

Identification of Cysteinylated Aroma Precursors of Certain Volatile Thiols in Passion Fruit Juice

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Together with 3-mercaptohexan-1-ol and 3-mercaptohexyl acetate, already known to contribute to the aroma of passion fruit (*Passiflora edulis*), 3-mercapto-3-methylbutan-1-ol and 3-mercapto-3-methylbutyl acetate have been identified for the first time in this fruit. 3-Mercaptohexan-1-ol and 3-mercapto-3-methylbutan-1-ol may be produced in vitro from nonvolatile extracts of this fruit by the enzymatic action of a cell-free extract of *Eubacterium limosum*, which has a β -lyase activity on *S*-cysteine conjugates (EC 4.4.1.13). This release strongly suggests that these volatile thiols are present in combined form, as *S*-cysteine conjugates. It was possible to identify the precursor of 3-mercaptohexan-1-ol as *S*-(3-hexan-1-ol)-L-cysteine, in the form of trimethylsilylated derivatives from the juice of this fruit, using GC/MS analysis. The presence of free and combined forms of these volatile thiols in this fruit has now been demonstrated.

Keywords: *passion fruit; S-cysteine conjugate, aroma precursor, 3-mercapto-3-methylbutan-1-ol, 3-mercapto-3-methylbutyl acetate, 3-mercaptohexan-1-ol.*

INTRODUCTION

The powerful characteristic aroma of passion fruit has already been the subject of much research. The free compounds and precursors involved in this aroma described until now were terpenes and norisoprenoids, present in both free and glycosylated form, glycosides of benzyl alcohol, 3-methyl-2-buten-1-ol, and mandelonitrile (Chassagne et al., 1996a,b; Winterhalter, 1990; Engel and Tressl, 1983). Sulfur compounds also contribute to passion fruit aroma (Werkhoff et al., 1998; Shibamoto and Tang, 1990) including two volatile thiols, 3-mercaptohexan-1-ol (3MH) and 3-mercaptohexyl acetate (A3MH), which play an important role (Engel and Tressl, 1991).

Wine tasters sometimes compare the aromas of certain grape varieties, particularly Sauvignon blanc, to that of passion fruit. This olfactory analogy is validated by the presence of 3MH and its acetate in wines made from this grape variety (Tominaga et al., 1996, 1998a). The identification of a cysteinylated precursor of 3MH in Sauvignon blanc grapes (Tominaga et al., 1998c) led us to verify whether this compound is also present in passion fruit, which contain high concentrations of 3MH.

This article reports on the identification of two volatile thiols, 3-mercapto-3-methylbutan-1-ol and its acetate as well as the precursor of 3MH, *S*-(3-hexan-1-ol)-L-cysteine, in passion fruit juice.

MATERIALS AND METHODS

Specific Extraction of Volatile Thiols from Passion Fruit Juice. Fifteen passion fruits from La Réunion were pressed in the laboratory. The untreated juice (approximately 300 mL) was clarified by centrifugation for 10 min at 3800g and extracted using dichloromethane (100 mL \times 2). The

volatile thiols in the organic extract were purified by extracting with *p*-hydroxymercuribenzoate (*p*-HMB), according to the method described by Tominaga et al. (1998b).

Preparing the Juice Extract. The crude extract containing sulfur flavor precursors (CESFPs) from passion fruit was obtained by adapting the method developed by Darriet et al. (1993). Following extraction with dichloromethane, as described above, the juice (250 mL) was centrifuged again (3800g, 10 min). It was then percolated through a grafted C₁₈ silica column (2.2 \times 10 cm) (Alltech; C-Gel C18 C-490). When the column had been rinsed with 50 mL of water, the fraction retained was eluted with 100 mL of ethanol at 10% (v/v) and evaporated to dryness in a vacuum at 35 °C. The residue was suspended in 1 mL of water. The CESFPs thus obtained were washed with dichloromethane (500 μ L \times 2) to remove any contaminant volatile compounds. It was then evaporated to dryness and the residue resuspended in 1 mL of water.

Enzymatic Release of the Volatile Thiols from the CESFPs of Passion Fruit Juice. The volatile thiols were enzymatically released from the CESFPs of passion fruit juice under the conditions described by Tominaga et al. (1998c). The CESFPs of passion fruit juice (200 μ L, corresponding to 50 mL of juice) were incubated with 100 μ L of a cell-free extract of *Eubacterium limosum* for 15 min in 500 μ L of a reagent medium consisting of potassium phosphate buffer (100 mM, pH 8.0) containing 1 mM EDTA, 0.1 mM pyridoxal phosphate, and 1 mM glutathione. The volatile products formed were extracted using 200 μ L of dichloromethane. The organic phase was collected by centrifugation. The combined organic phases from two consecutive procedures were concentrated to 25 μ L under nitrogen flow and analyzed by GC/FPD and GC/MS.

Purifying the CESFPs of Passion Fruit Juice and Preparing Trimethylsilylated Derivatives. The CESFPs corresponding to 100 mL of passion fruit juice was purified by affinity chromatography through a Chelating Sepharose 4B (Pharmacia; code 17-0575-01) column and trimethylsilylated under the conditions described by Tominaga et al. (1998c).

GC/FPD and GC/MS. The conditions under which the volatile thiols were analyzed by GC/FPD and GC/MS were identical to those described by Tominaga and Dubourdieu (1997). The trimethylsilylated derivatives were analyzed by GC/MS under the conditions described by Tominaga et al. (1998c).

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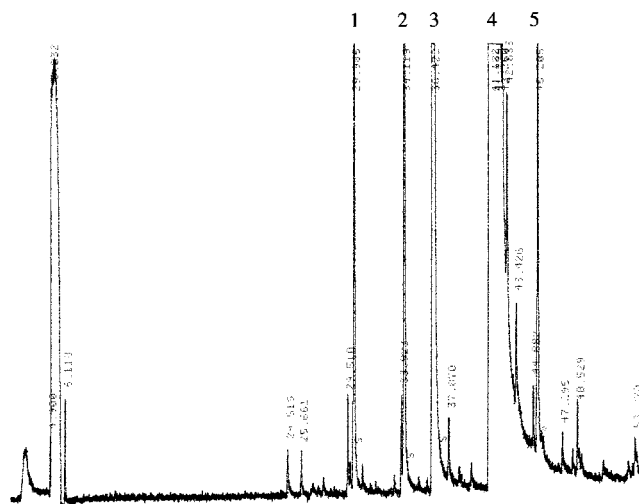


Figure 1. GC/FPD analysis of the volatile thiols extracted from passion fruit (peak 1, 3-mercapto-3-methylbutyl acetate; peak 2, 3-mercapto-3-methylbutan-1-ol; peak 3, 3-mercapto-hexyl acetate; peak 4, 3-mercaptohexan-1-ol; peak 5, 3-mercaptohexyl butanoate).

Syntheses. *3-Mercapto-3-methylbutyl Acetate.* Two drops of acetic anhydride were added to 100 nmol 3-mercapto-3-methylbutan-1-ol (kindly provided by Dr. Joulain, Société Robertet in Grasse, F-06130) in 1 mL of dichloromethane in the presence of anhydrous sodium sulfate. The acetylation reaction continued for 18 h at room temperature. To remove any excess acetic anhydride (acetic acid), the product was purified by selective extraction of the volatile thiols with *p*-HMB, as follows. The sample was diluted in 5 mL of dichloromethane and extracted (1.5 mL \times 3) for 2 min using 1 mM *p*-HMB in a 0.01 N NaOH solution. The three aqueous phases of *p*-HMB together were washed with 2 mL of dichloromethane and 100 mg of powdered cysteine was added. After 5 min, the sample was extracted using 1.5 mL of dichloromethane. One microliter extract was analyzed by GC/MS. *S*-3-(Hexan-1-ol)-L-cysteine: *S*-3-(hexan-1-ol)-L-cysteine was obtained by reducing the *S*-(3-hexan-1-ol)-L-cysteine prepared from *trans*-2-hexenal (Aldrich 13,265-9) and L-cysteine under the conditions described by Tominaga et al. (1998c).

RESULTS AND DISCUSSION

Identifying New Volatile Thiols in Passion Fruit Juice. The volatile thiols in passion fruit juice were specifically extracted using *p*-HMB and purified by percolating the *p*-HMB-thiol complex through a Dowex column. GC/FPD analysis showed five main peaks (Figure 1). The compounds corresponding to peaks 3, 4, and 5 (A3MH, 3MH, and 3-mercaptohexyl butanoate) had already been identified in passion fruit (Engel and Tressl, 1991). Two volatile thiols (peaks 1 and 2), 3-mercapto-3-methylbutyl acetate (A3MMB) and 3-mercapto-3-methylbutan-1-ol (3MMB), were identified for the first time. The latter compound is already known to exist in Sauvignon blanc wines (Tominaga et al., 1998a) and roasted coffee beans (Holscher et al., 1992). To our knowledge, the acetate had never been reported in nature. This compound has a reminiscent aroma of grapefruit peel. Its mass spectrum is presented in Figure 2. However, in our conditions, neither 3-mercaptohexyl pentanoate, recently identified by Werkhoff et al. (1998), nor 3-mercaptohexyl hexanoate (Engel and Tressl, 1991) were present in the extract analyzed.

Enzymatic Release of Volatile Thiols from CESFPs of Passion Fruit Juice. The CESFPs of passion fruit juice were prepared by percolation on a grafted C₁₈

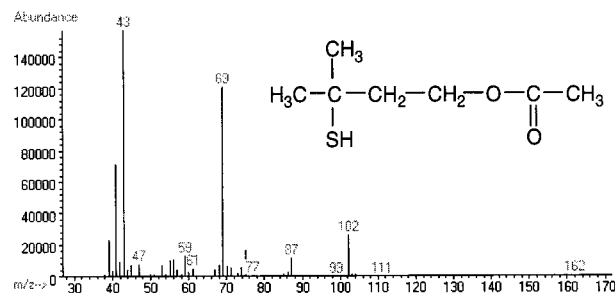


Figure 2. Mass spectrum of 3-mercapto-3-methylbutyl acetate (peak 1 in Figure 1).

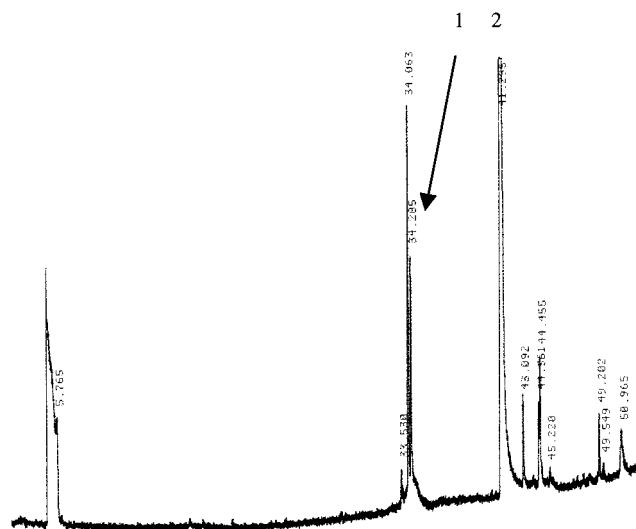


Figure 3. Enzymatic release of 3-mercapto-3-methylbutan-1-ol (peak 1) and 3-mercaptohexan-1-ol (peak 2) from a nonvolatile passion fruit extract in the presence of a cell-free extract of *Eubacterium limosum* which have a β -lyase activity on *S*-cysteine conjugates (EC 4.4.1.13).

silica column and eluted by ethanol. The CESFPs were then exposed to a cell-free extract of intestinal bacteria (*Eubacterium limosum*), which have a β -lyase activity on *S*-cysteine conjugates (EC 4.4.1.13) (Larsen and Stevens, 1986). After incubation for 15 min at 30 °C, the volatile compounds released were analyzed by GC/FPD (Figure 3). Peaks 1 and 2 were identified by GC/MS analysis. 3-Mercapto-3-methylbutan-1-ol (peak 1) and 3-mercaptohexan-1-ol (peak 2) were formed by the action of the bacterial extract on CESFPs of passion fruit juice. However, the acetates of these compounds were never observed among the substances released. If the bacterial homogenate was previously deactivated by heating, no volatile thiols were released (data not shown).

It was, therefore, likely that the two mercapto alcohols in passion fruit juice were also present in the form of *S*-cysteine conjugates, as previously identified in Sauvignon blanc grapes (Tominaga et al., 1998c).

Identifying *S*-3-(Hexan-1-ol)-L-cysteine in Passion Fruit Juice. The *S*-cysteine conjugate was directly identified by GC/MS in the form of trimethylsilylated derivatives. This process had already been used to identify this compound in Sauvignon blanc must (Tominaga et al., 1998c). The CESFPs of passion fruit juice (400 μ L, corresponding to 100 mL of juice), including the sulfur flavor precursors to be identified, were purified by percolating it through a Chelating Sepharose 4B column (Pharmacia). This system is capable of fixing certain

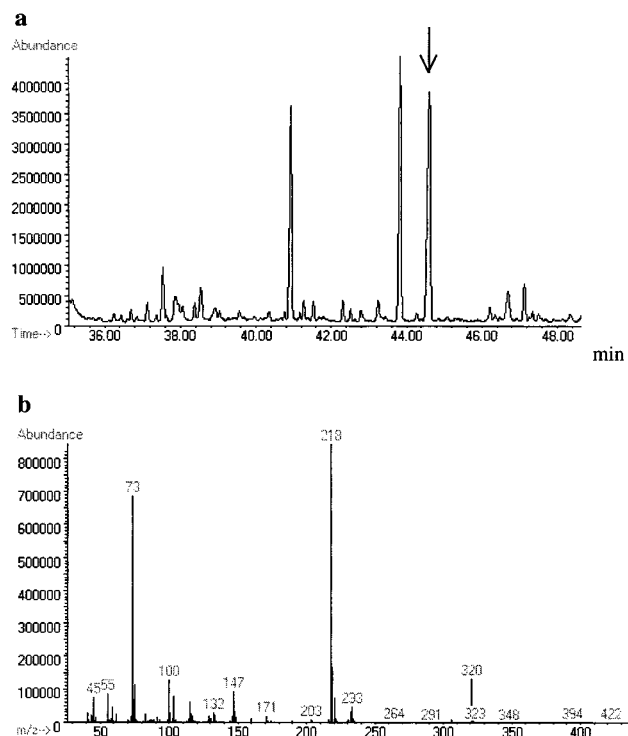


Figure 4. Identifying *S*-3-(hexan-1-ol)-L-cysteine in passion fruit juice. (a) Chromatogram obtained by trimethylsilylation of the purified juice extract. (b) Mass spectrum of the peak in Figure 4a indicated by an arrow.

amino acids (tryptophane, cysteine, etc.) via the intermediary of copper chelate (Belew and Porath, 1990). The purified fraction was trimethylsilylated and analyzed by GC/MS. A peak with retention time identical to that of synthetic *S*-3-(hexan-1-ol)-L-cysteine was obtained on a BPX-5 column (Figure 4a). Its mass spectrum had fragment characteristic of trimethylsilylated *S*-3-(hexan-1-ol)-L-cysteine (Figure 4b). It was not, however, possible to identify the cysteinylated precursor of 3MMB in this sample due to the very small quantities present.

Besides "felinine" [*S*-3-(3-methylbutan-1-ol)-cysteine, a combined form of 3-mercapto-3-methylbutan-1-ol found in cat urine] (Westall, 1953), *S*-cysteine conjugates were first identified in the plant kingdom in must made from *Vitis vinifera* L. cv. Sauvignon blanc (Tominaga et al., 1998c). We report here that this family of chemical compounds is also present in passion fruit juice. In grapes, 3-mercaptohexan-1-ol is mainly present in conjugate form, while in passion fruit juice this volatile thiol is present in both free and conjugate form. The presence of larger quantities of this volatile thiol in wine than grapes is due to the metabolic action of yeast. In passion fruit, the conjugates are probably converted into free thiols either by acid hydrolysis, or by an endogenous enzyme, such as a β -lyase.

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