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Enzymatic hydrolysis of edible *Passiflora* fruit glycosides

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Abstract

The combined action of Hemicellulase REG 2 and sweet almond glucosidase containing β-D-glucopyranosidase, α-L-rhamnopyranosidase, α-L-arabinopyranosidase and α-L-arabinfuranosidase activities allowed release of most of the volatile compounds bound as aglycones in edible *Passiflora* fruits. Great variability between the four species studied, *P. edulis*, *P. edulis* f *falvicarpa*, *P. ligularis*, *P. molissima,* was noticed. 2,5-Dimethyl-4-hydroxy-3-(2H) furanone (furaneol) was identified for the first time in bound form in purple and yellow passion fruit. Only geraniol was identified as the aglycone in *P. molissima*. and no terpenol is present in the hydrolysate obtained from *P. ligularis*. Several terpene diols: 2,6-dimethyl-1,8-octanediol, (E)- and (Z)-2,6-dimethylocta-2,7-diene-1,6-diol, 2,6-dimethylocta-3,7-dien-2,6-diol and 2,6-dimethylocta-1,7-dien-3,6-diol have been identified in both purple and yellow passion fruit and small amounts of (Z)-2,6-dimethylocta-2,7-diene-1,6-diol are present in *P. molissima*. α-Ionol derivatives oxygenated in position 3 seem to be characteristic of purple passion fruit whereas β-ionol compounds oxygenated in position 3 are the major norisoprenoids identified as the aglycone in yellow passion fruit. The bound norisoprenoid content of *P. ligularis* and *P. molissima* is low. Important concentrations of bound aromatic alcohols are found in purple and yellow passion fruit whereas phenolics can be considered as characteristic of purple varieties.

Keywords: Passion fruit; Glycosides; Enzymatic hydrolysis; Aglycones; Variability

1. Introduction

About 530 species have been identified in the Passifloraceae family; these plants grow essentially in tropical regions, but are also present in subtropical and temperate areas. The genus *Passiflora*, which constitutes the passiflora group, is the most important with 450–500 species. Among them, several are cultivated for their fruit: *P. edulis* Sims (purple passion fruit), *P. edulis* f *flavicarpa* Degener (yellow passion fruit or maracuja), the most important, but also *P. ligularis* Juss, *P. quadrangularis*, *P. molissima* Bailey (banana passion fruit).

Passion fruits develop an intense, floral, estery and exotic aroma, with a characteristic sulfury note for the yellow passion fruit.

About 294 volatile compounds have been identified in several passion fruit extracts (Shibamoto & Tang, 1990; Whitfield & Last, 1986). Recently Werkhoff, Güntert, Krammer, Sommer and Kaulen (1998) identified 180 new compounds in yellow passion fruits.

The presence of glycosidically bound volatile compounds in *Passiflora* (*P. edulis* and *P. edulis* f *flavicarpa*) has been ascertained by the release of volatile compounds during their acid or enzymatic hydrolysis (Chassagne & Crouzet, 1995; Engel & Tressl, 1983; Salles, Essaied, Chalier, Jallageas & Crouzet, 1988; Winterhalter, 1990).

Structural studies have allowed the identification of monoglucosides, and several diglycosides as aroma precursors. α-L-Rhamnopyranosyl-β-D-glucosides or rutinosides and α-L-arabinofuranosyl-β-D-glucosides are present in *P. edulis*, *P. edulis* f *flavicarpa* and *P. ligularis* (Chassagne, Crouzet, Bayonove & Baumes, 1997, 1998), whereas α-L-arabinopyranosyl-β-D-glucosides (Chassagne, Crouzet, Bayonove, Brillouet & Baumes, 1996a) have been detected in *P. edulis*. Moreover, β-D-glucopyranosyl-β-D-glucosides or gentiobiosides, and α-L-rhamnopyranosyl-β-D-glucosides of mandelonitrile, have been detected in the glycosidic fraction isolated from several passion fruit species glucosides (Chassagne, Crouzet, Bayonove & Baumes, 1996b; Chassagne & Crouzet, 1998).

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The mechanism of glycoside hydrolysis was described as sequential (Günata, Bitteur, Brillouet, Bayonove & Cordonnier, 1988); the (1→6) linkage is only cleaved by a specific glycosidase and a β-D-glucoside is released. The aglycone and glucose are liberated by the subsequent action of a β-D-glucosidase.

Under these conditions β-D-glucosidase, α-L-rhamnopyranosidase, α-L-arabinofuranosidase and α-L-arabinopyranosidase activities are needed for the hydrolysis of passion fruit glycosides. The purpose of the present work was to make a qualitative and quantitative study of the compounds released by the action of enzymatic preparations containing these enzymatic activities on glucosidic extracts obtained from several edible *Passiflora* fruits.

2. Materials and methods

2.1. Plant material

Purple passion fruits, *Passiflora edulis*, from Kenya, were purchased at Rungis market, France. Yellow passion fruits, *P. edulis* f *flavicarpa* and *P. ligularis*, from the Research Centre of Institut de Recherche Agronomique, Njombé, Cameroun and Colombia were respectively obtained from Rungis market, France, banana passion fruit, *P. molissima*, harvested on Reunion island, were kindly furnished by CIRAD. All fruits were at commercial maturity stage.

The pulp obtained as indicated in Chassagne et al. (1997), was centrifuged (30 min, 10 000*g*) at 4°C and the clear juices obtained were kept at −18°C until analysis.

2.2. Reagents and reference samples

Analytical reagent grade solvents (pentane, dichloromethane) were further purified by distillation before use. Amberlite XAD-2 resin from Röhm and Hass was washed successively with methanol, acetonitrile and diethyl ether (each for 8 h), then dried and stored in methanol according to Günata, Bayonove, Baumes and Cordonnier (1985).

p-NP-β-D-Glucopyranoside, *p*-NP-α-L-rhamnopyranoside, *p*-NP-α-L-arabinopyranoside and *p*-NP-α-L-arabinofuranoside were from Sigma.

Reference aroma compounds, 95–99% purity, were obtained from Fluka, Sigma, Aldrich or Merck, except for 2,6-dimethyl-octa-1,7-dien-3,6-diol, 2,6-dimethyl-octa-3,7-dien-2,6-diol and 3,7-dimethyl-octan-1,7-diol which were a gift from Professor C. Tapiero, Université Montpellier II; (E) and (Z) -2,6-dimethyl-octa-2,7-dien-1,6-diol, 3-oxo-α-ionol, (E) and (Z) -3-oxoretro-α-ionol, 4-oxo-β-ionone, 4-oxo-β-ionol, dehydrovomifiol and 4-oxo-7,8-dihydro-β-ionol which were gifts from P. Winterhalter, Universität of Braunschweig.

n-Paraffins C8-C30, purity >95.5% were from Fluka. Trifluoracetylation (TFA) reagent [*N*-methylbis-(trifluoroacetamide)] was from Pierce (Rockford, IL).

2.3. Enzymes

Hemicellulase REG-2 (Gist-Brocades) and sweet almond glucosidase (emulsin) (Boehringer Mannheim) were used.

β-D-Glucopyranosidase, α-L-rhamnopyranosidase, α-L-arabinopyranosidase and α-L-arabinofuranosidase were purified by Günata et al. (1988) and Günata, Bayonove, Tapiero and Cordonnier (1990) and by Reyné, Salles and Crouzet (1992).

2.4. Isolation of glycosidic compounds

Clear juice (50 ml) was poured onto a 9×1 cm i.d. column filled with solvent-washed XAD-2 at 1.5 ml min^{-1}. The column was rinsed with 50 ml of distilled water and the free volatile compounds were eluted with 50 ml of pentane-dichloromethane (2:1, v/v). The glycosidically-bound components were then eluted using 50 ml of methanol. The eluate was dried over anhydrous sodium sulfate, filtered and concentrated to dryness under vacuum at 45°C. The residue constituted the crude glycosidally bound fraction (Günata et al., 1985).

2.5. Enzyme assay

β-D-Glucopyranosidase, α-L-rhamnopyranosidase, and α-L-arabinopyranosidase and α-L-arabinofuranosidase activities were determined by spectrometric measurement of *p*-nitrophenol (pNP) liberated by hydrolysis of the corresponding pNP-glycoside. One volume of enzymatic solution (diluted if necessary) was added to one volume of substrate solution 4 mM in acetate buffer 100 mM, pH 4.4. The mixture was incubated at 40°C for 10 min and the reaction stopped by addition of 1 ml of sodium carbonate solution (1 M). The liberated *p*-nitrophenate ion was quantified by absorbance determination at 400 nm. The results are expressed as nanokatal per ml of enzymatic solution.

2.6. Enzymatic hydrolysis

The glycosidic extracts obtained from 0.5 ml of clear juice were concentrated under vacuum to dryness, redissolved in 0.3 ml of 0.2 M citrate-phosphate buffer (pH 5) and extracted five times using pentane-dichloromethane (2:1). To the residue were added:

Fifty microlitres of purified enzymatic preparation in 0.2 M citrate–phosphate buffer pH 5 (α-L-rhamnopyranosidase, 3.15 nkat/ml; α-L-arabinofuranosidase 3.37 nkat/ml; α-L-arabinopyrano-sidase, 1.57 nkat/ml and β-D-glucopyranosidase, 1.34 nkat/ml); or 0.3 ml of industrial

enzymatic preparations (12.5 mg of hemicellulase REG-2, 12.5 mg of hemicellulase REG-2 and 20 mg of almond glucosidase in 1 ml 0.2 M citrate–phosphate buffer pH 5).

The mixtures were incubated at 40°C for 16 h. After cooling to room temperature, 32 μg of 4-nonanol were added as internal standard and the mixtures extracted five times with 300 μl of pentane-dichloromethane (2:1). This aglycone extract was concentrated to a final volume of 300 μl by microdistillation at 37°C. Controls were run with heat-inactivated enzymes (30 min at 95°C).

2.7. Trifluoracetylation of glycosides

The bound fraction obtained from 0.5 ml of juice or 0.5 g of berry (*P. molissima*) or a mixture of synthetic glycosides was concentrated to dryness, in a small screw-capped vial at 60°C under nitrogen and derivatized with the TFA reagent (*N*-methyl-bis-trifluoro-acetamide) according to the method of Voirin, Baumes, Günata, Bitteur, Bayonove & Tapiero (1992). Phenyl β-D-glucopyranoside (10 μg) was used as an internal standard.

2.8. GC analysis

A Varian 3300 gas chromatograph equipped with split injector (1/10) and flame ionization detector was used. For aglycones, two fused silica capillary columns (J&W Scientific, Folson, CA) were employed: (a) DB Wax and (b) DB-5MS (30 m×0.25 mm i.d., film thickness = 0.25 μm).

The temperature programs were: (a) 3 min isothermal at 60°C and then increased at 2°C/min to 220°C; (b) increased from 40 to 200°C at 2°C/min. In the two cases the carrier gas was H_2 at 1.8 ml/min; N2 at 30 ml/min was used for the make-up. The injector temperature was maintained at 250°C and the detector temperature was (a) 250°C and (b) 300°C. Linear retention indices were calculated using n-paraffin standards (Van den Dool & Kratz, 1963).

For trifluoracetylated glycosides, a DB-5MS fused silica capillary column was used (30 m×0.25 mm i.d., film thickness = 0.25 μm, J&W Scientific, Folsom, CA). The column temperature was programmed at 3°C/min from 125 to 220°C, then increased at 2°C/min to 280°C and held at this temperature for 15 min. The flow rates for the carrier gas (H_2), and the makeup were the same as those mentioned above. The injector temperature was maintained at 280°C and the detector temperature at 300°C.

As indicated above, 4-nonanol and phenyl β-D-glucopyranoside were used as internal standard for quantification of aglycones and glycosides, respectively; it was assumed that the response factor was equal to one for all the compounds. Three analyses, extraction and measurement, were carried out to determine the variation coefficient of each aglycone identified.

2.9. GC–MS analysis

A Varian 3400 gas chromatograph coupled to an Automass 020 (Unicam, Argenteuil, France) mass spectrometer was used for electron impact (EI) spectra recording. The DB-5MS and the temperature program were the same as described above. Helium was used as carrier gas at 2 ml/min. The temperature of the ion source was 160°C and the ionization voltage 70 eV, the filament current was 0.376 mA and the mass range was 35–250 scanned at 1 s/decade.

2.10. Repeatability

Three analyses, extraction and measurement, were carried out on each aglycone extract to determine the variation coefficient for each component identified.

3. Results and discussion

3.1. Use of purified enzymes

Three samples of a glycosidic extract obtained from *P. edulis* were hydrolyzed by the following purified enzymes: α-L-rhamnosidase, α-L-arabinofuranosidase, α-L-arabinofuranosidase and β-D-glucosidase.

The trifluoracetylated glycosides present in the initial extract or unhydrolyzed sample after enzymatic action were analyzed by GC and the results are given Fig. 1.

The chromatographic profile obtained before any enzyme action [Fig. 1(a)] was modified by the action of the α-L-rhamnosidase [Fig. 1(b)], we observed a decrease for several peaks 5, 6, 8 corresponding to α-L-arabinopyranosyl β-D-glucopyranosides (retention time between 30 and 45 min) and an increase of peaks 1, 2, 3 corresponding to glucosides (retention time between 15 and 30 min). These modifications indicate a partial hydrolysis of the components present in the glycosidic extract. On the other hand, no sensible modification of the chromatographic profile was observed after the action of α-L-arabinofuranosidase [Fig. 1(c)]. This result is in good agreement with the identification of several arabinopyranosyl-glucopyranosides (Chassagne, et al., 1996a) and of only one arabinopyranosyl-glucopyranoside (Chassagne et al., 1998).

Under the combined action of α-L-rhamnosidase and β-D-glucosidase [Fig. 1(d)] most of the glycosides, except the compounds identified as linalyl β-D-glucoside and arabino-pyranosyl-glucopyranosides, were hydrolyzed.The presence of linalyl glucoside after hydrolysis can be explained by the poor substrate affinity of Aspergillus niger β-D-glucosidase for tertiary alcohol glycosides (Günata et al., 1990).

These results indicate that most of the glycosidically bound volatiles present in purple passion fruit can be

hydrolyzed by a mixture containing α-L-rhamnosidase, α-L-arabinofuranosidase, α-L-arabinopyranosidase and β-D-glucosidase activities.

3.2. Use of industrial enzymes

In a second step, we searched for industrial enzymes able to give the same result. Preliminary assays (Chassagne, Bayonove, Crouzet & Baumes, 1995) have shown that hemicellulase REG2 gives the best results for the hydrolysis of some passion fruit bound aroma compounds, linalool, geraniol, nerol and 2-phenylethanol. This enzyme is known to possess high β-glucosidase and α-L-rhamnosidase activities and weak α-L-arabinopyrano-sidase activity (Cordonnier, Günata, Baumes & Bayonove, 1989). The result obtained by the action of this enzyme on the passion fruit glycosidic extract is approximatively the same as that obtained by the conjugated action of pure β-glucosidase and α-L-rhamnosidase, vicianosides and linalyl β-D-glucoside are scarcely hydrolyzed (Fig. 2).

According to Cordonnier et al. (1989), sweet almond emulsin possesses an α-L-arabinopyranosidase activity tenfold greater than that of hemicellulase REG2. The addition of 40 nkat/ml of arabinopyranosidase, present in emulsin, to hemicellulase allowed total hydrolysis of benzyl and 3-methyl-but-2-en-1-ol glucoside (Fig. 3). However, only a partial hydrolysis of linalyl vicianoside was performed; 20% was hydrolyzed for an arabinopyranosidase activity equal to 80 nkat/ml. This enzyme mixture was used for the release of the aglycone moiety of several *Passiflora* fruits.

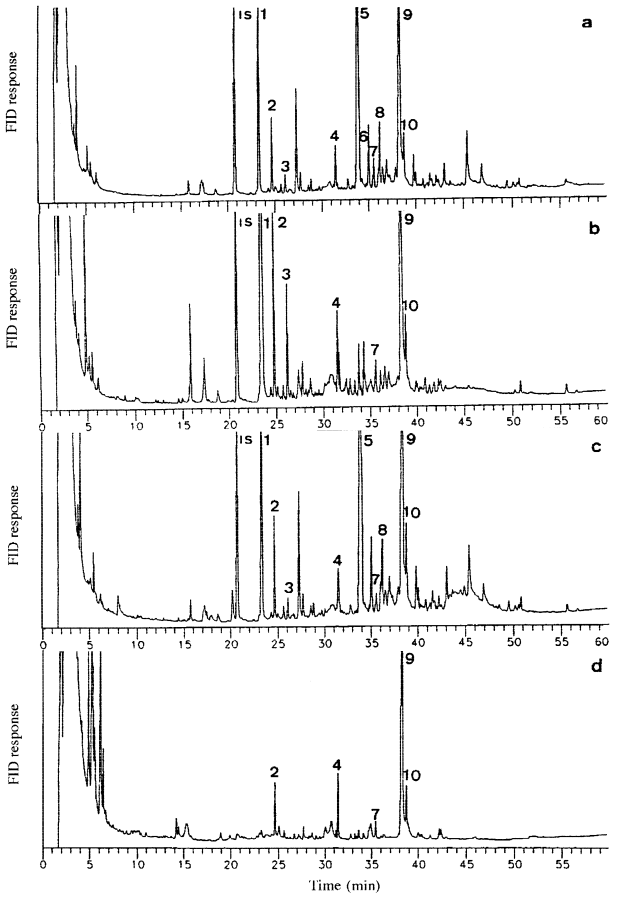

Fig. 1. Gas chromatogram of trifluroacetylated glycosides from *Passiflora edulis* (a) before enzymatic treatment, (b) after the action of α-L-rhamnopyranosidase, (c) after the action of α-L-arabinofuranosidase, (d) after the combined action of α-L-rhamnopyranosidase and β-D-glucosidase. IS: internal standard (phenyl β-D-glucopyranoside); 1: benzyl β-D-glucopyranoside; 2: (S)-linalyl β-D-glucopyranoside; 3: 2-phenylethyl β-D-glucopyranoside; 4: 3-methyl-but-2-en-1-yl α-L-arabinopyranosyl β-D-glucopyranoside; 5: benzyl α-L-rhamnopyranosyl β-D-glucopyranoside; 6: (S)-linalyl α-L-arabinopyranosyl β-D-glucopyranoside; 7: benzyl α-L-arabinopyranosyl β-D-glucopyranoside; 8: 2-phenylethyl α-L-rhamnopyranosyl β-D-glucopyranoside; 9: unidentified α-L-arabinopyranosyl β-D-glucopyranoside; 10: (S)-linalyl α-L-arabinopyranosyl β-D-glucopyranoside.

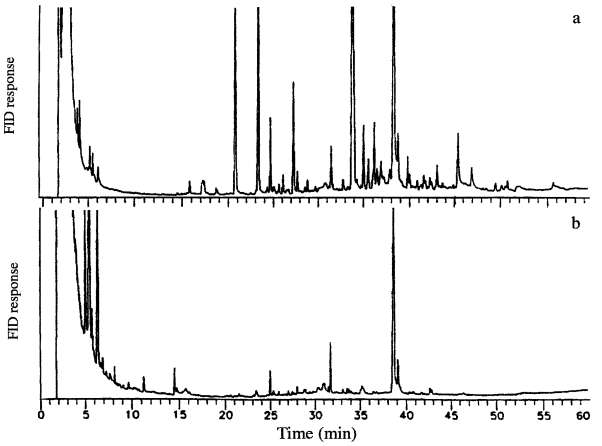

Fig. 2. Gas chromatogram of trifluroacetylated glycosides from *Passiflora edulis* (a) before enzymatic treatment, (b) after the action of hemicellulase REG2.

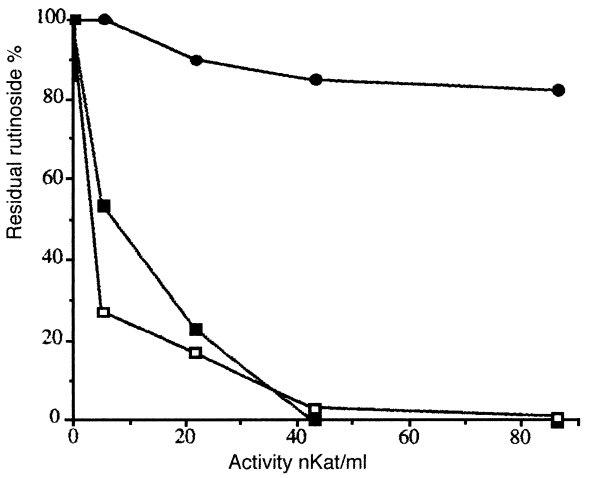

Fig. 3. Hydrolysis of: ●—● linalyl, □—□ benzyl, ■—■ 3-methyl-but-2-en-1-yl α-L-arabinopyranosyl-β-D-glucoside by hemicellulase REG2 and α-L-arabinopyranosidase enzymatic activities (from emulsin) varying from 0 to 85 nkat/ml.

3.3. Volatile compounds released by enzymatic hydrolysis

The volatile compounds released after enzymatic hydrolysis of heterosidic extracts obtained from *P. edulis*, *P. edulis* f *falvicarpa*, *P. ligularis*, *P. molissima*, were identified by GC–MS and GC using reference compounds. Two columns possessing different polarity, DB5 and DB Wax, were used.

Several alcohols, identified as alycones, were previously identified (Table 1) by Engel and Tressl (1983) in yellow passion fruits; however, heptan-2-ol, octan-1-ol (Z)-3-octen-1-ol and octan-1,8,-diol were identified for the first time and methyl and ethyl 3-hydroxyhexanoate were tentatively identified. A great variability between the four species was noticed; only a few alcohols were released from the glycosidic fraction of *P. ligularis* and *P. molissima*.

Although the alcohols present in *P. edulis* and *P. edulis* f. *flavicarpa* are qualitatively identical, quantitative differences appear between these two species. The quantities of (Z)-3-hexen-1-ol are higher in purple passion fruit than in yellow fruits whereas the reverse is true for octan-1-ol. Moreover, 2,5-dimethyl-4-hydroxy-3-(2H) furanone (furaneol®), 236 μg/kg in the purple passion fruit and 534 μg/kg in the yellow passion fruit, was identified for the first time in bound form in passion fruit. This compound was very recently reported as a free volatile compound of yellow passion fruit (Werkhoff et al., 1998). The two isomers of the TFA furaneyl glucoside in the enantiomeric ratio 57/43 have been detected in *P. flavicarpa* (Fig. 4). This glucoside has been previously identified in strawberry (Mayerl, Näf & Thomas, 1989) and mango (Sakho, Chassagne & Crouzet, 1997).

Most of the terpene alcohols present in the hydrolyzate obtained from yellow and purple passion fruits (Table 2). have been identified in bound form (Engel & Tressl, 1983; Winterhalter, 1990). Only geraniol was identified as aglycone in *P. molissima* and no terpenol was present in the hydrolyzate obtained from *P ligularis*.

Several terpene diols, reported by Winterhalter (1990) as purple passion fruit glycosidically bound compounds, e.g. 2,6-dimethyl-1,8-octanediol, (E)- and (Z)-2,6-dimethylocta-2,7-diene-1,6-diol, have been identified in both purple passion fruit and yellow passion fruit. Morever, 2,6-dimethylocta-3,7-dien-2,6-diol and 2,6-dimethylocta-1,7-dien-3,6-diol have been detected in the enzymatic hydrolysate of the glycosidic extract of these two species.

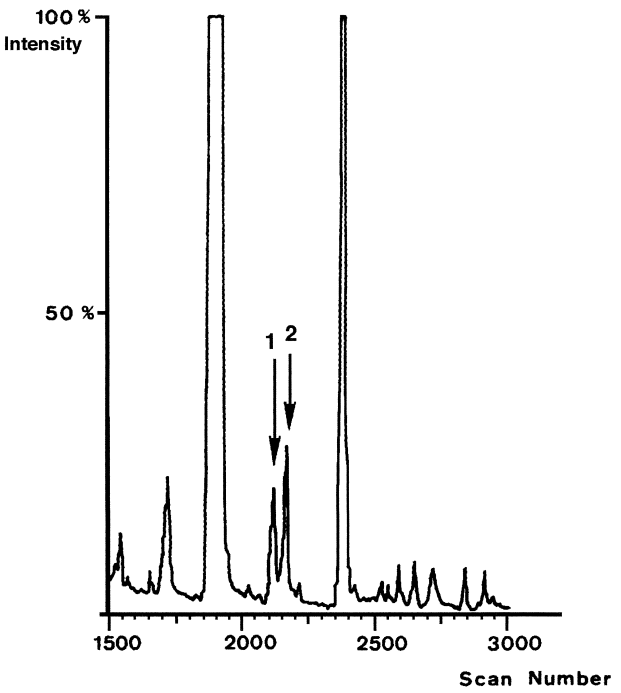

Fig. 4. Separation of the two diastereoisomers of 2,5-dimethyl-4hydroxy-3-(2H) furanone glucoside (furaneyl glucopyranosides) 1 and 2 in *Passiflora edulis* by GC–MS, total ionic current.

Table 1
Alcohols and hydroxy compounds enzymatically liberated from a glycosidic extract from edible *Passiflora* fruits (μg/kg)

Aglycone	RI[a]	RI[b]	*P. edulis*	*P. edulis f. flavicarpa*	*P. ligularis*	*P. molissima*
3-Methyl-but-3-en-1-ol	1246	–	137 ± 2	20 ± 10	–	–
3-Methyl-but-2-en-1-ol	1322	800	887 ± 43	677 ± 30	133 ± 5	103 ± 13
Hexan-1-ol	1355	863	717 ± 28	696 ± 48	–	–
(Z)-3-hexen-1-ol	1384	848	475 ± 14	72 ± 4	–	1034 ± 13
Heptan-2-ol	1322	900	35 ± 14	45 ± 1	3 ± 1	–
(Z)-3-octen-1-ol	–	1049	–	44 ± 8	–	tr
Octan-1-ol	1559	1071	239 ± 13	1235 ± 62	–	136 ± 8
Octan-1,8-diol	2366	1373	53 ± 10	–	–	–
Methyl 3-hydroxyhexanoate[c]	1640	1048	118 ± 25	–	199 ± 30	–
Ethyl 3-hydroxyhexanoate[c]	1670	1126	tr	–	20 ± 10	–
2,5-Dimethyl-4-hydroxy-3-(2H) Furanone	2024	1056	236 ± 93	534 ± 73	–	–

[a] Retention indices on DB Wax.
[b] Retention indice on DB 5MS.
[c] Tentatively identified (MS).

These two compounds have been identified in free form in the water-soluble fraction obtained after liquid-liquid extraction of yellow passion fruit pulp using $CHCl_3$ as solvent (Engel & Tressl, 1983). 2,6-Dimethylocta-7-ene-2,6-diol, reported by Winterhalter (1990) among the aglycones of purple passion fruit, was not found. Small amounts of (Z)-2,6-dimethylocta-2,7-diene-1,6-diol are present in *P. molissima*. These polyols were previously found in free or bound form in several other fruits such as grape (Rapp & Knipser, 1979; Williams, Strauss, & Wilson, 1980a; Williams et al., 1980b; Rapp, Mandery & Ullemeyer, 1983; Strauss, Wilson & Williams, 1988), papaya fruit (Winterhalter, Katzenberger & Schreier, 1986) or Lulo fruit (Wintoch, Morales, Duque & Schreier, 1993). They are known to produce, by acid catalyzed rearrangement, several monooxygenated compounds such as linalool oxide, nerol oxide, hotrienol, linalool or α-terpineol (Engel & Tressl, 1983; Strauss et al., 1988; Williams et al., 1980b).

The most important differences with regard to previously-reported data are the quantities of linalool and α-terpineol liberated after SDE at pH 3 by Engel and Tressl (1983); these authors reported 4800 μg/kg for linalool in yellow passion fruit whereas our results indicate only 500 μg/kg. This difference can result from the total hydrolysis of linalyl glycosides and from the rearrangement of the free polyhydroxylated compounds identified by Engel and Tressel (1983) during heating at the native pH of juice.

For purple passion glycosides the quantity of linalool liberated by enzymatic hydrolysis is 1400 μg/kg whereas Winterhalter (1990) found only 250 μg/kg. It can be assumed that only partial hydrolysis was performed in the conditions used by this author whereas a greater yield was obtained when the optimized mixture: hemicellulase REG2 and emulsin, was used.

Concerning α-terpineol, our results are in good agreement with those of Winterhalter (1990) for purple passion fruits whereas there is an important discrepancy in the results obtained for yellow passion fruits. Engel and Tressl (1983) reported 2000 μg/kg of this compound whereas we found only traces. This difference can be explained by the degradation of polyols and by the rearrangement reactions of terpene compounds occurring during the acid hydrolysis of glycosides (Engel & Tressl, 1983). α-Terpinyl glycosides were not detected among the glycosidic fraction obtained from the four species studied (Chassagne et al., 1998).

The variability of the aglycone content of edible *Passiflora* is clearly apparent when the C_{13} norisoprenoids are considered (Table 3). The norisoprenoid content of the hydrolyzate obtained from *P. edulis* f. *flavicarpa* (10 mg/kg) was threefold greater than that obtained from *P. edulis* (3 mg/kg). Moreover, unidentified compounds possessing the mass spectra characteristics of norisoprenoids were detected in the glycosidic extract hydrolyzate obtained from *P. edulis* f. *flavicarpa*. The norisoprenoid content in the hydrolyzate obtained from *P. molissima* (1360 μg/kg) and from *P. ligularis* (588 μg/kg) was less important than that determined for purple and yellow passion fruits.

The results for the norisoprenoid aglycones detected in purple passion fruit are in good agreement with those reported by Winterhalter (1990); however, 4-hydroxy-7,8-dihydro-β-ionol was not detected, whereas 3,4-dihydro-3-oxo-edulan was tentatively identified. This compound can be a precursor or a derivative of edulan (Werkhoff et al., 1998). Only four of the norisoprenoid compounds identified in the aglycone mixture obtained from purple passion fruit were also identified in the similar extract from yellow passion fruit: 3-oxo-α-ionol, 4-oxo-β-ionol, 4-oxo-7,8-dihydro-β-ionol and 3-oxo-7,8-dihydro-β-ionol. α-Ionol derivatives oxygenated in position 3 seem to be characteristic of purple passion fruit whereas β-ionol compounds oxygenated in position 3 are the major norisoprenoids identified as aglycones in yellow passion fruit. The concentration of 4-oxo-7,8-dihydro-β-ionol in the aglycone mixture isolated from *P. edulis* f. *flavicarpa* was 7-fold greater than the concentration of this compound isolated from *P. edulis* or *P. ligularis*.

Table 2
Terpenic alcohols and diols enzymatically liberated from a glycosidic extract from edible *Passiflora* fruits (μg/kg)

Aglycone	RI[a]	RI[b]	*P. edulis*	*P. edulis f. flavicarpa*	*P. ligularis*	*P. molissima*
Linalool	1549	1100	1442 ± 49	498 ± 15	–	–
α-Terpineol	1689	1189	78 ± 12	tr	–	–
Citronellol	1760	1225	112 ± 21	430 ± 221	–	–
Nerol	1800	1220	113 ± 15	53 ± 7	–	–
Geraniol	1849	1249	367 ± 63	381 ± 38	–	35 ± 1
2,6-Dimethylocta-3,7-dien-2,6-diol	1949	1176	163 ± 27	–	–	–
2,6-Dimethylocta-2,7-dien-3,6-diol	2135	1270	130 ± 31	–	–	–
2,6-Dimethyl-1,8-octanediol	2216	–	314 ± 133	350 ± 212	–	–
(E)-2,6-Dimethylocta-2,7-dien-1,6-diol	2267	1333	121 ± 48	152 ± 49	–	–
(Z)-2,6-Dimethylocta-2,7-dien-1,6-diol	2317	1358	782 ± 229	753 ± 90	–	81 ± 12

[a] Retention indices on DB Wax.
[b] Retention indices on DB 5MS.

If we eliminate the compounds resulting from the hydrolysis of cyanogenic glycosides—benzaldehyde and mandelonitrile—the main aromatic compounds liberated by the enzymatic hydrolysis are aromatic alcohols, aromatic acids and phenols (Table 4).

Benzyl alcohol is the most abundant aromatic alcohol, principally in the aglycone fraction isolated from *P. edulis* (13 mg/kg) and *P. edulis* f. *flavicarpa* (6 mg/kg); lower concentrations are found in *P. ligularis* and *P. molissima* (Whitfield & Last, 1986).

Bound aromatic alcohols are frequently reported in fruits (Stahl-Biskup, Intert, Holthuijzen & Stengele, 1993); however their concentration is generally lower than that found in purple and yellow passion fruits. Except for eugenol and methyl salicylate, phenolic compounds can be considered characteristic of purple passion fruit. According to the results reported by Winter and Kloti (1972) and Whitfield and Last (1986) concerning the low contribution of phenols to passion fruit aroma, it can be postulated that these compounds, which are phytotoxic, are principally present in bound form.

Cinnamic acid was detected in yellow and purple extracts, whereas benzoic acid and cinnamic alcohols are present only in the aglycone fraction isolated from purple passion fruit. Cinnamic alcohol has recently been reported in free form in yellow passion fruit (Werkhoff et al., 1998).

Table 3
C13 norisoprenoids enzymatically liberated from a glycosidic extract from edible *Passiflora* fruits (μg/kg)

Aglycone	RI[a]	RI[b]	*P. edulis*	*P. edulis f. flavicarpa*	*P. ligularis*	*P. molissima*
3,4-Dihydro-3-oxo-edulan[c]						
3-Hydroxy-β-damascone	1958	1445	194 ± 50	–	–	–
3-Hydroxy-β-ionol[c]	2518	–	–	84 ± 2	–	–
3-Oxo-α-ionol	2540	1631	–	3158 ± 729	–	–
4-Oxo-β-ionol	2613	1627	597 ± 227	35 ± 6	54 ± 39	231 ± 39
4-Oxo-β-ionone	2620	1659	710 ± 268	169 ± 23	51 ± 18	131 ± 32
4-Oxo-7,8-dihydro-β-ionol	2632	–	92 ± 34	–	–	–
3-Hydroxy-7,8-dihydro-β-ionol[c]	2653	1700	164 ± 78	1134 ± 215	173 ± 26	29 ± 10
3-Hydroxy-β-ionone[c]	2656	1646	–	917 ± 163	–	–
3-Oxo-7,8-dihydro-β-ionol[c]	2661	1675	–	4176 ± 852	–	482 ± 13
3-Oxoretro-α-ionol (1)	2685	1684	443 ± 183	482 ± 93	310 ± 39	367 ± 27
3-Oxoretro-α-ionol (2)	2716	1692	601 ± 227	–	–	70 ± 14
Dihydrovomifoliol	2860	1749	241 ± 103	–	–	55 ± 9
Vomifoliol	–	1769	69 ± 13	–	–	–
	–	1774	45 ± 2	–	–	–

[a] Retention indices on DB Wax.
[b] Retention indices on DB 5MS.
[c] Tentatively identified (MS).

Table 4
Aromatic compounds enzymatically liberated from a glycosidic extract from edible *Passiflora* fruits (μg/kg)

Aglycone	RI[a]	RI[b]	*P. edulis*	*P. edulis f. flavicarpa*	*P. ligularis*	*P. molissima*
Benzaldehyde	1500	954	16039 ± 539	16261 ± 499	209 ± 73	–
Methyl salicylate	1747	1182	761 ± 6	tr	306 ± 28	75 ± 37
Benzyl alcohol	1860	1031	13609 ± 1434	6039 ± 273	753 ± 80	–
2-Phenylethanol	1893	1107	1269 ± 159	722 ± 61	175 ± 12	91 ± 7
Eugenol	2145	1343	915 ± 5	593 ± 57	267 ± 19	86 ± 12
4-Ethylphenol	2164	1163	281 ± 53	–	–	594 ± 71
Cinnamic alcohol	2252	–	90 ± 6	–	–	–
4-Allylphenol[c]	2327	1249	1049 ± 82	tr	–	–
Isoeugenol	2330	1428	46 ± 4	–	–	–
Benzoic acid	2405	–	255 ± 21	–	–	69 ± 7
Mandelonitrile	–	1477	10612 ± 288	11234 ± 2030	–	–
Cinnamic acid	2845	1435	908 ± 316	3659 ± 42	–	–
Methyl 2,5-dihydroxybenzoate	2985	1519	± 41	–	–	–

[a] Retention indices on DB Wax.
[b] Retention indices on DB 5MS.
[c] Tentatively identified (MS).

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