

Study of methods for the extraction of volatile compounds from fermented grape must

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Abstract

The main objective of the present study was to analyze the volatile compounds extracted from fermented grape musts by two extraction methods in order to characterize the samples. The purge and trap system provided the necessary sensitivity for the analysis of low-boiling point compounds, such as acetaldehyde and ethyl acetate, and the liquid–liquid extraction method allowed for the analysis of a great number of medium to high boiling point volatile compounds, such as phenylethyl alcohol, hexanoic and octanoic acids, ethyl hexanoate and ethyl octanoate. Ethyl propionate and propyl acetate, extracted by the liquid–liquid method, characterized the sample of grape must fermented by *Kloeckera apiculata*. The sample of grape must fermented by *Pichia membranaefaciens* was characterized by 2-propanol and 2-hexanone, extracted by the purge and trap system and liquid–liquid method, respectively. These results show that the purge and trap/dynamic headspace system and liquid–liquid extraction method are complementary in the determination of the aroma profiles of fermented grape musts and characterization of the samples.

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1. Introduction

Wine aroma is composed of more than 800 compounds, which have been identified in the volatile fraction. The volatile compounds show variable polarity, solubility, volatility, pH and concentration, and are very unstable. They are easily oxidised by contact with air or degraded by heat. Therefore extraction procedures to qualitatively represent the original wine aroma are very complicated. Several extraction methods have been developed and used, each presenting some advantages

and some disadvantages (Blanch, Reglero, Herraiz, & Tabera, 1991; Etievant, 1996). It is usually necessary to combine different methods to obtain a complete extraction of all the volatile compounds, resulting in extracts truly representative of the sample aroma.

Liquid–liquid extraction is one of the methods used (Gonzales-Viñas, Perez-Coelho, Salvador, Cabezudo, & Martin-Alvarez, 1996; Lamikanra, Grimm, & Inyang, 1996; Villén, Señoráns, Reglero, & Herrariz, 1995). An advantage of this method is that all volatile compounds (low, medium and high volatility) can be analysed in one extraction step, but the method may require solvent evaporation, which, in some cases, results in the loss or degradation of some compounds and formation of others not present in the original wine.

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The purge and trap/dynamic headspace system is a modern instrumental technique suitable for the extraction and concentration of volatile compounds from wine. Dynamic headspace analysis is a technique, making it possible to analyse the volatile fraction without necessarily destroying it. The method involves purging the sample in an inert gas in much the same way as we breathe in the natural flavour of a product, and permits a correlation with sensory studies. The system has been widely used by some authors to analyze the volatile compounds of fruits and beverages (Lubbers, Verret, & Voilley, 2001; Mamede & Pastore, 2004; Rosillo, Salinas, Garijo, & Alonso, 1999) and to correlate the results with sensory analyses (Mamede, Cardello, & Pastore, 2005; Noble, Flath, & Forrey, 1980).

Many volatile flavour compounds are formed during the alcoholic fermentation of grape must (Margalith & Schwartz, 1970). In addition to *Sacharomyces cerevisiae*, other yeasts, such as *Kloeckera apiculata*, *Candida valida* and *Pichia membranaefaciens*, can initiate fermentation in winemaking and (Fleet & Heard, 1994) and contribute to the bouquet of the wine (Margalith & Schwartz, 1970).

This objective this work was to apply two techniques for the extraction of the volatile compounds present in the grape musts fermented by yeasts from the “Serra Gaúcha” and central regions of the State of Rio Grande do Sul (RS-Brazil) to verify the aromatic profile of each sample.

2. Material and methods

2.1. Grape material

White and red 2001 vintage grape must samples (*Vitis vinifera* var. Chardonnay and *Vitis vinifera* var. Pinot Noir) were obtained from the “Serra Gaúcha” region (RS-Brazil).

2.2. Yeast

Four pure cultures of the yeasts were used in the experiments. *Kloeckera apiculata*, *Candida valida* and *Saccharomyces cerevisiae* were isolated from the “Serra Gaúcha” region. *Pichia membranaefaciens* was isolated from the central region of Brazil. All these yeasts are part of the Laboratory of Bioaromas collection.

2.3. Fermentation

The fermentation of the grape musts were started after the inoculation of 10^7 cells/mL into 125 ml conical flasks containing 25 ml of must. The samples were incubated at 15 °C, with shaking at 100 rpm, for 7 days. After 168 h of fermentation the musts were filtered

through a Millipore membrane (0.22 µm pore). The cell free must was frozen at –10 °C until the chromatographic analyses were performed.

2.4. Analytical methods

2.4.1. General

Two techniques were used for the extraction of volatile compounds from the wine samples.

2.4.2. Purge and trap concentrator/dynamic headspace system

The volatile compounds were isolated using the purge and trap concentrator/dynamic headspace system; model HP- G1900-60500 (Hewlett-Packard, USA), equipped with a Tenax trap. A purge vessel containing 2 ml of the cell-free must was connected to the purge and trap unit. The carrier gas was helium, at a flow rate of 30 ml/min through the sample. The following parameters for the isolation and concentration of the volatile compounds were: sample temperature, 30 °C, purge time, 15 min, flow-rate, 30 ml/min, desorption temperature, 180 °C, desorption time, 10 min. The temperature transfer line used was 180 °C.

2.4.3. Liquid–liquid extraction

Twenty-five millilitre samples of each fermented grape must and pure grape must were used according to Bertrand (1981) methodology. Five grams of NaCl were added to each sample, followed, successively, by 2, 2 and 2 ml of the hexane/ethyl ether (1:1, v/v) mixture for extraction. After phase separation, the organic phase was concentrated with nitrogen (gas) to 3 ml and stored at –20 °C until the chromatographic analyses were performed.

2.5. Gas chromatography–mass spectrometry conditions

Gas chromatography was carried out using a Shimadzu 17A gas chromatograph coupled to a QP-5000 – Shimadzu – EM mass spectrometer. An HP- INNOWax (Hewlett-Packard, USA) 30 m × 0.25 mm I.D. capillary column coated with a 0.25 µm layer of cross-linked polyethylene glycol, was used. The carrier gas was helium (1 ml/min) and the temperature was programmed as follows: initial temperature, 35 °C (5 min); 3 °C/min ramp to 140 °C; 10 °C/min ramp to 180 °C, and 5 min hold at 180 °C. The detector temperature was 250 °C and the injector temperature 200 °C, split (100:1). The injection form used to identify the volatile compounds extracted by liquid–liquid method was splitless. The ionization voltage applied was 70 eV and the mass spectra were obtained in a scan range from 35 to 350 *m/z*. The analyses were carried out in triplicate.

3. Results and discussion

3.1. Purge and trap system

Using the purge and trap extraction system, 25 volatile compounds were identified in the aroma of the Chardonnay and Pinot Noir fermented grape musts (Table 1).

The purge and trap/dynamic headspace technique is a suitable method for the normal analysis and quantification of most of the volatile compounds in wine, because the sample preparation is very simple and the extraction and analysis completely automated.

One of the advantages of this method versus liquid–liquid extraction is that the compounds are extracted from the sample matrix without the use of an organic solvent so, in the chromatogram, the solvent peak does not interfere in the analysis. This is very important in the detection of some interesting peaks that elute with the solvent peak, such as acetaldehyde (bp \cong 38.8) and other low boiling point compounds.

However, the liquid–liquid method is only sensitive for the detection of high or medium boiling point volatile components, such as 3-methyl butanol (bp \cong 112.0) and 2-ethyl hexanoic acid (bp \cong 227.6), when present in high concentrations. The extraction of the volatile compounds by dynamic headspace can depend on the nature of the non-volatile components, the increase or decrease in the volatility of the aromas greatly influencing the overall wine aroma (Lubbers & Voilley, 1998).

The technique of purge and trap is only efficient for low boiling point volatile compounds.

Interactions between volatile compounds and non-volatile compounds play a role dynamic headspace analysis. Ethanol is the major volatile organic component of alcoholic beverages. The dynamics of volatility of the aroma compounds of the wine can be modified for ethanol. In ethanol solution, the activity coefficient of the volatile compounds is lower than those observed in water. Furthermore, ethanol leads to modification in protein conformation which tends to reduce the number of bindings sites of the aroma compounds (Druaux, Lubbers, Charpentier, & Voilley, 1995; Lubbers, Voilley, Charpentier, & Feuillat, 1994) Probably this interaction is the reason for the low extraction of the volatile compounds.

3.2. Liquid–liquid extraction

Liquid–liquid extraction allowed for the identification of 40 volatile compounds in the aroma of the fermented grape musts.

As can be seen in Table 1, this method was the best of the two procedures tested in terms of extraction efficiency for the high, medium and low volatility components, allowing for the identification of most of the

known esters, alcohols, acids and aldehydes. The main disadvantage of this method is the use of a toxic organic solvent (diethyl ether/hexane). However, the solvent volume is very small when compared with other extraction methods and, since the extraction is carried out in a closed vessel, the risk of solvent mixture losses is minimized. In this work it was possible to detect ethyl acetate using this method, this being a very low boiling point compound, usually not identified due to solvent interference. Ethyl acetate was not detected in recent research using liquid–liquid extraction method with dichloromethane (Ortega-Heras, González-SanJosé, & Beltrán, 2002).

The compounds 2-hexen-1-ol, 3-hexen-1-ol, heptanol, decanol, benzaldehyde, ethyl hexanoate, ethyl octanoate, hexanoic and octanoic acids were all identified in non-fermented grape must. Fermentation did not influence the detection of these volatile compounds.

Ethyl hexanoate and ethyl octanoate were characteristic of Pinot Noir grape must.

3.3. Characterization of the samples

The application of two methods allowed for the characterization of the samples, based on the detection of the volatile compounds. Many volatile aroma compounds were detected in all the samples, such as 2-methyl butanol, 3-methyl butanol, 1-hexenol, ethyl acetate, isoamyl acetate, acetaldehyde and propanoic, butanoic, hexanoic and octanoic acids, but some compounds were characteristic of specific samples.

The Pinot Noir and Chardonnay grape musts fermented by *Saccharomyces cerevisiae* were characterized by the presence of propanal, butanal, isobutyraldehyde and 3-methyl butanal, without conversion to their respective alcohols. During fermentation, the aldehydes are usually converted to their respective alcohols by the enzyme aldehyde dehydrogenase (Boulton, Singleton, Bisson, & Kunkee, 1996). This characterization was only possible using the purge and trap extraction system, since it was not possible to detect these compounds using liquid–liquid extraction.

The fermentation of these grape musts by *Candida valida* and *Pichia membranaefaciens* led to the formation of butyl acetate and phenethyl acetate, compounds also extracted by the purge and trap system. This last volatile compound was only extracted by the liquid–liquid extraction method. Fermentation, carried out by *Pichia membranaefaciens*, resulted in the production of 2-hexanone, a compound only extracted by the liquid–liquid method. Samples of grape must fermented by *Kloeckera apiculata* showed no difference from the other samples with respect to the presence of propyl acetate and ethyl propionate, compounds with a pleasant aroma. In a previous study (Mamede et al., 2005), the authors found that the samples of grape musts fermented by *Pichia*

Table 1
Volatile compounds identified by each method

Volatile compounds	Reliability of identification*	Extraction method	
		Purge and Trap	Liquid–liquid
		Occurrence**	Occurrence**
<i>Alcohols</i>			
Ethanol ⁽¹⁾	a/b	+	+
1-Propanol ⁽¹⁾	b	+	–
2-Propanol ^(2 and 3)	b	+	–
1-Butanol ^(7 and 8)	b	+	+
1-Hexenol ⁽¹⁾	a/b	+	+
2-Hexen-1-ol ⁽¹⁾	b	–	+
3-Hexen-1-ol ⁽¹⁾	b	–	+
2-Methyl propanol ⁽¹⁾	a/b	+	+
3-Methyl butanol ⁽¹⁾	a/b	+	+
Heptanol ⁽¹⁾	a	–	+
Decanol ⁽¹⁾	a	–	+
Phenylethyl alcohol ⁽¹⁾	b	–	+
<i>Esters</i>			
Methyl acetate ^(2,3,4,5,6 and 9)	a	+	–
Ethyl acetate ⁽¹⁾	a/b	+	+
Ethyl propionate ^(4 and 9)	a	+	–
Ethyl butyrate ^(3,4,6 and 8)	a/b	+	–
Ethyl lactate ^(7 and 8)	a	–	+
Ethyl hexanoate ^(2,5,7 and 9)	a/b	–	+
Ethyl octanoate ^(2,5,7 and 9)	a/b	–	+
Propyl acetate ^(4 and 9)	a/	+	+
Propionate isobutyl ⁽¹⁾	b	–	+
Isopropyl acetate ^(2,3,4,5,6 and 9)	a	+	–
Butyl acetate ^(2,3,5 and 6)	a/b	+	–
Butyl Isobutyrate ^(3,4,6 and 8)	b	+	–
Isoamyl acetate ⁽¹⁾	a/b	+	+
Acetate phenethyl ^(5,6,7 and 8)	b	–	+
Phenethyl acetate ^(2,3,5 and 6)	a/b	+	+
<i>Aldehydes</i>			
Acetaldehyde ⁽¹⁾	a/b	+	–
Propanal ^(7 and 8)	b	+	–
Butanal ^(7 and 8)	b	+	–
Isobutyraldehyde ^(7 and 8)	b	+	–
3-Methyl butanal ^(7 and 8)	b	+	–
Benzaldehyde ⁽¹⁾	a/b	–	+
<i>Acids</i>			
Acetic acid ⁽¹⁾	a/b	+	–
2-Ethyl hexanoic acid ⁽¹⁾	b	+	–
3-Methyl butyric acid ⁽¹⁾	b	–	+
Isopropanoic acid ⁽¹⁾	b	–	+
Lactic acid ^(7 and 8)	b	–	+
Propanoic acid ⁽¹⁾	a/b	–	+
Butanoic acid ⁽¹⁾	a/b	–	+
Hexanoic acid ⁽¹⁾	a/b	–	+
Octanoic acid ⁽¹⁾	a/b	–	+
<i>Miscellaneous</i>			
2-Hexanone ^(2 and 3)	a/b	–	+
3-Hydroxy-3-butanone ^(7 and 8)	a/b	+	+

Compound detected: (1) presented in all the samples; (2) P. Noir must fermented by *P. membranaefaciens*; (3) Chardonnay must fermented by *P. membranaefaciens*; (4) Chardonnay must fermented by *K. apiculata*; (5) P. Noir must fermented by *C. valida*; (6) Chardonnay must fermented by *C. valida*; (7) P. Noir must fermented by *S. cerevisiae*; (8) Chardonnay must fermented by *S. cerevisiae*; (9) P. Noir must fermented by *K. apiculata*.

* Identification: (a) mass spectrum in agreement with spectra found in the NIST mass spectral library and the retention times are the same as those of the pure substances available in the laboratory, estimated on the same column; (b) identification based on the compound assigned by the NIST mass spectral library with a similar index (SI) > 90%.

** Occurrence: (+) compound identified; (–) not detected.

membranaefaciens and *Kloeckera apiculata* presented the highest means for aroma acceptance, similar to that of sparkling wine.

4. Conclusions

Probably the low extraction of the high and medium boiling point compounds by the purge and trap/dynamic headspace system is due to the interaction among aroma compounds, water and ethanol.

The liquid–liquid extraction method require more handling of the sample and is time consuming. The purge and trap system is automated, practical leading to minimal volatile loss.

Compounds, such as propanal, butanal, isobutyraldehyde and 3-methyl butanal, were found in the samples fermented by *Saccharomyces cerevisiae*. These compounds were extracted in this study only by purge and trap system, while 2-hexanone, detected only in the grape musts fermented by *Pichia membranaefaciens*, was extracted exclusively by the liquid–liquid extraction method. These results show that the purge and trap/dynamic headspace system and liquid–liquid extraction method are complementary in the determination of the aroma profiles of fermented grape musts and characterization of the samples.

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References

- Bertrand, A. (1981). Formation des substances volatiles au cours de la fermentation alcoolique. Incidence sur la qualité des vins. *Colloque Société Française Microbiologie*, Reims, 251–267.
- Blanch, G. P., Reglero, G., Herraiz, M., & Tabera, J. (1991). A comparison of different extraction methods for the volatile components of graper juice. *Journal Chromatography Science*, 29, 11–15.
- Boulton, R. B., Singleton, V. I., Bisson, I. F., & Kunkee, R. E. (1996). *Principles and practices of winemaking* (pp. 102–181). Davis: Chapman & Hall.
- Druaux, C., Lubbers, S., Charpentier, C., & Voilley, A. (1995). Effects of physico-chemical parameters of a model wine on the binding of decalactone on bovine serum albumin. *Food Chemistry*, 53, 203–207.
- Etievant, P. X. (1996). Artifacts and contaminants in the analysis of food flavor. *Critical Review Food Science Nutrition*, 36, 733–745.
- Fleet, G. H., & Heard, G. M. (1994). Growth during fermentation. In G. H. Fleet (Ed.), *Wine microbiology and biotechnology* (pp. 27–54). Chur, Switzerland: Harwood Academic Publishers.
- Gonzales-Viñas, M. A., Perez-Coelho, M. S., Salvador, M. D., Cabezudo, M. D., & Martin-Alvarez, P. J. (1996). Changes in gas-chromatographic volatiles of young Arien wines during bottle storage. *Food Chemistry*, 56, 399–403.
- Lamikanra, O., Grimm, C. C., & Inyang, I. D. (1996). Formation and occurrence of flavor components in Noble muscadine wine. *Food Chemistry*, 56, 373–376.
- Lubbers, S., Verret, C. O., & Voilley, A. (2001). The effect of glycerol on the perceived aroma of a model wine and a white wine. *Lebensmittel, Wissenschaft und Technologie*, 34, 262–265.
- Lubbers, S., & Voilley, A. (1998). Flavor–matrix interactions in wine. In A. L. O. Watherhouse & E. Ebeler (Eds.), *Chemistry of wine flavor* (pp. 217–229). Oxford University Press.
- Lubbers, S., Voilley, A., Charpentier, C., & Feuillat, M. (1994). Influence of monoproteins from yeast on the aroma intensity of a model wine. *Lebensmittel-Wissenschaft und-Technologie*, 27, 108–114.
- Mamede, M. E. O., & Pastore, G. M. (2004). Avaliação da produção dos compostos majoritários da fermentação de mosto de uva por leveduras isoladas da região da “Serra Gaúcha” (RS). *Ciência e Tecnologia de Alimentos*, 24(3), 453–458.
- Mamede, M. E. O., Cardello, H. M. A. B., & Pastore, G. M. (2005). Evaluation of an aroma similar to that of sparkling wine: Sensory and gas chromatography analyses of fermented grape musts. *Food Chemistry*, 89, 63–68.
- Margalith, P., & Schwartz, Y. (1970). Advances in applied microbiology. In D. Perlman (Ed.), *Flavor and microorganisms*. New York: Academic Press.
- Noble, A. C., Flath, R. A., & Forrey, R. R. (1980). Wine headspace analysis. reproducibility and application to varietal classification. *Journal Agricultural and Food Chemistry*, 28, 346–353.
- Ortega-Heras, M. O., González-SanJosé, M. L. O., & Beltrán, S. (2002). Aroma composition of wine studied by different extraction methods. *Analytical Chemical Acta*, 458, 85–93.
- Rosillo, L., Salinas, M. R., Garijo, J., & Alonso, G. L. (1999). Study of volatiles in grapes by dynamic headspace analyses. Application to the differentiation of some *Vitis vinifera* varieties. *Journal Chromatography*, 847, 155–159.
- Villén, J., Señoráns, F. J. D., Reglero, G., & Herraiz, M. (1995). Análisis of wine aroma by direct injection in gas chromatography without previous extraction. *Journal Agricultural and Food Chemistry*, 43, 717–722.