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Analytical Methods

Application of gas chromatography–olfactometry (GC–O) in analysis and quality assessment of alcoholic beverages – A review

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Abstract

In recent years, intensive studies have been carried out regarding the sensory activity of the individual components of the odours of various alcoholic beverages and the dependence between the odour and the chemical composition of the volatile fraction of these products, using gas chromatography with olfactometric detection (GC–O). GC–O is a technique based on sensory evaluation of the eluate from the chromatographic column. Quantitative and qualitative odour evaluation is possible thanks to the presence of a specially constructed attachment, a so-called olfactometric port.

Olfactogram appearance depends on the analyte isolation procedure and the quantitative method used. In this work, a discussion and comparison of the most often used methods of alcoholic beverage sample preparation are presented, including solvent and solventless methods, as well as quantitative methods, such as the detection frequency methods, dilution to threshold methods and direct intensity methods.

Specific focus is placed on the utilization of the techniques discussed in the analysis and evaluation of the quality of alcoholic beverages. The paper presents numerous examples of studies aimed at determining the dependence between the composition and content of volatile compounds and the organoleptic properties of products such as beer, wine and spirits, as well as the identification and comparison of compounds responsible for the aroma of various alcoholic beverages or those responsible for unwanted odours.

Keywords: Gas chromatography–olfactometry; Alcoholic beverages; Odour; Volatile compounds

Contents

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1. Introduction

The composition and content of odour compounds determine the quality of alcoholic products. The smell of an alcoholic beverage is the effect of a large number of chemical compounds with different properties (such as polarity or volatility) occurring at widely differing concentrations. The chemical composition of the odours depends on the quality and type of the raw materials, as well as the conditions of the fermentation process.

Alcoholic fermentation conducted under industrial conditions leads to a series of byproducts in addition to ethanol. They include carbonyl compounds, alcohols, esters, acids and acetals, all of them influencing the quality of the finished product. The composition and concentration levels of the byproducts can vary widely. Some compounds appear in high concentrations (hundreds of mg/l); however, a large part appear at significantly lower levels (even as low as ng/l). The influence of the individual compounds on the odour profile can be very different. Quite frequently, compounds appearing in trace quantities have a greater influence on the sensory properties of alcoholic products than those which appear in high concentrations.

In recent years, intensive studies have been carried out regarding the sensory activity of the individual components of food and alcoholic beverage odours, and the dependence between the odour and the chemical composition of the volatile fraction of these products. The majority of the accomplishments within this area can be attributed to the combination of gas chromatography with olfactometric detection.

2. Overall characteristics of the GC–O technique and instrumentation

Gas chromatography with olfactometric detection is based on sensory evaluation of the eluate from the chromatographic column aimed at discovering the active odour compounds. The role of the detector is played by a properly educated person or a team of evaluating personnel. Qualitative and quantitative evaluation of the odour is carried out for each analyte leaving the chromatographic column. This allows establishing whether a given compound is sensory active at a given concentration (i.e. whether it appears in the sample at a level higher than the threshold of sensory detection) and what its smell is, as well as the determination of the time of sensory activity and the intensity of the odour. Determination of the analyte's odour is possible thanks to the presence of a special attachment, a so-called olfactometric port, connected in parallel to conventional detectors, such as flame-ionization detector (FID) or mass spectrometer (MS) (Fig. 1). The flow of

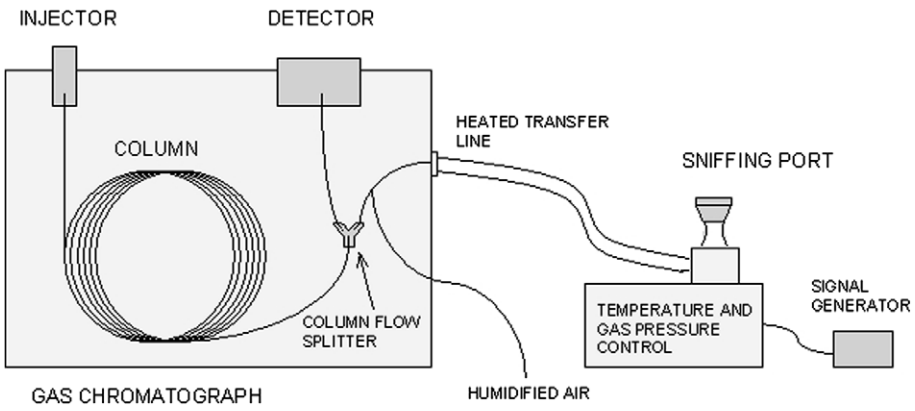

Fig. 1. Scheme of the gas chromatograph equipped with the olfactometric detector.

the eluate is split in such a way that the analytes reach both detectors simultaneously, owing to which both signals can be compared. A combination of the olfactometric detector with a mass spectrometer is particularly advantageous, as it makes the identification of odour-active analytes possible. However, since the mass spectrometer works under vacuum conditions, while the olfactometric detector works under atmospheric pressure conditions, the retention times of the analytes might differ for the two detectors (typically shorter for the mass spectrometer). This difficulty can be overcome by installing a restrictor (in the form of a narrow bore capillary) before the mass spectrometer to increase the pressure drop between the interface and the flow splitter, as well as through careful selection of the flows of the carrier and auxiliary gases (Hochereau & Bruchet, 2004).

The design of all commercially available olfactometric ports is very similar. The eluate delivered to the port through a dedicated transfer line is smelled in a glass or a PTFE conical port fitted to the shape of a nose. The transfer line is heated to prevent the condensation of semivolatile analytes on the walls of the capillary. Auxiliary gas (moist air) is added to the eluate to prevent the drying of the nose mucous membranes of the evaluating personnel, as this could cause discomfort, especially in longer analyses. The transfer line length can vary widely, but it has to be long enough to ensure a comfortable sitting position for the evaluator during detection and to avoid discomfort due to the vicinity of hot chromatograph components. If the extract analyzed is sufficiently concentrated, the eluate stream can sometimes be separated into several streams delivered simultaneously to individual olfactometric ports, with the detection conducted by several people at the same time. The most representative results can be obtained in this manner (Debonneville, Orsier, Flament, & Chaintreau, 2002).

3. Sample preparation methods

Determination of odour substances using instrumental techniques consists of two stages. The first stage of the analysis, isolation of the analytes from the matrix, is particularly important. The appearance of the olfactograms depends to a large extent on the isolation procedure, as numerous comparative studies revealed that the use of different sample preparation techniques (even using different solvents in the case of liquid–liquid extraction) might affect the composition and contents of the isolated compounds (Bonino et al., 2003; Lopez & Gomez, 2000; Nonato, Carazza, Silva, Carvalho, & Cardeal, 2001). The extract isolated should be representative, hence the selection of a proper sample preparation procedure is crucial (Plutowska & Wardencki, 2007; Sides, Robards, & Helliwell, 2000). Isolates obtained using exhaustive extraction methods, including solvent extraction and distillation, do not always reflect the composition of the odour reaching the odour and taste receptors during eating and drinking. One should remember that only some of the volatile odour compounds contribute to the fragrance of beverages and food. The composition of the volatile fraction of the products can change depending on the solubility of the components and the properties of the matrix (e.g. sugar content). Consequently, it is more advantageous to use isolation methods which reflect the release of the volatile components from the matrix rather than determining the overall contents of these components, as this facilitates the correlation with sensory analysis results. Both static and dynamic headspace methods can be used for this purpose; however, because of the possibility of analyte enrichment, dynamic methods are used more often (Pollien et al., 1997).

3.1. Solvent extraction methods

Solvent extraction methods are usually time consuming and involve many stages. This is due among others to the need to rinse the organic extract with aqueous solutions of different pH to remove acids and non-volatile compounds which might get into the poorly selective extraction solvents. The removal of non-volatile substances from the isolate is crucial not only because of the risk of chromatographic column contamination, but also because of the possible artifact creation in the hot injector, which could falsify the results. In addition, the odour of fatty acids is intense and long-lasting enough in the olfactometric port that it might hinder the detection of analytes eluting directly after them (Ferreira, Lopez, Escudero, & Cacho, 1998). Problems related to the low selectivity of the extraction solvents towards alcoholic beverages constituents can be partially overcome by using adsorption resins subsequently extracted with solvents. This is an efficient method for the extraction of odour compounds from alcoholic products. Amberlite resins of the XAD type are often used as the sorbent (Aznar, Lopez, Cacho, & Ferreira, 2001; Lermusieau, Bulens, & Collin, 2001; Vermaulen, Guyot-Declerck, & Collin, 2003) because of their relatively low selectivity and high efficiency towards polar substances. Extraction with solid sorbents can be carried out by shaking the sample with resin particles; however, solid-phase extraction in SPE columns (Cullere, Escudero, Cacho, & Ferreira, 2004; Escudero et al., 2004; Lopez, Ortin, Perez-Trujillo, Cacho, & Ferreira, 2003) or glass columns (Aznar et al., 2001; Ferreira, Ortin, Escudero, Lopez, & Cacho, 2002) is much easier. Despite the benefits of solid sorbent extraction, it has been found that dynamic headspace techniques produce simpler and "cleaner" olfactograms for beverages such as wine (Campo, Ferreira, Escudero, & Cacho, 2005).

Conventional solvent extraction is usually followed by extract concentration through partial or complete removal of the solvent via distillation. This not only lengthens the analysis time and increases the risk of analyte loss, but also increases the risk of odour component degradation and artifact creation as a result of oxidation processes. While solvent removal through distillation is usually conducted under reduced pressure and temperature conditions, it is practically impossible to avoid the contact between the extract and the air during the multi-step sample prepara-

tion procedures. Gas chromatographic studies with olfactometric detection demonstrated the need for the protection of odour compounds during solvent extraction through the addition of antioxidants such as 2-*tert*-butyl-4-methoxyphenol (BHA), especially when studying oxidation processes in alcoholic beverages during their production and storage. Without the antioxidant, the extract obtained might not be representative because of oxidation reactions of higher alcohols, amino acids, some lactones, terpenes and esters (Escudero & Etievant, 1999).

3.2. Headspace methods

Purge and trap methods used for the extraction of volatile compounds from alcoholic beverages might vary widely. Sorption traps with porous polymers such as Tenax TA or Porapak Q, as well as resins such as Lichrolut EN, are most often used (Campo et al., 2005). Analyte liberation from the traps is carried out using thermal desorption or solvent elution (Fur, Mercurio, Moio, Blanquet, & Meunier, 2003; Garruti, Franco, Silva, Janzantti, & Alves, 2006; Pollien et al., 1997). Porous polymer sorbents, especially Porapak Q, trap only small amounts of ethanol, which is advantageous as it reduces the possibility of trap breakthrough and the width of the solvent peak on the chromatogram. Compared to conventional exhaustive extraction techniques, headspace methods have the benefit of usually not causing the loss of the most volatile compounds, which often have the greatest influence on the odour of the sample. In addition, headspace techniques enable chromatographic analysis of these compounds, which is often difficult with solvent methods due to the presence of the solvent peak (Grosch, 2001).

3.3. Solid-phase microextraction (SPME)

Solid-phase microextraction (SPME) is often used in headspace analysis (Guerche, Dauphin, Pons, Blancard, & Darriet, 2006; Gurbuz, Rouseff, & Rouseff, 2006; Ong & Acree, 1999). Especially successful is its utilization for dilution to threshold methods (Deibler, Acree, & Lavin, 1999), as it allows for significant simplification of the analytical procedure. Most importantly, instead of diluting the extract, it is possible to directly dilute the sample, which is usually a lot less time consuming (Fan & Qian, 2005; Marti, Mestres, Sala, Busto, & Guasch, 2003). Furthermore, SPME allows for almost complete elimination of the sample preparation step. The simplicity of the utilization of this technique relies on the possibility of using different thicknesses of solid-phase extraction fiber coatings instead of conducting a series of sample dilutions. The downside of such an approach is the small number of commercially available fibers of different thicknesses. The time-consuming task of preparing solutions with different concentrations of the odour compounds can be eliminated by using different split ratios of the carrier gas in the split/splitless injector (Deibler, Llesca, Lavin, & Acree, 2004), or through the use of various lengths of the fibers (Deibler et al., 1999). These solutions make it possible to achieve even a 50-fold sample dilution.

Initial optimization of the conditions is required when SPME is used, as the qualitative and quantitative composition of the isolate might change depending on the kind of the solid-phase microextraction fiber used, temperature, extraction time and/or the volume of the sample (Fan & Qian, 2005). Literature studies indicate that in most cases a two centimeter mixed DVB/CAR/PDMS fiber (divinylbenzene/carboxen/polidimethylsiloxane) ensures satisfactory yield of the largest amounts of the odour compounds from alcoholic beverages.

3.4. Extract fractionation

When the aroma of the alcoholic beverage analyzed is characterized by a very complicated qualitative composition, its fractionation is sometimes advisable (Lee & Noble, 2003; Lopez, Ferreira, Hernandez, & Cacho, 1999). This often allows for the determination of odour compounds appearing in trace or ultratrace amounts in the vicinity of other fermentation byproducts occurring in significant amounts, such as fusel alcohol or fatty acid esters. For example, fractionation of the isolate of the odour compounds present in the Chinese alcoholic beverage Yanghe Daqu obtained by solvent extraction with Freon 11 into an acidic fraction and four neutral-alkaline fractions enabled effective separation and identification of over 70 odour-active compounds (Fan & Qian, 2006a, 2006b). The neutral-alkaline fraction was obtained by extraction of the freon isolate with water at elevated pH, and the acidic fraction was obtained through extraction of the former with diethyl ether at a low pH. Because of the complex composition of the neutral-alkaline fraction, it was further fractionated using normal-phase preparative liquid chromatography. An analogous method was used to determine the odour compounds in young, red grape wines (Ferreira et al., 1998). Similarly, the odour compounds of Pinot Noir wine were divided into two fractions, with the extract for fractionation obtained by solvent extraction followed by solvent-assisted flavour evaporation (SAFE) (Fang & Qian, 2005).

Chromatographic fractionation can also be carried out in reversed phase mode, with the mobile phase consisting of water and ethanol. This eliminates the use of toxic and sensory active solvents, but unfortunately renders significant analyte enrichment impossible, as a result of which additional solvent extraction of the fractions obtained is usually required (Aznar et al., 2001). Fifteen odour fractions were obtained from Chardonnay wine in this way, which allowed for the identification of over 70 sensory active components of its aroma (Ferreira, Hernandez-Orte, Escudero, Lopez, & Cacho, 1999); 50 fractions were obtained from Spanish Rioja wine, which enabled the identification of over 50 odour compounds (Aznar et al., 2001). It should be stressed that preparative liquid chromatography is a conservative

method which does not cause any qualitative or quantitative changes in the composition of the sample.

3.5. Selective extraction techniques

One of the goals of gas chromatographic analysis with olfactometric detection is the identification and quantitative evaluation of specific analytes from a given class of organic compounds. Selective extraction methods are used for this purpose (Fretz, Kanel, Luisier, & Amado, 2005; Janusz et al., 2003). For example, Bouchilloux et al. used a combination of vacuum distillation and selective thiol trapping through the reaction with *p*-hydroxy mercurobenzoic acid (*p*-HMB), which made is possible to obtain odour profiles of red Bordeaux wines resulting only from the presence of sulfur compounds, without the influence of the wide array of other odour compounds (Bouchilloux, Darriet, Henry, Lavigne-Cruege, & Dubourdieu, 1998). An additional benefit of this method was the elimination of polyphenols from the extract. These compounds appeared in the extracts obtained by conventional solvent extraction, making chromatographic analysis more difficult.

3.6. Selecting the appropriate extraction technique

The usefulness of a given extraction technique and the representativeness of the sample are often verified through initial conventional sensory analysis and a comparison of the odour properties of the alcoholic beverage samples and their corresponding extracts (Aznar et al., 2001; Bernet, Dirninger, Claudel, Etievant, & Schaeffer, 2002; Ferreira, Hogg, & Guedes de Pinho, 2003; Ferreira et al., 2002; Priser, Etievant, Nicklaus, & Brun, 1997). For example, similarity tests on samples of lager beer and fragrance compounds extracted using different solvents, such as hexane, diethyl ether, ethyl acetate and dichloromethane, demonstrated that the dichloromethane extract was the most representative (Soares da Costa et al., 2004). Sensory analysis of champagne odour compound extracts obtained using three different methods, adsorption with XAD resins, dichloromethane extraction and extraction through ethanol demixing using the profile descriptive method (Baryłko-Pikielna, 1975) demonstrated that the odour of the isolate obtained using the last method was the closest to the original (Priser et al., 1997).

Unfortunately, the similarity testing is not directly possible in the case of extraction using solvents with intense odours or headspace methods, including solid-phase microextraction (SPME). In the case of SPME, the most disadvantageous is that the extracts might differ both qualitatively and quantitatively depending on the type of the fiber used.

4. Factors influencing the quality of results

A number of factors affect the quality of results. The volatile compound extraction technique is particularly important, as it affects the representativeness of the isolate and the composition of the eluate subject to sensory evaluation. Sample storage is also critical from the point of view of sample representativeness. To prevent auto-oxidation, wine extracts should be stored in a carbon dioxide atmosphere at low temperature (Bernet et al., 2002). Dividing the extract into small, individual portions is recommended to avoid changes in the composition caused by repeated opening of the container with the sample. From the point of view of qualitative odour evaluation, the quality of chromatographic separation is important, hence the conditions of the separation must be carefully optimized. Finally, one cannot avoid the effect of human involvement in this technique, and the limitations related to it. To minimize the deviations, one should strive to ensure constant analysis conditions for each evaluator, such as a consistent sample order for the samples analyzed, or the same scale used for evaluating odour intensity (Delahunty, Eyres, & Dufour, 2006).

4.1. Detection conditions

It should be remembered that a human is the detector in the technique discussed, thus minimizing all factors which can influence the evaluator and consequently affect the analysis results is very important, similar to conventional sensory analysis. For example, the environment in which olfactometric determination is being carried out is very important. Most importantly, the laboratory must be free of all foreign odours and sounds, and must allow for the maintenance of a consistent temperature and pressure.

Unlike in conventional methods of sensory analysis, the person evaluating the sample has to deal with many different odours appearing for only a few seconds at irregular time intervals during a chromatographic analysis which usually lasts tens of minutes at a time. Despite this, errors typical of sensory analysis can also appear here, related to the ability of a person to remember odour profiles and the ability to foresee or assume the composition and content of the odour compounds in later samples. This is particularly true when the individual samples do not significantly differ qualitatively or quantitatively and are analyzed repeatedly. The probability of making this type of error can be reduced by analyzing the samples in random order and/or by randomly adding blank samples or samples that are completely different from the series undergoing analysis to the sequence.

Alcoholic beverage samples, especially wine samples, are most often complicated mixtures of many compounds, whose separation requires the use of long chromatographic programs. To prevent the reduction in detection sensitivity caused by fatigue, especially in analyses lasting upwards of 25 min, the entire analysis should be divided into several parts, and the evaluator should be changed for each part (Bernet et al., 2002). It has also been found that increased auxiliary gas flow in the transfer line of the olfactometric port reduces the sensory sensitivity, but also the delay

between the olfactometric detector signal and the signal of the detectors used in parallel, thus this parameter should be carefully optimized (Hanaoka, Sieffermann, & Giampaoli, 2000).

4.2. Chromatographic conditions

Very important from the point of view of olfactometric determination is the choice of proper chromatographic conditions, such as temperature and injection mode, as well as the type of the stationary phase of the chromatographic column. Thermal desorption is typically used with headspace methods, most often in splitless mode. Solvent extracts, on the other hand, can be injected at low temperature directly on-column, which avoids decomposition of thermally labile analytes.

The stationary phase of the chromatographic column should ensure not only high selectivity, but also separation efficiency, as the shape of the peak is also important, especially in the case of methods which directly measure intensity. If the mixture of odour compounds is very complicated or if the compounds are isomers, it is sometimes necessary to use two-dimensional and/or chiral chromatography, in order to obtain satisfactory selectivity (Wanikawa, Hosoi, Kato, & Nakagawa, 2002). Despite the fact that such an approach is usually enough to obtain satisfactory chromatographic separation of the analytes, it is not always sufficient for olfactometric detection by the evaluator, especially when the odours are long lasting, e.g. for sulfur compounds. To alleviate this problem, a special interface has been developed for multi-dimensional chromatography, with so-called double-cool-strand interface, in which analytes leaving the first column are cryogenically trapped in transfer capillary loops and sequentially injected into a chiral column, at intervals of a few seconds to a few minutes (Begnaud, Starkenmann, Waal, & Chaintreau, 2006).

5. Quantitative methods

Several quantitative methods exist for the evaluation of the intensity of odours and their relative influence on the odour of the sample (Ruth, 2001). These methods can be categorized into three groups based on the method of determination:

- detection frequency methods (Ferrari et al., 2004; Pollien, Fay, Baumgartner, & Chaintreau, 1999; Pollien et al., 1997),
- dilution to threshold methods,
- direct intensity methods.

The second group consists of universally used methods, such as the so-called CHARM method (Combined Hedonic Aroma Response Measurement) (Kishimoto, Wanikawa, Kono, & Shibata, 2006; Mariaca & Bosset, 1997) and AEDA (Aroma Extract Dilution Analysis) (Aznar et al., 2001; Benn & Peppard, 1996; Darriet et al., 2002; Escudero et al., 2004; Ferreira, Aznar, Lopez, & Cacho, 2001; Ferreira, Petka, & Aznar, 2002; Gijs, Chevance, Jerkovic, & Collin, 2002; Guth, 1997; Guyot-Declerck, Francois, Ritter, Govaerts, & Collin, 2005; Kotseridis, Razungles, Bertrand, & Baumes, 2000; Lermusieau et al., 2001; Lopez et al., 1999; Marti et al., 2003), while the group of methods which directly measure the intensity of odour includes posterior intensity methods (Cullere et al., 2004; Lopez et al., 2003; Petka, Ferreira, & Cacho, 2005) as well as time–intensity methods: OSME (Deibler et al., 2004; Hanaoka et al., 2000) and the fingerspan method (Bernet et al., 2002; Etievant, Callement, Langlois, Issanchou, & Coquibus, 1999).

5.1. Frequency detection methods

In frequency detection methods, a team consisting of 6–12 people analyzes the same sample. The percentage of people who sensed the odour compound at a given retention time is counted (Fur et al., 2003). Compounds which are sensed more frequently than others are acknowledged as having the most important influence on the odour of the given sample. Sometimes the results for each odour region are quantified using so-called olfactometric indices, including NIF (Nasal Impact Frequency) values or SNIF (Surface of Nasal Impact Frequency) (Fig. 2). The NIF value is set to a value of one when each of the evaluators sensed a given odour, and to zero when no-one sensed any odour at a given retention time (Ferrari et al., 2004), and corresponds to the total height of the olfactometric signal (vertical axis in the figure). The SNIF values take into account the olfactory stimulation time (shaded areas in Fig. 2) (Pollien et al., 1997). Fig. 3 presents an example of an olfactogram obtained using a frequency detection method. The result obtained is only related to the intensity of the odour sensed at a given concentration of the analyte in the sample, which is the main limitation of this group of methods. If an analyte always appears at concentrations higher than the detection threshold, so that it is sensed by all evaluators, then regardless of its concentration the results of the analysis of a given sample might be the same.

The fundamental benefit of methods based on detection frequency is their simplicity, owing to which qualified evaluators are not required. The methods are repeatable, and the results reflect the differences in sensitivity between the evaluators, which can be related to differences within a given population. Frequency detection methods are the least time consuming and the easiest to conduct while dilution to threshold methods are the most time consuming and direct intensity methods are the hardest to conduct properly (Delahunty et al., 2006).

5.2. Dilution to threshold methods

Dilution to threshold methods are used the most frequently in the analysis of the odours of alcoholic beverages. They provide a quantitative description of the odour

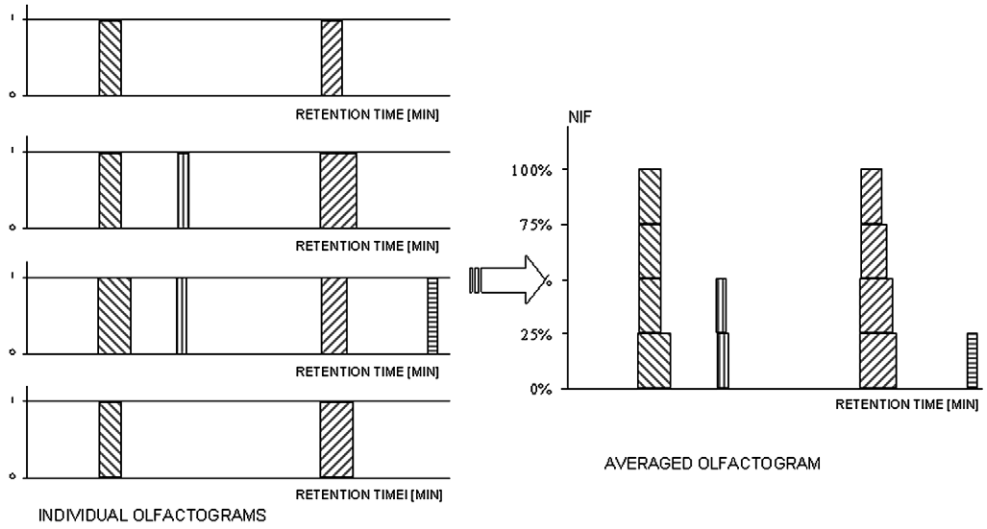

Fig. 2. Scheme of olfactogram forming in detection frequency methods, when four evaluators participate in the experiment.

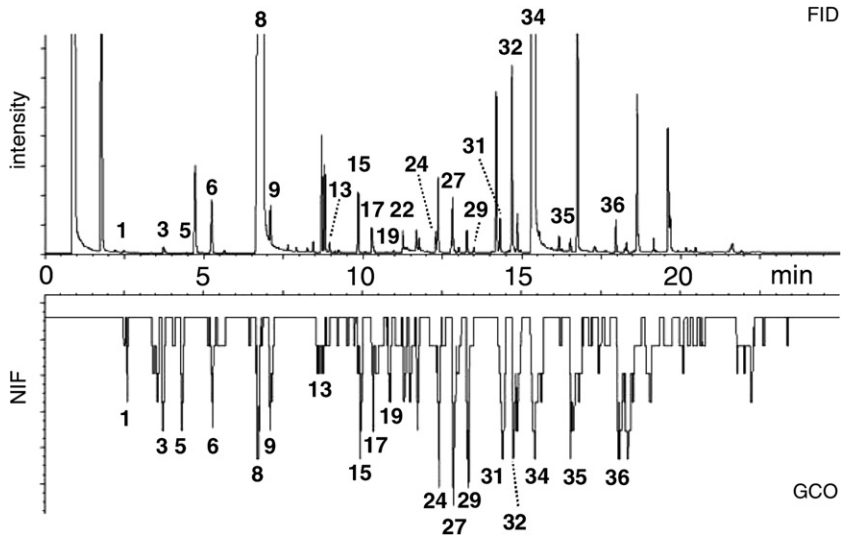

Fig. 3. Comparison of the aroma profiles of Croatian Rhine Riesling wine obtained by gas chromatography with flame-ionization detector (FID) (upper) and olfactometry detector (GCO) (lower) using detection frequency method (reproduced from Komes et al., 2006).

potential of a given compound based on the ratio between its concentration in the sample to the sensory threshold in air (Delahunty et al., 2006). These methods consist of preparing a series of dilutions of the extracts of odour compounds, most often using twofold, threefold, fivefold or 10-fold dilution levels (R) and then evaluating each sample using an odour detector (Ferreira, Petka, et al., 2002) (Fig. 4). The evaluator states under which dilution the compound analyzed can still be sensed, and usually describes the type of smell. The odour potential can be described as so-called aroma values or odour values, as well as odour units or flavour units (Delahunty et al., 2006). The most frequently counted are so-called odour activity values (OAVs) (Aznar et al., 2001; Cullere et al., 2004; Guth, 1997; Lopez et al., 2003; Ong & Acree, 1999), which represent the ratio of the concentration of a given substance in the sample to the sensory detection threshold.

The AEDA method measures the highest sample dilution at which the odour of the analyzed compound is still detectable. This value is used to calculate the so-called odour factor dilution (FD) (Ruth, 2001). If the last dilution under which the analyte was still detectable is equal to P ($P = 0, 1, 2, 3, \ldots$), then its dilution factor is R^P (Ferreira, Petka, et al., 2002). It follows from the definition of FD that in order for the dilution factor to be treated as a quantitative measure, one should maintain the consistency of the conditions for the determination of the odour compounds in the analyzed products, especially when it comes to the extraction process.

Compared to the AEDA method, the CHARM method requires an additional determination of the duration of the odour sensation in the column eluate, and allows for the determination of specific chromatographic peaks. The peak areas are expressed in dimensionless "Charm" values,

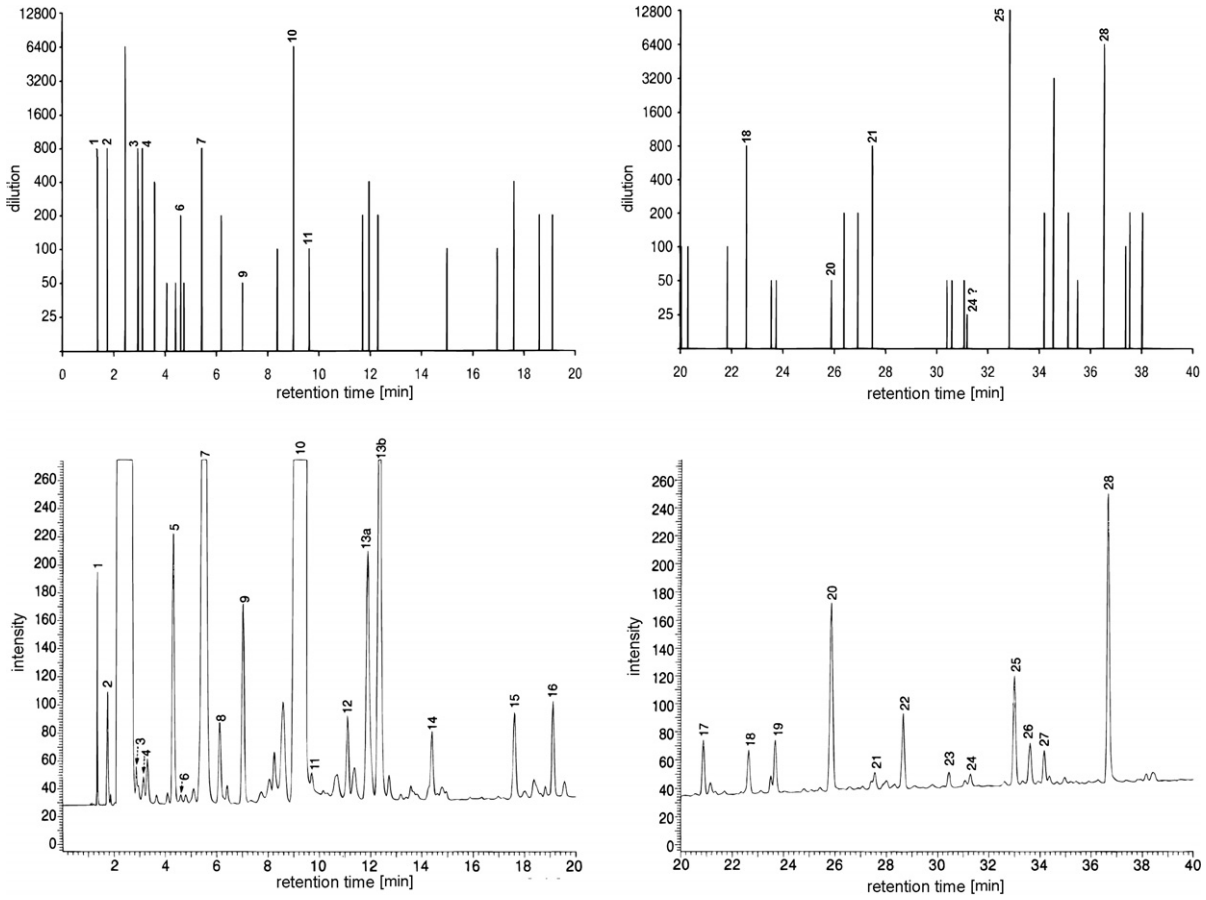

Fig. 4. Comparison of the aroma profiles of tequila obtained by gas chromatography with olfactometry detector (GCO) using AEDA method (upper) and flame-ionization detector (FID) (lower) (reproduced from Benn & Peppard, 1996).

which are proportional to the amount of the analyte in the extract, and inversely proportional to the sensory detection threshold (Acree, Barnard, & Cunningham, 1984). While the odour dilution factor responds only to the height of the peak, CHARM also considers the width and shape of the peak, thus low and wide peaks of analytes characterized by higher detection thresholds, but appearing in the samples at higher concentrations, might get the same CHARM values as narrow and tall peaks of compounds with low detection thresholds present in low concentrations. Thus, CHARM is suitable for the determination of the significance of the individual odour compounds in a given sample, at a cost of lower precision.

The dilution methods have also some drawbacks. The total analysis time is long, especially in the case of large evaluator panels, therefore the number of evaluators taking part in the analysis is usually limited. This, in turn, increases the probability of obtaining low-precision and subjective results. In addition, the results depend on the sensory detection threshold of the analytes rather than on the realistic intensity of the analyte odour in a given sample (Etievant et al., 1999). Dilution to threshold methods are also criticized for the underlying false assumption that the odour intensity increases in parallel with the concentration for all odour components in a sample (Petka et al., 2005).

5.3. Direct intensity methods

In frequency detection and dilution to threshold methods, each of the evaluators states only the presence or absence of odour stimuli, while in direct intensity methods, the intensity of the stimuli and its duration are measured. While dilution to threshold methods render the measurement of the intensity of the odour stimuli impossible, direct intensity measurement methods use different kinds of quantitative scales to measure the intensity of the odour of the eluting compound. Depending on the method, the measurement can performed in different ways. These include a single, time-averaged measurement (Fan & Qian, 2006b), a measurement registered after the elution of the analyte (posterior intensity evaluation methods), or, most frequently, a dynamic measurement, where the appearance of an odour, its maximum intensity and decline are registered in a continuous manner (OSME, fingerspan method) (Fu, Yoon, & Bazemore, 2002). In the first case, the evaluator assigns each compound appearing in the eluate an appropriate value from a previously defined intensity scale

(similarly to conventional sensory analysis), while in the second case, peaks similar to chromatographic peaks are determined in response to the analytes. The olfactogram obtained is similar to chromatograms obtained with the use of conventional detectors and represents odour intensity as a function of the retention time (Fig. 5). The height of the peak corresponds to the maximum odour intensity of a given analyte, while the width corresponds to odour duration.

Attempts at correlating quantitative odour compound determination results with odour intensity measurements in Spanish red wines indicated that the individual types of wines could be differentiated based on differences in odour intensity as well as differences in OAVs of some aroma components (Cullere et al., 2004). It has been found, however, that the differentiation possibilities were significantly limited in cases when the analyzed olfactometric signal appeared in close proximity to a large number of other signals, as well as when odour intensity was very high in all analyzed samples. This indicates that the choice of a proper sample preparation technique is crucial. One should strive to obtain simple olfactograms, limiting the possibility of extraction of compounds with little relevance to the sensory properties of the sample, as well as compounds appearing in concentrations close to the detection threshold (Baryłko-Pikielna, 1975).

Little information is available on quantitative determination of the odour compounds using olfactometric detectors. Nevertheless, a significant correlation was found between the logarithm of the area of a peak obtained using the OSME method and the logarithm of the analyte concentration in the sample (Petka et al., 2005), i.e. between the intensity of the odour and the logarithm of the concentration of a substance, as described by the Steven's Law (Rossiter, 1996).

To plot the olfactograms, specially designed guides (connected to differential resistors) controlled with a computer mouse, spring-buttons or sliders (fingerspan method) are used most frequently (Etievant et al., 1999; Gurbuz et al., 2006; Hanaoka et al., 2000). The only practical downside of this group of methods is the requirement that the group of evaluators has certain expertise; nevertheless, an experienced panel usually obtains fast, repeatable and generally consistent results (Delahunty et al., 2006). The fingerspan

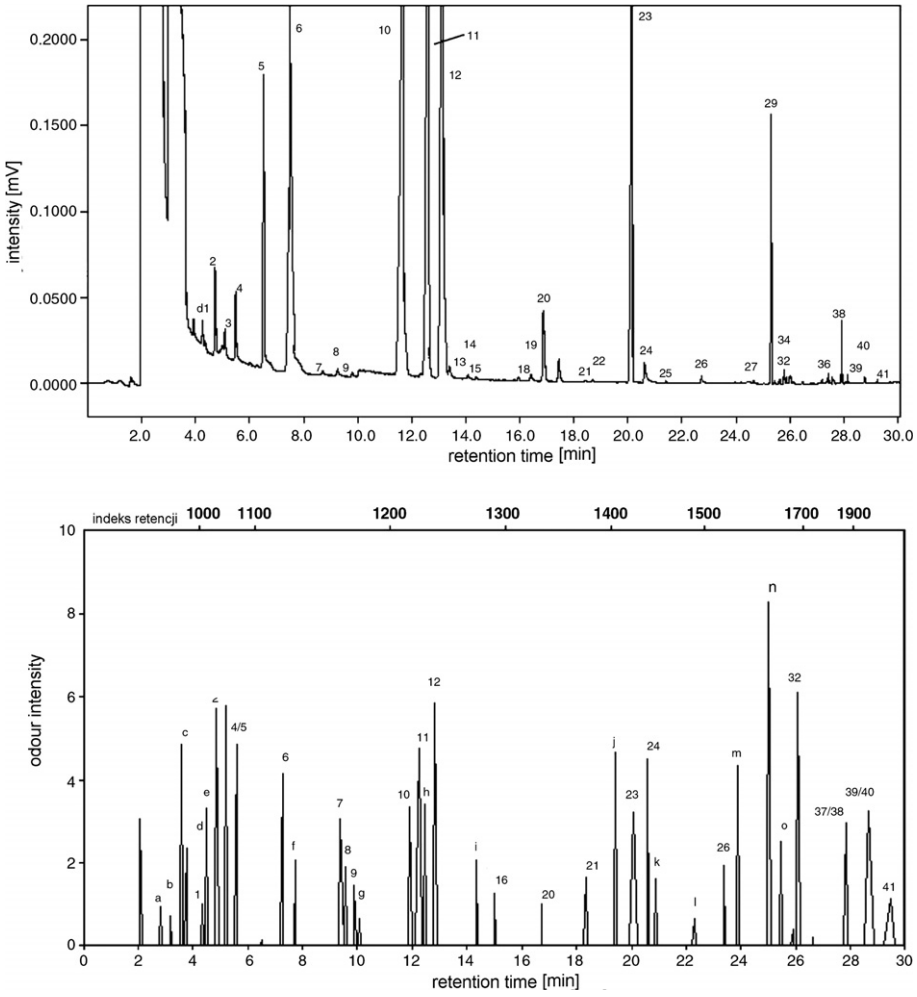

Fig. 5. Comparison of the aroma profiles of a cashew apple-based alcoholic beverage obtained by gas chromatography with flame-ionization detector (FID) (upper) and olfactometry detector (GCO) (lower) using OSME method. Compounds marked with the letters were not detected by FID (reproduced from Garruti et al., 2006).

method requires relatively little preparation from the evaluators. It was used to evaluate the possibility of differentiating Alsatian Gewurztraminer wines originating from different wineries by an inexperienced panel of evaluators (Bernet et al., 2002). The simplicity of this method, according to the authors of the publication, results from the intuitive method of registering the olfactograms, based on the conversion of odour stimuli to the movement of fingers, i.e. moving the potentiometer slider using the thumb and the index or middle finger. The distance between the two is proportional to the intensity of the odour, and the time of sliding corresponds to the duration of the odour in the olfactometric port. Studies demonstrated that even a completely unprepared team of evaluators was able to repeatedly perform olfactometric measurements. The evaluators became familiar with the instrumentation and a kind of autocalibration of the potentiometer scale used to measure the intensity of odour stimuli occurred already after about two chromatographic analyses. The results of earlier studies indicating that the measurement of odour intensity was more reproducible than the measurement of its duration have also been confirmed, with the determination of the end of odour stimulus being much harder than the determination of its beginning.

6. Examples of applications of gas chromatography with olfactometric detection

Gas chromatography–olfactometry studies on odour of alcoholic beverages usually have the goal of determining the relationship between the composition and the content of volatile compounds and the organoleptic properties of products such as beer (Lermusieau & Collin, 2003; Soares da Costa et al., 2004), wines (Campo et al., 2005; Lee & Noble, 2003) and whiskeys (Wanikawa et al., 2002), as well as identification and comparison of the compounds entering the aroma of different alcoholic beverages, such as wines (Cullere et al., 2004; Ferreira et al., 2001; Gurbuz et al., 2006; Guth, 1997; Kotseridis et al., 2000; Lopez et al., 1999; Petka, Ferreira, Gonzales-Vinas, & Cacho, 2006; Schneider, Baumes, Bayonove, & Razungles, 1998), cognacs (Ferrari et al., 2004; Lablanquie, Snakkers, Cantagrel, & Ferrari, 2002), beer with and without hops (Kishimoto et al., 2006; Lermusieau et al., 2001) and tequila (Benn & Peppard, 1996). Another goal might be the determination of compounds responsible for undesired odours (Darriet et al., 2002; Darriet, Pons, Lamy, & Dubordieau, 2000; Guerche et al., 2006; Simpson, Capone, & Sefton, 2004).

The GC–O results are often correlated with the results of conventional sensory evaluation conducted in parallel, usually using typical methods, especially difference detection methods (Baryłko-Pikielna, 1975) such as the triangle method (Campo et al., 2005; Kotseridis et al., 2000; Lermusieau et al., 2001; Souza, Vasquez, Mastro, Acree, & Lavin, 2006), the duo-trio method (Escudero et al., 2004) or the descriptive sensory analysis (Souza et al., 2006). Sensory analysis of the individual samples yields descriptors characterizing their smell. Chromatographic analysis with olfactometric detection makes it possible to determine which compounds are responsible for the individual descriptors. If the correlation between the descriptors and the individual analytes is more complicated, sometimes it is advisable to use appropriate chemometric methods, such as regression analysis methods (e.g. the least square method (Campo et al., 2005; Lee & Noble, 2003) or procrustes analysis (Fur et al., 2003)).

6.1. Using GC–O to identify odour compounds in alcoholic beverages

Gas chromatography enables the separation and identification of most components entering the volatile fraction of the products. However, because of the differences in sensory detection thresholds and the course of the psychometric function of the individual components, it is not possible to analyze the sensory activity using any of the conventional detectors. A comparison of olfactograms with chromatograms obtained with the use of detectors such as the flame-ionization detector (FID) or mass spectrometer (MS) often reveals that compounds producing large signals with the conventional detectors are weakly detectable in the eluate from the column, and vice versa. It should be emphasized that sometimes compounds detected with the nose as peaks on an olfactogram are not at all detectable with conventional detectors, which shows the enormous sensitivity of the human nose (Benn & Peppard, 1996; Ferreira et al., 1998; Marin, Acree, & Barnard, 1988).

When the concentration of an analyzed odour compound is so small that detection and identification with a conventional detector is impossible, sometimes an introductory olfactometric analysis can help determine the retention time of a given analyte for the purposes of its selective enrichment. One possibility is cryotrapping of the eluate in an empty deactivated capillary with the use of liquid nitrogen as the cooling agent (Callemien, Dasnoy, & Collin, 2006). An eluate stream splitter allowing for electronic switching of the flow in the trap in a selected range of retention times allowed for selective enrichment of the unidentified components of beer aroma responsible for the undesirable phenolic-tobacco odour created during the ageing of beer. It should be pointed out that analyte identification by mass spectrometry in an ether extract became possible only after capturing the fraction from thirty consecutive chromatographic analyses, while the analytes were detectable in the olfactometric detector without sample preconcentration.

Gas chromatography with olfactometric detection is often used to create odour profiles of traditional alcoholic beverages characteristic of any given country or region, which are usually distinguished by atypical aromas resulting from specific raw materials or technologies used during their production. Among numerous examples, studies can be pointed out which had as their aim the determination of the odour components of the aroma of traditional

Chinese distillates including Yanghe Daqu (Fan & Qian, 2005; Fan & Qian, 2006b), Wuliangye and Jiannanchun (Fan & Qian, 2006a), Pinot Noir wine from Oregon (Fang & Qian, 2005), Petite Arvine wine from Switzerland (Fretz et al., 2005), Rhine Riesling wine from Croatia (Komes, Ulrich, & Lovric, 2006), young white wines from the Canary Islands (Lopez et al., 2003), Turkish Kalecik Karasu wine (Selli et al., 2004) or Cachaca, a Brazilian sugarcane distillate (Souza et al., 2006).

Sometimes grape varieties from which given wines are produced are analyzed. Studies indicated that a significant portion of odour compounds entering the aroma of wines appear already during the processing of fruits as a result of chemical and enzymatic reactions, and the final composition mainly depends on the variety of grapes. For example, Schneider et al. determined volatile monoterpenes and norisoprenoids in *Vitis vinifera* L. Cv. Melon B. grapes, formed as a result of enzymatic degradation of glycosides and responsible for the specific aroma of Muscat wine (Schneider, Rzaungles, Augier, & Baumes, 2001). Serot et al. identified the main active odour components in musts from French–Romanian grape hybrids, obtaining consistent results for three olfactometric methods used (Serot, Prost, Visan, & Burcea, 2001).

6.2. Application of GC–O for the quality control of the raw materials used in the production of alcoholic beverages

Low quality of alcoholic beverages, especially wines, often results from low-quality raw materials used in their production. Fermentation of rotting or mouldy fruit or grains results in low organoleptic quality as a result of the presence of the degradation products of the natural components of the raw materials, as well as undesirable microbial metabolites. Mould development is particularly undesirable because of the earthy, fungi or musty smells which appear in the aroma. These smells are caused by compounds which usually have very low detection thresholds, such as geosmine, 2-methyl isoborneol, 1-octen-3-one or chloroanisoles (Darriet et al., 2000; Goj, 1998). The possibility of discovering and identifying these compounds by gas chromatography with olfactometric detection during the early stages of raw material rot, as well as during fermentation or in a ready product is very important. Current studies aim not only at the identification of sensory-active metabolites of undesirable microorganisms appearing in alcoholic beverages, but also at linking lower organoleptic quality with the composition of the microflora, which could help avoid the development of fungi and other microorganisms during raw material storage and during fermentation (Darriet et al., 2002; Guerche et al., 2006).

6.3. Using GC–O to control alcoholic beverage production processes

Numerous volatile odour compounds are formed during the production of alcoholic beverages. Their quantitative and qualitative composition depends strictly on the conditions of the process and is the result of enzymatic, microbiological and/or thermal processes. Gas chromatography with olfactometric detection can be used to study the formation of the aroma of alcoholic beverages, as well as the influence of individual factors, such as the type of yeast used for fermentation. Studies indicated that different yeast varieties have different abilities to produce and transform various odour compounds in wines, and that other than the type and maturity of grapes (Ebeler, Terrien, & Butzke, 2000), they determine the final odour of the wine (Delfini et al., 2001). Odour profile of an alcoholic beverage can provide important information for the improvement and optimization of the conditions of the production process. Such studies can be especially useful during the pre-production stages of new beverages made of previously unused raw materials, such as apple cashew, which thus far have been considered waste byproducts of the popular nuts (Garruti et al., 2006).

Determination of volatile odour substances using gas chromatography with olfactometric detection can be used to control technological processes. For example, determination of the increasing content of aldehydes, such as methional or phenylacetaldehyde, formed as a result of the degradation of amino acids according to Strecker's mechanism during the maturation and storage of beer, can be an indicator of the ageing and the lowering of its organoleptic quality (Soares da Costa et al., 2004). Gijs et al. found that the determination of the level of odour compounds such as β-damascenone or dimethyl trisulfide can be a helpful indicator in controlling and optimizing the pH during beer brewing, as the dependence between the pH of beer and the production of these compounds during brewing has been established (Gijs et al., 2002). Studies of a wider scope were conducted by Guyot-Declerc et al. regarding the influence of maturation time and the pH of beer on its organoleptic properties. These authors stressed the dependence of the concentrations of other compounds, such as *trans*-2-nonenal and methional, on the pH of maturing beer. They also claimed that the intensity of dimethyl trisulfide odour increases during maturation regardless of pH, and that the β-damascenon odour does not depend on pH or maturation time.

Another example is the determination of volatile products of Maillard non-enzymatic browning reactions in oak wood extracts coming from barrels used for fermentation and storage of high quality wines and spirits (Cutzach, Chatonnet, Henry, & Dubordieu, 1997). Odour compounds formed during thermal processing of wood can influence the aroma both positively and negatively, therefore barrel production conditions can have a significant influence on the quality of the finished product.

A significant problem, especially in southern countries, is premature degradation of wine aromas because of oxidation, which in these regions proceeds faster as a result of higher temperatures, pH and enzymatic activity in fruits during grape collection and the production of wine. From

the point of view of organoleptic properties, one observes in this case lowered impressions of freshness and fruitiness, accompanied by the appearance of odours resembling paper, cooked, rancid or rotten food, as well as the smell of fodder, hay or wood. Such phenomena might shorten the expiry date, which is why the technique discussed was used to analyze changes in odour profiles of young, white wines, related to the processes of oxidation (Escudero, Cacho, & Ferreira, 2000). The emergence of nineteen compounds likely having a significant influence on sensory changes in wine aromas, as well as the disappearance or lowering of the concentration of fifteen desirable aroma components were observed in the study. The effect of the individual odour components was determined using the aroma extract dilution analysis method (AEDA). This method was also used to identify undesirable odour compounds related to oxidation changes in white, Portuguese wines (Ferreira et al., 2003). The authors, basing on odour comparison of spoiled wine and samples of wine spiked with the analyzed compounds, identified three compounds, among which 3-methylthiopropionic aldehyde was selected as the most characteristic indicator of wines spoiled as a result of oxidation.

6.4. Identifying compounds responsible for aftertaste of alcoholic beverages

An interesting application of the olfactometric detector is GC–O analysis of odour compounds in Chardonnay wines responsible for the aftertaste, the sensation received during sensory evaluation after swallowing the sample. For the determination, polydimethylsiloxane-coated stir bars were used, as in the Stir Bar Sorptive Extraction (SBSE) technique. The stir bars enclosed in glass, perforated capsules were placed in the oral cavities of evaluators who had previously conducted sensory analyses of wines (Buettner & Welle, 2004). Chromatographic analysis of the compounds desorbed from the stir bars yielded odour profiles of compounds which remain in the mouth the longest during wine drinking and are responsible for the aftertaste. A comparison of the results of olfactometric analysis of the extracts obtained using the above technique, named "Buccal Odour Screening System" (BOSS) by the authors, with those for the isolates obtained by dichloromethane extraction and vacuum distillation, indicated that most of the compounds comprising wine aromas, except the most polar ones which interact with saliva, were responsible for the emergence of the aftertaste. However, the holding times of the individual compounds in the oral cavities of the evaluators differed significantly (Buettner, 2004).

In similar studies (Petka et al., 2006), a specially designed extractor imitating the oral cavity was used for isolation of compounds taking part in retronasal perception. The extractor was based on the purge-and-trap principle. The sorption trap was placed at the top of a flask containing a mixture of wine and articial saliva, through which a stream of nitrogen was bubbled.

6.5. Using GC–O to reconstruct alcoholic beverage odours

Literature often emphasizes that the main drawback of gas chromatography with olfactometric detection is the ability to only evaluate the contribution of individual substances on the odour of a given product, neglecting the mutual influence of the odour compounds on the summary perception of scent. Nevertheless, this technique presents a key stage for the reconstruction of food product odours. The most common approach relies on determining the individual odour activity values (OAV) or flavour dilution (FD) values for each identified compound and reproducing the odour composition with the use of substances with the highest OAV or FD values (Ferreira, Petka, et al., 2002; Lorrain et al., 2006). Quite frequently, satisfactory simulations of realistic odours come from mixtures of a few compounds with the highest sensory activity. However, excessive simplification can be counterproductive. For example, Ferreira et al. found that models based on mixtures of odour extracts with OAV values over 0.5 bore the closest resemblance to Spanish Grenache wines; the addition of compounds with OAV values below 0.5 had no practical influence on the aroma. On the other hand, compositions made exclusively from compounds with OAV values over 10 had completely different sensory properties than the original samples (Ferreira, Petka, et al., 2002). Other studies indicated that the specificity of the aroma of a given alcoholic beverage can also be decided by individual compounds with significantly lower OAVs, but characterized by odour different than the other components (Escudero et al., 2004).

To identify key odour components, different kinds of tests are used, such as omission tests (Ferreira, Petka, et al., 2002; Guth, 1997) or addition tests, which evaluate changes in odour caused by omission of individual compounds in a reproduced composition, or changes caused by the addition of individual odour substances (Escudero et al., 2004). Only tests aiming at realistic recreation of the aroma composition can yield information on the summation effects or mutual buffering of compounds entering the aroma of alcoholic beverages (Lorrain et al., 2006).

7. Conclusions

Despite the fact that odour detectors have already been in use for over 40 years, literature indicates that in recent years they have been used more frequently, finding applications especially in the analysis of food and beverages (including alcoholic beverages) (Ruth, 2001). The further investigations are still conducted in order to improve GC–O technique, i.e. to achieve a higher sensitivity and better reliability and repeatability of the results.

It can be clearly concluded from this review that these aims may be principally realized by applying broader range of sample preparation techniques, particularly so-called solventless ones, mainly headspace analysis. These techniques are preferably applied because they combine

extraction and preconcentration in one step, not requiring initial sample preparation, and - contrary to solvent techniques – needing only small amounts of sample. Important advantage of a such approach is also fact that they are preservative techniques, allowing preservation of natural composition and sample characteristics, therefore, the obtained extracts are very representative. It should be added that the newest investigations described in the literature, using GC–O for the analysis of samples taken *in vivo* during alcoholic beverages consumption, are based on solventless sample preparation techniques.

Another perceptible tendency in the development of GC–O is aimed at development of highest repeatability and reliability of the obtained results by unifying, simplifying and shortening of applied procedure. It can be done by improving the instrument use in olfactometric detection. For example, direct intensity method are used universally, particularly time–intensity method, because due to an appropriate detector construction, dynamic continuous measurements with simultaneous registration of aroma stimulus are possible. In the effect, the obtained olfactograms are similar to the chromatograms obtained from conventional detectors. Better repeatability and reliability can be also obtained by using simultaneous detection by several parallel connected olfactometers, performed at the same time by several investigators. There is available information describing modification attempts of GC-coupled with olfactometer, i.e. application of specially cooled interface between two chromatographic columns, which enables detaining eluates from column, increasing chromatographic selectivity, which is described in the review.

Beside the fact that GC–O is known and used since many years there is still need for improvement of familiar techniques and the investigations on using them for quantitative analysis of odour compounds. The further research is also needed because studies on the optimization of working parameters and on the quality and reliability of the obtained results, which is very important considering feasibility of implementing GC–O technique to industrial practice, have not been taken on board very well.

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References

Acree, T. E., Barnard, J., & Cunningham, D. G. (1984). A procedure for the sensory analysis of gas chromatographic effluents. *Food Chemistry, 14*, 273–286.

Aznar, M., Lopez, R., Cacho, J. F., & Ferreira, V. (2001). Identification and quantification of impact odorants of aged red wines from Rioja. GC–olfactometry, quantitative GC–MS, and odor evaluation of HPLC fractions. *Journal of Agricultural and Food Chemistry, 49*, 2924–2929.

Baryłko-Pikielna, N. (1975). *Zarys analizy sensorycznej żywności*. Warszawa: Wydawnictwa Naukowo-Techniczne, pp. 241–295.

Begnaud, F., Starkenmann, C., Waal, M., & Chaintreau, A. (2006). Chiral multidimensional gas chromatography (MDGC) and chiral GC–Olfactometry with a double-cool-strand interface: Application to Malodors. *Chemistry and Biodiversity, 3*, 150–160.

Benn, S. M., & Peppard, T. L. (1996). Characterization of tequila flavor by instrumental and sensory analysis. *Journal of Agricultural and Food Chemistry, 44*, 557–566.

Bernet, C., Dirninger, N., Claudel, P., Etievant, P., & Schaeffer, A. (2002). Application of finger span cross modality matching method (FSCM) by naïve assessors for olfactometric discrimination of Gewurztraminer wines. *Lebensmittel-Wissenschaft und- Technologie, 35*, 244–253.

Bonino, M., Schellino, R., Rizzi, C., Aigotti, R., Delfini, C., & Baiocchi, C. (2003). Aroma compounds of an Italian wine (Ruché) by HS-SPME analysis coupled with GC–ITMS. *Food Chemistry, 80*, 125–133.

Bouchilloux, P., Darriet, P., Henry, R., Lavigne-Cruege, V., & Dubourdieu, D. (1998). Identification of volatile and powerful odorous thiols in Bordeaux red wine varieties. *Journal of Agricultural and Food Chemistry, 46*, 3095–3099.

Buettner, A. (2004). Investigation of potent odorants and afterodor development in two Chardonnay wines using the buccal odor screening system (BOSS). *Journal of Agricultural and Food Chemistry, 52*, 2339–2346.

Buettner, A., & Welle, F. (2004). Intra-oral detection of potent odorants using a modified stir-bar sorptive extraction system in combination with HRGC–O, known as the buccal odour screening system (BOSS). *Flavour Fragrance Journal, 19*, 505–514.

Callemien, D., Dasnoy, S., & Collin, S. (2006). Identification of a stale-beer-like odorant in extracts of naturally aged beer. *Journal of Agricultural and Food Chemistryry, 54*, 1409–1413.

Campo, E., Ferreira, V., Escudero, A., & Cacho, J. (2005). Prediction of the wine sensory properties related to grape variety from dynamic-headspace gas chromatography–olfactometry data. *Journal of Agricultural and Food Chemistry, 53*, 5682–5690.

Cullere, L., Escudero, A., Cacho, J., & Ferreira, V. (2004). Gas chromatography–olfactometry and chemical quantitative study of the aroma of six premium quality Spanish aged red wines. *Journal of Agricultural and Food Chemistry, 52*, 1653–1660.

Cutzach, I., Chatonnet, P., Henry, R., & Dubordieu, D. (1997). Identification of volatile compounds with a "Toasty" aroma in heated oak used in barrelmaking. *Journal of Agricultural and Food Chemistry, 45*, 2217–2224.

Darriet, P., Pons, M., Henry, R., Dumont, O., Findeling, V., Cartolaro, P., et al. (2002). Impact odorants contributing to the fungus type aroma from grape berries contaminated by powdery mildew (Uncinula necator); incidence of enzymatic activities of the yeast *Saccharomyces cerevisiae*. *Journal of Agricultural and Food Chemistry, 50*, 3277–3282.

Darriet, P., Pons, M., Lamy, S., & Dubordieau, D. (2000). Identification and quantification of geosmine, an earthy odorant contaminating wines. *Journal of Agricultural and Food Chemistry, 48*, 4835–4838.

Debonneville, C., Orsier, B., Flament, I., & Chaintreau, A. (2002). Improved hardware and software for quick gas chromatography–olfactometry using CHARM and GC-"SNIF" analysis. *Analytical Chemistry, 74*, 2345–2351.

Deibler, K. D., Acree, T. E., & Lavin, E. H. (1999). Solid phase microextraction application in gas chromatography/olfactometry dilution analysis. *Journal of Agricultural and Food Chemistry, 47*, 1616–1618.

Deibler, K. D., Llesca, F. M., Lavin, E. H., & Acree, T. E. (2004). Calibration of gas chromatography inlet splitting for gas chromatography olfactometry dilution analysis. *Flavour Fragrance Journal, 19*, 518–521.

Delahunty, C. M., Eyres, G., & Dufour, J.-P. (2006). Gas chromatography–olfactometry. *Journal of Separation Science, 29*, 2107–2125.

Delfini, C., Cocito, C., Bonino, M., Schellino, R., Gaia, P., & Baiocchi, C. (2001). Definitive evidence for the actual contribution of yeast in the

transformation of neutral precursors of grape aromas. *Journal of Agricultural and Food Chemistry, 49*, 5397–5408.

Ebeler, S., Terrien, M. B., & Butzke, C. E. (2000). Analysis of brandy aroma by solid-phase microextraction and liquid–liquid extraction. *Journal of the Science of Food and Agriculture, 80*, 625–630.

Escudero, A., Cacho, J., & Ferreira, V. (2000). Isolation and identification of odorants generated in wine during its oxidation: A gas chromatography-olfactometric study. *Eur. Food Res.* Techno.

Escudero, A., & Etievant, P. (1999). Effect of antioxidants on the flavor characteristics and the gas chromatography/olfactometry profiles of champagne extracts. *Journal of Agricultural and Food Chemistry, 47*, 3303–3308.

Escudero, A., Gogorza, B., Melus, M. A., Ortin, N., Cacho, J., & Ferreira, V. (2004). Characterization of the aroma of a wine from Maccabeo. Key role played by compounds with low odor activity values. *Journal of Agricultural and Food Chemistry, 52*, 3516–3524.

Etievant, P. X., Callement, G., Langlois, D., Issanchou, S., & Coquibus, N. (1999). Odor intensity evaluation in gas chromatography–olfactometry by finger span method. *Journal of Agricultural and Food Chemistry, 47*, 1673–1680.

Fan, W., & Qian, M. C. (2005). Headspace solid phase microextraction and gas chromatography–olfactometry dilution analysis of young and aged Chinese "Yanghe Daqu" liquors. *Journal of Agricultural and Food Chemistry, 53*, 7931–7938.

Fan, W., & Qian, M. C. (2006a). Characterization of aroma compounds of Chinese "Wuliangye" and "Jiannanchun" liquors by aroma extract dilution analysis. *Journal of Agricultural and Food Chemistry, 54*, 2695–2704.

Fan, W., & Qian, M. C. (2006b). Identification of aroma compounds in Chinese "Yanghe Daqu" liquor by normal phase chromatography fractionation followed by gas chromatography/olfactometry. *Flavour Fragrance Journal, 21*, 333–342.

Fang, Y., & Qian, M. (2005). Aroma compounds in Oregon Pinot Noir wine determined by aroma extract dilution analysis (AEDA). *Flavour Fragrance Journal, 20*, 22–29.

Ferrari, G., Lablanquie, O., Cantagrel, R., Ledauphin, J., Payot, T., Fournier, N., et al. (2004). Determination of key odorant compounds in freshly distilled cognac using GC–O, GC–MS, and sensory evaluation. *Journal of Agricultural and Food Chemistry, 52*, 5670–5676.

Ferreira, A. C. S., Hogg, T., & Guedes de Pinho, P. (2003). Identification of key odorants related to the typical aroma of oxidation-spoiled white wines. *Journal of Agricultural and Food Chemistry, 51*, 1377–1381.

Ferreira, V., Aznar, M., Lopez, R., & Cacho, J. (2001). Quantitative gas chromatography–olfactometry carried out at different dilutions of an extract. Key differences in the odor profiles of four high-quality spanish aged red wines. *Journal of Agricultural and Food Chemistry, 49*, 4818–4824.

Ferreira, V., Hernandez-Orte, P., Escudero, A., Lopez, R., & Cacho, J. (1999). Semipreparative reversed-phase liquid chromatographic fractionation of aroma extracts from wine and other alcoholic beverages. *Journal of Chromatography A, 864*, 77–88.

Ferreira, V., Lopez, R., Escudero, A., & Cacho, J. F. (1998). The aroma of Grenache red wine: Hierarchy and nature of its main odorants. *Journal of the Science of Food and Agriculture, 77*, 259–267.

Ferreira, V., Ortin, N., Escudero, A., Lopez, R., & Cacho, J. (2002). Chemical characterization of the aroma of Grenache rose wines: Aroma extract dilution analysis, quantitative determination, and sensory reconstitution studies. *Journal of Agricultural and Food Chemistry, 50*, 4048–4054.

Ferreira, V., Petka, J., & Aznar, M. (2002). Aroma extract dilution analysis. Precision and optimal experimental design. *Journal of Agricultural and Food Chemistry, 50*, 1508–1514.

Fretz, C., Kanel, S., Luisier, J.-L., & Amado, R. (2005). Analysis of volatile components of Petite Arvine wine. *European Food Research and Technology, 221*, 504–510.

Fu, S.-G., Yoon, Y., & Bazemore, R. (2002). Aroma-active components in fermented bamboo shoots. *Journal of Agricultural and Food Chemistry, 50*, 549–554.

Fur, Y., Mercurio, V., Moio, L., Blanquet, J., & Meunier, J. M. (2003). A new approach to examine the relationships between sensory and gas chromatography–olfactometry data using generalized procrustes analysis applied to six French Chardonnay wines. *Journal of Agricultural and Food Chemistry, 51*, 443–452.

Garruti, D. S., Franco, M. R. B., Silva, M. A. A. P., Janzantti, N. S., & Alves, G. L. (2006). Assessment of aroma impact compounds in a cashew apple-based alcoholic beverage by GC–MS and GC–olfactometry. *LWT, 39*, 372–377.

Gijs, L., Chevance, F., Jerkovic, V., & Collin, S. (2002). How low pH can intensify β-damascenone and dimethyl trisulfide production through beer aging. *Journal of Agricultural and Food Chemistry, 50*, 5612–5616.

Goj, T. (1998). Zanieczyszczenia chemiczne występujące w spirytusach. *PFiOW, 4*, 18–21.

Grosch, W. (2001). Evaluation of the key odorants of foods by dilution experiments, aroma models and omission. *Chemical Senses, 26*, 533–545.

Guerche, S., Dauphin, B., Pons, M., Blancard, D., & Darriet, P. (2006). Characterization of some mushroom and earthy off-odors microbially induced by the development of rot on grapes. *Journal of Agricultural and Food Chemistry, 54*, 9193–9200.

Gurbuz, O., Rouseff, J. M., & Rouseff, R. L. (2006). Comparison of aroma volatiles in commercial Merlot and Cabernet Sauvignon wines using gas chromatography–olfactometry and gas chromatography-mass spectrometry. *Journal of Agricultural and Food Chemistry, 54*, 3990–3996.

Guth, H. (1997). Quantitation and sensory studies of character impact odorants of different white wine varieties. *Journal of Agricultural and Food Chemistry, 45*, 3027–3032.

Guyot-Declerck, C., Francois, N., Ritter, C., Govaerts, B., & Collin, S. (2005). Influence of pH and ageing on beer organoleptic properties. A sensory analysis based on AEDA data. *Food Quality and Preference, 16*, 157–162.

Hanaoka, K., Sieffermann, J.-M., & Giampaoli, P. (2000). Effects of the sniffing port air makeup in gas chromatography–olfactometry. *Journal of Agricultural and Food Chemistry, 48*, 2368–2371.

Hochereau, C., & Bruchet, A. (2004). Design and application of a GC-SNIFF/MS system for solving taste and odour episodes in drinking water. *Water Science and Technology, 49*, 81–87.

Janusz, A., Capone, D. L., Puglisi, C. J., Perkins, M. V., Elsey, G. M., & Sefton, M. A. (2003). (*E*)-1-(2,3,6-Trimethylphenyl)buta-1,3-diene: A potent grape-derived odorant in wine. *Journal of Agricultural and Food Chemistry, 51*, 7759–7763.

Kishimoto, T., Wanikawa, A., Kono, K., & Shibata, K. (2006). Comparison of the odor-active compounds in unhopped beer and beers hopped with different hop varieties. *Journal of Agricultural and Food Chemistry, 54*, 8855–8861.

Komes, D., Ulrich, D., & Lovric, T. (2006). Characterization of odor-active compounds in Croatian Rhine Riesling wine, subregion Zagorje. *European Food Research and Technology, 222*, 1–7.

Kotseridis, Y., Razungles, A., Bertrand, A., & Baumes, R. (2000). Differentiation of the aromas of Merlot and Cabernet Sauvignon wines using sensory and instrumental analysis. *Journal of Agricultural and Food Chemistry, 48*, 5383–5388.

Lablanquie, O., Snakkers, G., Cantagrel, R., & Ferrari, G. (2002). Characterization of young cognac spirit aromatic quality. *Analytica Chimica Acta, 458*, 191–196.

Lee, S.-J., & Noble, A. C. (2003). Characterization of odor-active compounds in Californian Chardonnay Wines using GC–olfactometry and GC–mass spectrometry. *Journal of Agricultural and Food Chemistry, 51*, 8036–8044.

Lermusieau, G., Bulens, M., & Collin, S. (2001). Use of GC–olfactometry to identify the hop aromatic compounds in beer. *Journal of Agricultural and Food Chemistry, 49*, 3867–3874.

Lermusieau, G., & Collin, S. (2003). Volatile sulfur compounds in hops and residual concentrations in beer – A review. *Journal of the American Society of Brewing Chemists, 61*, 109–113.

Lopez, E. F., & Gomez, E. F. (2000). Comparison of solvents for determination of monoterpenes in wine using liquid–liquid extraction. *Chromatogr., 52*, 798–802.

Lopez, R., Ferreira, V., Hernandez, P., & Cacho, J. F. (1999). Identification of impact odorants of young red wines made with Merlot, Cabernet Sauvignon and Grenache grape varieties: A comparative study. *Journal of the Science of Food and Agriculture, 79*, 1461–1467.

Lopez, R., Ortin, N., Perez-Trujillo, J. P., Cacho, J., & Ferreira, V. (2003). Impact odorants of different young white wines from the Canary Islands. *Journal of Agricultural and Food Chemistry, 51*, 3419–3425.

Lorrain, B., Ballester, J., Thomas-Danguin, T., Blanquet, J., Meunier, J. M., & Fur, Y. (2006). Selection of potential impact odorants and sensory validation of their importance in typical chardonnay wines. *Journal of Agricultural and Food Chemistry, 54*, 3973–3981.

Mariaca, R., & Bosset, J. O. (1997). Instrumental analysis of volatile (flavour) compounds in milk and dairy products. *Lait, 77*, 13–40.

Marin, A. B., Acree, T. E., & Barnard, J. (1988). Variation in odor detection thresholds determined by charm analysis. *Chemical Senses, 13*, 435–444.

Marti, M. P., Mestres, M., Sala, C., Busto, O., & Guasch, J. (2003). Solid-phase microextraction and gas chromatography olfactometry analysis of successively diluted samples. A new approach of the aroma extract dilution analysis applied to the characterization of wine aroma. *Journal of Agricultural and Food Chemistry, 51*, 7861–7865.

Nonato, E. A., Carazza, F., Silva, F. C., Carvalho, C. R., & Cardeal, Z. L. (2001). A headspace solid-phase microextraction method for the determination of some secondary compounds of Brazilian sugar cane spirits by gas chromatography. *Journal of Agricultural and Food Chemistry, 49*, 3533–3539.

Ong, P. K. C., & Acree, T. E. (1999). Similarities in the aroma chemistry of Gewurztraminer variety wines and Lychee (*Litchi chinesis* Sonn.) Fruit. *Journal of Agricultural and Food Chemistry, 47*, 665–670.

Petka, J., Ferreira, V., & Cacho, J. (2005). Posterior evaluation of odour intensity in gas chromatography–olfactometry: Comparison of methods for calculation of panel intensity and their consequences. *Flavour Fragrance Journal, 20*, 278–287.

Petka, J., Ferreira, V., Gonzales-Vinas, M. A., & Cacho, J. (2006). Sensory and chemical characterization of the aroma of a White wine made with Devin grapes. *Journal of Agricultural and Food Chemistry, 54*, 909–915.

Plutowska, B., & Wardencki, W. (2007). Aromagrams – Aromatic profiles in the appreciation of food quality. *Food Chemistry, 101*, 845–872.

Pollien, P., Fay, L., Baumgartner, M., & Chaintreau, A. (1999). First attempt of odorant quantitation using gas chromatography-olfactometry. *Analytical Chemistry, 71*, 5391–5397.

Pollien, P., Ott, A., Montigon, F., Baumgartner, M., Munoz-Box, R., & Chaintreau, A. (1997). Hyphenated headspace-gas chromatography-sniffing technique: Screening of impact odorants and quantitative aromagram comparisons. *Journal of Agricultural and Food Chemistry, 45*, 2630–2637.

Priser, C., Etievant, P. X., Nicklaus, S., & Brun, O. (1997). Representative Champagne wine extracts for gas chromatography olfactometry analysis. *Journal of Agricultural and Food Chemistry, 45*, 3511–3514.

Rossiter, K. J. (1996). Structure–odor relationships. *Chemical Reviews, 96*, 3201–3240.

Ruth, S. M. (2001). Methods for gas chromatography–olfactometry: A review. *Biomolecular Engineering, 17*, 121–128.

Schneider, R., Baumes, R., Bayonove, C., & Razungles, A. (1998). Volatile compounds involved in the aroma of sweet fortified wines (Vins Doux Naturels) from Grenache Noir. *Journal of Agricultural and Food Chemistry, 46*, 3230–3237.

Schneider, R., Rzaungles, A., Augier, C., & Baumes, R. (2001). Monoterpenic and norisoprenoic glycoconjugates of *Vitis vinifera* L. cv. Melon B. as precursors of odorants in Muscadet wines. *Journal of Chromatography A, 936*, 145–157.

Selli, S., Cabaroglu, T., Canbas, A., Erten, H., Nurgel, C., Lepoutre, J. P., et al. (2004). Volatile composition of red wine from cv. Kalecik Karasu grown in central Anatolia. *Food Chemistry, 85*, 207–213.

Serot, T., Prost, C., Visan, L., & Burcea, M. (2001). Identification of the main odor-active compounds in musts from French and Romanian hybrids by three olfactometric methods. *Journal of Agricultural and Food Chemistry, 49*, 1909–1914.

Sides, A., Robards, K., & Helliwell, S. (2000). Developments in extraction techniques and their application to analysis of volatiles in foods. *Trends in Analytical Chemistry, 19*, 322–329.

Simpson, R. F., Capone, D., & Sefton, M. A. (2004). Isolation and Identification of 2-ethoxy-3,5-dimethylpyrazine, a Potent Musty Compound from Wine Corks. *Journal of Agricultural and Food Chemistry, 52*, 5425–5430.

Soares da Costa, M., Goncalves, C., Ferreira, A., Ibsen, C., Guedes de Pincho, P., & Silva Ferreira, A. C. (2004). Further insights into the role of Methional and Phenylacetaldehyde in Lager Beer Flavor stability. *Journal of Agricultural and Food Chemistry, 52*, 7911–7917.

Souza, M. D. C. A., Vasquez, P., Mastro, N. L., Acree, T. E., & Lavin, E. H. (2006). Characterization of Cachaca and Rum Aroma. *Journal of Agricultural and Food Chemistry, 54*, 485–488.

Vermaulen, C., Guyot-Declerck, C., & Collin, S. (2003). Combinatorial synthesis and sensorial properties of Mercapto primary alcohols and analogues. *Journal of Agricultural and Food Chemistry, 51*, 3623–3628.

Wanikawa, A., Hosoi, K., Kato, T., & Nakagawa, K. (2002). Identification of green note compounds in malt whisky using multidimensional gas chromatography. *Flavour Fragrance Journal, 17*, 207–211.