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Contribution to aroma potential of Andean blackberry (*Rubus glaucus* Benth.)

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ABSTRACT

Free and glycosidically bound volatiles of purees from Andean blackberry (*Rubus Glaucus* Benth.) fruit were determined. Free volatile compounds were extracted by two techniques: solvent extraction and Solid-Phase Micro-Extraction (SPME). The glycosidically bound volatiles were isolated using Solid Phase Extraction (SPE) technique. The analysis of free volatiles and enzymatically released aglycons were performed by GC-MS. Fifty five volatile compounds were identified and quantified from solvent extraction. The major class was the carboxylic acids (68.8%), dominated by palmitic acid (16.8%) and benzoic acid (15.0%). Alcohols were highly represented (23.4%), with 2-heptanol (10.6%) and terpinen-4-ol (5.7%) being prevalent compounds of this class. Using HS-SPME, among the seventy-one identified compounds, alcohols (47.3%) including 2-heptanol (17.9%) and terpinen-4-ol (20.0%), and esters (39.8%) with ethyl and methyl benzoate (33.9 and 3.8% respectively) were the two predominant classes. β-damascenone, a potent flavour compound was detected using SPME method. Fifty-three aglycons were identified, acids (57.4%), norisoprenoids (15.4%), terpenic alcohols (10.3%) and some aliphatic and shikimic alcohols (15.0%) predominated in the glycosidically bound fraction. Acetylenic precursors of β-damascenone were present at noticeable level in the glycosidic fraction.

1. Introduction

Blackberry fruits (*Rubus* spp.) and derived products are highly esteemed by consumers because of their pleasant flavour and taste. Andean blackberry (*Rubus glaucus* Benth) is a native fruit from the north of the Andes in South America. It has also been planted in many other tropical areas and widely cultivated in Ecuador, Costa-Rica and Colombia. The berry presents small drupes on a receptacle about 1–2.5 cm long. Ripen berries are dark red or purple and have a pleasant aroma and sweet–sour taste. This fruit is nowadays widely cultivated in South America and the annual production is surpassing 10,000 tons in Colombia. Fruits are essentially consumed as jams, juices, and for ice-cream and yoghurt flavouring (Morales et al., 1996).

Aroma potential of fruits is made of free volatiles and volatile precursors such as glycosides that often occur at higher levels than their free counterparts (Chassagne, Boulanger, & Crouzet, 1999). Acid or enzymatic hydrolysis of glycosides release odor-active aglycons which can be potent odorants in several cases. Indeed, the contribution of the released aglycons to the flavour of several fruits such as grape (Günata, Bayonove, Baumes, & Cordonier, 1985), acerola (Boulanger & Crouzet, 2001), pineapple (Wu, Kuo, Hartman, Rosen, & Ho, 1991) including the fruits from *Rubus* genus, such as Andean blackberry from Colombia (Duque et al., 2005), raspberry (*R. idaeus*) (Pabst, Barron, Etievant, & Schreier, 1991) or blackberry (*R. lacinata* L.) (Humpf & Schreier, 1991) has been reported.

Free volatile composition of Andean blackberry (Morales et al., 1996) and effect of freeze-concentration and enzymatic clarification by pectinase (Ramos, Delgado, Bautista, Morales, & Duque, 2005) on juice flavour were studied. Among 44 volatile compounds identified alcohols and esters were the main free volatile compounds (Morales et al., 1996). Enzymatic treatment of pulp led to increase in the concentration of several volatiles including some alcohols (Ramos et al., 2005).

The aim of this work was to contribute to the characterisation of Andean blackberry fruit coming from Ecuador with the objective of better using aroma potential during fruit processing.

2. Materials and methods

2.1. Fruit material

10 kg of blackberries (*Rosaceae* family, *Rubus Glaucus* Benth.) were obtained from a local market in Ecuador (Ambato). Intact and mature fruits (Brix/acid ratio 4.0) were carefully selected and air-shipped to France in ice-box (+4 °C). After washing, the fruits were rapidly crushed in a Waring blender (Waring®, USA) for 3 min. Enzymatic or chemical oxidations that could occur during crushing were not prevented to have the samples close to those obtained in industrial conditions. The resulting puree was kept at −20 °C until analysis.

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2.2. Reagents and chemicals

All solvents used were of high analytical grade. Dichloromethane and methanol were purchased from Carlo Erba (Rodano, Italy), diethyl ether (purity more than 99.8%) was obtained from Fluka (Buchs, Switzerland) and n-pentane (purity 99%) from Sigma (Steinheim, Germany). Aroma standards were mainly from Sigma and Fluka. Cartridge Supelclean™ LC-18 was from Supelco (France) and enzymatic preparation AR2000 from DSM (France).

2.3. Extraction of aroma compounds

2.3.1. Extraction of free volatile compounds

After 5 min of defrosting, the fruit puree (10 g) was spiked with 15.4 µg of n-octanol in ethanol as internal standard. Volatiles were extracted with 50 mL of a pentane/ether mixture (1:1, v/v) using a Potter Elvejhem (5 min, room temperature). The upper organic phase was recovered, dried over anhydrous sodium sulphate, concentrated (Vigreux column) at 37 °C to ca. 1 mL. The organic extract was stored at −20 °C until analyzed. Each extraction was triplicated.

2.3.2. Headspace Solid-Phase Micro-Extraction (HS-SPME) of free volatiles

Three SPME fibers (Supelco), PDMS (polydimethylsiloxane, 100 µm), PDMS–DVB (polydimethylsiloxane–divinlybenzene, 65 µm) and CAR-PDMS-DVB (Carboxen-polydimethylsiloxane–divinlybenzene, 50 µm) respectively were tested for the extraction of the volatiles from fruit puree (unpublished data). Qualitative differences among the fibers were not clearly evidenced. PDMS–DVB fiber was chosen since it yielded chromatograms with fewer artefacts.

Different parameters such as time, sampling temperature, puree dilution rate, effect of salt and GC injection conditions were studied. The best conditions were as follows: 1.25 g puree added with 3.75 mL NaCl solution (42%) was placed into a 10 mL glass vial. The vial was tightly capped with a PTFE-faced silicon septum and magnetically stirred. The equilibrium lasted for 10 min at 60 °C and the adsorption of analytes by SPME fiber for 20 min at 60 °C. Each extraction was replicated three times.

2.3.3. Extraction of glycosidically bound volatile compounds and enzymatic release

20 g puree was added with 50 mL methanol containing 10 mM of β-D-gluconolactone to inhibite β-glucosidase activity (Sarry & Günata, 2004). Magnetically stirred for 90 min at 4 °C, the mixture was then centrifuged (23,800 g, 4 °C, 20 min) and the supernatant concentrated under vacuum to dryness. The dry residue was dissolved in 6 mL distillated water.

The extract was then subjected to solid phase extraction (SPE) on C_{18} reversed phase column (LC-18, 0.5 g, Supelco) successively pre-conditioned with 10 mL methanol and water. After loading 6 mL of extract, the resin was rinsed with 25 mL water followed by 25 mL pentane/ether (1:1, v/v) to remove the free volatile fraction. The glycosidically bound compounds were eluted with 25 mL methanol. Methanol fraction was concentrated under vacuum to dryness at 40 °C. The extract was re-solubilised in 3 mL phosphate–citrate buffer (0.2 M, pH 5) and washing four times with pentane/ether (1:1, v/v) mixture. After addition of 800 µL of glycosidase rich enzyme preparation (AR2000, 70 mg mL^{-1} in previous phosphate–citrate buffer) the mixture was incubated at 40 °C for 18 h. Released aglycons were then extracted (5×1 mL) with pentane/ether (1:1, v/v). The organic layer was spiked with 3.08 µg of n-octanol in ethanol as internal standard, dried over anhydrous Na_2SO_4, filtered and concentrated (Dufton column) at 40 °C to ca. 600 µL prior to GC–MS analysis. All extractions were performed in triplicate.

2.4. GC–MS analysis

GC-MS analyses were carried out using an Agilent 6890 gas chromatograph coupled with Agilent Mass Spectrometer (5973 N). Volatile compounds were separated on a DB-Wax (J&W Scientific, Folsom, CA, USA) fused silica capillary column (30 m×0.25 mm i.d.×0.25 µm film). Helium was used as carrier gas at a constant flow-rate of 1 mL min^{-1}.

The injector temperature was 250 °C. On-column injection (2 µL) was performed for organic extracts. The initial oven temperature was 40 °C and then increased at 3 °C min^{-1} to 250 °C and maintained for 20 min. For SPME analysis, the injection (250 °C) was performed in splitless mode for 1 min. The initial oven temperature was 40 °C and then increased at 3 °C min^{-1} to 160 °C, then from 160 °C to 240 °C at 10 °C min^{-1}. Mass spectrometer operated in EI mode (70 eV) with mass range from 40 to 350 Da. Source, interface and quadruple temperatures were 230 °C, 280 °C and 150 °C respectively.

2.5. Compounds identification and quantification

Identifications were performed using either NIST (2002 version) or Wiley 275 spectra library, spectra data from authentic compounds and comparison of linear retention index (determined by injection of a series of n-alkanes) with those found in literature. *n*-Octanol was used as internal standard for semi-quantification. Amounts were expressed as µg *n*-octanol equivalent kg^{-1} of fresh weight.

3. Results and discussion

3.1. Composition in free volatile compounds

Purees from Andes berry fruits were subjected both to solvent and HS-SPME extractions. Solvent extraction was used for semi-quantitative determinations of volatiles. SMPE technique was used for qualitative purposes and comparison of both extraction methods. Quantification by SPME technique was not possible in a non homogenous matrix like puree without the use of isotope analogues or standard addition technique. Since a great number of targeted volatile compounds were not available, SPME technique for the quantification was abandoned.

The number of free volatiles identified by SPME technique (71) was significantly higher than the solvent extraction one (55) (Table 1). Particularly, 5 aliphatic aldehydes were detected by SPME while absent in organic extract. 14 terpenes and 11 terpenic alcohols were detected by SMPE but only 6 for each of them by solvent extraction. Sesquiterpene hydrocarbons such as α-and β-phellandrene, and α- and β-caryophyllene were only detected in SPME while absent in organic extract. β-damascenone, a potent flavour compound with low odor threshold (2 ppt in water, (Buttery, Teranishi, Ling, & Turnbaugh, 1990) and fruity and floral notes was only detected by SPME analysis. Such qualitative differences between liquid–liquid extraction and SPME have been extensively reported in the literature. They are due to the selectivity of SPME fiber and that of the organic solvent and also to the matrix effect that could be different when sampling the headspace or sample matrix (Yang & Peppard, 1994; Psillakis & Kalogerakis, 2002).

When relative abundance of volatiles was considered by both extraction techniques, the most abundant classes were organic acids (68.8%), aliphatic and shikimic alcohols (16.0%), terpenic alcohols (7.4%) and esters (6.0%) by solvent extraction contrary to esters (39.8%), terpenic alcohols (28.8%), aliphatic and shikimic alcohols (18.5%) and terpenes (8.7%) by SPME technique. Interestingly by both extractions techniques the predominant compounds in main volatile classes were the same (*i.e.* benzoic acid, 2-heptanol, ethyl benzoate and terpinen-4-ol).

Morales et al. (1996) using a similar solvent extraction technique for Andean blackberry volatiles from Colombia detected 44 volatiles,