analyst has to deal with decomposition of labile compounds, loss of highly volatile compounds and heat-induced artifact formation. The latter drawback can be contrasted by the use of a modified system, an SDE under static vacuum (SDE-SV), which allows extractions at 30–35 °C. In addition, the use of SDE-SV, although being more time-consuming, eliminates the concentration step prior to sample injection.

In general, the extracts obtained by SE can be very complex, so that many co-elutions may occur in GC–O, making the identification of individual flavour compounds difficult. The fractionation of these extracts is a time-consuming, but reasonable, mode to overcome this problem. It may be accomplished in several manners, such as by washing the extract with dilute acid, dilute base, and either sodium metabisulfite or 2, 4-dinitrophenylhydrazine, promoting the elimination of acids, bases or carbonyl compounds from the extract, respectively. If each wash solution is then re-extracted with solvent, the fractions containing only acids, bases or carbonyls may be recovered, even though multiple manipulations of the extract may cause the loss of highly volatile compounds. Further aspects should be considered with regards to SE methods, as the possible loss of the more volatile compounds during solvent removal, the need of large sample amounts in order to attain concentrated extracts, and the presence of non-volatile and high-boiling compounds in the extract, or impurities from the solvent. Furthermore, in GC–O analysis the solvent peak may cover early eluting odour-active volatiles.

On the other hand, samples extracted by means of SD methods are commonly free of non-volatile or high-boiling compounds, and therefore do not contaminate liners and columns of the GC system. Moreover, the extract may be concentrated, thus enabling the detection of trace components. However, poor extraction of highly polar or hydrophilic compounds (acids and alcohols) may occur, as also artifact formation due to thermal degradation. With regards to odour assessment analyses, SD methods may not be suitable for fresh material extraction, such as fruits and vegetables, as the extract will elicit an odour that is more similar to a cooked, rather than fresh fruit or vegetable odour.

A further very popular method is SAFE, which may be applied after SE techniques or be used as an individual extraction method for aqueous foods, such as milk, fruit pulps or matrices with high oil content. The technique, which may be applied to a solvent extract or a food matrix, removes volatiles under low temperature and high vacuum conditions. The extract is then collected into flasks which are cryogenically cooled with liquid nitrogen. The attained material should be representative of the original sample; uncooked, but without high-boiling compounds and colour. Some attention and time should be devoted to the cleaning of the SAFE apparatus, in order to avoid contamination of liners and columns.

HS methods, which are also frequently applied, may be divided into static (SHS) and dynamic (DHS) headspace analyses; the former is characterised by the sampling of the atmosphere around the headspace of a food matrix, located in a vial, after equilibrium has been achieved; the latter removes larger amounts of volatiles due to a constant sweeping of the matrix by a flow of carrier gas; a concentration step is required prior to GC analyses. HS techniques are a valuable tool for GC–O analysis combining simplicity, solvent-free procedures, requirement of small sample amounts, and no artifact formation. However, the relative concentration of flavour components in the headspace does not correspond to the concentration in the sample due to the differences in volatility of flavour compounds.

A further technique, worthy of note is SPME, a widely applied solvent-free method which exploits the high adsorption power of a fused silica fibre coated with a specific extraction phase, which is selected according to the type of matrix. However, the use of SPME as isolation method prior to GC–O analysis, presents some limits due to the possible non-representative nature of the extracts. The chemical profile of the collected volatiles depends upon the type, thickness and length of the fibre, as well as on the sampling time and temperature. Reviews dedicated to SPME in food analysis have been published by Kataoka et al. [16] and Wardencki et al. [17].

Although a series of flavour isolation methods are known, the most appropriate way to attain an optimum recovery of the flavour chemicals is the employment of more than one extraction technique. Sample preparation for food flavour analysis has been exhaustively reviewed by Wilkes et al. [18].

With regards to artifact formation, it must be highlighted that the thermal breakdown of analytes may not only occur during the sample isolation procedure, but also in the heated inlet during the sample introduction process, causing total or partial

loss of some components. This may result in the generation of new peaks which will be assessed by GC–O and, possibly, be odour-active. For example, sulphur-containing compounds are particularly susceptible to thermal decomposition, and readily decompose in contact with metal, such as the heated injector block, forming artifacts [19]. The chromatographic behaviour of a compound varies, obviously, according to its chemistry and to the column stationary phase, and might affect GC–O data. The GC injection process should meet the sample characteristics, in order to avoid heat-induced decomposition (e.g. by maintaining the inlet temperature as low as possible).

### 2.2. GC–O data measurement methods

During the years, the training of panellists, more precisely of the human noses, and data handling methods began to include some of the practices commonly used in sensory testing. Over the last decades, GC–O has been largely used in combination with sophisticated olfactometric methods which were developed to collect and process GC–O data, and hence, to estimate the sensory contribution of a single odour-active compound. These methods are commonly classified in four categories: dilution, time-intensity, detection frequency, and posterior intensity methods [10,20–22]. Dilution analysis, the most applied method, is based on successive dilutions of an aroma extract until no odour is perceived by the panellists. This procedure, usually performed by a reduced number of assessors, is mainly represented by CHARM (combined hedonic aroma response method) [23], developed by Acree et al., and AEDA (aroma extraction dilution analysis), first presented by Ullrich and Grosch [24]. In AEDA, samples are evaluated by the panellists in increasing dilution order and the impact of an odour-active compound is given by its dilution factor (FD) value. The latter is calculated by dividing the largest volume analysed by the lowest volume in which the respective odour-active compound was still detectable. The overall results are reported in an aromagram presenting the FD value, or its logarithm, against the retention index, as exemplified in Fig. 1, or simply by listing the FD values. On the other hand, in CHARM analysis the dilutions are presented to the panellists in a randomised order, avoiding bias introduced by the knowledge of the dilution being analysed. The panellists record the start and end of each detected odour; the detection duration for each individual is then compiled, and an aromagram is generated by plotting the duration of the odour sensation against the dilution value (Fig. 2). CHARM values can be calculated according to

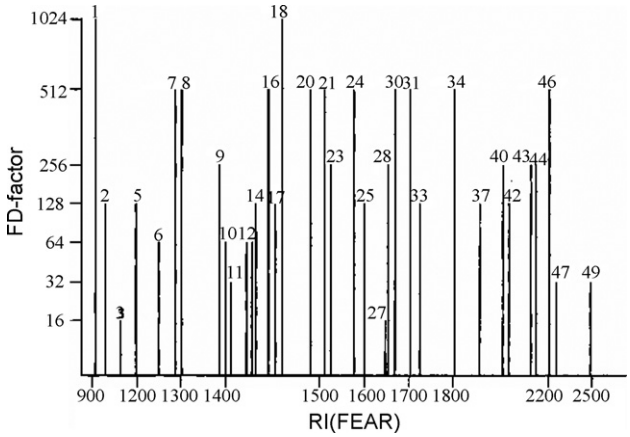


Fig. 1. Example of AEDA results attained from the analysis of neutral/basic volatiles of milk chocolate. The most potent odour-active compounds were 3-methylbutanal (No. 1) eliciting a malty odour and 2-ethyl-3,5-dimethylpyrazine responsible for a potato chip-like note (No. 18). (2) 2,3-butandione, (3) hexanal, (5) unknown, (6) (*Z*)-4-heptenal, (7) 5-methyl-(*E*)-2-hepten-4-one, (8) 1-octen-3-one, (9) dimethyl trisulphide, (10) nonanal, (11) trimethylpyrazine, (12) unknown, (14) (*E*)-2-octenal, (16) 2-ethyl-3,6-dimethylpyrazine, (17) unknown, (20) 2,3-diethyl-5-ethylpyrazine, (21) (*Z*)-2-nonenal, (23) (*E*)-2-nonenal, (24) (*E*,*Z*)-2,6-nonadienal, (25) (*Z*)-2-decenal, (27) (*E*)-2-decenal, (28) phenylacetaldehyde, (30) 2-methyl-3-(methyldithio)furan, (31) (*E*,*E*)-2,4-nonadienal, (33) unknown, (34) (*E*,*E*)-2,4-decadienal, (37) 2-phenylethanol, (40) $\delta$-octenolactone, (42) $\gamma$-nonalactone, (43) ethyl cinnamate, (44) $\gamma$-decalactone, (46) *R*-$\gamma$-decalactone, (49) skatole (Reprinted from [45] with permission of the American Chemical Society, © 1997).

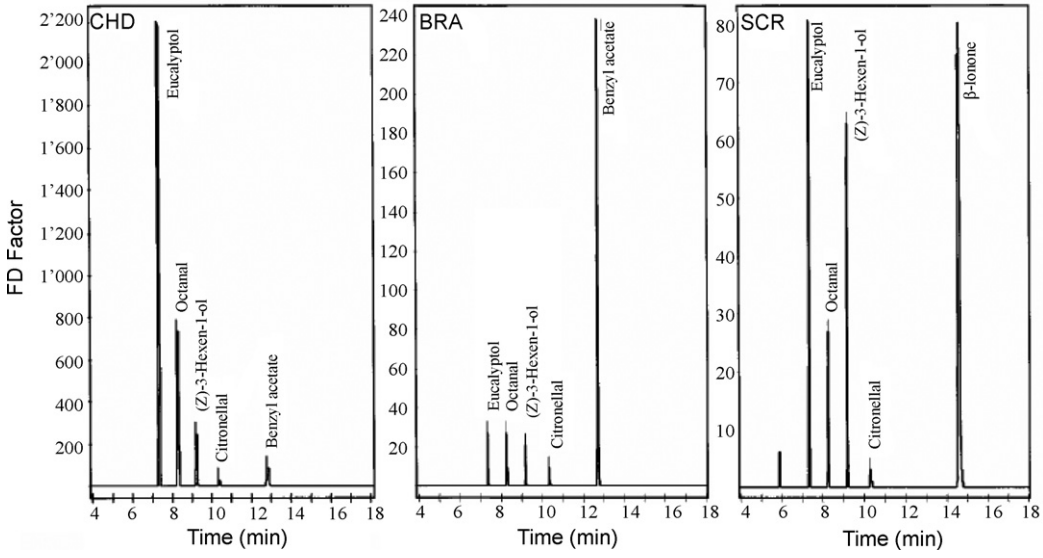


Fig. 2. Flavour model CHARM analysis performed by three panellists (the letters refer to the initials of the panellists which performed the analyses) (Reprinted from [93] with permission of the American Chemical Society, © 2002).

Eq. (1), where $n$ is the number of coincident responses between panellists and $d$ is the dilution value. The later is analogous to the FD value in AEDA.

$$c = d^{n-1} \quad (1)$$

AEDA presents limitations, such as controversial statistical data manipulation, the non-consideration of odourant losses during the isolation procedure and of synergistic or suppressive effects of distinct compounds in a flavour mixture. With regards to CHARM, limitations can be observed in quantification analyses, which require the replication of the experiment by at least three different trained assessors.

Time-intensity methods, such as OSME (Greek word for odour), are based on the immediate recording of the intensity as a function of time by moving the cursor of a variable resistor [25]. In OSME odour-active compounds intensity information is attained in a single run, although the results are reliable only if trained assessors are used. A further approach, the detection frequency method [26], uses the number of evaluators detecting an odour-active compound in the GC system's effluent as a measure of its intensity. This GC–O method is performed with a panel composed of numerous and untrained evaluators; 8–10 assessors are a good compromise between low variation of the results and analysis time. It must be added that the results attained are not based on real intensities and are limited by the scale of measurement. At a particular concentration and odour intensity, a compound may be perceived by all assessors, but as the concentration and the odour intensity may continue to increase, however, the detection frequency cannot. Based on the latter method, the nasal impact frequency (NIF) has been developed [27,28]. Fig. 3 illustrates the NIF profile of a sample odour. The NIF technique does not require a trained panel, no intensity scale has to be learned by the evaluators, and therefore no intensity measurement is performed. Consequently, peak intensities are not related to compound's odour intensity, but to their detection frequency. Commonly, peak heights and areas are defined as NIF and SNIF (surface of nasal impact frequency), respectively. Each panellist participates in $1/n$ of the final results (n stands for the number of panellists); if NIF is 100%, all $n$ panellists detected the odourant [27]. Another GC–O technique, is the posterior intensity method [29], proposes the measurement of a compound odour intensity, and its posterior scoring on a previously determined scale. This posterior registration of the perceived intensity may cause a considerable variance between assessors. The attained results may generally be well correlated with detection frequency method results, and to a lesser extent, with dilution methods.

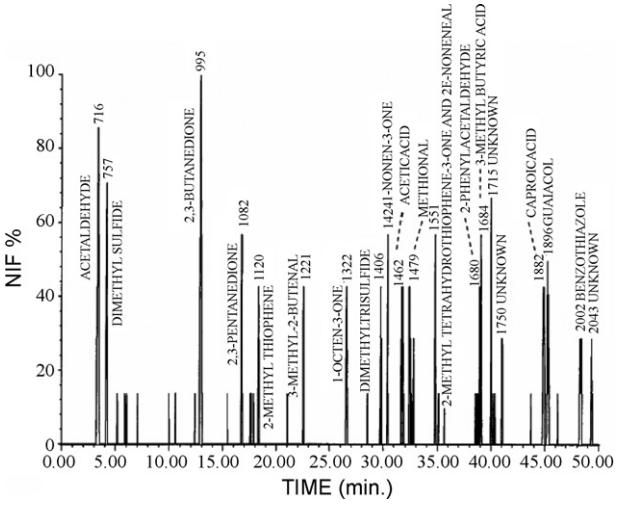


Fig. 3. NIF profile of the volatiles isolated from yogurt headspace. Retention indices are presented at each peak apex (Reprinted from [28] with permission of the American Chemical Society, © 1997).

Odour panels consist of individuals that are selected and screened for specific anosmia; in the case no insensitivities are found, the panellists are trained by sniffing different dilutions of standard compounds selected according to the matrix to be analysed. Considering that the olfactory ability between humans may be significantly different, the quality description is generally based on glossaries of olfactive descriptors, with the aim of normalising the language between panellists. Furthermore, if intensity measurements are to be carried out, the panellists have to learn a scale. The number of evaluators which shall be comprised in a panel is a rather controversial matter; dilution methods are often performed using only 1–3 assessors, while in detection frequency techniques higher reliability is attained with 8–10 assessors. A large number of trained panellists is also required for intensity evaluations since a high variability may be commonly observed within and between panellists.

## 3. Relationship between odourant concentration and odour intensity

The screening of significant odourants in food has not only been extensively made by performing GC–O dilution methods, but also through the odour activity value (OAV) concept. As described in Section 2.2, in dilution methods a dilution series of the original aroma extract from a particular food are evaluated and the key odourants are ranked in order of potency. The highest dilution at which a substance is sniffed is represented by its FD value; the latter value is considered as proportional to the OAV evaluated in air Audouin et al. [30]. Both methodologies are applied in the determination of the aroma compounds which most likely contribute to the overall odour of a food.

Some flavour chemicals may present an increased intensity in odour-activity according to a proportional increment of their concentration, while with others the change in intensity may be the opposite or just less marked. The dependence of intensity upon concentration regards a constant which quantifies odours,

and is denominated as slope. The idea of slope as a constant, which is characteristic of a substance, assumes the validity of the Stevens's power law [31]. This law states that equal changes in stimulus magnitude ($\Phi$) produce the corresponding change in perceived intensity ($\Psi$); $k$ is a constant and $n$ is the Steven's exponent, as presented in Eq. (2).

$$\Psi = k\Phi^n \quad (2)$$

It has to be noted that the slope depends upon the method by which it was determined. Generally, a relatively high value for the slope indicates a strong dependence of intensity upon concentration, while a low-slope odourant is typically not very powerful when assessed in the undiluted form.

In 1957, Patton and Josephson [32] first proposed an estimation of the importance of a flavour chemical to a food based on the ratio of its concentration in that food to its threshold concentration in that same matrix. In connection to this approach, in 1963, Rothe and Thomas [33] derived the odour activity values (OAVs), with the aim to better correlate the concentration of odourant with its detection threshold value; the latter is defined as the lowest concentration or intensity that is perceived by the panellist [30]. It is clear that the theoretical intensity of an odourant under any specific set of conditions could be roughly expressed in terms of its OAV. However, the difficult and time-consuming determination of threshold values, which vary among and within panellists [34], led to controversies related to the use of OAVs as indicators of the percent contribution to the overall intensity of a sample.

With regards to the aforementioned odour quantification parameter, threshold concentration, it has to be emphasised that not only the detection threshold is involved in the description of an odour. Further two levels are known, namely recognition and difference thresholds; the former is the lowest concentration or intensity at which a substance or an olfactive quality attribute can be identified and described, while the latter is the magnitude of a stimulus above which there is no increase in the perceived intensity of the appropriate quality for that stimulus [12]. Moreover, the synergistic or suppressive effects of different odourants in a food matrix are not considered in the determination of OAVs and GC–O analysis. The sample preparation steps may deprive the real food matrix of some of its characteristics. The compounds detected as odour-active in GC–O are most likely to be significant. However, the investigated extract could be too concentrated and so present odour-active compounds in GC–O, but not in the food, or compounds that might not be odour-active in GC–O due to an insufficient concentration of the extract and still contribute to the odour of the food matrix.

In order to attain a complete analysis of key odourants, subsequent sensorial analysis should be performed, such as studies of recombination models and omission experiments. In the former, the aroma model system for a specific food sample is prepared based on the combination of previously achieved AEDA or CHARM values, and/ or OAVs. Odourants showing higher values are used to formulate a recombined model, which is then compared to the real food product for similarity or difference. The preparation of such models is simple for liquid food matrices, attaining a homogeneous blend of odourants; satisfactory examples of this procedure have been shown in studies on the aroma of sour cream butter [34], stewed beef juice [35], coffee brew [36], strawberry juice [37], wine [38], and olive oil [39]. Difficulties arise when aroma models are prepared for solid foods, as it is not simple to simulate the composition and structure of the non-volatile fraction of the food and to imitate their odourant's distribution. In order to overcome this, limit suitable inert alternative bases are applied in solid food aroma models, such as cellulose or sunflower oil. The omission experiments, on the other hand, deal with the preparation of an aroma model for a specific food sample in which one or more odourants are omitted. In this experiment, the panellists are asked to perform duo and triangle tests to compare the reduced model with the complete one and indicate the perceived sensorial differences [40].

## 4. Analysis of dairy products: milk and cheese

One of the earliest applications of formal laboratory sensory analysis was made in the dairy products industry. In the early 1990s, techniques for the judgement of these products were developed to stimulate the interest and educate consumers. The attributes considered were appearance, flavour and texture, in relation to the presence or absence of predetermined defects. It is natural, therefore, that the dairy industry has acquired a broad knowledge on sensory defects, including their causes and consequences.

In sensorial analysis, the verbal expression of quality is of great importance in order to achieve normalised responses from the human perception, hence, the development of dedicated glossaries of olfactive descriptors adequate for each food matrix, of great relevance. Generally, panels generate their own list to describe the notes of the product under investigation. Several different flavour lexicons have been developed to study the flavour profiles in food products [41,42], the influence of different starters and addition of bacteria, and also the effects of fat reduction. In this respect, cheeses are an excellent example, Drake et al. [43] compiled a comprehensive descriptive sensory language for Cheddar cheese flavour. This language resource, known as the cheese lexicon, was generated from the analysis of 220 Cheddar cheese samples and 70 other cheeses varying in age, fat content and geographical regions. Other Cheddar cheese flavour lexicons are also known [42]. However, so far, a comprehensive list of cheese key odourants determined by GC–O, has not yet been established.

The application of GC–O to dairy products enabled the characterisation of the impact flavour compounds of several matrices, including not only milks and cheeses, which will be discussed in this section, but also sweet cream butter [44], yogurt [28,34], milk chocolate [45], and milk powder [46]. The subject has been briefly reviewed by Friedrich and Acree in 1998 [47], while Curioni and Bosset published an overview focused on GC–O of various types of cheeses [48].

It is well-known that the flavouring chemicals vary according to the sample's state, so that raw milk elicits a distinct odour when compared to that of heated or processed milk; with dif-

ferent classes of compounds responsible for the characteristic odour of distinct samples. For example, esters are responsible for the flavour of raw milk samples, while lactones and heterocyclic compounds for that of heat-treated and pasteurised milk. On the other hand, fermented dairy products, such as cheese and yogurt, are characterised by the presence of fatty acids [47].

Milk has been studied in depth by Moio et al. [49–51], who analysed the matrix under distinct conditions. The analyses of several raw milk samples led to the observation that dimethylsulphone, ethyl butanoate, ethyl hexanoate, heptanal, indole, nonanal, and 1-octen-3-ol, were commonly reported as odour-active compounds. Even though similarities were indicated, the overall odour of each milk variety sample presented a different aroma profile, with ethyl butanoate and ethyl hexanoate the most potent in the cow, sheep and goat milk, while water buffalo milk flavour was better characterised by nonanal and 1-octen-3-ol [49]. When investigating high temperature/short time (HTST) and ultra-high temperature (UHT) pasteurised cow milks, dimethylsulphone and 2-heptanone were found to be the most potent odour-active compounds, replacing ethyl butanoate and ethyl hexanoate, observed in raw cow milk. Both of these odourant couples can be used as aroma quality indicators, for heated and raw milk, respectively [50]. The same above-mentioned research group also performed GC–O and CHARM analyses to determine the key odourants of ovine milk, derived from animals subjected to distinct diets (natural pasture, grass meadow, and on mixed grain rations), investigating, as such the effects of the diet on milk flavour. The key-odour notes were similar for all three samples, although differing in intensity. Ethyl butanoate and ethyl hexanoate were reported as potent for all three diet types, while heptanal, octanal and nonanal were more relevant for the milk obtained after the mixed grain ration diet. Compounds such as 1-octen-3-ol, dimethylsulphone and indole presented higher odour potency in the milk produced by the ewes fed with natural pasture and grass meadow [51].

Cheeses, by definition, are fermented milk-based food products, with more than 500 different types, each presenting complex, variety and type-specific flavour profiles, which are originated from the degradation of the major milk constituents, such as lactose, citrate, lipids and proteins [52]. The odour-active compounds belong to several chemical classes, such as alcohols, aldehydes, ketones, esters, lactones, furans, nitrogen-containing compounds, as also pyrazines and sulphur-containing compounds, terpenes and their derivatives, aromatic compounds and free fatty acids.

In general, little is known about the characteristic impact flavour of most cheese varieties. Distinction has to be made between key odourants and odour-active compounds, since as well-known, only a small fraction of the volatile substances of a food matrix is responsible for its characteristic odour. Cheddar cheese is one of the best studied varieties; GC–O was applied to this hard cheese for the first time in 1995, when Christensen and Reineccius [53] performed an investigation on the odour impact compounds present in 3-year-old cheeses by means of AEDA. The components which presented a higher impact, based on their dilution factor, were ethyl acetate, 2-methylbutanal, 3-methylbutanal, 2,3-butanedione, α-pinene, ethyl butyrate, ethyl caproate, 1-octen-3-one, acetic acid, and methional, followed by several acids. According to the authors, AEDA was not the optimal method, since it did not enable a complete determination of the volatile odour fraction, which would include the assessment of hydrogen sulphide, acetaldehyde, and methanethiol. Moreover, Milo and Reineccius [54] investigated and quantified the chemicals responsible for the flavour of regular-fat and low-fat Cheddar cheeses applying GC–O analysis of the static headspace of the samples, denominated by them as GCO-H. A gas chromatograph was connected to a purge and trap system, and equipped with a non-polar column. AEDA was also carried out on a cheese flavour fraction isolated by high-vacuum distillation. The latter analyses were performed on a GC system equipped with a splitter with two retention gaps, one of which connected to a flame ionisation detection (FID) system and the other to the transfer line (sniffing port), with the effluent splitted 1:1; three stationary phases of distinct polarities were used. Two panellists performed AEDA and the OAVs of the odourants described as potent were calculated on the basis of quantitative data and on sensory thresholds in oil and water. The authors suggested that acetic acid, butyric acid, methional, diacetyl, and homofuraneol were primarily responsible for the pleasant mild aroma of Cheddar cheese. Additionally, highly volatile sulphur compounds, such as methanethiol and dimethyl sulphide, contributed significantly to the aroma. Furthermore, the meaty, brothy odour characteristic of low-fat Cheddar was considered to be related to the high concentrations of methional, DMHF [2,5-dimethyl-4-hydroxy-3(2*H*)-furanone or Furaneol], and mainly, homofuraneol. The higher water content in low-fat cheese, combined with a possible increased microbial activity, was assumed to be the reason for the elevated concentrations of the latter compounds. Moreover, a comparison of the volatile compositions of full- and reduced fat Cheddar showed that the levels of methanethiol are highly correlated with the flavour grade. This observation indicated that the lack of aroma in reduced-fat Cheddar can be mainly related to the absence of methanethiol. However, a combination of the latter and decanoic acid or butanoic acid in all cheeses gave a better correlation with Cheddar flavour than methanethiol alone [55].

As previously mentioned, the flavour of Cheddar cheese comprises a wide variety of substances belonging to several chemical classes, and therefore, sophisticated extraction methods are constantly applied. O'Riordan and Delahunty [56] investigated the volatile fraction of the mature Cheddar cheese buccal headspace analysis (BHA) extracts, in order to determine its odour profile during human consumption. In addition, the BHA extract was compared to that obtained by vacuum distillation; both analyses were performed by means of GC–O. Vacuum distillation yields extracts that are not necessarily representative of the compounds that are perceived by a person during the eating process, while the BHA extracts comprises the volatile compounds which are displaced during the mastication of a food matrix, in concentration ratios that stimulate the olfactory epithelium.

The analyses were performed applying a method adapted from OSME, which enabled the estimation of odour intensity. The sample's BHA and vacuum distilled aromagrams, illustrated in Figs. 4 and 5, respectively, report statistically treated time-

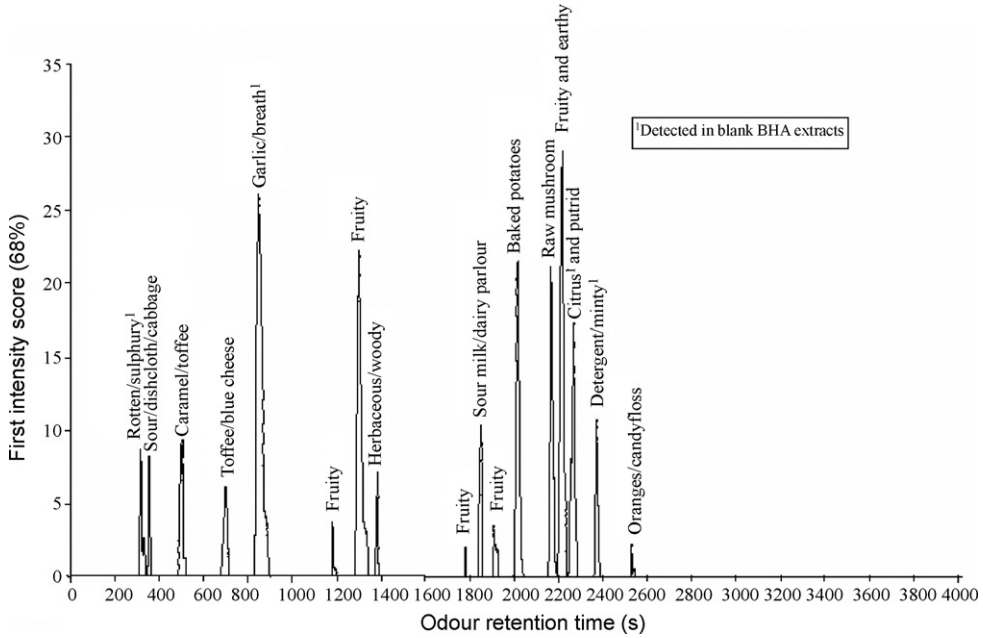


Fig. 4. Cheddar cheese buccal headspace analysis (BHA) principal aromagram (Reprinted from [56] with permission from ©Wiley-VCH, Verlag GmbH & Co., 2001).

intensity data. The first intensity score represents the underlying shape of the panellist's time-intensity curves, enabling data comparison within and between panellists. The application of this OSME derived method, rather than AEDA, represented an additional feature of Cheddar cheese flavour analysis. Moreover, it is worth to note that the authors mention the use of a GC–O system; although GC/MS-O was used. The analyses were performed by two evaluators, who determined the contribution of individual compounds to the aroma of the mature Cheddar cheese, followed by principal components analysis (PCA). The gas chromatograph column's effluent was split 1:20 to the MS and sniffing port, respectively, and time-intensity data were recorded on a module using a 100 mm unstructured line scale and a modified computer mouse that incorporated a resistance to movement. The modified mouse allowed the assessors to relate the perceived odour intensity to the physical stimulus of hand pressure. According to a principal aromagram intensity measurement, the BHA extract was characterised mainly by ethyl hexanoate, ethyl butyrate, methional, ethyl hexanoate, octanal, and dimethyl trisulphide. Furthermore, compounds described by the authors as "unknowns" were considered to be relevant for the odour of the matrix, giving an earthy, garlic-like and raw mushroom contribution to the odour profile. In general, all the compounds detected in the BHA extract have been associated to the volatile profile of Cheddar cheese in former works. On the other hand, by means of vacuum distillation a near-complete volatile extract was attained,

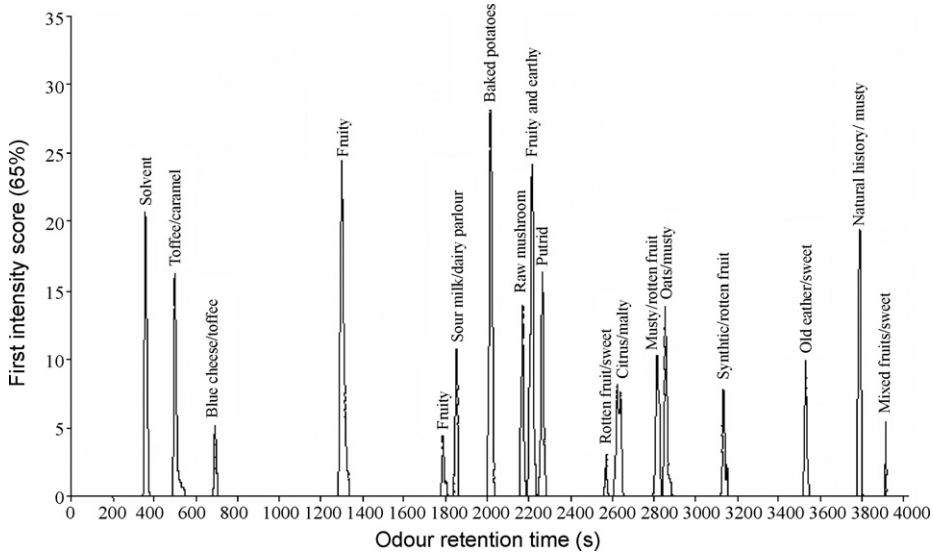


Fig. 5. Cheddar cheese vacuum distillation principal aromagram (Reprinted from [56] with permission from ©Wiley-VCH, Verlag GmbH & Co., 2001).

being distinguished from the BHA extracts by the presence of low vapour pressure odour-active volatile compounds. The odour-potency of this fraction was mainly characterised by the presence of methional, ethyl butyrate, ethyl hexanoate, dimethyl trisulphide, and 3-methylbutanal. The contribution of the earthy note was likewise representative, as also a musty one.

Further representative odourants were determined by Zehentbauer and Reineccius [57] through the application of dynamic headspace dilution assay (DHDA) on a mild Cheddar variety. Dilution was made through a stepwise decrease of the purge time; starting with 30 min, which was equivalent to FD 1, and ending with 20 s, corresponding to FD 64. Each dilution was sniffed by a single evaluator on three distinct GC columns. The authors accomplished GC–O analysis replicates through analyses on capillary columns coated with three distinct stationary phases, and not by performing duplicate analysis on a given column. The identified odourants were then reported on at least two stationary phases. The GC column effluent was split 1:1 between the FID system and the sniffing port. The DHDA results showed that, in addition to the odourants previously identified by AEDA and GCOH [55], other key aroma components were (*Z*)-4-heptenal, 2-acetyl-1-pyrroline, dimethyl trisulphide, 1-octen-3-one, (*Z*)-1,5-octadiene-3-one, and (*E*)- and (*Z*)-2-nonenal. These had been underestimated or not even perceived previously by using AEDA. Moreover, as supported by other authors [52,56], it was pointed out that single volatiles eliciting characteristic Cheddar cheese notes were not identified, confirming that the global aroma results from a balance between the odourants present in different concentrations in the matrix. Another interesting research was performed on the volatile aroma fraction of two sharp Cheddar cheeses of British Farmhouse origin [58]. The analyses by means of GC–O and AEDA, produced quite interesting results: the identification of some specific flavour notes was successfully performed, and *p*-cresol was characterised as the main responsible for a so-called cow resembling and phenolic note, whereas an intense soil-like odour could be related to 2-isopropyl-3-methoxypyrazine. At much lower odour intensity, 2-isobutyl-3-methoxypyrazine contributed to the earthy, bell pepper-like odour elicited by the samples. Remarkably, the concentrations of *p*-cresol and 2-isopropyl-3-methoxypyrazine were lower in the core than in the rind of the same wedge of cheese. Further studies also dealt with the investigation of a characteristic nutty flavour of some Cheddar cheeses by means of GC–O, applying AEDA and posterior intensity method. The solvent extracts (evaluated by three panellists) and DHS sampling (analysed by two panellists) of young and aged, nutty and not nutty, cheese models were compared. The solvent extracts were analysed using capillary columns of distinct polarities, while for the DHS samples the GC–O system was equipped with a polar column. For both analyses, a FID system/sniffing port split of 1:1 was applied. The DHS recovery technique enabled an optimised investigation, revealing that the Strecker aldehydes, such as 2-methylpropanal, 2-methylbutanal and 3-methylbutanal, imparted a nutty note to that matrix, especially in aged cheeses [59]. On the other hand, in Swiss-type cheese, also classified as a hard cheese, propionic acid was the key compound considered as responsible for the nutty note [60]. Another curious odour is the floral, rosy note identified in Cheddar cheese [61], which can be classified as an unclean off-flavour. A GC–O technique AEDA was applied, using both, polar and non-polar capillary columns, a FID system/sniffing port split of 1:1 and a panel composed of two evaluators. Phenylacetaldehyde and phenylacetic acid, originated from the catabolism of aromatic amino acids, were reported as responsible for the undesired note of the Cheddar cheese flavour. This floral, rosy note had been previously reported also in Camembert [62] and Swiss Gruyére [63].

Although the Cheddar cheese flavour has been extensively studied, it is worthy of note, once more, that a standard list of that cheese's odour-active molecules responsible for its overall odour does not exist. The same is, obviously, true for other dairy matrices. A characteristic odour is defined by the so-called component balance theory [52,64], which is ruled by a wide range of parameters, such as cheese age, microflora, biochemistry, as also odour extraction methods.

Several other cheese types have been investigated through GC–O and were reported in literature. The aforementioned hard cheese, Swiss Gruyére, presented a series of flavourings responsible for its typical odour, such as 2- methylbutanal, 3-methylbutanal, methional, dimethyl-trisulphide, phenylacetaldehyde, 2-ethyl-3,5-dimethylpyrazine, 2,3-diethyl-5-methylpyrazine, methanethiol, as well as a variety of acids [63]. Also investigated was the origin of a potato-like off-flavour on that cheese, which could be attributed to 2-ethyl-3,5-dimethylpyrazine and 2,3-diethyl-5-methylpyrazine. DHS-GC/MS analysis and GC–O, applying AEDA, were performed using a non-polar column. The gas chromatograph column's effluent was split to the FID system/sniffing port in a 1:1 proportion. The origin of the potato-like odour remained undetermined, but the association of the sweaty odour of the cheese to 2-methyl butyric acid, 3-methyl butyric acid and butyric acids was achieved. Furthermore, the well-known shortcomings of AEDA were reported, such as the non-consideration of synergistic and suppressive effects of distinct odourants in a matrix.

Indeed, AEDA is the most frequently applied method for cheese flavour analysis. In this respect a further method, aroma extract concentration analysis (AECA) [65], was developed and applied to Camembert cheese analysis [62]. In AEDA, the extract is strongly concentrated by distillation procedures, possibly leading to the loss of volatiles, while AECA, on the other hand, is started with GC–O analysis of the original volatile extract, which is then concentrated stepwise by distilling off the solvent and then, after each step, an aliquot is subjected to analyses. 2,3-Butanedione, 1-octen-3-one, 1-octen-3-ol, β-phenethyl acetate, 2-undecanone, δ-decalactone, butyric and isovaleric acid were found to be fundamental for Camembert aroma. Remarkable was the olfactometric detection of 1-octen-3-one and 1-octen-3-ol, which co-eluted when performing GC–O on a non-polar column, but were separated on a polar one and analysed by AECA. It was observed that the odour intensity of 1-octen-3-ol might have been enhanced by the corresponding ketone, 1-octen-3-one. Since both compounds elicited a mushroom-like note, their co-elution resulted in the percep-

tion of an enhanced intensity. However, in order to elucidate which compound enhanced the odour of which, the compound's threshold values and concentrations in the matrix should have been considered. From the AECA results presented, it was concluded that 1-octen-3-ol is more intense than 1-octen-3-one, however the threshold value of the latter, although in water, is known to be lower [66–68]. Furthermore, additional analyses carried out on the static headspace sample, by using GC–O, indicated that methanethiol and dimethyl sulphide might also play a significant role. The mushroom, floral and garlic notes in Camembert aroma, as described by Dumont et al. [69], were related to 1-octen-3-ol, β-phenethyl acetate and dimethyl sulphide, respectively.

An investigation on Parmigiano Reggiano cheese was carried out through the employment (and comparison) of OSME and AEDA GC–O methods [70]. The former was performed on a GC–O system equipped with a polar, while the latter was carried out on a polar and on a non-polar column. Acetaldehyde, 2-methylpropanal, 3-methylbutanal, ethyl hexanoate, dimethyl trisulphide, and methional were identified as intense odourants by OSME and as possessing the highest FD values by AEDA. The latter method also enabled the determination of further potent odourants, such as ethyl butyrate, diacetyl, DMHF, 2-methylbutanal, 2,6-dimethylpyrazine, 2-heptanone, and 2,4-hexadienal.

Although a series of key odour-active compounds have been identified by means of GC–O and reported in literature, in general fermented dairy products are characterised by four common potent odourants; 1-octen-3-one, methional, 3-methylbutanal, and butyric acid. These and further key-compounds are reported in Table 1.

Table 1
Odour-active compounds commonly detected by means of GC/O in dairy products and the respective olfactive description

| Compound | Odour description | Reference |
|---|---|---|
| (*E,E*)-2,4-Nonadienal | Fatty, floral | [57,61] |
| (*Z*)-4-Heptenal | Fatty, oily, creamy | [59,61,63] |
| 1-Nonen-3-one | Pungent, mushroom-like | [29] |
| 1-Octen-3-ol | Mushroom-like | [58,62] |
| 1-Octen-3-one | Mushroom-like, metallic | [57–59,61,62,75] |
| 2,3-Butanedione | Buttery, creamy | [57,59,61,62,79] |
| 2-Heptanone | Fruity, spicy, cinnamon | [63,70,77] |
| 2-Nonanone | Floral, fruity, green, oily | [62,77] |
| 2-Undecanone | Floral, rosy, citric | [62] |
| 3-Methylbutanal | Malt-like, green | [57,61–63,70] |
| 6-Dodecen-γ-lactone | Fatty | [59,61–63,75] |
| Acetaldehyde | Ethereal, pungent | [70,75] |
| Acetic acid | Pungent, vinegar-like | [61,62,75] |
| Butyric acid | Sharp, cheesy, rancid, sour, sweaty | [62,70,75] |
| Diacethyl | Buttery | [75] |
| Dimethyl sulphide | Sulphurous, boiled-cabbage | [75,77] |
| Dimethylsulphone | Sulphurous | [49,51] |
| Ethyl butanoate | Fruity, sweet | [58] |
| Ethyl butyrate | Ethereal, fruity, banana, pineapple | [59,61,70] |
| Ethyl caproate | Fruity, winy, pineapple, banana | [58,59,61,62,70,76] |
| DMHF | Sweet, caramel, fruity, strawberry-like | [61,70,74] |
| Heptanal | Oily, fatty, sweet, nutty | [70,77] |
| Hexanal | Green, grassy | [59,61,70,75] |
| Homofuraneol | Sweet, caramel | [61,75] |
| Indole | Fecal, putrid, musty, floral on high dilution | [63] |
| Isovaleric acid | Rancid, cheesy, sweaty, fecal, putrid | [62] |
| Methional | Boiled potato-like | [57,59,61–63,70,75,77] |
| Nonanal | Floral, green, waxy | [57,61,75] |
| Propionic acid | Pungent, rancid | [59,63] |
| δ-Decalactone | Coconut, creamy, peach, buttery on dilution | [58,59,61–63,74] |

## 5. Coffee flavour analysis

The pleasant aroma released during the grinding of roasted coffee beans is as attractive as that of fresh brewed coffee. With respect to the matrix itself, there are over 25 varieties of coffee plants, although only two are exploited in economically significant quantities; these are Arabica (*Coffea arabica*) and Robusta (*Coffea canephora*). The former, considered to be more highly valued, is characterised by a less bitter and more aromatic flavour when compared to Robusta. Commonly, the roasted coffee sold in the markets consists of a blend of both types of beans.

Several aroma constituents of coffee are products of Maillard reaction, a thermally induced reaction between amino acids and reducing sugars, which will be better illustrated in Section 6 of this review.

Research dedicated to the potent odour-active compounds of the flavour of raw and, particularly, of roasted beans has been extensive throughout the years. The green bean aroma profile is certainly the less complex, while the roasted bean is characterised by 800 components present in a vast concentration range [71]. Commonly, fatty, green, lactonic and terpene-like notes characterise green coffee beans, while sweet, sulphurous, and amino odours develop during roasting [72]. Some of the main classes of compounds which have been identified in roasted coffee beans are sulphur compounds, pyrazines, pyridines, pyrroles, oxazoles, furans, aldehydes, ketones, and phenols.

As well-known, the coffee bean's chemical composition depends on several factors, such as species and variety of bean, geographic origin, soil conditions, and storage of the beans, as well as time and temperature of the roasting procedure [72]. Further influence, as mentioned previously could derive from the sample preparation process. The differences in the flavour profile of food matrices are not only dependent on the applied GC–O method, but also on the solute extraction procedures. Several different methods have been applied, including the use of a gas-tight syringe to sample the headspace volatiles arising from roasted coffee; a static headspace sampler, investigating the effects of time and temperature on the compounds being released from ground roasted Arabica coffee; SPME is also among the most applied sampling methods.

GC–O is commonly employed for the analysis of roasted beans and brewed coffee volatiles, especially using AEDA and CHARM methods. Semmelroch et al. [73] employing the former

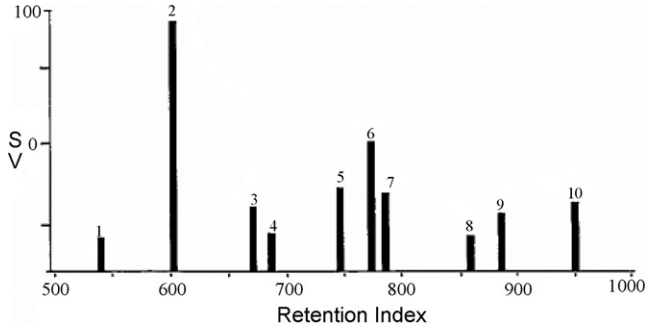


Fig. 6. Odour spectrum of CHARM analysis performed on brewed coffee by means of SPME-GC–O. (1) 2,4,5-trimethylthiazole, (2) 3-methoxy-2-isobutyl-pyrazine, (3) sotolone, (4) abhexone, (5) vanillin, (6) fuaneol, (7) 4-vinylguaiacol, (8) 4-ethylguaiacol, (9) 2-isopropyl-3-methoxypyrazine, (10) 2-furfurylthiol (reprinted from [74] with permission of the American Chemical Society, © 1999).

method, identified 14 compounds as responsible for the overall odour of roasted ground Arabica coffee, while Deibler et al. [74] by means of CHARM indicated 30 potent odourants in brewed coffee, however, amongst these 18 could be reliably identified through MS library matching, linear retention indices (*I*) and olfactive impressions. An odour spectrum of brewed coffee is presented in Fig. 6.

An interesting aspect of coffee flavour regards the diversity between the aroma impact of the two most widespread varieties, Arabica and Robusta. Blank et al. [75] investigated by means of GC–O applying AEDA the key-odour compounds of the powder and brew of both types of coffee. Thirty-nine odour-active compounds were detected and out of these 32 identified. The profile of the flavour chemicals responsible for the overall aroma was similar, differing mainly in their odour activity value. The difference in flavour of powder and brew presented to be related to the predominance of enoloxo compounds, e.g. 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (sotolone), 5-ethyl-3-hydroxy-4-methyl-2(5*H*)-furanone (abhexone), DMHF, 3,4-dimethylcyclopentenol-1-one in Arabica, and of 3,5-dimethyl-2-ethylpyrazine, 2,3-diethyl-5-methylpyrazine, 4-ethylguaiacol and 4-vinylguaiacol in Robusta. With respect to the powder, preparation of the brews enhanced the flavour difference, as the concentration levels of water-soluble odourants (DMHF, sotolone, abhexone) responsible for the sweet-caramel note increased more in the Arabica than in the Robusta coffee. On the other hand, the alkylpyrazines and guaiacols were responsible for the spicy, harsh, earthy odour of the Robusta coffee.

The potent odourants responsible for the characteristic coffee flavour have already been the subject of a great deal of research, much of which performed by means of aroma model systems, aroma simulations, and sensory assessment of odourants in oil/water mixtures, omission experiments, and triangle tests. An interesting work, worth of mention, carried out by Semmelroch and Grosch [36], was focused on the quantification, through dilution experiments, of the key-odour compounds present in coffee brews prepared from roasted Arabica and Robusta. Both brews presented 17 potent odourants, which based on OAV, differed in their rankings. 2-Furfurylthiol, 3-mercapto-3-methylbutyl formate, methanethiol, β-damascenone, methylpropanal, and 3-methylbutanal were amongst the most potent odourants. Even though the analyses were not performed by GC–O, the overall results attained by these authors are comparable in qualitative levels with those aforementioned attained by Blank et al. [75].

Akiyama et al. [71] performed an interesting research on the volatile compounds released during the grinding of roasted Arabica coffee beans. These volatiles were sampled by means of SPME under static and dynamic conditions, and then analysed by GC–O, applying CHARM. Preliminary, static headspace samplings were made using three different fibre types; PDMS (polydimethylsiloxane), PDMS/DVB (divinylbenzene), and CAR (Carboxen)/PDMS. However PDMS/DVB was selected due to its higher sensitivity towards a greater number of pyrazines, sulphur compounds, and phenols. The aim of the investigation was to evaluate the effects of the different techniques on the isolated aroma profiles.

Sample dilution, required by CHARM, was enabled through static and dynamic SPME sampling, out using the fibre in four different exposure lengths (10, 5, 2.5 and 1.3 mm). Dynamic sampling was carried out with nitrogen gas flow variations; the fibre was exposed to the effluent nitrogen gas from the sampling apparatus during the grinding of roasted coffee beans (150 g) at 200, 400, 600, 800, and 1000 mL/min for 8 min. On the other hand, static sampling, based on dilution analysis, was achieved by using varying thicknesses and exposure lengths of the three types of fibres. After sampling, the fibre was placed into the injection port of the GC–O system, and thermally desorbed. The analyses were performed on a polar column, and the olfactometric results were expressed as CHARM values and odour spectrum value (OSV). The former indicates the true odour activity measurement and is a linear function of concentration, while the latter is a normalised CHARM value modified with an approximate Steven's law exponent ($n = 0.5$), expressing, therefore, the relative importance of an odourant independently from its concentration. The attained results indicated that the dynamic SPME headspace sampling generated an enhanced release of volatiles, noted particularly by exacerbated nutty-roast and smoke-roast impressions. With regards to olfactive language, the adopted descriptors were derived from the results of a single preliminary free choice GC–O analysis using a lexicon of commonly used words for coffee evaluation. The descriptors acidic, buttery-oily, green-black currant, green-earthy, nutty-roast, phenolic, smoke-roast, soy sauce, sweet-caramel, and sweet-fruity were used in all GC–O experiments to classify the potent odourants. Highly volatile compounds, eliciting buttery-oily odour, such as 2-methylbutanal, 3-methylbutanal, 2,3-butanedione, and 2,3-pentanedione, were more abundant in the dynamic than in the static headspace, while the opposite was observed for (*E*)-2-nonenal, more abundant in the static one. With respect to the nutty-roast aroma, 2-methyl-3-furanthiol and pyrazines were, as indicated by their CHARM values, more abundant in the dynamic headspace. In particular, 2-methyl-3-furanthiol could be indicated by its high OSV and CHARM value in the dynamic condition as a significant candidate for the nutty-roast aroma released during the grinding of roasted coffee beans. With regards to the smoke-roast aroma, 2-furanmethanethiol presented high CHARM values in both static and dynamic

headspace and was indicated as an odour-active component by its OSVs. Noteworthy are (*E*)-β-damascenone which presented identical CHARM values in both headspace sampling types, accompanied by an extremely high OSV value in the static condition; and 4-ethenyl-2-methoxyphenol, which presented the highest CHARM value and OSV in the dynamic condition. Further compounds also presented high CHARM values, especially under the dynamic sampling condition. On the basis of CHARM values and OSVs, the sulphur compound 3-methyl-2-butene-1-thiol, was the strongest contributor for the smoke-roast odour released during the grinding of roasted coffee beans. The authors emphasised that sotolone and abhexone were not detected in this study; both are commonly found in the solvent extracts of roasted powder and brewed Arabica coffee as potent odourants, possessing a seasoning-like aroma quality [76].

The contribution of 3-mercapto-3-methylbutyl formate to the overall flavour of coffee is well-known, differently to 3-mercapto-3-methylbutyl acetate which was first identified by Kumazawa and Masuda [77] in the volatile fraction of roasted coffee brew, isolated by steam distillation under reduced pressure. According to the results attained by means of AEDA, the acetate contribution to the flavour varied, depending on the degree of the coffee beans roasting. Considering the synthetic pathway of 3-mercapto-3-methylbutyl formate, it was assumed that the corresponding acetate is also formed during the roasting process. GC–O analyses were performed on a polar column, and the glass sniffing port was connected to the outlet of a TCD. AEDA revealed 3-mercapto-3-methylbutyl formate to be a potent contributor to the coffee flavour even at low degrees of roasting, with only small differences in FD factors at different roasting levels. On the other hand, 3-mercapto-3-methylbutyl acetate was described as potent solely in the highly roasted brew; even though with an FD factor lower than that observed for the corresponding formate. These results indicated that the effect of the acetate on the impact flavour of coffee brew was dependent on the beans roasting degree, enabling a possible use as a marker (see Fig. 7).

A further headspace GC–O method, applied to evaluate impact aroma compounds of coffee, was GC-SNIF; the extracted sample was representative of a coffee cup in equilibrium with the surrounding air and was detected by a panel of 6–10 assessors instead of 1 or 2, as used for other methods [78]. In this method, developed by Pollien et al. [27], the intensities of the aromagram peaks were based on the detection frequencies of odourants perceived at the sniffing port. Due to the use of several assessors, these frequencies appeared to be repeatable, and reproducible by two independent panels without training prior to analysis. This approach allowed the calculation of standard deviations and least significant differences, therefore aromagrams could be compared on a more quantitative basis than with previous GC–O methods. The method was applied to the comparison of impact flavourings of a coffee brew and to the corresponding instant coffee, both prepared from the same beans. The two profiles exhibited little qualitative differences, but variations in intensity. In addition, a new key odourant, 1-nonen-3-one, was detected in the coffee aroma.

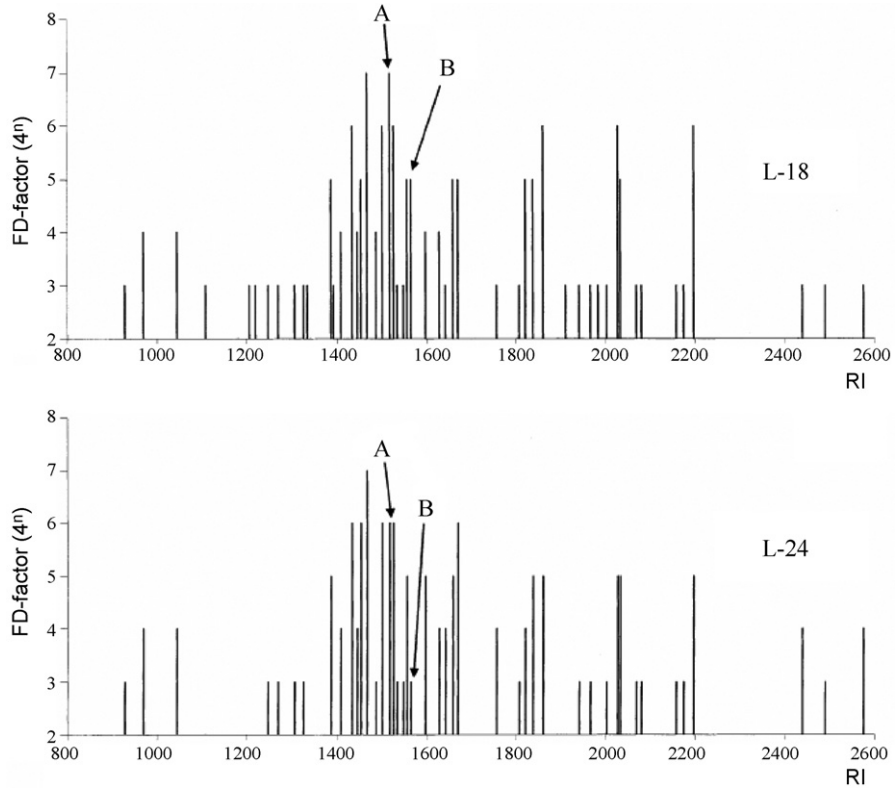


Fig. 7. Aromagram of coffee brews prepared from beans of different roasting degrees; high (L-18) and low (L-24) degree of roasting, respectively, top and bottom. (A) 3-mercapto-3-methylbutyl formate and (B) 3-mercapto-3-methylbutyl acetate (reprinted from [77] with permission of the American Chemical Society, © 2003).

Much is known about the key-odour compounds responsible for the coffee flavour, however as previously mentioned; several parameters influence the sampling procedure and the analytical and sensorial methods. Hence, it is possible to observe that one investigation by means of GC–O and AEDA of the headspace volatiles revealed 2-ethyl-3,5-dimethylpyrazine and 2,3-diethyl-5-methylpyrazine as odourants with the highest FD factors, and eliciting an earthy note [76], while another group reported both these compounds among the 14 more potent ones, but with 2-furfurylthiol and β-damascenone as being more representative [73]. A concise compilation of potent odourants commonly detected by means of GC–O and reported as responsible for the flavour of coffee are given in Table 2, along with their respective olfactive description.

Table 2
Potent odourants responsible for the flavour of coffee commonly detected by means of GC/O and the respective olfactive description

| Compound | Odour description | Reference |
|---|---|---|
| (*E*)-2-Nonenal | Buttery, oily | [71] |
| (*E*)-β-Damascenone | Sweet, fruity | [71,74,75] |
| 2,3-Butanedione | Buttery, creamy | [36] |
| 2,3-Diethyl-5-methylpyrazine | Nutty, roasted | [36,71] |
| 2,3-Pentanedione | Buttery, oily | [36,71] |
| 2,4,5-Trimethylthiazole | Chemical, plastic-like | [36,74] |
| 2-Ethyl-3,5-dimethylpyrazine | Nutty, roasted | [36,71] |
| 2-Ethyl-4-hydroxy-5-methyl-3(2*H*)-furanone | Sweet, caramel | [36,71] |
| 2-Furanmethanethiol | Smoked, roasted | [71] |
| 2-Furfurylthiol | Roasted | [36,74] |
| 2-Isopropyl-3-methoxypyrazine | Green, herbaceous | [74] |
| 2-Methoxy-3-(2-methylpropyl)pyrazines | Green, earthy | [71] |
| 2-Methoxyphenol | Phenolic | [71] |
| 2-Methyl-3-furanthiol | Nutty, roasted | [71] |
| 2-Methylbutanal | Buttery, oily | [36,71] |
| 3-(Methylthio)propanal | Soy sauce-like | [71] |
| 3-Hydroxy-4,5-dimethyl-2(5*H*)-furanone (sotolone) | Cotton-candy, spicy | [36,74–76] |
| 3-Mercapto-3-methylbutyl formate | Blackcurrant, catty, roast | [36,71] |
| 3-Methoxy-2-isobutylpyrazine | Green | [36,74] |
| 3-Methyl-2-butene-1-thiol | Smoked, roasted | [71] |
| 3-Methylbutanal | Buttery, oily | [36,71] |
| 3-Methylbutyric acid | Acidic | [71] |
| 4-Ethenyl-2-methoxyphenol | Phenolic | [71] |
| 4-Ethylguaiacol | Spicy | [36,74] |
| 4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone | Sweet, caramel | [36,71] |
| 4-Vinylguaiacol | Spicy, eugenol-like | [36,74] |
| 5-Ethyl-3-hydroxy-4-methyl-2(5*H*)-furanone (abhexon) | Sweet, spicy | [36,74–76] |
| 6,7-Dihydro-5methyl-5*H*-cyclopentapyrazine | Nutty, roasted | [71] |
| DMHF | Sweet, caramel, fruity, strawberry-like | [74,75] |
| Guaiacol | Spicy, harsh, earthy | [36,74] |
| Methional | Boiled potato-like | [36,74] |
| Vanillin | Sweet, vanilla-like | [36] |
| Methanethiol | Sulphurous, gasoline, garlic | [36] |
| 3,5-Dimethyl-2-ethylpyrazine | Roasted | [75] |
| 2-Ethyl-3,5-dimethylpyrazine | Nutty, roasted | [71] |

## 6. Investigations on meat flavour

Flavour development in cooked meat is a well-studied subject, presenting several key aspects which can be representative for other cooked food matrices. The flavour of raw meat is relatively mild, presenting few volatile compounds, while over 1000 flavouring chemicals have been reported in cooked meat [79]. The flavour of the latter is predominantly derived from the well-known Maillard reaction and the degradation and oxidation of lipids. The Maillard reaction is of extreme importance for the formation of most cooked flavours, and can be briefly described as a thermally induced reaction between reducing sugars and amino groups. A recent and concise review on this topic has been made by van Boekel [80]. A further relevant reaction is the Strecker degradation, previously cited in the present review in relation to cheese flavour analysis. This reaction consists in the decarboxylation and deamination of an amino acid in the presence of a dicarbonyl compound forming a Strecker aldehyde and an aminoketone. The former may be a reactive carbonyl and take part in the Maillard reaction, while the latter may undergo a cyclisation reaction producing heterocycles (pyrazines).

Cooking methods, such as roasting, frying or grilling, as also boiling, smoking or reheating, influences flavour formation. Moreover, the temperature reached during the cooking process may have considerable effects on the profile of the formed compounds, influencing the overall flavour. Further aspects to be considered are the meat's origin, since each animal possesses a unique ratio of amino acids, fatty acids, and sugars, generating so distinct flavours; the breed and the animal's diet, since both can influence meat chemical composition, especially with regards to fat content and fatty acid composition. In beef, lamb and pork the lipids consist mainly in saturated fatty acids, which do not react as easily as the unsaturated ones. Fish and fowl, on the other hand, present many unsaturated lipids [77], that generate flavours, and other small reactive molecules which may interact, due to the Maillard reaction, producing even more complex flavours. Unsaturated lipids generate more rapidly in fish and fowl, than in beef [81].

GC–O has been often used to determine the flavour profile of several meat related matrices, such as boiled [82], roasted [83], and fried beefs [84], ham [85,86], sausages [87,88], turkey breast meat [89] etc. Specht and Baltes [84] investigated the key-odour compounds of shallow pan-fried beef by means of GC–O and AEDA. The volatiles were trapped during two distinct frying processes; on a hot plate at 280 °C, 3 min per beef side or at 300 °C, 1 min per side, both were oil free. The analyses were performed on a non-polar column, retrofitted with a quick-seal splitter, dividing the effluent 1:1. AEDA was performed by a panel composed of five trained assessors. Twelve peaks in the 280 °C extract and nine peaks in the 300 °C extract, were characterised by high aroma values, and therefore, were important contributors to fried beefsteak aroma. Furthermore, the 280 °C extract was mainly represented by a stronger pleasant flavour, that was related to 2-ethyl-3,5-dimethylpyrazine

and 2-propyl-3-methylpyrazine, which co-elute on the stationary phase employed. Methional was the only sulphur-containing compound indicated as potent odourant. Octanal, 2,3,5-trimethylpyrazine, 2-ethyl-5-methylpyrazine, 2-ethenyl-3,6(5)-dimethylpyrazine, 4,5-dihydro-5-propyl-2(3*H*)-furanone, 2(*E*)-nonenal, and 4,5-dihydro-5-butyl-2(3*H*)-furanone were identified as key-odourants for both extracts. Moreover, the majority of compounds with high aroma values, aldehydes and ketones, were reported to be responsible for fatty, sweet, or roasted flavour qualities contributing to the roasted meat character of shallow fried beef.

The juice formed during beef stewing, and presenting a similar flavour to the stewed piece of meat, is also amongst the vast variety of studied meat matrices [35]. This juice was analysed by GC–O and AEDA, with the acidic fraction of the juice extract analysed on a polar column and the neutral fraction on a non-polar column. According to the attained FD factors, AEDA revealed 16 compounds as responsible for the overall stewed beef juice flavour; of these, out of these six presented the highest FD values; acetic acid, methional, butyric acid, 12-methyltridecanal, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, and 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone.

In general, the odourants of cooked meats have been widely investigated by means of GC–O, however not only in the determination of a meat's sample key-odour compounds, but also as screening approach for the identification of odour-active substances. For example, Machiels et al. [90] investigated the odour-active compounds derived from model mouth isolation of two commercial and cooked, Irish beefs, and identified them by GC–O and GC/MS. One meat was labelled as organic and the other as conventional. The samples were cooked in an oil bath at 145 °C for 20 min, cooled and stored at 48 °C overnight. The headspace of the cooked meat samples was flushed with a 100 mL/min flow of purified nitrogen and the volatile compounds were trapped on a Tenax TA trap while a plunger made up and down screwing movements to simulate mastication. Desorption of the volatile compounds from the trap was performed on a thermal desorption device retrofitted to the GC–O system. GC–O analyses were performed on a non-polar column, and the GC column's effluent was split between the FID system (40%) and two sniffing ports (30% each). The system was also supplied with helium, being added as make-up gas to the splitter (25 mL/min), in order to increase velocity, and with air (500 mL/min), used to create a Venturi effect, employing the hot air from the oven to heat the transfer line between the GC oven and the sniffing ports. The detection frequency method was performed in duplicate by eight assessors, previously trained to use a glossary of descriptors defined by the group during the training period. The glossary included notes such as: air, boiled, burnt, caramel, chemical, chicken, chocolate, fatty, fruity, gas, gravy, green, roasted, stewed, and sweet. A total of 81 compounds could be identified in both samples, 72 compounds in the organic sample and 62 in the conventional one. However, amongst these, methanethiol, dimethyl sulphide, 2-butanone, ethyl acetate, 2- and 3-methylbutanal, an unknown compound, 2-octanone, decanal, and benzothiazole were identified as odour-active in the organic and in the conventional sample. Furthermore, two unknown substances were among the odour-active compounds detected in the organic sample, while 2,3-pentanedione, 4-methyl-3-penten-2-one, 2-heptanone, dimethyl trisulphide, and nonanal were active in the conventional beef's flavour. Some of these compounds (2- and 3-methylbutanal, 2,3-pentanedione, decanal, dimethyl trisulphide and nonanal) had already been identified in the aforementioned shallow fried beef sample [84]. It must be added though, that a reduced number of key volatile flavourings, such as sulphur-containing compounds, pyrazines and other heterocyclic compounds, were detected. Moreover, in contrast to other investigations, the key odourants were mainly represented by low boiling point compounds. The rather unexpected flavour profile, attained in this work, was related to the cooking method and the artificial mouth isolation combined with the addition of artificial saliva, effecting the flavour release from the matrix.

A further work, carried out by Machiels et al. [91], was focused on the investigation of cooked beef originating from animals of three different breeds, Belgian Blue, Limousin and Aberdeen Angus, and fed with two distinct fattening diets. The beef volatiles of cooked beef meat were extracted likewise [90] by using the model mouth apparatus, and being afterwards assessed by GC–O. The detection frequency method was performed by a panel composed of twelve assessors, which after GC–O training sessions compiled the glossary of olfactive descriptors to be used. The following notes were included: sulphurous, chocolate, caramel, fruity, burnt, sweaty, earthy, fermented, green, fresh, chemical, oily, buttery, onion, nutty, and meaty. The signal to noise level of the assessors was determined by using Tenax tubes without adsorbed volatile compounds as blank. A total of 30 odour-active compounds were detected in the aroma of all three cooked beef meats, out of these 16 derived from Maillard reactions (sulphur and nitrogen containing compounds) and Strecker rearrangement products (thiazols and 2,3-diethyl-5-methylpyrazine), 7 from lipid degradation (saturated and unsaturated aldehydes and ketones), and the other 7 were not identified. Again, it must be noted that the applied GC–O methods enabled the screening of odour-active compounds, and not the assessment of the odourants responsible for the impact flavour of the samples. The odour activity of some of these compounds had already been described in cooked meat flavour: The olfactive description of each one of these compounds is described in Table 3, along with other odour-active compounds commonly detected in meat flavour.

Hydrogen sulphide (rotten eggs, sewage), methanethiol (rotten eggs, meat, cheesy) and 2-methylpropanal (toasted, fruity, pungent) have also been identified in the flavour of Iberian Ham [86] by means of the detection frequency method (Fig. 8); however, their odour activity was reported for the first time in cooked beef meat. Carbon disulphide was also reported for the first time as an odour-active compound in this matrix; however it has also been identified in the aroma profile of dry sausage [92]. Moreover, Belgian Blue-derived meat was richer in odour-active compounds, and also more influenced by diet differences.

Along time, several studies were focused on a precise class of compounds. One peculiar approach, worth of mention, was based on the investigation of in-oven roast beef top note [83],

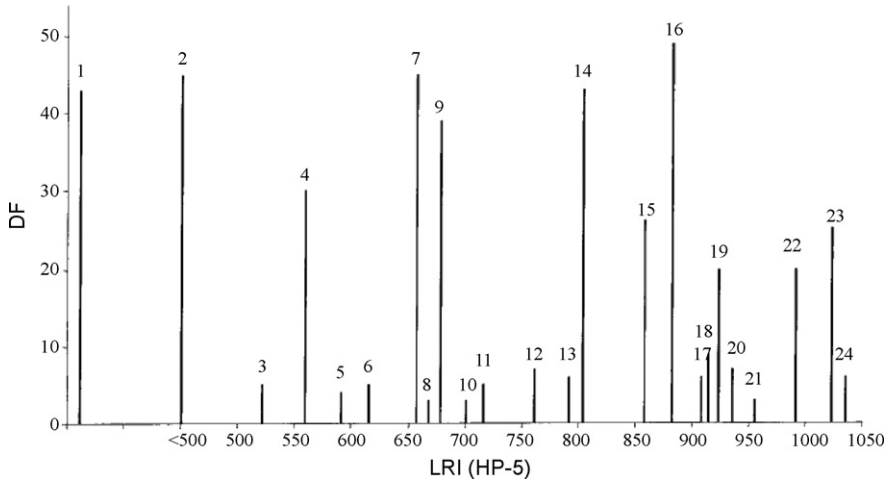


Fig. 8. Aromagram attained by performing detection frequency method on Iberian ham volatiles. DF- detection frequency; (1) hydrogen sulphide, (2) methanethiol, (3) unknown, (4) 2-methylpropanal, (5) 2,3-butanedione, (6) unknown, (7) 3-methylbutanal, (8) 2-methylbutanal, (9) 1-penten-3-one, (10) 2-pentanone, (11) pentanal, (12) ethyl 2-methylpropanoate, (13) unknown, (14) hexanal and (Z)-3-hexenal, (15) ethyl 2- methylbutyrate and (E)-2-hexenal, (16) 2-methyl-3-furanthiol and 2-heptanone, (17) heptanal and 3-mercapto-2-pentanone, (18) methional and 2-furfurylthiol, (19) 2-acetyl-1-pyrroline, (20) (E)-2-heptenal, (21) dimethyl trisulphide, (22) 1-octen-3-one and 1-octen-3-ol, (23) 2-propionyl-1-pyrroline and octanal, (24) (E)-2-octenal (reprinted from [86] with permission of the American Chemical Society, © 2002).

which is nothing else than the tracing of the most volatile notes perceived when the meat is roasting in a traditional oven. The work was focussed on carbonyl compounds, since as previously observed [84], aldehydes and ketones are the most involved compounds in fried beef's odour. The volatiles were extracted by cooking the meat (not salted, oil-free) in a modified domestic electric oven connected to a Tenax cartridge and an air pump. This trap was then analysed by a GC/MS-O multisniffing system [93] (Fig. 9), equipped with a polar column. A reliable compound's identification was enabled by the microderivatisation of carbonyl compounds with (2,3,4,5,6-pentafluorophenyl)hydrazine leading to the formation of pentafluorophenylhydrazones. The MS spectra of the aldehyde and methyl ketone hydrazones presented an abundant

Table 3
General meat odour-active compounds detected by means of GC/O and their respective olfactive description

| Compound | Odour description | Reference |
|---|---|---|
| 1-Octen-3-one | Metallic | [83] |
| 2,3-Butanedione | Sweet, buttery | [35,84] |
| 2,3-Diethyl-5-methylpyrazine | Earthy, roasty, meaty | [35,84,91] |
| 2,3-Pentanedione | Buttery, lemon, sweet, fruity | [35] |
| 2,4-Nonadienal | Fatty | [83,89] |
| 2-Acetyl-2-thiazole | Roasty | [35,84] |
| 2-Butanone | Chemical, burnt | [35,90] |
| 2-Ethyl-3,5-dimethylpyrazine | Burnt, meaty, green | [84] |
| 2-Methylbutanal | Pungent, green, sweet, roasty | [35,82] |
| 2-Methylpropanal | Toasted, fruity, pungent | [86] |
| 2-Nonenal | Tallowy, fatty | [83,85,89] |
| 2-Octanone | Fruity, musty | [90] |
| 2-Propyl-3-methylpyrazine | Bread crust- like | [84] |
| 3-Methylbutanal | Pungent, green, sweet, roasty | [35,84] |
| Benzothiazole | Pyridine-like, metallic | [35] |
| Dimethyl disulfide | Mouldy, pungent, rubbery, onion | [35,84] |
| Dimethyl trisulphide | Cabbage-like, sulphurous | [35,90] |
| Ethyl acetate | Caramel, sweet | [35,90] |
| Heptanal | Fatty, rancid, citric | [35,84] |
| Hexanal | Green | [35,84,85] |
| Hydrogen sulphide | Rotten eggs, sewage | [86,91] |
| Methanethiol | Rotten eggs, meat, cheesy | [35,86,91] |
| Methional | Cooked potato | [35,84] |
| Octanal | Fruity, green | [35,84] |

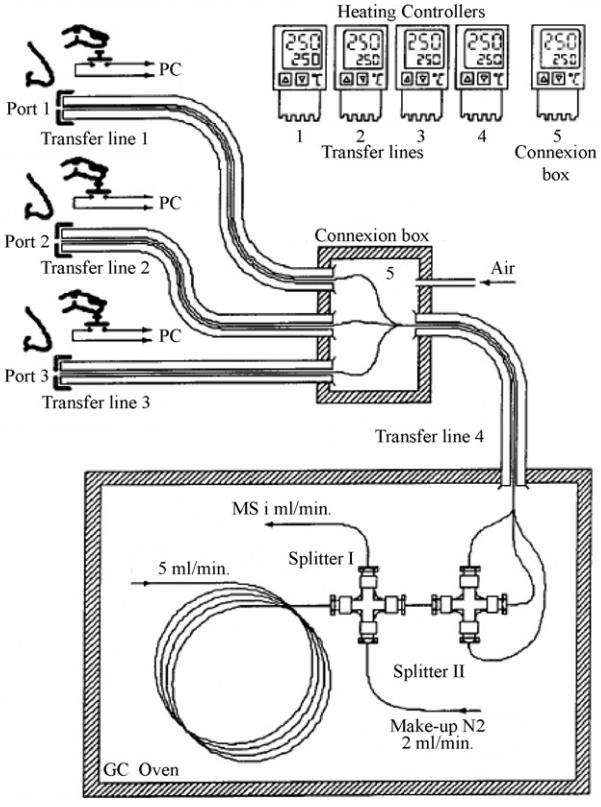


Fig. 9. Scheme of the GC/MS-O multisniffing system (reprinted from [93] with permission of the American Chemical Society, © 2002).

molecular ion and characteristic fragmentation facilitating peak assignment. Olfactometric analyses were made by nine panellists applying GC-SNIF method. Panel's detection frequency revealed identical odour-active compounds, however differing in intensity. According to the calculated NIF units percentage, nonanal, 1-nonen-3-one, (*E*)-2-nonenal, (*E*,*E*)-2,4-nonadienal, propanal, 2-propanone, 1-octen-3-one, and phenylacetaldehyde were amongst the most intense compounds. A total of 23 compounds were identified as odour contributors, and most of them had already been reported in cooked beef samples.

Although, as aforementioned, over 1000 flavouring chemicals have been reported in cooked meat, research related to compound odour-activity and to elucidate the odourant development processes continues. The flavour industry is constantly searching for new chemicals and methods, with the aim of increasing the consumer acceptance of a product's flavour. Elmore et al. [82] evaluated the role of novel thiazoles and 3-thiazolines reported for the first time in the headspace of pressure-cooked beefsteaks. The odour activity potential of these molecules was screened by means of GC–O, and was found not to be as representative as was expected to be. However, it was confirmed that lipid degradation products may undergo Maillard reaction during the cooking process. Such works may be useful for the creation of new cooked meat flavour chemicals, through the modification of Maillard reaction products.

Similarly to coffee flavour analyses, Maillard model reactions were also used to investigate flavour formation. Hofmann and Schieberle [94] elucidated by means of AEDA the most potent odour-active compounds formed in a thermally treated aqueous cysteine and ribose solution, by means of AEDA. Heated mixtures containing cysteine commonly elicit odours resembling meat flavour. Amongst the 20 odour-active chemicals formed, 2-furfurylthiol (FFT) and 2-methyl-3-furanthiol (MFT), in particular, were established as key-odourants, followed by 2-thenyl mercaptan and ethyl mercaptan. These volatiles were responsible for the overall roasty, meat-like, and sulphurous notes of the model system.

Processed meat flavour analysis has also been the subject of several GC–O investigations, such as fermented and non-fermented sausages, ham etc. One well-studied matrix is fermented sausage, for which the odour plays a great role in the establishment of its quality, hence triggering considerable flavour profile investigation, as also the determination of key-odour compounds. It was believed that the final aroma of a sausage resulted from its diversity in terms of ingredients and fermentation parameters, leading to the development of rather specific flavourings. A research carried out on 18 distinct dry fermented sausages, though, showed that the odour-active compounds profile itself was similar, but the differences in the ratio of odour-active compounds were decisive to the overall flavour [87]. A more recent work confirmed these findings by means of GC–O and detection frequency method, for sausages prepared with nitrite or nitrate as curing agents [88].

The effect of dry curing has also been investigated with regards to ham flavour. Flores et al. [85] studied the Spanish variety, named Serrano dry-cured ham or White ham, by GC–O applying the detection frequency method using a panel composed of four assessors. The GC–O system was equipped with a packed column coated with a stationary phase of medium polarity. In general, packed columns support larger sample size ranges, from tenths of a microliter up to 20 μL, and thus the dynamic range of the analysis can be enhanced. Components present at trace levels can be easily separated and determined quantitatively without preliminary fractionation or concentration. Furthermore, packed columns are more efficient than the open tubular columns, due to the much smaller particle diameter. On the other hand, the use of packed columns leads to lower resolution due to the higher pressure drop per unit length. In addition, the operation of packed columns has to be made at higher column flow rates. The column effluent was split 1:1 to the FID system and sniffing port device. Prior to analyses, the volatiles were isolated through a dynamic headspace technique. Seventy-seven compounds were identified, with 44 of these characterised as being odour-active compounds by the four panellists. Ketones, esters, aromatic hydrocarbons, and pyrazines were reported as the volatile compounds which generated the pleasant aroma of the hams, while hexanal, 3-methylbutanal, 1-penten-3-ol, and dimethyl disulphide were related with the short ripening drying stage. The volatile fraction of the similar French product was studied by Berdagué et al. [95] using vacuum distilled extracts and GC–O, the latter technique named by the authors as flavour tests. Analyses were performed on a non-polar column with the effluent being split 20:80, respectively, to the FID system and the sniffing port. Aldehydes, ketones, and alcohols were amongst the odour-active compounds. Further, remarkable, odour-active compounds were γ-nonalactone, pyrazines, and 1-methyl-2-pyrrolidinone.

A further ham type worth of mention is the exquisite Iberian Ham, also known as Black ham, which constitutes solely 5% of the ham produced in Spain. The production process consists in a prolonged dry-curing stage, forming its characteristic flavour. The flavour of Iberian hams, processed in the traditional way, has been studied recently by Carrapiso et al. [86], by means of the detection frequency approach; the characteristic flavour of the samples was associated mainly to the presence of 2-methyl-3-furanthiol (MFT), 2-heptanone, 3-methylbutanal, methanethiol, 1-penten-3-one, and 2-methylpropanal. Unfortunately, scarce information is available on the key-odours responsible for the flavour of the aforementioned ham varieties, and the application of AEDA or CHARM to this matrix is rarely reported.

Another commercially important meat type, worthy of mention, is poultry meat. An interesting work was carried out by Brunton et al. [89] on turkey breast meat flavour. SPME sampling, GC–O and AEDA were performed on freshly cooked and oxidised cooked turkey samples. The sampling was performed on a series of increasingly diluted (in bi-distilled water) cooked turkey homogenates heated at 80 °C for 30 min. GC–O analyses were made on a polar column with the effluent being split 1:2, to the FID system and the sniffing port, respectively. AEDA enabled the characterisation of the main odour-active compounds; 1-octen-3-one, an unidentified compound, and (*E*,*E*)-2,4-decadienal were the most potent in freshly cooked turkey, followed by 2-phenyltiophene and several dienals. Except for 2-phenyltiophene and the unknown compound,

the odour potency of all compounds increased in the turkey sample stored for 3 days. In both samples 1-octene-3-one and (*E*,*E*)-2,4-decadienal were indicated as the most potent odourants, while (*E*,*Z*)-2,6-nonadienal was included amongst the most potent aroma constituents in oxidised turkey samples.

It is well-known that dienals exert an important role in the flavour of poultry [96,97], as well as other meats. For example, (*E*,*E*)-2,4-decadienal has been reported as a key odourant in freshly roasted turkey, boiled chicken and pressure cooked chicken [98]. Likewise is true for 1-octene-3-one, previously reported as a contributor to the aroma of chicken [99], however this ketone had not been previously identified as a key-odour in turkey. According to the authors, the increase in carbonyl compounds during the storage process can be associated to the formation of off-flavours in turkey breast meat.

## 7. Fruit flavour determination

Fruit flavours are a subtle blend of characterizing volatile compounds, combined with carbohydrates (sugars as glucose, fructose, and sucrose), organic acids (citric and malic acids), and commonly, non-characterizing volatile esters. As well-known for other food matrices, the complex volatile fraction is composed of a wide variety of compounds belonging to several chemical classes. Thus, an individual fruit may have well over a hundred different volatile compounds, which also differ according to the fruit's ripening stage. Fortunately, the fact that fruit volatiles are present in higher concentrations if compared to other foodstuffs, often more than 30 ppm, has simplified analytical research in this field.

The odour-active compounds of essential oils extracted from citrus fruits (*Citrus* sp.), such as orange, lime, and lemon, were among the first character impact compounds identified by flavour chemists [100]. Those fruits are known to be rich in mono- and sesquiterpenes and their oxygenated derivatives, whereas the odour relative to most non-citrus fruits is characterised mainly by esters and aldehydes, as is the case for strawberry, banana, cranberry, apple etc. Some odour-active compounds commonly detected in fruit matrices and their respective olfactive description are reported in Table 4.

A widely studied fruit matrix is strawberry (*Fragraria* sp.), the aroma of which is a combined perception of many aromatic notes, such as fruity, sweet, caramel-like, floral, and buttery [37,101]. The volatile fraction of several varieties of strawberries has been well studied by means of various techniques in the past 30 years, leading to the assumption that more than 360 volatile compounds are related to their characteristic flavour [102]. Despite the great knowledge on strawberry flavour, GC–O has been rarely applied to this fruit. Commonly, model mixtures and stable isotope dilution assays have been performed, followed by direct sensory evaluations including odour threshold determination [37].

Schieberle [103] reported the use of GC–O and AEDA to determine the character impact compounds in fresh strawberry juice, verifying that although a large number of compounds had been identified in the aroma of strawberries, only 15 were reported to be the most important contributors to strawberry aroma. Amongst these and, with decreasing FD values, 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (DMHF), butanoic acid, acetic acid, (*Z*)-3-hexenal, and a variety of esters were listed.

Table 4
Some odour-active compounds detected by means of GC/O in distinct fruit matrices and their respective olfactive description

| Compound | Odour description | Reference |
|---|---|---|
| DMHF | Sweet, caramel, fruity, strawberry-like | [100,103–105, 108,117] |
| 2,5-Dimethyl-4-methoxy-3(2*H*)-furanone | Strawberry-like | [100,103–105] |
| γ-Decalactone | Green, fruity, peach-like | [101,105] |
| γ-Dodecalactone | Green, fruity, peach-like | [101,105] |
| Ethyl butyrate | Fruity, juicy, pineapple-like | [103] |
| *iso*-Amyl acetate | Sweet, fruity, banana-like | [100] |
| β-Damascenone | Sweet, fruity, rose | [105,107,108] |
| 4-(*p*-Hydroxyphenyl)-2-butanone | Sweet, raspberry-like | [107,108] |
| Sotolone | Cotton candy, spice, maple | [107,108] |
| Linalool | Floral, lavender-like, citric, sweet | [105,107] |
| Dodecanal | Citric, lemon-like, green | [114] |
| (*Z*)-3-Hexenal | Green | [107] |
| Ethyl cinnamate | Sweet, spicy, basamic | [117] |
| Ethyl 3-methyl butyrate | Fruity | [100,116] |
| Benzaldehyde | Sweet, almond-like | [100] |
| Methyl anthranilate | Floral, sweet | [100] |
| 4-Mercapto-4-methyl-2-pentanone | Grapefruit-like | [100] |
| Nootkatone | Citric, grapefruit-like, orange-like | [100] |
| Methyl N-methylanthranilate | Floral, sweet | [100] |
| 3-Methylthio-1-hexanol | Green, vegetable-like | [100] |
| 1-Octen-3-one | Mushroom-like, metallic | [105] |
| (*E*)-2-Hexenal | Green | [105] |

Noteworthy is the controversy regarding the attribution of DMHF, also named as Furaneol (a trademark of Firmenich), strawberry furanone or pineapple furanone, and the corresponding methoxy compound, also known as mesifurane, as strawberry key-odour compounds. These volatiles, which elicit caramel-like, sweet, cotton-candy resembling, fruity notes, might be considered as the most important odour-active compounds in strawberry aroma [100,103–105]. On the other hand, even though these compounds have been identified in several studies, their concentration alters in relation to the strawberry cultivars, either wild or cultivated, or according to the maturation stages; in some cultivars they have not been found at all [104,106].

A further work on the aroma profile determination of strawberries applying GC–O reported γ-dodecalactone as possessing an intense strawberry-like note [101]. However, curiously, the technique was generally defined as sensorial evaluation. Two sample preparation methods were applied, purge-and-trap and group-oriented argentation chromatographic fractionation. The isolated extracts were injected in a GC system equipped with a needle valve to split the column's flow at a ratio of approximately 1:1 to the FID system and the sniffing port, with the effluent

assessed by three evaluators. Purge-and-trap sampling followed by GC analysis enabled the identification of 119 compounds, with 40 of these being odour-active. Argentation chromatography, instead, led to the acquisition of three fractions—ester, with an intense floral, fruity odour; aldehyde, dominated by a pungent, green note; and lactonic, mainly represented by γ-decalactone and γ-dodecalactone. Both lactones have also been identified by Schulbach et al. [105], however being reported to elicit a peach-like note. The latter research group analysed five strawberry varieties by GC–O using two distinct columns, and an effluent split of 1:2 to the FID system and to the sniffing port, respectively. Two assessors detected, by means of GC–O, 17 compounds as presenting moderate to strong ratings (scale from 0 to 100) for at least one of the five analysed cultivars. The most significant odour-active compounds were reported to be ethyl butyrate, β-damascenone, linalool, 1-octen-3-one, (*E*)-2-hexenal, and γ-dodecalactone. Other key-odour compounds, such as hexanal, DMHF, and γ-decalactone were found to be variety specific.

Raspberry (*Rubus idaeus* L.) flavour has also been widely investigated, presenting more than 200 identified volatiles, out of these, 10 have been indicated as key-odour compounds; α-ionone, β-ionone, (*Z*)-3-hexenol, geraniol, linalool, benzyl alcohol, acetoin, acetic and hexanoic acids, and 4-(*p*-hydroxyphenyl)-2-butanone (raspberry ketone) [107]. With regards to this matrix, a work worth of mention was performed by Roberts and Acree [108] on the determination of odour-active compounds released from a food matrix under simulated mouth conditions by combining a retronasal aroma simulator (RAS) with GC–O (RAS-GC–O) and CHARM. The experiments were focussed on the influence of heating and cream addition on raspberry aroma. Dynamic headspace samples of different volumes of gas simulating a dilution series, were attained from the RAS system, which was kept at 37 °C, and filled with synthetic saliva and the samples. The latter comprised fresh, heated, or with mixed-with-cream raspberries. During analysis, the evaluators were asked to indicate the start and end of the olfactive sensation, as well as to describe the odour quality. The GC system was equipped with a non-polar column coupled to a polar one; an initial 20 cm loop was immersed in liquid nitrogen, which was removed after injection and prior to the oven temperature program start. A total of 14 character impact compounds were identified: β-damascenone, diacetyl, sotolone, 1-hexen-3-one, 1-nonen-3-one, 1-octen-3-one, (*Z*)-3-hexenal, vanillin, raspberry ketone, 2 unidentified compounds, DMHF, octanal, and ethyl 2-methylbutyrate. The authors pointed out that the absence of both ionones, recognised as raspberry key-odours [107], could have been dependent on the variety and time of harvest. The heating process promoted an increase in β-damascenone, sotolone, 1-nonen-3-one, 1-octen-3-one, vanillin, and raspberry ketone. The heated matrix was characterised by the classic, or commonly known, raspberry odour, with raspberry ketone indicated as the major responsible. On the other hand, the addition of cream led to a decrease in the berries odour perception. All heated raspberry key-odour compounds presented decreased CHARM values, while that related to diacetyl increased. The authors reported a loss in volatility caused by the addition of fat to the matrix, changing its viscosity and mass transfer.

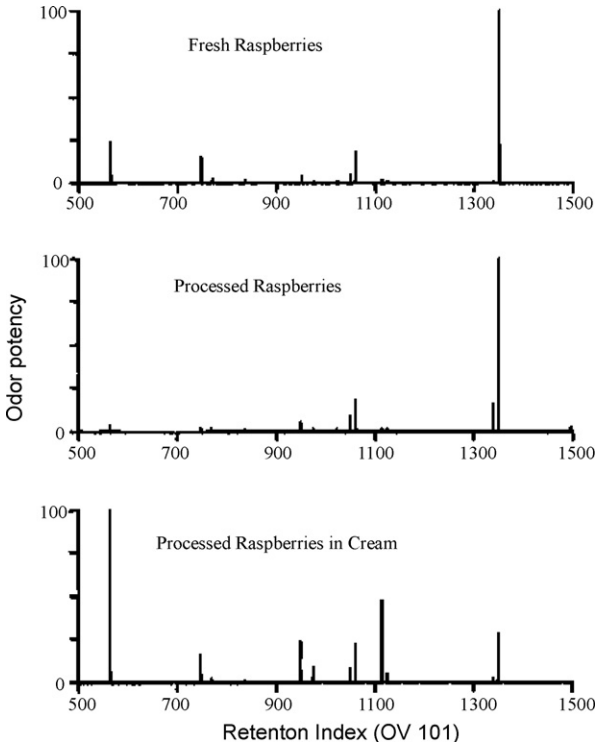


Fig. 10. Odour spectra comparison of raspberry samples analysed by means of RAS-GC–O and CHARM (reprinted from [108] with permission of the American Chemical Society, © 1996).

For the sake of comparison, the odour spectra method was applied. The latter enabled the standardisation of GC–O data by converting dilution analysis results using Stevens' exponent [refer to Eq. (2)], to odour potency values. A potency of 100 was assigned to the compound with the highest value, while the other values were then consecutively normalised. In the spectra, the derived values (*x*-axis) were plotted against the compound's retention index (*y*-axis), enabling this way a clearer comparison between similar or distinct samples, even when assessed by different GC–O methods (see Fig. 10).

An aspect of great importance, related to food flavour complexity, is the extensive number of co-elutions that may occur both on non-polar and polar stationary phases, leading to the inaccurate identification of odour-active compounds. The possible masking of odour-active trace-level compounds by major interferences or agglomeration of olfactive impressions resulting in unreliable olfactive characterisation often occurs, as reported by Klesk et al. [109] when performing AEDA of raspberry odour. On a polar column 59 compounds were detected, with 53 of these identified, while on a non-polar column 48 out of 53 detected compounds were identified. Amongst these volatiles, 27 were detected solely on the polar column, while 22 only on the non-polar one. The aforementioned drawback may be overcome by using multidimensional GC combined with olfactometry (MDGC–O). However, attention should be paid to the MDGC–O subject, since some articles described multidimensional GC/GC–O systems, in which two columns with identical

stationary phases were installed [110,111]. These investigations, carried out on unpasteurised fresh puree of kiwi [110] and guava [111], were performed using two identical non-polar columns, defined as preparative and analytical columns, located in two distinct GC systems, coupled through a cryotrapping device. The proposed approach may be considered as a valuable and sophisticated enrichment process for trace-level compounds, although it is still a monodimensional separation.

In MDGC [112], or rather MDGC–O, key fractions of the entire sample, are selected from the first column and re-injected onto the second one, where ideally, they should be fully resolved. Two chromatographic columns of differing polarities, but generally of identical dimensions, are employed. Furthermore, when the heart-cut operations are not carried out, the primary column elutes normally in the first dimension GC system, while the previously re-injected fractions are analysed in the second dimension GC system, equipped with a detector, and in the case of a MDGC–O, also with a sniffing port.

As mentioned previously, citrus peel essential oil, more specifically its volatile fraction, is the major responsible for the characteristic odour of these fruits. The volatile fraction constitutes more than 90% of the oil [113], and although it has been extensively studied, it is still subject of sophisticated investigations for rather specific purposes. The work carried out by Elston et al. [114] may be held as an interesting example of this: the authors carried out an MDGC/MS-O experiment in order to evaluate the use of valencene's (a sesquiterpene) concentration as an index for estimating the commercial value of orange (*Citrus sinensis* Osbeck) oil, as also its effective role in the overall flavour of this matrix. The information on the odour-activity of this abundant sesquiterpene in diverse orange matrices, such as oil and juice, is not only scarce, but also controversial. The use of it as a key predictor, on the other hand, is well recognised [115], and its concentration level may be correlated to the oil's odour quality due to maturity effects. The MDGC/MS-O system was equipped with a polar column in the first dimension (1D) and a non-polar column in the second (2D) (both 30 m × 0.53 mm I.D. × 0.5 μm $d_f$), connected via a cryofocussing device, based on Dean's switching method [112]. When no first dimension fraction was subjected to heart-cutting, a bypass select valve, installed before the FID system, splitted the primary column effluent to the FID system and the sniffing port, in a 1:1 ratio. The latter configuration enabled the performance of monodimensional GC–O. During the valencene region heart-cutting, the fraction was cryotrapped for the selected time interval and then released onto the 2D column for separation. The secondary column effluent was split to the MS and to the sniffing port in a proportion of 1:5, respectively. The detection frequency method was applied by two panellists. Monodimensional GC–O analysis on the polar column led to the detection of a citric, lemon-like, green odour in the region expected for valencene, while through MDGC/MS-O a single compound, dodecanal, was indicated as presenting the aforementioned olfactive impressions. The results attained by means of MDGC/MS-O demonstrated that in the concentration in which valencene was present in the orange oil it could not be detected by the panellists, although it could be representative for the oil. Nevertheless, it must be emphasised that the method applied proved to be valuable for reliable odour-activity determination, clearing the role of valencene on the orange oil's odour, even though the attained results on dodecanal and valencene could have been probably achieved by using a monodimensional GC–O, on a non-polar column. As reported in the manuscript, dodecanal and valencene present retention indices of 1722 and 1726, respectively, on a polar column, and of 1411 and 1513 on a non-polar one. As a polar column has been used in the 1D, the former values might indeed be representative for a co-elution, since valencene is very abundant in that sample.

Tropical fruits are one of the most important topics in food flavour research, since their attractive flavour adds economical value to several products. This subject has been concisely reviewed by Franco and Janzantti [116]. The odour-active compounds sampled from the static cryogenic headspace of dehydrated banana (*Musa cavendishii* cv. *nanica*), defined as banana passa, have also been analysed by using MDGC/MS-O [117]. However, the latter was not composed of two hyphenated GC instruments, but of a single GC/MS equipped with a dedicated transfer system and retrofitted with a sniffing port device. In the case, the first dimension separation was less than satisfactory, the analytes were heart-cut and released onto the second dimension. The heart-cutting of the region eliciting the most potent olfactive impression enabled the identification of 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone. According to the authors, this compound could not have been detected through GC analysis on a polar column, as its olfactive impression was masked by odour-active co-eluting interferences. A further confirmation of the compound potency was enabled through its concentration in a $CO_2$/ acetone cold trap. After five collecting runs, the column was washed with dichloromethane and the collected material injected in the MDGC/MS-O system. All regions presenting potent odour-activity were also subjected to heart-cutting and the key-odour compounds isolated. AEDA, performed by 12 panellists, revealed 4-hydroxy-2,5-dimethyl-3(2H)-furanone (sweet, caramel note) and ethyl cinnamate (fruity, banana passa-like odour) as the main character impact compounds, followed by isovaleric acid, ethyl butanoate, 2-methylpropan-1-ol, 3-methylbutan-1-ol, (*Z*)-3-hexen-1-ol, and 3-nonen-2-ol.

A topic, which must not be overlooked, is the analysis and resolution of optically active compounds present in food flavours. Distinct enantiomers may impart different flavours, have distinct degradation pathways and often be characterised by different biological activities. Many natural flavour materials occur as specific chiral isomers, and their odour can be very distinctive and characteristic [118]. In general, enantiomers may differ either in odour quality, e.g. eliciting different odour notes; or in odour intensity, that can be described by the odour threshold value.

Enantioselective GC–O (Enantio-GC–O) is a valid method for the correct determination of sensory properties. Since the enantiomeric ratio determination of individual components in fruits and similar natural products has developed into a routine, several food industries often try to improve the quality of a product, such as, for example, the enhancement of fruit

juice's flavour through the addition of synthetically derived terpenes, lactones, and similar components. Iwabuchi et al. [119] analysed the odours of white peach (*Prunus persica*) by means of GC–O applying CHARM, and also by Enantio-GC–O, using a broadly selective chiral stationary phase. The odour of white peach was characterised by (*Z*)-2-hexenol (green, hay notes), (*Z*)-6-dodecen-4-olide (peach-like odour) and 3-methyl-(*E*)-5-decen-4-olide (sweet, fresh, juicy note). The absolute structure of the latter compound was elucidated, with the (3*S*, 4*R*)-(−)-enantiomer identified as the prevailing isomer. The (−)-enantiomer was characterised by a sweet, fresh, juicy odour, presenting a lower threshold value than its (+)-antipode.

It is worth noting, once again, that differences in the volatile composition of fruits may occur in relation to the applied extraction methodology, as also due to differences in the cultivar or the geographical origin. The use of heat during extraction can also result in modifications; moreover, odour-active compounds in fruits often present glucosidic precursors which are converted to the free aroma compound during heating processes, or at low pH values.

## 8. Remarks on food flavour analysis

It is a known fact that the research for new flavour compounds is an ever-growing field, being continuously influenced by consumer demands. For this reason, the investigation and identification of odour-active compounds, especially key-odour notes, in food samples, as also the determination of their relevance and release from the matrix, are of extreme importance for the characterisation of a food. In this respect, GC–O is considered a useful analytical and sensorial tool, with a vast number of investigations carried out on food flavour. The introduction of the technique was a breakthrough in analytical aroma research and marked the beginning of the discrimination of a multitude of volatiles in odour-active and non odour-active, in relation to their existing concentrations in the matrix under investigation. On the basis of the results attained, new flavour creations emerged.

It is certain that the references cited in this review represent a very small part of the studies performed, by means of GC–O, on food flavours. Furthermore, many extraction techniques have been developed, in combination, to enhance the quality of the flavour results, even though none of the commonly used extraction methods alone is able to give a complete reproduction of a flavour's profile. The application of diverse extraction procedures, on an identical matrix, appears to be the best choice, enabling to achieve a more extensive screening. Likewise is true for the GC–O methods, where the exploitation of different methods may give complementary information on a given matrix.

It should also be highlighted that, unfortunately, apart from the information available in the open literature, a considerable amount of GC–O research is carried out in the food industries.

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