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Original article

Quality, typicity and potential valorization of *Piper borbonense*, a poorly known wild pepper from Reunion Island

M. Weil[1,a], R. Boulanger[2], G. Morel[2], A. Servent[2], A. Shum Cheong Sing[3] and P. Bohuon[4]

[1] CIRAD UMR Qualisud, F-97410 Saint-Pierre, Réunion, France. Qualisud, Université Montpellier, CIRAD, Montpellier SupAgro, Université d'Avignon, Université de La Réunion, Montpellier, France

[2] CIRAD, UMR QualiSud, F-34398 Montpellier, France. Qualisud, Université Montpellier, CIRAD, Montpellier SupAgro, Université d'Avignon, Université de La Réunion, Montpellier, France

[3] Université de la Réunion, Faculté des Sciences et Technologies, Laboratoire de Chimie des Substances Naturelles et des Sciences des Aliments (LCSNSA), 15, avenue René Cassin, Moufia, Réunion, France

[4] Montpellier SupAgro, UMR QualiSud, F-34093 Montpellier, France. Qualisud, Montpellier SupAgro, CIRAD, Université Montpellier, Université d'Avignon, Université de La Réunion, Montpellier, France

Summary

Introduction – ***Piper borbonense* from Reunion Island is an overlooked wild pepper that remains unutilized today. The purpose of this multidisciplinary work was to study its anatomy, morphology and biochemical composition with a view to its possible commercial development.** *Materials and methods* – **We determined its biochemical composition using, notably, gas and liquid chromatography plus spectroscopic methods.** *Results and discussion* – **This pepper differs from *Piper nigrum* through the pedicel, which forms an integral part of the peppercorn. It can be distinguished from other tailed peppers, such as *Piper cubeba* and wild peppers from Madagascar, through its ovoid shape. Its compounds of interest, essential oil and piperine, are mostly present in the perisperm. Starch (41% db) is its main constituent. *Piper borbonense* has low pungency (piperine content: 0.2% db) and high aroma potential (essential oil content: 9.8% db), distinguishing it from *Piper nigrum* and bringing it closer to the tailed peppers, such as *Piper cubeba* and the wild peppers of Madagascar. Its aroma composition, very rich in monoterpenes, notably limonene (27%), can be considered as that of a good quality pepper.** *Conclusion* – **The typicity of *Piper borbonense* affords an interesting potential for domestication and valorization.**

Keywords
Indian Ocean, composition, piperine, essential oil, aroma compounds

Significance of this study

What is already known on this subject?

- The wild peppers from Madagascar (*Piper* spp.), named Tsiperifery, are used and consumed locally. If not domesticated, they are exported and sold at high prices, notably to Europe. The wild pepper *Piper borbonense* from Reunion Island is not cultivated and is little consumed; it has not been described in the scientific literature.

What are the new findings?

- The low pungency (piperine, 0.2%) and high aroma potential (essential oil, 9.8%) of *Piper borbonense* bring it closer to some other tailed peppers such as *Piper cubeba* and the wild peppers of Madagascar, but sets it apart from *Piper nigrum*.

What is the expected impact on horticulture?

- The aroma composition of *Piper borbonense* suggests a pepper of good quality which, associated with its typicity (high essential oil content and very low piperine content), affords an interesting potential for its domestication and valorization.

Introduction

There are around 700 species of pepper worldwide (Sumathykutty *et al.*, 1999). In 2017, the production of this spice amounted to 690,000 tons, with 32% supplied by Vietnam, the leading producer ahead of Indonesia and India (FAO Statistics Division, 2019). Although several species are domesticated today, *Piper nigrum* accounts for the overwhelming majority of production. Pepper, which is particularly consumed and appreciated for its ability to enhance the taste and aroma of food (Dhas and Korikanthimath, 2003; Schweiggert *et al.*, 2007), is also known and used for its functional properties (Nisha *et al.*, 2009; Suresh *et al.*, 2007). The same authors explain that the culinary and medicinal (anti-microbial and antioxidant) virtues of pepper come from different constituents, such as piperine, volatile compounds of essential oil, polyphenols and carotenoids.

Black pepper (*Piper nigrum*) is well documented in the scientific literature (Jayashree *et al.*, 2009; Menon and Padmakumari, 2005a; Zachariah *et al.*, 2010) and an international standard (International Standard Organization, 1998) sets out commercial specifications. For other species of pepper that are less common but are grown and consumed today, such as *Piper cubeba* (Bos *et al.*, 2007; Jirovetz *et al.*, 2002) or *Piper longum* (Varughese *et al.*, 2016), the literature primarily focuses on their aroma composition. The wild peppers from Madagascar (*Piper* spp.), named Tsiperifery, are used and consumed locally. They are also exported and sold

[a] Corresponding author: mathieu.weil@cirad.fr.

at high prices, notably to Europe. The processing methods for these Malagasy peppers have been described (Weil *et al*., 2014). *Piper borbonense* (Miq.) C. DC. Piperaceae, which is also a wild pepper, is common in the low and medium-altitude rainforests of the Reunion Island (Inventaire National du Patrimoine Naturel, 2017); work is under way to prove its endemic nature. This hand-picked and non-cultivated plant was described in 1857 as a "fortifying and depurative" infusion effective to treat mouth diseases and "cure scurvy" (Lavergne, 2016). *Piper borbonense* is very little used nowadays apart from the vine which is prepared by some traditional tea makers for its medicinal properties. Although this pepper is well distributed throughout the island, being found for example along the Langevin river, at Grand Etang, or at Anse des Cascades, it is yet to be utilized, though it seems to offer worthwhile potential. It is not cultivated and is little consumed, it has not been described in the scientific literature, apart from a single article on how its quality is affected by processing (Weil *et al*., 2014). The aim of this study was to provide a detailed morphological, anatomical and biochemical characterization of *Piper borbonense*, with the hypothesis to determine its typicity and potential interest for valorization.

Materials and methods

Plant material

Piper borbonense was authenticated by Christian Fontaine from the Conservatoire Botanique National de Mascarin. The specimen (internal reference number: 1002054) is kept in the herbarium of the CBNM in Reunion Island. We collected, at the end of 2015, wild mature *Piper borbonense* (Miq.) C. DC. Piperaceae, in a limited area of around 3,000 square meters, from a place called Rivière Langevin (DMS: -21°2'04.49''S; 55°38'33.07''E) in the very south of Reunion Island. La Réunion is a French overseas department and an island in the Indian Ocean, 600 km east of Madagascar and 175 km southwest of Mauritius. Pepper spikes were picked from vines that could climb 10 meters high on their live supports. Equal quantities of the different collections of spikes were frozen at -80 °C (Froilabo freezer, Bio Memory, 690 L) before being pooled to form one single batch. The frozen peppercorns with their pedicels were separated from the spikes by hand prior preparation for description and analyses.

Sample preparation

According to needs (whole pepper or milling) for future measurements and analysis, the pepper was defrosted for two hours at room temperature or ground (still frozen) for 10 seconds at 10,000 rpm in a mill (Retsch – Grindomix GM200, Retsch GmbH, Germany) for all the analyses.

Analytical methods

1. Peppercorn mass, length and diameter. The mass was determined with a precision balance (Scaltec SBC 22 model, Scaltec GmbH, Germany). The precision of the balance was ± 0.1 mg. The length and diameter were determined with a digital calliper (Absolute Digimatic, CD-15CPX model, Mitutuyo Corporation, Sakado, Japan). The precision of the equipment was ± 0.2 mm.

2. Dry matter content. The dry matter content (mean "essential oil-free dry matter") was obtained by drying 5 g of ground pepper in an aluminium cup in the oven (ULE 400, Memmert GmbH, Germany) at 105 °C for 30 h (*i.e*., until constant weight). The initial and final masses were determined with a precision balance (Scaltec SBC 22 model, Scaltec GmbH, Germany). The mean relative standard deviation of repeatability was ± 0.84% ($n = 3$). Water content expressed on a dry basis was deduced from the essential oil and dry matter content.

3. Piperine content. The piperine content, expressed on a dry basis, was determined by the spectrophotometric method described in ISO 5564 (International Standard Organization, 1982). The spectrophotometer used was a Thermospectronic Helios α v4.60 (Thermo Fisher Scientific, USA). The mean relative standard deviation of repeatability was ± 7.3% ($n = 3$).

4. Essential oil content. The essential oil content, expressed on a dry basis, was determined using a method adapted from standard ISO 6571 (International Standard Organization, 2008). One single modification made to the applied method was the elimination of xylene. The mean relative standard deviation of repeatability was ± 2.2% ($n = 3$).

5. Identification and quantification of essential oil compounds.

Separation on a polar column. Volatile compounds were analysed on a GC (HP 6890), equipped with a Supelco-Wax polar column (Supelco, 60 m × 320 µm × 0.25 µm) coupled to a MS detector. Aliquots (0.1 µL) of concentrated essential oil (obtained as described above) were injected into the GC–MS in split mode (1:30). The temperature of the transfer line was 250 °C and the flow rate of the gas carrier (Helium) was 0.8 mL min^{-1}. The temperature programme was as follows: initial temperature 60 °C, heating rate of 4 °C min^{-1} until a final temperature of 230 °C was reached and maintained constant for 20 min. The molecules were identified using a GC/MS (HP 6890) functioning in electron impact (70 eV) mode. The mass range was between 25 and 350 m/z.

Separation on a non-polar column. Volatile compounds were analysed with a GC (HP 6890), equipped with an SPB-5 non-polar column (Supelco, 60 m × 320 µm × 0.25 µm) coupled to a MS detector. Aliquots (0.2 µL) of concentrated essential oil (obtained as described above) were injected into the GC–MS in split mode (1:50). The temperature of the transfer line was 250 °C and the flow rate of the gas carrier (Helium) was 0.7 mL min^{-1}. The temperature programme was as follows: initial temperature 60 °C, heating rate of 4 °C min^{-1} until a final temperature of 250 °C was reached, then maintained constant for 50 min. The molecules were identified using a GC/MS (HP 6890) functioning in electron impact (70 eV) mode. The mass range was between 20 and 400 m/z.

Identification. The aroma compounds separated on the two columns were identified by comparing their mass spectra to those available in commercial libraries (NIST02, Wiley) or by comparison with commercial standards and by comparison of their retention indices calculated relative to those available in the literature (Adams, 1995; Jennings and Shibamoto, 1980; Kondjoyan and Berdagué, 1996) and internet databases (2014).

Quantification on a non-polar column. The aroma compounds were quantified by a GC (HP 5890), equipped with an SPB-5 non-polar column (Supelco, 60 m × 320 µm × 0.25 µm) coupled to a FID detector. Aliquots (0.3 µL) of a mixture of concentrated essential oil (obtained as described above) and an internal standard terpinolene (20:2; v/v) were injected into the GC–FID in split mode (1:33). The flow rate of the gas carrier (Helium) was 0.7 mL min^{-1}. The oven temperature programme was as follows: initial temperature 60 °C, rate of 4 °C min^{-1} until a final temperature of 250 °C was reached,

then maintained constant for 20 min. The mean relative deviation of repeatability was ± 3.39% (n = 3).

6. Carbohydrate contents. Soluble and insoluble carbohydrates were extracted according to Clegg (1956). Separations were carried out by alcohol extraction; the residual starch was then hydrolysed. 0.2 g of ground pepper was weighed and then transferred into 5 mL of hot ethanol 80%. After 10 min, the mixture was filtered in a fritted glass material and extracted again with hot ethanol to reach a final volume of 10 mL. The Anthrone reagent was made by dissolving 1 g of Anthrone in 1 L of sulphuric acid solution containing 760 mL of concentrated H_2SO_4. The reaction was conducted on diluted extracts with a reaction time of 12 min in boiling water and read at room temperature on a Specord 600 spectrophotometer (Analytiks Jena, Jena, Germany) at 630 nm. Starch was hydrolysed with 52% perchloric acid for 20 min. Anthrone reactions were carried out on the diluted solution resulting from the hydrolysis. Soluble and insoluble carbohydrate contents were expressed in g 100 g^{-1} of dried matter. The mean relative standard deviations of repeatability were ± 12.73% for starch and ± 7.36% for soluble carbohydrates (n = 6).

7. Glucose and fructose contents. The aqueous extraction of sugars was performed by adding 100 mL of milli-Q water to 100 mg of sample. After 1 h of shaking, samples were filtered through a 0.45 µm filter (Millipore) and placed in a vial before analysis. The remaining glucose and fructose were monitored by a Shimadzu HPLC equipped with LC-20AB model pumps and a SIL-20A autosampler (Shimadzu, Kyoto, Japan), coupled with a PDA Decade 2 detector (Antec Leyden, the Netherlands). The sugars were separated in a 4 × 250 mm CarboPac MA1 Column (Dionex, Germany). The eluent used was a degassed NaOH 800 mM solution pumped at a flow rate of 0.4 mL min^{-1}. A freshly prepared solution of D-glucose and D-fructose was used to calibrate the system. The mean relative standard deviation of repeatability was ± 10.72% for glucose (n = 4) and ± 10.93% for fructose (n = 4).

8. Lipid content. The lipid content was determined on ground pepper according to the Soxhlet gravimetric method. A Soxtec-Avanti 250 semi-automatic device (Foss, Hillerød, Denmark) was used for fat extraction with petroleum ether as the solvent. The extraction time was 90 min at 110 °C. The fatty extracts were then kept for 16 h at 110 °C in order to remove traces of solvent. Fat content was expressed in g 100 g^{-1} of dry matter. The mean relative standard deviation of repeatability was ± 3.39% (n = 6).

9. Polyphenol content. The polyphenol content, expressed on a dry basis, in Gallic Acid Equivalent, was determined according to the colorimetric method (using Folin-Ciocalteu reagent) described in ISO 14502-1 (International Standard Organization, 2005). The spectrophotometer used was a Specord 600 (Analytik Jena AG, Jena, Germany). The mean relative standard deviation of repeatability for total polyphenols was ± 5.8% (n = 3).

10. Carotenoid content. Carotenoids were extracted from 200 mg of ground pepper mixed in a tube containing 1 mL of distilled water for 2 min. Then 10 mL of ethanol/hexane (4/3 v/v) was added before homogenization for 60 seconds in a Fastprep 24 (MpBiomedical, Santa Ana, USA) using sand as a lysing matrix and a ceramic ball as a mortar. The hexane phase was recovered and ethanol residues were mixed again with 5 mL of hexane. This operation was repeated three times. All organic phases were collected together and dried with anhydrous sodium sulphate. After evaporation on a Genevac HZ plus (Genevac, Warminster, USA), extracts were recovered in 0.5 mL of dichloromethane and 0.5 mL of methanol/methyl tert-butyl ether (80/20 v/v) and analysed by HPLC.

Carotenoids were then analysed according to the method described by Dhuique-Mayer *et al*. (2016). The HPLC system used was an Agilent 1100 photodiode array detector (Agilent, Massy, France). The Column was a C_{30} column (250 × 4.6 mm i.d., 5 µm: YMC Europe GmbH, (YMC, Dinslaken, Germany). Carotenoids were quantified by calibrating β-carotene at 450 nm. The mean relative standard deviations of repeatability for total carotenoids was ± 6.8% (n = 9).

11. Cellulose, hemicellulose and lignin contents. The fibre contents, expressed on a dry basis, were determined according to the Van Soest principle, following the method described in FD U44-162 (AFNOR, 2016). The mean relative deviations of repeatability were ± 4.66% for cellulose, ± 8.44% for hemicellulose, and ± 7.65% for lignin (n = 4).

12. Amino acid determination. Free amino acids were analysed following the method used by Moore (1958). Total amino acid analysis was performed using a Biochrom 30 amino acid analyzer (Biochrom Ltd., Cambridge, UK). Amino acid separation along the cationic column was obtained with a succession of four sodium citrate buffers of increasing pH (2.6–8.6), ionic strength (0.2–0.5 M) and increasing temperature gradient (52–95 °C). Amino acids were derivatized with the ninhydrin reagent (135 °C) and detected simultaneously at 570 nm and 440 nm. The entire process lasted 90 min per sample, including the resin regeneration phase. Quantification was performed by comparing peak areas with a standard including 26 acidic, neutral and basic amino acids (Sigma, St. Louis, Missouri, USA). Norleucine (250 nmol mL^{-1} in sodium citrate buffer, 0.2 M, pH 2.2) was also used as an internal standard. The mean relative deviation of repeatability was ± 5.00%.

13. Mineral compound determination. 500 mg of pepper was mineralized by two successive calcinations for 1 h 30 min and 30 min in an oven (Thermolyne Muffle Furnace 6000, Thermofisher, Waltham, USA) at 500 °C. The ashes were then solubilized prior to analysis by inductively coupled plasma atomic emission spectrometry ICP-AES (Agilent 720 series, Agilent Technologies, Santa Clara, USA).

Results and discussion

Description of the plant and its fruit

1. Plant morphology. The plant is a dioecious vine with a stem that becomes woody reaching a diameter of 4–5 cm at the base and climbing to a height of 5–10 m on support trees. It displays sterile creeping or climbing branches, adhering to the support by claspers forming at the nodes; its broadleaf leaves are deeply cordate, sometimes attenuated in a sharp point at the tip, without differentiated acumen, with a pubescent or glabrous petiole, reaching 2.5 cm in length; its stipules are deciduous. The branches, which are fertile, are more or less trailing, swollen at the nodes, without adventitious roots. The leaf lamina is glabrous, narrowly oval or elliptic, rounded or obtuse at the base, asymmetrical and acuminate at the tip. The species displays leaf dimorphism in adult plants. The inflorescences form in single spikes and are leaf-opposed.

The fruiting of *Piper borbonense* plants observed in Reunion Island takes place from July to November, depending on the years and the places where the plant grows. Fruiting on the same vine is staggered over several weeks, up to two months. Consequently, the spikes on a given vine never reach

Figure 1. Whole mature peppercorn and longitudinal section of a corn from *Piper borbonense* (Weil, 2017).

their ripe stage at the same time; the ripening of peppercorns on the same spike is also staggered. Fully ripe pepper fruits are red.

2. Fruit morphology and anatomy. The fruit is ovoid in shape (Figure 1); it is extended in the form of a pedicel (commonly known as the "tail" in the appellation "tailed pepper") by which it is attached to the spike. A peppercorn measures around 10.7 mm in length (pedicel included) and has a diameter of 3.6 mm for a mass of around 47 mg (Table 1). The fruit comprises the perisperm (or kernel) which accounts for around a third (17 mg) of the total mass. The endocarp separates the perisperm from the mesocarp (or pulp), which is itself surrounded by an exocarp (or envelope) (Figure 1). *Piper borbonense* is easily distinguished from *Piper nigrum* which is spherical and does not have a tail, as it remains on the spike when black pepper is threshed. *Piper borbonense* is also easily distinguished from *Piper cubeba*, another tailed pepper but which is spherical in shape and not ovoid, as shown in the photos proposed by Khan (2015). The morphology of *Piper borbonense*, apart from its ovoid shape, is quite similar to that of the wild Malagasy peppers (Weil *et al*., 2014).

Distribution of the main constituents and compounds of interest in the different parts of the fruit (wb)

In our analyses, fresh pepper was found to comprise 65% water and 32% dry matter (Table 2). The mesocarp was very rich in water (93%) while the dry matter (77%) was the major constituent of the perisperm and mostly present there. Piperine (0.061% of the whole peppercorn) was distributed equitably between the perisperm (49%) and the mesocarp (51%). Essential oil (3% of the peppercorn) was mostly present in the perisperm (92%) as shown in Table 2 and Figure 2.

Table 1. Main characteristics of a fresh mature *Piper borbonense* corn (mean values ± 95% confidence interval with *n* noted in brackets).

Part of pepper	Weight ($\times 10^{-6}$ kg)	Length ($\times 10^{-3}$ m)	Diameter ($\times 10^{-3}$ m)
Whole peppercorn	47.3[1] ± 0.9 (429)	10.69[1] ± 0.66 (10)	3.63 ± 0.24 (10)
Perisperm (kernel)	17.1 ± 0.9 (25)	3.87 ± 0.21 (10)	2.16 ± 0.15 (10)
Mesocarp (pulp)	30.2[2] ± 1.8	NA	NA

[1] Including tail 5.09 ± 0.43 × 10^{-3} m.
[2] Estimated from whole peppercorn minus kernel weight data.
NA: Not applicable.

Table 2. Dry matter, water, essential oil and piperine contents in different parts (whole peppercorn, kernel and pulp) of the fresh mature *Piper borbonense* corn (mean values ± 95% confidence interval with $n = 3$).

Component	Content (g 100 g^{-1} wb)		
	Whole peppercorn	Perisperm (kernel)	Mesocarp (pulp)[2]
Dry matter	32.0 ± 1.0	77.0 ± 0.9	6.5 ± 5.2
Essential oil	3.04 ± 0.19	7.77 ± 0.24	0.36 ± 0.75
Water	65.0 ± 1.1	15.2 ± 0.7	93.1 ± 4.5
Piperine[1]	0.061 ± 0.001	0.082 ± 0.002	0.049 ± 0.007

[1] Included in dry matter.
[2] Estimated from quantities in whole peppercorn minus kernel.

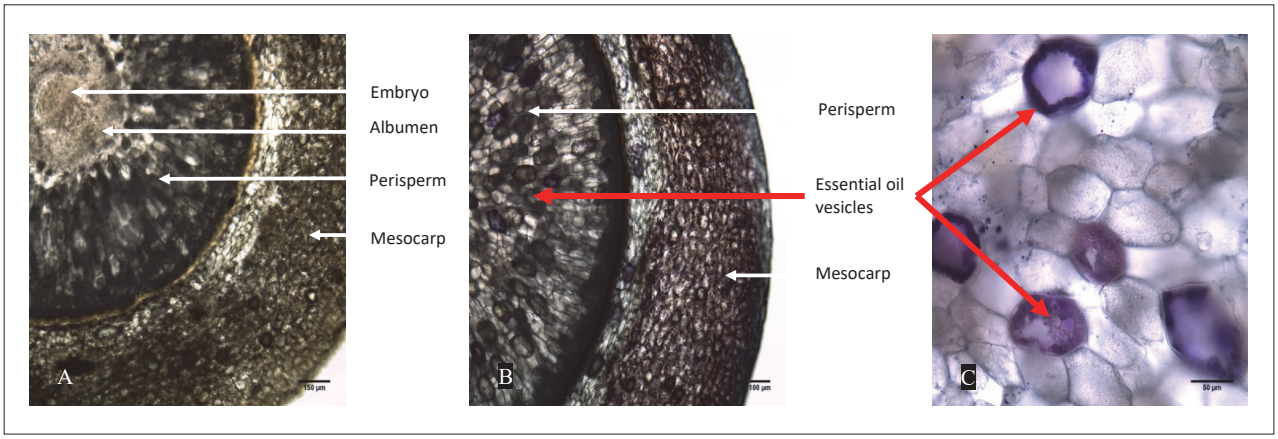

Figure 2. Histological sections of *Piper borbonense* showing the essential oil vesicles stained with Nadi reagent, Figure 2C (Sanier, 2016).

Peppercorn global composition (db)

According to Table 3, carbohydrates, with 41% starch and 7% soluble sugars (of which 2.50% glucose and 3.32% fructose), are the main compounds of the peppercorn. The starch content of *Piper borbonense* is similar to that of black pepper (38 and 45%) determined by Zachariah *et al*. (2010) and Jayashree *et al*. (2009) respectively for different cultivars or origins. Fibres (cellulose 9%, lignin 4% and hemicellulose 3%) accounted for 16%. Proteins accounted for 12%, identical to that described by Jayashree *et al*. (2009) but three times as high as that (4%) found by Zachariah *et al*. (2010) in *Piper nigrum.* The lipid content (9.5%) was relatively high in comparison to those (1.9 to 9%) reported by Ravindran (2000) for black pepper.

Piperine and essential oil contents (db)

Piperine and essential oil contents (Table 3) are of major interest as they respectively confer to pepper its hot and spicy taste and flavour.

Table 3. Composition of fresh mature *Piper borbonense* (mean values ±5% confidence interval with *n* noted in brackets).

Component	Content (g 100 g^{-1} db)
Starch	40.64 ± 5.43 (6)
Soluble carbohydrates (sugars)	7.35 ± 0.57 (6)
Glucose	2.50 ± 0.43 (4)
Fructose	3.32 ± 0.58 (4)
Proteins	11.87 ± 0.20 (4)
Essential oil	9.78 ± 0.32 (3)
Lipids	9.48 ± 0.48 (6)
Cellulose	8.65 ± 0.46 (3)
Lignin	3.79 ± 0.64 (3)
Hemicellulose	3.36 ± 0.99 (3)
Mineral compounds	3.37
Polyphenols[1]	1.56 ± 0.08 (3)
Piperine	0.20 ± 0.05 (3)
Carotenoids[2]	0.031 ± 0.004 (9)

[1] g eq gallic acid 100 g^{-1}
[2] g eq β-carotene 100 g^{-1}

1. Piperine content. The piperine content (0.20%) was barely higher than that (0.15%) found by Khan (2015) in *Piper cubeba* but 15 to 20 times less than the contents (3 and 4%) found by Jayashree *et al*. (2009) and Zachariah *et al*. (2010), respectively, in black pepper. *Piper borbonense* was also less rich in piperine than the wild peppers of Madagascar studied by Weil *et al*. (2014) which exhibited contents of between 0.5 and 3%.

2. Essential oil content. The essential oil content of *Piper borbonense* (almost 10%) was similar to that (11.8%) found by Bos *et al*. (2007) and higher than that (4.8%) found by Khan (2015) in *Piper cubeba*; it was well over that (around 3%) found by Jayashree *et al*. (2009) and Zachariah *et al*. (2010) in different varieties of black pepper. This value of 10% was within the range (2.8 to 13.1%) of that found for the wild peppers of Madagascar by Weil *et al*. (2014).

The piperine (0.2%) and essential oil (9.8%) contents of *Piper borbonense* were far off the commercial specifications given by standard ISO 959-1 (International Standard Organization, 1998) for black pepper, which are 4% for piperine and 2% for essential oil. This low pungency and high aroma potential of *Piper borbonense* bring it closer to some other tailed peppers such as *Piper cubeba* and the wild peppers of Madagascar, but sets it apart from *Piper nigrum.*

Volatile compounds: composition, specificity and quality of the essential oil

Twenty-four aroma compounds were identified amounting in all to almost 97% of the essential oil of *Piper borbonense* (Table 4). These compounds belonged to three distinct families: monoterpenes (69%), phenylpropanoids (25%) and sesquiterpenes (4%). The majority compounds of the essential oil were as follows: limonene (27%), alpha phellandrene (14%) and asaricin (13%). Alone, these three compounds accounted for over 50% of the total. Then came the two pinenes (alpha and beta), present in equal proportions and accounting together for 14% of the total essential oil. A third of the 24 compounds identified were present at under 1% in the essential oil.

The same major families of aroma compounds (monoterpenes, sesquiterpenes and phenylpropanoids) were found in the essential oils of *Piper borbonense* and *Piper nigrum* (Jagella and Grosch, 1999; Jirovetz *et al*., 2002; Pino *et al*., 1990). The proportion of limonene (27%) in *Piper borbonense* was similar to that (20% on average) found by several authors in black pepper (Jayashree *et al*., 2009; Menon and Padmakumari, 2005b; Zachariah *et al*., 2010). Likewise, pinenes

Table 4. Aromatic compounds in essential oil of fresh mature *Piper borbonense* (mean values ± 95% confidence interval with $n = 3$).

Compound	KI (Supelcowax)		KI (SPB5)		% (v/v) in essential oil (spb5)
	Experimental	Literature*	Experimental	Literature**	
Limonene [ms]	1,180	1,188	1,034	1,029	27.31 ± nd
Alpha-phellandrene [ms]	1,140	1,152	1,010	1,003	14.47 ± 0.20
Beta-pinene [ms]	1,082	1,073	982	979	6.81 ± 1.07
Alpha-pinene [ms]	1,015	1,007	931	939	6.78 ± 2.46
Delta-3-Carene [ms]	1,120	1,134	1,016	1,031	3.42 ± 0.07
Eucalyptol [ms]	1,192	1,189	1,037	1,031	2.77 ± nd
Para-cymene [ms]	1,245	1,248	1,028	1,025	1.92 ± 0.94
Beta-myrcene [s]	1,130	1,138	990	991	1.72 ± 0.07
Camphene [ms]	1,047	1,050	949	954	1.60 ± 0.50
Sabinene [s]	1,092	1,098	975	975	1.43 ± 0.01
Alpha-Terpineol [ms]	1,676	1,685	1,197	1,189	0.59 ± 0.19
Total for monoterpenes	-	-	-	-	*68.82 ± 5.50*
Asaricin [ms]	2,180	2,137	1,508	1,496	13.47 ± 1.50
Dillapiole [s]	2,354	2,350	1,636	1,621	4.12 ± 0.57
Safrole [ms]	1,855	1,830	1,298	1,287	3.55 ± 1.27
Elemicin [s]	2,205	2,217	1,559	1,557	1.89 ± 0.14
Myristicin [s]	2,246	2,254	1,535	1,519	1.39 ± 0.14
Methyl-eugenol [ms]	1,522	nd	1,413	1,404	0.46 ± 0.07
Total for phenyl-propanoïds	-	-	-	-	*24.88 ± 3.69*
Delta-elemene [ms]	1,447	1,444	1,352	1,138	1.32 ± 0.03
Germacrene D [ms]	1,690	1,708	1,500	1,485	0.79 ± 0.10
Caryophyllene (E) [ms]	1,577	1,583	1,437	1,419	0.56 ± 0.04
Alpha-cadinene [ms]	1,733	1,724	1,546	1,539	0.44 ± 0.47
Alpha-ylangene [ms]	1,461	1,460	1,385	1,375	0.32 ± 0.06
Alpha-cubebene [ms]	1,470	1,460	1,366	1,351	0.19 ± 0.03
Alpha-humulene [ms]	1,634	1,640	1,469	1,455	0.12 ± 0.00
Total for sesquiterpenes	-	-	-	-	*3.74 ± 0.73*
Undetermined compounds	-	-	-	-	*3.48 ± 0.93*

nd: Means not determined.
ms: Identified by comparison with published mass spectra.
s: Identified by comparison with commercial standards.
* Jennings and Shibamoto (1980), Kondjoyan and Berdagué (1996) and Internet databases (2014).
** Adams (1995) and Internet databases (2014).

(alpha and beta) were present in relatively similar proportions in both pepper species. However, for other compounds, the proportions differed much more from one species to the next. For instance, in *Piper borbonense* caryophilene accounted for only 0.6% and sabinene 1.5% of the total essential oil, while those compounds amounted to 23% and 16% respectively on average in the *Piper nigrum* studied by the same authors. Some other differences are noteworthy. For instance, asaricin, which was largely present (13%) in the essential oil of *Piper borbonense,* was not identified by those authors and was only identified in a very small proportion (under 1%) by Jirovetz *et al.* (2002) in black pepper. On the other hand, alpha amorphene and alpha copaene, which each accounted for around 2% of the total essential oil of *Piper nigrum* characterized by Menon and Padmakumari (2005a) were not identified in the essential oil of *Piper borbonense*. If one now compares the essential oil composition of *Piper borbonense* with that of *Piper cubeba* which is also a tailed pepper, some significant differences can be seen. Indeed, limonene and alpha phellandrene, which accounted for 27% and 14% respectively of the essential oil of *Piper borbonense* only accounted for 2.3% and 0.4% in the *Piper cubeba* analysed by Bos *et al.* (2007). As for asaricin, the third majority compound in *Piper borbonense* according to our analyses, it was not characterized in *Piper cubeba* by those same authors.

According to Schulz *et al.* (2005) who worked on black pepper, optimum pepper aroma ("top-peppery-note") is obtained if monoterpene (excluding alpha- and beta-pinene) content is high but at the same time, the pinene content is low. As the essential oil analysed in our study contained 69% of monoterpenoids excluding pinenes, which amounted to only 14% of the total, we can conclude that the aroma of the wild pepper *Piper borbonense* is of good quality. According to Jirovetz *et al.* (2002), limonene, beta-pinene, alpha-phellandrene, delta-carene, asaricin and elimicine give black pepper its characteristic aroma. In our study these compounds accounted for more than 67% of the total essential oil of *Piper borbonense* wild pepper. For Jagella and Grosch (1999),

alpha-pinene, alpha-phellandrene, myrcene, and limonene are key odorants in *Piper nigrum*. These four compounds amounted to 50% of *Piper borbonense* essential oil in our study. Safrole, the seventh compound in order of importance in *Piper borbonense*, has been identified as a carcinogen by several authors (Auerbach *et al*., 2010; Van den Berg *et al*., 2011). While this compound, and several other volatile compounds present in this wild pepper, such as limonene and methyleugenol, are subject to restrictions in cosmetology (AFSSAPS, 2010), it is not the case for food.

Antioxidant compounds

Carotenoids and polyphenols represent around 2% of the peppercorn (Table 3). Phytomicronutriments are naturally present in the majority of fruits, vegetables and spices; they share certain characteristics, including anti-oxidant properties, and are involved in colour (Renard C., 2014).

1. Carotenoids. The total carotenoid content of *Piper borbonense* was 31 mg eq β-carotene for 100 g (db) of pepper (Table 3), while 9.5 mg 100 g^{-1} is found in *Piper longum* (Veeru *et al*., 2009) and 500 mg 100 g^{-1} in chilli pepper, which is known to be particularly rich in carotenoids (Schweiggert *et al*., 2007). In our case, we settled for quantifying total carotenoids while the main carotenoids identified in black pepper are beta-carotene, lycopene and lutein (Variyar and Bandyopadhyay, 1990). Carotenoids could give its red colour to *Piper borbonense*. Indeed, several authors (Deli *et al*., 2001; Variyar and Bandyopadhyay, 1990) described carotenoids such as lycopene, beta-carotene and capsorubin, as responsible for the red color of mature *Piper nigrum*.

2. Polyphenols. The polyphenol content (Table 3) of *Piper borbonense*, 1.56 g GAE 100 g^{-1} (db), was slightly higher than that (1.2 g 100 g^{-1}) measured by Agbor *et al*. (2006) or that (1.3 g 100 g^{-1}) measured by Cheng (2015) in *Piper nigrum*. Although this pepper is considered as a spice and not as a foodstuff, it is still a fruit and, as such, its polyphenol content is as high as that of fruits considered to be rich in polyphenols, such as strawberry (Brat *et al*., 2006) or mango (Murillo *et al*., 2012). As suggested by Guyot S. (2014), the degradation of polyphenols could explain the browning observed for pepper after drying.

Amino acids

The amino acid content (Table 5) of *Piper borbonense* was around 10 g for 100 g of pepper, dry basis, of which 0.4 g 100 g^{-1} of free amino acids. The three amino acids that were most present were glutamic acid (1.5 g 100 g^{-1} db), leucine (1 g 100 g^{-1} db) and aspartic acid (1 g 100 g^{-1} db).

Although pepper is not especially consumed for its nutritional value, it should be noted that it contains 7 of the 8 amino acids considered to be essential; in fact, only tryptophane has not been identified. The presence of free amino acids, some of which (lysine, arginine, asparagine, glutamic acid and proline) are able to combine with reducing sugars (including glucose and fructose which alone account for almost 6% of the dry matter) could lie behind Maillard reactions occurring during drying and/or storage.

Mineral elements

The mineral salts in fully ripe fresh pepper amounted around 3% (db). Potassium, at almost 2.5%, was the most abundant compound (Table 6).

Conclusion

The aroma composition of *Piper borbonense* suggests a pepper of good quality which, associated with its typicity (high essential oil content and very low piperine content), affords an interesting potential for its domestication and valorization. While the presence and/or proportion of certain volatile compounds of the essential oil seem to differentiate it from black pepper and from other peppers such as *Piper cubeba* and *Piper longum*, more investigation is required to confirm that such differences are indeed due to species rather than to abiotic factors such as climate or *terroir*, growing conditions, or processing methods. Work seeking to identify and validate some chemical authentication keys remains to be done if we wish to make use of these keys to distinguish and valorize the *Piper borbonense* of Reunion from other peppers, either domesticated or wild, notably from the Indian Ocean.

Table 5. Amino acid contents in fresh mature *Piper borbonense*.

Amino acid	Concentrations (g 100 g^{-1} db)	
	Total amino acid	Free amino acid
Glutamic acid	1.551	0.058
Leucine*	1.053	0.011
Aspartic acid	1.002	0.030
Proline	0.774	0.008
Tyrosine	0.718	0.008
Alanine	0.677	0.021
Glycine	0.524	0.005
Serine	0.517	-
Valine*	0.492	0.004
Phenylalanine*	0.455	0.004
Isoleucine*	0.407	0.004
Lysine	0.345	0.010
Arginine	0.321	0.005
Threonine*	0.299	0.008
Histidine*	0.263	0.016
Methionine*	0.225	0.002
Cysteine	0.135	0.001
Gaba	0.087	0.062
Asparagine	-	0.169
Total	9.845	0.434

* Essential amino acids.

Table 6. Mineral contents in fresh mature *Piper borbonense*.

Mineral compounds	Concentration	
P	0.24	g 100 g^{-1} db
K	2.35	
Ca	0.43	
Mg	0.29	
Na	0.06	
Cu	14.25	ppm db
Fe	35.35	
Mn	13.05	
Zn	7.9	
Al	11.1	

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