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2-Methylbutyl acetate in wines: Enantiomeric distribution and sensory impact on red wine fruity aroma

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ABSTRACT

Enantiomers of 2-methylbutyl acetate were assayed in red and white commercial wines from various vintages and origins, using chiral gas chromatography (γ-cyclodextrin), revealing the exclusive presence of the S-enantiomeric form. Results also confirmed that (S)-2-methylbutyl acetate levels were generally higher in red than white wines of the same age, and that acetate levels increased gradually during ageing. Olfactory threshold of (S)-2-methylbutyl acetate was evaluated at 313 μg/L in dilute alcohol solution (12% v/v) and 1083 μg/L in a fruity aromatic reconstitution, reflecting its presence in wines at subthreshold concentrations. At concentrations considerably lower than its olfactory threshold, 2-methylbutyl acetate was associated with blackberry-fruit and banana notes. It was also revealed that, even at subthreshold concentrations, this compound had a modification on the perception of fruity aromas in the matrices studied. Sensory profiles highlighted, for the first time, its specific contribution to black-, fresh-, and jammy-fruit notes, despite its subthreshold concentrations.

1. Introduction

Various beverages and fruits are known to contain 2-methylbutyl acetate. In wine, its presence has been reported at concentrations from 129 to 200 μg/L in white wines and from 181 to 359 μg/L reds (Baumes, Cordonnier, Nitz, & Drawert, 1986). This compound is usually associated with banana and fruity descriptors and its olfactory threshold was evaluated at 5 μg/L in water and at 160 μg/L in wine (Molina, Swiegers, Varela, Pretorius, & Agosin, 2007; Siebert et al., 2005; Swiegers et al., 2009).

In apples, together with ethyl 2-methylbutanoate, it is a major contributor to the flavour of many cultivars and its presence is correlated with good maturity (Mattheis, Fellman, Chen, & Patterson, 1991). Moreover, 2-methylbutyl acetate concentrations are used to predict the harvest period as it appears a few weeks before ethylene production (Mattheis et al., 1991; Molina et al., 2007). It has also been identified in various fruits, such as bananas (Wyllie & Fellman, 2000), melons (Fallik et al., 2001), and jackfruit (Maia, Andrade, & Zoghbi, 2004).

Acetates may be formed via the Ehrlich reaction from the corresponding amino acid, L-isoleucine (2S, 3S) in this case, during alcoholic fermentation by *Saccharomyces cerevisiae*. They are transformed into α-oxo acids, which are then decarboxylated to produce aldehydes, which are, in turn, reduced to form the corresponding higher alcohol or oxidized into carboxylic acid (Antalick, 2010; Styger, Jacobson, & Bauer, 2011). The 2-methylbutyl acetate synthesis pathway from L-isoleucine (2S, 3S) is illustrated in Fig. S1, Supplementary material.

2-Methylbutyl acetate has one asymmetrical carbon atom, indicating the possible presence of two enantiomers. Previous research into the distribution of these two possible configurations in apples revealed that the (S)-form was totally dominant, with only 0.3% of the (R)-enantiomer detectable (Matich & Rowan, 2007; Schumacher et al., 1998). A very recent study in various fermented foods reported that 2-methylbutyl acetate occurred almost exclusively in the (S)-enantiomeric form, with the exception of few cheeses where the (R)-enantiomeric form was dominant in a 57/43 ratio (R/S, m/m) (Matheis, Granvogl, & Schieberle, 2016).

The goal of this research was to assay 2-methylbutyl acetate in red and white wines from various vintages and origins, using chiral gas chromatography, and evaluate its qualitative and quantitative organoleptic impact on a fruity mixture composed of thirteen ethyl esters and acetates.

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2. Materials and methods

2.1. Chemical compounds

Absolute ethanol (analytical grade, 99.97%) and sodium sulphate (99%) were supplied by Scharlau Chemie S.A, Barcelona, Spain. Microfiltered water was obtained using a Milli-Q Plus water system (resistivity: 18.2 MΩ cm, Millipore, Saint-Quentin-en-Yvelines, France). Tartaric acid and sodium hydroxide were purchased from VWR-Prolabo, Fontenay-sous-Bois, France. Standard-grade purity compounds were obtained from commercial sources as follows: ethyl propanoate, ethyl 2-methylpropanoate, ethyl butanoate, ethyl hexanoate, ethyl octanoate, ethyl 3-hydroxybutanoate (racemic mixture 50:50, m/m), 2-methylpropyl acetate, butyl acetate, ethyl 3-methylbutanoate, hexyl acetate, 2-methylbutyl acetate (racemic mixture 50:50, m/m), and (S)-2-methylbutan-1-ol from Sigma–Aldrich, Saint-Quentin-Fallavier, France; 3-methylbutyl acetate from VWR-Prolabo, Fontenay-sous-Bois, France. Ethyl (2S)-2-methylbutanoate, (S)-2-methylbutyl acetate, ethyl (2R)-2-hydroxy-4-methylpentanoate, and ethyl (2S)-2-hydroxy-4-methylpentanoate were synthesized by Hangzhou Imaginechem Co., Ltd. (Hangzhou, China).

2.2. Samples

(S)- and (R)-2-methylbutyl acetate and (S)- and (R)-2-methylbutan-1-ol were assayed in 78 red and 28 white wines from several vintages and origins (Table 1). The origins of the red wines were as follows: 48 Bordeaux, 15 Burgundy, 4 Rhone Valley, 2 Loire Valley, and 9 Languedoc. The origins of the white wines were as follows: 10 Bordeaux, 8 Burgundy, 1 Rhone Valley, 4 Loire Valley, and 5 Alsace.

2.3. Aromatic reconstitutions

Dilute alcohol solution was prepared with ethanol and microfiltered water, to obtain an ethanol level of 12% vol. (v/v) and 5 g/L of tartaric acid (pH adjusted to 3.5 with sodium hydroxide).

The fruity aromatic reconstitution (FAR) was prepared in dilute alcohol solution, using thirteen ethyl esters and acetates at the average concentrations found in red wines (Lytra, Cameleyre, Tempere, & Barbe, 2015), excluding 2-methylbutyl acetate (Table 2).

Diluted fruity aromatic reconstitution (D-FAR) was used for the particular olfactory threshold establishment, and corresponded to the FAR diluted in 50 mL matrix (from 0.4 mL to 50 mL), with a dilution factor of 2.

2.4. Gas Chromatography-Olfactometry (GC-O) analyses

GC-O analysis was performed as described by Cameleyre, Lytra, Tempere, and Barbe (2015). GC-O analyses were carried out to ensure that the high-purity reference compounds did not contain any odouriferous impurities and to ascertain that the compound considered was responsible for the odour properties identified. Olfactometry analyses were carried out using an HP-6890 gas chromatograph (HP, Wilmington, DE, USA), equipped with a flame ionization detector (FID) and a sniffing port (ODO-I SGE, Ringbow, Australia), both connected to the column exit by a flow-splitter. GC effluent was combined with humidified nitrogen (Air Liquide, France) at the bottom of the glass-sniffing nose (SGE, Victoria, Australia) to avoid nasal dehydration. A micro-volume of each ethyl ester and acetate listed in Table 2, as well as the racemic solution and (S)-enantiomer of 2-methylbutyl acetate, was directly injected in splitless-split mode (injector temperature, 240 °C; splitless time, 30 s; split flow, 50 mL/min). The column was a BP21 (SGE, Ringwood, Australia), 50 m × 0.32 mm i.d., and film thickness was 0.25 μm. The oven was programmed at 40 °C for the four first minutes and the temperature increased at a rate of 10 °C/min up to a final isotherm at 230 °C for 10 min. The carrier gas was hydrogen 5.5 (Air Liquide, France) with a column head pressure of 15 psi.

Table 1
Concentration of (S)-2-methylbutyl acetate in wines.

Ageing time (years)	No. of wines analysed	Average (S)-2-methylbutyl acetate concentrations (μg/L)[a]
Red wine		
2–5	18	54.8 ± 6.2
6–10	28	76.5 ± 7
11–15	17	73.9 ± 6.1
15–22	15	81.4 ± 7
White wine		
2–5	12	15.9 ± 2.4
6–11	14	23.8 ± 4

[a] Average concentrations ± standard deviation.

2.5. Quantitation and separation

2.5.1. Sample preparation

Samples were prepared as described by Lytra et al. (2015). A 100 mL wine sample was spiked with 100 μg/L octan-3-ol as an internal standard. It was then extracted using 8, 4, and 4 mL dichloromethane, with magnetic stirring (700 rpm), for 5 min each and separated in a separating funnel for 5 min. The organic phases were blended, dried over sodium sulphate, and concentrated under nitrogen flow (100 mL/min) to obtain 250 μL wine extract.

2.5.2. Chromatographic conditions

GC–MS analysis was performed as described by Lytra et al. (2015). Gas chromatography analyses were carried out on an HP 5890 GC system coupled to an HP 5972 quadrupole mass spectrometer (Agilent Technologies, Les Ulis, France), equipped with a Gerstel MPS2 autosampler. Injections were in split mode (Split ratio: 30: 1), using a 2 mm i.d. non-deactivated direct linear transfer (injector temperature, 200 °C; interface temperature, 280 C). The column was a Chiraldex Gamma-TA (50 m × 0.25 mm i.d., film thickness: 0.12 μm Astec, Whippany, NJ, USA) for quantitation and separation of 2-methylbutan-1-ol and 2-methylbutyl acetate. The oven temperature was programmed at 40 °C for 1 min, then increased at a rate of 1 °C/min to 70 °C, and finally raised by 3 °C/min to a final isotherm at 200 °C, and maintained for 5 min. The total run time was 93 minutes. The carrier gas was helium N55 (Air Liquide, France) with a constant flow of 1 mL/min. The mass spectrometer was operated in electron impact mode at 70 eV with selected-ion-monitoring (SIM), using 3 characteristic ions for (R)- and (S)-2-methylbutan-1-ol: *m/z* 70 as quantifier and *m/z* 56 and 57 as qualifiers, as well as 3 characteristic ions for (R)- and (S)-2-methylbutyl acetate, *m/z* 70 as quantifier and *m/z* 73 and 55 as qualifiers ((S)-2-methylbutyl acetate LRI, 1208; (R)-2-methylbutyl acetate LRI, 1211). (R)- and (S)-2-methylbutan-1-ol and (R)- and (S)-2-methylbutyl acetate (racemic mixture) were characterized by comparing their linear retention indices and mass spectra with those of standards. For the internal standard two characteristic ions have been selected; *m/z* 83 as quantifier and *m/z* 59 and 101 as qualifiers.

Calibration curves using the racemic mixture of 2-methylbutyl acetate were established in dilute alcohol solution (12%, v/v), using a representative range of its average concentrations in wines (Linear range: 10–600 μg/L racemic mixture). These samples were then extracted under the conditions described above and analysed by GC-MS in SIM mode. The calibration curves were plotted as relative peak areas (analyte versus internal standard), as a function of