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Étude des procédés de transformation de poivres sauvages d'îles de l'océan indien : impact sur la qualité (piquant, arôme et couleur)

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Glossaire

AFNOR : Agence Française de Normalisation
ANOVA : Analysis of variance
ASTA : American Spice Trade Association
B : Blanching (blanchiment)
BS ou bs : Base sèche
BH ou bh : Base Humide
CE : Commission Européenne
CIE : Commission Internationale de l'Eclairage
CIRAD : Centre de Coopération Internationale en Recherche Agronomique pour le Développement
CoA : Coenzyme de transfert de groupements acyle
D : Drying (séchage)
DB ou db : Dry base
DP : Dry process (voie sèche)
ESA : European Spice Association
FAO : Food and Agriculture Organization
F : Fresh (frais)
GC-FID : Chromatographie Gazeuse couplée à un Détecteur à Ionisation de Flamme
GC-MS : Chromatographie Gazeuse couplée à un Spectromètre de Masse
HACCP : Hazard Analysis Critical Control Point (Analyse des dangers, maîtrise des points critiques)
5-HMF : 5-Hydroxyméhylfurfural
HPLC : High performance Liquid Chromatography
IPC : International Pepper Community
ISO : International Standard Organization
KI : Kovats Index
POD : Péroxydase
PPO : Polyphénoloxydase
PPS : Pepper Production System
S : Sweating (étuvage)
SHU : Scoville Heat Units
Tsiperifery : Poivre sauvage en langue malgache
USDA : United States Département of Agriculture
WB ou wb : Wet base
WP : Wet process (voie humide)

« La découverte d’un épice ou d’un aromate nouveau fera plus pour le bonheur du genre humain que la découverte d’une étoile »

Brillat – Savarin, magistrat, gastronome et auteur culinaire français (1755 – 1826)

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Introduction Générale

Introduction générale

Depuis l'antiquité, les épices ont été utilisées dans le monde entier pour aromatiser et préserver les aliments, ainsi qu'à des fins médicales et cosmétiques. Le poivre, également appelé « or noir » par les commerçants de l'époque, est aujourd'hui utilisé dans les cuisines de tous les continents. Il existe environ 700 espèces de poivre dans le monde (Sumathykutty*, et al.*, 1999). 463 000 tonnes ont été produites en 2014 dont 150 000 tonnes par le Vietnam, premier pays producteur au monde (FAO Statistics Division, 2017). Si plusieurs espèces sont aujourd'hui domestiquées, une seule d'entre elles, le *Piper nigrum,* représente l'écrasante majorité de la production. Les iles de l'Océan Indien occidental sont riches de différentes espèces de poivres sauvages. À la Réunion, le taxon *Piper borbonens*e (Miq.) C. DC, considéré comme endémique de l'ile, a été déterminé en 2011 par le Conservatoire National Botanique de Mascarin. Seules ses feuilles sont utilisées en pharmacopée et ce poivre méconnu, n'est pas exploité jusqu' à ce jour. À Madagascar les poivres sauvages *Piper spp.* dont le nom vernaculaire est Tsiperifery (littéralement poivre sauvage), font depuis une dizaine d'années, l'objet d'une exploitation grandissante. En 2015, le volume exporté, notamment vers l'Europe et en France en particulier, était estimé à 50 tonnes de poivre sec. Alors que ces poivres malgaches sont très appréciés par la haute gastronomie et les connaisseurs, la pérennité de la filière est aujourd'hui mise en péril, d'une part du fait d'une exploitation anarchique qui tend à faire disparaître la ressource, et d'autre part du fait d'une qualité peu maîtrisée des produits mis en marché, qui pourrait, à terme, conduire les utilisateurs à se détourner de ces poivres. Afin d'assurer la durabilité de la filière, à travers la préservation de la ressource et l'amélioration de la qualité, plusieurs projets de recherche menés actuellement à Madagascar et à la Réunion, s'intéressent à la domestication et à la transformation de ces poivres sauvages.

Les procédés de transformation du poivre sont assez peu décrits dans la littérature bien qu'il existe des savoir-faire traditionnels (notamment à Madagascar) ou industriels (par exemple en Inde). Ce déficit de littérature scientifique est accentué pour les poivres sauvages, en particulier pour les espèces *Piper spp*. de Madagascar et *Piper borbonense* de la Réunion.

L'objectif de cette thèse est d'acquérir des connaissances nouvelles sur les caractéristiques de ces poivres sauvages, les procédés de transformation et leurs impacts sur la qualité du poivre, évaluée à travers le piquant, l'arôme et la couleur. Les connaissances scientifiques et techniques acquises pourront servir de base à la mise en place de filières maîtrisées et hauts de gamme attendues par les acteurs à Madagascar et à la Réunion et par les consommateurs du monde entier.

Les questions de recherche posées sont les suivantes :

- Quels sont les procédés de transformation des poivres sauvages mis en œuvre à Madagascar ?
- Quelles sont les caractéristiques du *Piper borbonense* et quelles sont les opérations unitaires critiques pour sa qualité ?
- Quels sont les mécanismes biochimiques et physico-chimiques qui interviennent et expliquent l'expression ou la dégradation de la qualité du poivre lors des opérations de transformation ?

Étude Bibliographique

1. Étude bibliographique

Les poivres sauvages de l'océan indien, et en particulier le *Piper borbonense* de la Réunion, n'ayant fait l'objet d'aucune publication scientifique, l'étude bibliographique qui suit concerne essentiellement le *Piper nigrum* (ou poivre noir), référence mondiale des poivres, et quelques autres poivres domestiqués.

1.1. Le poivre

1.1.1. Généralités

Le poivre est une épice originaire de la côte ouest de l'Inde (côte de Malabar) dans l'état du Kerala. Au moyen âge, les épices, rares et recherchées étaient utilisées comme monnaie d'échange. C'est de là que vient l'expression « payer en espèces (épices) ». Le poivre, alors considéré comme le roi des épices, était autrefois échangé contre de l'or et des pierres précieuses (Schweiggert, Carle*, et al.*, 2007). Si le poivre n'est plus considéré comme un produit de luxe, il reste aujourd'hui l'épice la plus populaire et la plus largement utilisée au monde pour ses qualités aromatiques et de conservateur ainsi que pour ses propriétés médicinales (Mamatha*, et al.*, 2008). 463 000 tonnes de poivre ont été produites en 2014 dont près du tiers par le seul Vietnam, premier pays producteur devant l'Indonésie et l'Inde. Madagascar avec 7 100 tonnes produites la même année, arrive en 8ème position (**figure 1**). L'ensemble de la production de 2014 représentait une valeur de plus de 950 millions de dollars (FAO Statistics Division, 2017).

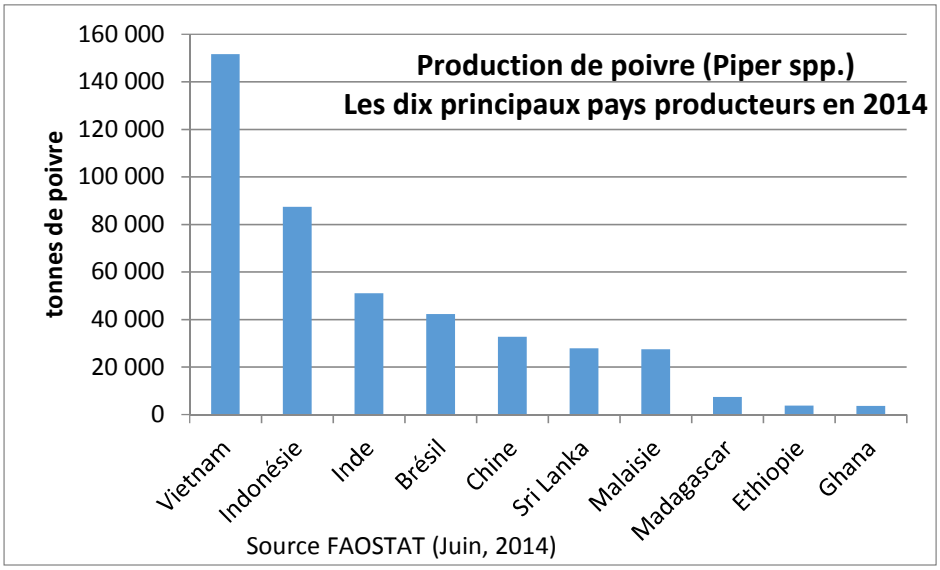


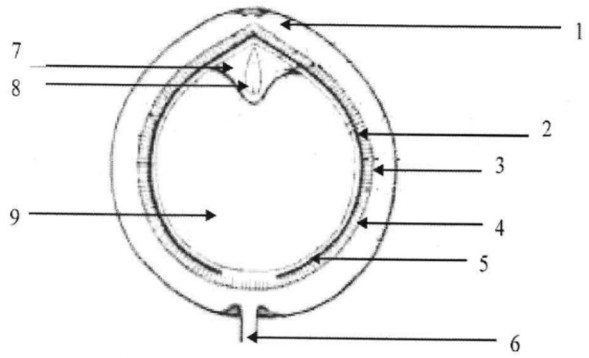
Figure 1 : Les 10 principaux pays producteurs de poivre (*Piper spp.*) FAOSTAT (Juin, 2014).

Le poivrier est une liane tropicale du genre *Piper*, commune en Asie (83 % de la production mondiale), en Amérique du Sud (11 %) et en Afrique (6 %). D'un point de vue botanique, sa classification est la suivante : Règne : Végétal ; Embranchement : Spermaphytes ; Sous-embranchement : Angiospermes ; Classe : Dicotylédones ; Sous-classe : Magnolides ; Ordre : Piperales ; Famille : Piperaceae ; Genre : *Piper.* Parmi les 700 espèces recensées du genre *Piper* (Sumathykutty*, et al.*, 1999), le poivre noir ou *Piper nigrum* est la plus commune. Il s'agit d'un poivre domestiqué, comme l'est également le *Piper cubeba*, poivre à queue d'Indonésie. Cependant, la grande majorité des espèces demeure sauvage, c'est-à-dire non cultivée et souvent même non exploitée ou de façon marginale. Plusieurs espèces de poivres sauvages poussent dans les Iles du Sud-Ouest de l'Océan Indien. Si les poivres sauvages (Tsperifery) malgaches sont aujourd'hui exploités, le *Piper borbonense*, poivre à queue sauvage réunionnais ne l'est pas ; seules ses feuilles sont parfois utilisées en pharmacopée alors que ses fruits ne sont pas connus et très peu consommés.

Le poivre est principalement consommé et apprécié pour ses vertus culinaires, c'est-à-dire sa capacité à rehausser le goût et l'arôme des plats (Dhas*, et al.*, 2003; Schweiggert, Carle*, et al.*, 2007). Il est également connu et utilisé pour ses propriétés fonctionnelles antimicrobiennes et anti-oxydantes (Nisha*, et al.*, 2009; Suresh*, et al.*, 2007).

1.1.2. Anatomie et structure du grain de poivre (Piper nigrum)

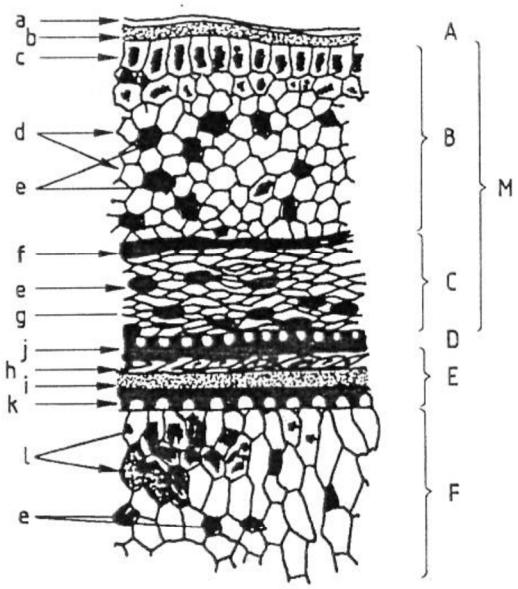
Le grain de poivre est de forme ronde (**figure 2**). Il peut être décomposé en trois parties : le mésocarpe (1, 2, 3, 4), le péricarpe (mésocarpe + 5, 6) et la graine (7, 8, 9) proprement dite.



1. Epicarpe
2. Mésocarpe interne
3. Mésocarpe externe
4. Faisceaux libéro-ligneux
5. Endocarpe
6. Pseudo-pédoncule
7. Endosperme
8. Embryon à deux cotylédons
9. Périsperme

Figure 2 : Coupe transversale d'une baie de *Piper nigrum* (Pham, 2007).

La structure du « grain » de *Piper nigrum* qui est à proprement parler une baie (un fruit charnu) contenant une graine, est bien décrite dans la norme ISO 959 (International Standard Organization, 1998). Le grain de poivre est donc composé, depuis l'extérieur vers l'intérieur de la baie, de cinq parties distinctes : épicarpe, mésocarpe, endocarpe, téguments séminaux et périsperme. Chacune de ses parties a sa propre structure telle qu'illustrée dans la **figure 3**.



A Épicarpe — a cuticule; b épiderme

M Mésocarpe — B mésocarpe externe; C mésocarpe interne
- c couche de cellules scléreuses à parois très épaisses, ponctuées, jaunes
- d cellules du mésocarpe externe à parois minces
- e cellules à essences
- f vaisseaux fibro - vasculaires
- g cellules à parois minces, aplaties, du mésocarpe interne,

D Endocarpe
- j cellules en fer à cheval de l'endocarpe

E Téguments séminaux
- h, i, k trois assises de cellules formant les téguments séminaux

F Périsperme (albumen nucellaire)
- l cellules de périsperme remplies d'amidon (ce dernier n'est représenté que dans quelques-unes des cellules).

Figure 3 : Dessin d'une coupe microscopique longitudinale de la baie de *Piper nigrum* (ISO 959-1).

1.1.3. Composition du grain de poivre et évolution au cours de la maturité

Le grain de poivre frais contient une quantité importante d'eau, qui varie selon sa maturité et les conditions climatiques avant cueillette ; elle est en général comprise entre 70 et 80 % (bh). Alors que de nombreux articles décrivent les constituants aromatiques du poivre noir et même de différentes espèces de poivre, peu de références concernent la constitution globale du *Piper nigrum*. Quelques auteurs (Dhas*, et al.*, 2003; Jayashree*, et al.*, 2009; Zachariah*, et al.*, 2010) fournissent néanmoins une composition grossière et incomplète (en base sèche) du poivre noir (**tableau 1**). L'amidon en est le composé principal.

Tableau 1. Composition du poivre (*Piper nigrum*) selon Dhas (2003), Jayashree(2009) et Zachariah (2010).

Composés	Concentration en g/100 g (bs)		
	Dhas (2003)	**Jayashree (2009)**	**Zachariah (2010)**
Amidon	28,0 - 49,0	34,7 - 52,3	32,1 - 42,8
Sucres solubles dans l'eau	-	-	2,3 - 8,0
Fibres	8,7 - 18,0	6,8 - 14,6	-
Protéines	-	9,6 - 14,1	2,1 - 6,0
Pipérine	1,7 - 7,4	2,8 - 4,4	1,8 - 4,2
Huile essentielle	-	2,4 - 3,3	1,4 - 5,2
Oléorésine	3,9 - 11,5	7,9 - 12,2	5,9 - 13,9
Acides aminés libres	-	-	0,3 - 0,8
Phénols	-	-	0,3 - 0,6
Minéraux	3,6 - 5,7	-	-

La composition du poivre évolue au cours de sa maturité. La concentration en amidon augmente alors que les teneurs en composés d'intérêts que sont la pipérine et l'huile essentielle diminuent en conséquence et dans le même temps (Jansz*, et al.*, 1984; Mathai, 1981; Rathnawathie*, et al.*, 1984).

1.2. Les procédés de transformation du *Piper nigrum*

1.2.1. Principales étapes mise en œuvre

Plusieurs auteurs parmi lesquels Dhas*, et al.* (2003), Ravindran (2000), Schweiggert, Mix*, et al.* (2005), Schweiggert, Carle*, et al.* (2007) ont décrit les différentes étapes de transformation du *Piper nigrum*. Les étapes et les paramètres appliqués varient selon les pays, les acteurs ainsi qu'en fonction des produits et marchés visés.

1.2.1.1. Récolte

Les baies en grappes sont cueillies à différentes maturités, jugées par la couleur, en fonction des produits finis et des rendements massiques espérés (Ravindran, 2000) :

- immatures (vertes) pour le poivre vert et l'extraction d'huile essentielle et d'oléorésine ;
- presque matures (jaunes-oranges) pour le poivre noir ;
- matures (rouges) pour le poivre rouge ;

- sur-matures (rouge foncé) pour le poivre blanc et le poivre gris (mouture de poivre).

La cueillette manuelle des grappes est réalisée depuis le sol ou en hauteur (échelle). Ceci dépend de la hauteur à laquelle la liane est enroulée sur son tuteur ; ce dernier peut être vivant (arbre ou arbuste) ou mort (bois, métal ou ciment).

1.2.1.2. Egrappage

Il permet de séparer les fruits de la grappe et peut être réalisé soit par foulage aux pieds, soit manuellement ou encore à l'aide de bâtons ; plus rarement à l'aide de séparateurs mécaniques (Dhas*, et al.*, 2003). Il est parfois plus aisé à mettre en œuvre sur poivre séché.

1.2.1.3. Séchage

Opération unitaire très courante en technologie alimentaire, le séchage est avant tout un procédé de transformation. Il s'agit d'une opération d'élimination d'eau d'un produit par ébullition ou par entrainement. Il vise à diminuer la teneur en eau du poivre frais (70 à 80 % bh), à des valeurs comprises entre 10 et 13 % (bh) afin de permettre sa conservation en limitant, notamment, le développement microbien. Il est, le plus souvent, réalisé au soleil ; la propreté du support, l'épaisseur et la fréquence de retournement des tas/couches, la protection lors de périodes de pluies influent sur le rendement massique et sur la qualité du poivre séché. De nombreux types de séchoirs, à combustibles ou solaires, artisanaux ou industriels ont été développés (Dhas*, et al.*, 2003). Ils sont cependant rarement accessibles de façons pérennes aux petits producteurs du Sud.

1.2.1.4. Nettoyage / Tri

Le nettoyage et le tri visent à éliminer les matières étrangères, impuretés, les baies légères avortées ou cassées. Ils peuvent être manuel, avec une vanne ou à l'aide de séparateurs mécaniques type cyclone ou table vibrante (Dhas*, et al.*, 2003). Le tri densimétrique permet également de classer le poivre par catégories commerciales.

1.2.1.5. Conditionnement et stockage

Le conditionnement du poivre doit être adapté à la qualité du produit et aux conditions

(durée, température, humidité et propreté des locaux) de stockage. Ainsi un poivre contenant moins de 10 % (bh) d'eau, stocké dans un local bien aéré pourra être conservé plusieurs mois dans un simple sac en toile de jute (Ravindran, 2000). Les sacs opaques, en polyéthylène et le conditionnement sous-vide partiel sont aujourd'hui souvent mis en œuvre pour protéger le poivre respectivement de la lumière, de la reprise d'humidité et de l'oxygène.

Les différentes étapes décrites ci-dessus, systématiquement mises en œuvre dans la filière poivre noir (**figure 4**) sont assez bien décrites dans la littérature. D'autres opérations unitaires telles que le décorticage (filière poivre blanc) ou la décontamination (filière export) bien que non systématiquement mises en œuvre, le sont également. D'autres étapes sont moins bien décrites ; leurs mises en œuvre technologiques ainsi que leurs intérêts économiques, sanitaires et sensoriels apparaissent donc comme moins évidents. C'est notamment le cas pour le blanchiment.

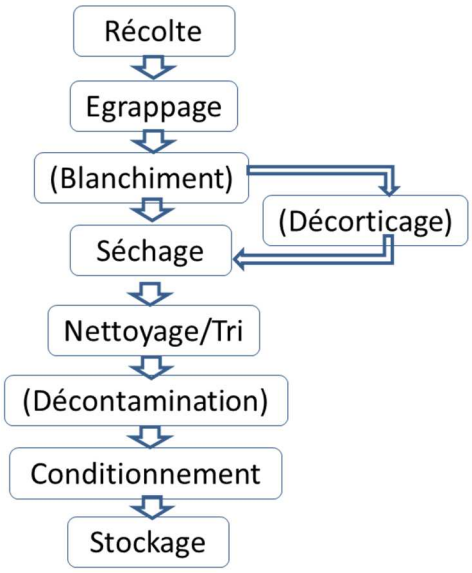


Figure 4 : Procédé de transformation du *Piper nigrum* établi à partir des descriptions faites par Dhas et Korikanthimath (2003), Ravindran (2000), Schweiggert *et al*. (2005) et Schweiggert, Carle, *et al*. (2007). Les étapes entre parenthèse ne sont pas systématiquement mises en œuvre.

1.2.2. Quelques étapes spécifiques

Les étapes décrites ci-dessous ne sont pas systématiquement mises en œuvre.

1.2.2.1. Le décorticage

Le décorticage mis en œuvre pour se débarrasser du péricarpe et obtenir du poivre

blanc peut être réalisé de différentes manières. Le décorticage mécanique des grains de poivre, et le dépulpage après un blanchiment des baies à 100°C durant une quinzaine de minutes sont deux méthodes possibles. Le rouissage est une autre alternative ; c'est la méthode la plus couramment employée : il s'agit d'un dépulpage par trempage. Le poivre est immergé dans l'eau (avec une circulation) durant une huitaine de jours. Le péricarpe et la pulpe sont ensuite éliminés par frottement manuel ou mécanique. Dans cette dernière technique, la fragilisation enzymatique recherchée du péricarpe est d'origine bactérienne. Ce développement bactérien peut s'accompagner de la synthèse d'arômes spécifiques qui peuvent être indésirables (Gopalam, 1990; Thankamani*, et al.*, 2004).

1.2.2.2. La décontamination

La décontamination/désinsectisation est mise en œuvre notamment pour les produits destinés à l'export. La fumigation à l'aide d'oxyde d'éthylène historiquement utilisée pour les épices est maintenant – parce que potentiellement cancérigène - interdite par l'Union Européenne. Des techniques de traitement à la vapeur ou d'irradiations (rayons X ou gamma, électrons accélérés) sont parfois appliquées (Schweiggert, Carle*, et al.*, 2007) mais elles font peur aux consommateurs et doivent être mentionnées sur l'étiquetage. La technologie micro-ondes est parfois employée. Pour Waje, Kim *et al*. (2008), les traitements à la vapeur, même si bien acceptés par les consommateurs puisque n'utilisant pas de produits chimiques, sont à proscrire d'une façon générale pour les épices dans la mesure où ils les décolorent, diminuent la teneur en huile essentielle et augmentent la teneur en eau. Selon les travaux de ces auteurs, l'irradiation donne de bons résultats en éliminant les micro-organismes sans affecter la qualité sensorielle du poivre.

1.2.2.3. Le blanchiment

Le blanchiment, parfois également appelé échaudage, consiste à immerger le poivre (généralement avant égrappage) quelques minutes dans de l'eau chaude. Il peut également être réalisé à la vapeur saturée d'eau. Le blanchiment est fréquemment employé dans la transformation des fruits et légumes notamment pour inactiver les enzymes et parfois faciliter l'épluchage. Selon Dhas (2003), un blanchiment de quelques minutes dans de l'eau à 100 °C permettrait non seulement d'éliminer les impuretés (poussières), de réduire significativement la charge microbienne et la durée

séchage, mais favoriserait également l'apparition d'une couleur brune uniforme. Pour Hong-Wei Xiao*, et al.* (2017), le blanchiment a également pour intérêt de diminuer les éventuelles contaminations chimiques tels que les pesticides (par lavage et dégradation thermique) et de désorber l'air des tissus. Les préconisations de temps et températures varient beaucoup selon les auteurs et l'effet recherché. Lorsque les conditions (durée et température) de l'échaudage appliquées permettent l'inactivation, partielle ou totale, des enzymes endogènes, notamment de la polyphénol oxydase et de la peroxydase, on parle de blanchiment (Hong-Wei Xiao*, et al.*, 2017; Renard C., 2014).

1.3. La qualité du poivre

1.3.1. Définitions, normes et règlementation

La qualité est définie par la norme ISO 9000 (International Standard Organization, 2015) de la façon suivante : « aptitude d'un ensemble de caractéristiques intrinsèques d'un objet à satisfaire des exigences ». Ces exigences peuvent être implicites ou explicites. Parmi les exigences explicites, les exigences règlementaires sont des exigences d'application obligatoire ; il y en a peu pour le poivre. Le règlement CE 2073/2005 (Commission of the European Communities, 2005) donne des exigences très générales sur les critères microbiologiques applicables aux denrées alimentaires ; le poivre doit notamment être exempt de salmonelles. Il existe en revanche quelques exigences concernant les teneurs maximales en mycotoxines. Ainsi le règlement CE 1881/2006 (Commission of the European Communities, 2006) limite la teneur en aflatoxines totales à 10 ppb et en aflatoxines B1 à 5 ppb tandis que le règlement CE 105/2010 (Commission of the European Communities, 2010) limite la teneur en ochratoxines A à 15 ppb.

Plusieurs organismes - l'European Spice Association, la Commission du Codex, Alimentarius, l'International Pepper Community, l'United States Département of Agriculture, l'American Spice Trade Association ou encore l'International Standard Organization, ont établi des normes pour le *Piper nigrum*. Ces normes fournissent des spécifications sanitaires, sensorielles et chimiques. Bien que non règlementaires et d'application volontaire, ces normes sont pourtant souvent imposés lors des transactions commerciales. Les spécifications qui y sont données concernant la qualité sanitaire : corps étrangers, teneurs maximum autorisées pour les bactéries

pathogènes (Salmonella et E. Coli) et les mycotoxines (aflatoxines et ochratoxines) ainsi que la qualité sensorielle sont souvent incomplètes, subjectives et parfois divergentes. Néanmoins trois référentiels (European Spice Association, 2011; International Pepper Community, 2015; International Standard Organization, 1998) donnent pour le poivre noir, des caractéristiques chimiques précises et assez homogènes ; elles sont reportées dans le **tableau 2**.

Tableau 2 : Spécifications chimiques (teneurs en %) commerciales pour le *Piper nigrum* selon l'European Spice Association, l'International Pepper Community et l'International Standard Organization

Composés	**valeur**	**Concentration en g/100 g (bh ou bs)**		
		ESA (2011)	**IPC (2015)**	**ISO959-1 (1998)**
Eau (bh)	maximum	12	12	13
Cendres totales (bs)	maximum	7	6	7
Cendres insolubles dans l'acide (bs)	maximum	1,5	-	1,2
Pipérine (bs)	minimum	-	4	4
Huile essentielle* (bs)	minimum	2	2	2
Oléorésine (bs)	minimum	7	-	6

*ml/100g (bs) pour l'huile essentielle

1.3.2. Déterminants de la qualité et composés d'intérêts

La définition de qualité(s) pour un produit alimentaire peut varier selon les contextes et les acteurs considérés. Elle peut prendre en compte : la qualité sanitaire, la qualité nutritionnelle, la qualité sensorielle ainsi que la qualité commerciale (aptitude à la vente) et la qualité technologique (aptitude à la transformation). Dhas*, et al.* (2003) énumèrent les nombreux critères qualitatifs qui peuvent être pris en compte à différents stades de la filière : taille des baies, couleur, densité, taux de baies légères, baies abîmées, teneur en eau, charge en microorganismes, présence de corps étrangers tels qu'excréments animaux ou insectes etc…Du point de vue du consommateur, les qualités sanitaire, nutritionnelle et sensorielle sont importantes. La qualité sanitaire, qui est de la responsabilité de chaque opérateur, demeure une exigence implicite pour les consommateurs. Si le poivre est également consommé pour ses vertus santé (par

exemple antiinflammatoires), c'est une épice essentiellement employée pour rehausser le goût des plats ; c'est donc sa qualité sensorielle qui prime. Cette qualité sensorielle est systématiquement évaluée à travers son piquant et son arôme (Mamatha*, et al.*, 2008; Schulz*, et al.*, 2005). Une troisième composante est également souvent prise en compte ; il s'agit de sa couleur (Pino*, et al.*, 1990; Schweiggert, Mix*, et al.*, 2005).

1.3.2.1. Le piquant : la pipérine et les composés analogues

Le piquant ou sensation de pseudo-chaleur du poivre est principalement conféré par la pipérine (**figure 5**). La pipérine a été découverte en 1819 par Oersted. C'est une substance cristalline, incolore, insoluble dans l'eau mais soluble dans l'éthanol.

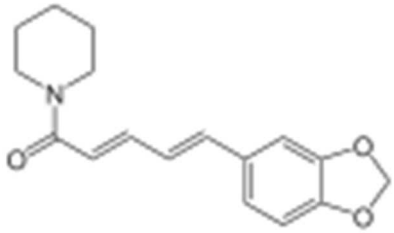


Figure 5. Structure de la pipérine

Sa synthèse enzymatique se réalise à partir de piperoyl-CoA et de pipéridine en présence de pipérotransférase. C'est un alcaloïde, dont la formule brute est $C_{17}H_{19}NO_3$. La pipérine représente en moyenne 84 % de l'ensemble des pipéramides, dont pipéranine, piperdardine, piperettine (Friedman (2008)). Les isomères de la pipérine (l'isopipérine, la chavicine et l'isochavicine) présentent un faible pouvoir piquant. Le pharmacologue Scoville (début du XX^{e} siècle) a défini une échelle (d'unité SHU) qui compare le pouvoir « piquant » des aliments/condiments : une valeur de 10 SHU est attribuée lorsqu'une dilution au 1/10 du produit fait disparaître la sensation de brûlure en bouche. La pipérine à 100 000 SHU se positionne entre la capsaïcine (composé piquant des piments) à 16 000 000 SHU et le gingérol (composé piquant du gingembre) à 60 000 SHU. Plusieurs auteurs, dont Zarai*, et al.* (2013) ont montré les propriétés anti-inflammatoires, anti-oxydantes et antibactériennes de la pipérine. La pipérine aurait également des propriétés antifongiques (Madhyastha *et al*. (1984), puisqu'elle permet de limiter la croissance d'Aspergillus parasiticus ainsi que la synthèse, par ce même champignon d'Aflatoxine B1.

1.3.2.2. L'arôme : huile essentielle et composés d'arôme

L'arôme et l'odeur du poivre sont en grande partie conférés par l'huile essentielle. Par définition, une huile essentielle est le liquide concentré et hydrophobe des composés volatils d'une plante obtenu par entraînement à la vapeur d'eau. Dans le cas particulier des agrumes l'huile essentielle est obtenue par expression (à froid) tandis que des méthodes plus couteuses telles que l'extraction au CO_2 supercritique peuvent être employées pour des produits à haute valeur ajoutée. Pour Sumathykutty*, et al.* (1999), dans la mesure où les teneurs (par exemple 1,6 % dans *Piper attenuatum* et 14,5 % dans *Piper cubeba*) et les compositions varient significativement d'une espèce à l'autre, l'étude des huiles essentielles peut être considérée comme un outil de caractérisation taxonomique des différents poivres. Pour Jirovetz*, et al.* (2002), le limonène, le β-pinène, l'α-phellandrène, le delta-carène, l'asaricin et l'élimicine confèrent au poivre noir son arôme caractéristique. Pour Jagella*, et al.* (1999), l'α-pinène, l'α-phellandrène, le myrcène, et le limonène sont les composés aromatiques clefs du *Piper nigrum*. La composition de l'huile essentielle est également un critère permettant de juger de la qualité des poivres. Ainsi, pour Schulz*, et al.* (2005) un arôme de poivre optimal est obtenu si la teneur en monoterpènes (en excluant α et β-pinène) est élevée alors que dans le même temps la teneur en pinènes est faible.

1.3.2.3. La couleur : composés et mécanismes impliqués

Différentes déclinaisons de poivre existent : vert, noir, blanc, gris, et rouge (plus rare). Ces déclinaisons (**figure 6**) peuvent être obtenues à partir de la même espèce, en l'occurrence, le *Piper nigrum*.

Poivre vert Poivre noir Poivre blanc Poivre gris Poivre rouge

Figure 6. Cinq déclinaisons du *Piper nigrum* (Weil, 2017).

Le poivre vert : Ses baies, cueillies immatures sont le plus souvent mises en saumure mais peuvent également être déshydratées (par lyophilisation ou friture sous vide) ;
Le poivre noir : Ses baies récoltées avant maturité complète, sont parfois blanchies

(on dit aussi échaudées) puis séchées ;

Le poivre blanc : Ses baies cueillies à maturité, sont débarrassées de leur péricarpe avant d'être séchées ;

Le poivre gris : Il s'agit d'un poivre noir broyé qui doit sa couleur au mélange du péricarpe noir et de l'amande blanche ;

Le poivre rouge : Ses baies récoltées à maturité (voir sur-matures) sont parfois blanchies puis séchées.

La dégradation de la couleur qui est un sérieux problème dans l'industrie des épices (Schweiggert, Kurz*, et al.*, 2007), l'est également pour la filière poivre. Plusieurs mécanismes, pouvant survenir après la cueillette, lors de la transformation et du stockage, sont à l'origine de la dégradation de la couleur du poivre. Ces mécanismes dépendent notamment de la température, de la présence d'oxygène et de la lumière ; il peut s'agir de dégradations oxydatives enzymatiques ou purement chimiques (Renard C., 2014) ou encore de réactions de Maillard (Horvathova*, et al.*, 2007).

L'évolution de la couleur est directement reliée à la composition biochimique. La diminution de la concentration en chlorophylle (donnant la couleur verte) et l'augmentation de concentration en caroténoïdes (rouge) au cours de la maturité des fruits a bien été décrite par Deli*, et al.* (2001). De leur côté, Variyar*, et al.* (1990) ont caractérisé les caroténoïdes majoritairement présents dans le *Piper nigrum* mature (rouge) ; il s'agit du β-carotène, du lycopène et de la lutéine. Agbor*, et al.* (2006) ont, eux, montré que l'activité anti-oxydante du poivre noir était essentiellement due à son importante concentration en polyphénols. Caroténoïdes et polyphénols, phytomicronutriments bien présents dans le poivre sont protégés dans le tissu végétal intact. En revanche, sous l'effet des procédés tels que les traitements thermiques qui engendrent la dégradation des membranes plasmiques et des cellules pariétales, les enzymes et l'oxygène accèdent plus facilement à leurs substrats ; caroténoïdes et polyphénols sont alors sujets à des dégradations enzymatiques et chimiques affectant la couleur (Renard C., 2014).

1.3.2.3.1. Evolution des caroténoïdes

Les caroténoïdes sont responsables de la couleur jaune, orange ou rouge de nombreux végétaux. Le rouge de la tomate provient du lycopène tandis que la couleur orange de la carotte est principalement due au β-carotène. Par leur structure chimique,

et en particulier la présence d'une chaine carbonée polyconjuguée, les caroténoïdes sont des molécules sensibles à l'oxygène, à la lumière, et à la chaleur, qui peuvent subir des réactions d'isomérisation et d'oxydation (Penicaud*, et al.*, 2011). Les réactions d'oxydation peuvent être auto activées, induites par la lumière ou catalysées par des enzymes. Ces réactions d'oxydation des caroténoïdes induisent non pas un brunissement comme c'est le cas pour les polyphénols mais une décoloration ou un ternissement de la couleur (Delia*, et al.*, 2004).

1.3.2.3.2. Évolution des polyphénols

Le brunissement enzymatique, largement décrit dans la littérature scientifique, consiste en une oxydation enzymatique des composés polyphénoliques par les phénolases (polyphénoloxydases et peroxydases) en quinones très réactives qui se polymérisent ensuite pour donner, fréquemment, des composés bruns (Guyot S., 2014). En plus des concentrations en enzymes et en phénols, le pH, l'activité de l'eau, la concentration en éléments minéraux tels que fer ou cuivre ainsi que temps et température influencent la vitesse de réaction. Or, les barèmes de traitement thermique (temps et températures) appliqués varient considérablement selon les sources : de 2 min à 82°C (Ravindran, 2000) à 10 min à 100°C (Schweiggert, Mix*, et al.*, 2005). On comprend donc que le blanchiment pourra, selon les cas, favoriser la réaction de brunissement enzymatique ou la limiter. En effet, selon Dhas (2003), un blanchiment modéré permettrait l'apparition d'un brunissement uniforme en favorisant (par la mise en contact enzyme – substrat) l'oxydation des phénols par la phénolase alors qu'un blanchiment plus intense désactiverait au contraire les enzymes impliqués limitant du même coup le brunissement du poivre. De même, selon Mangalakumari (1983), la catalyse non observée à température ambiante, serait optimisée vers 75 °C et inactivée à une température de 100 °C pendant quelques minutes. De son côté, Ravindran (2000), observe le même noircissement brillant et uniforme sur le poivre, quels que soient les barèmes de blanchiment appliqués : 2,5 min à 80 °C ou 100 °C. Gu (2013) suggère que le brunissement ne serait pas uniquement d'origine enzymatique. Il pourrait également provenir d'une oxydation purement chimique des polyphénols (Renard C., 2014) ou de réactions de Maillard (Horvathova*, et al.*, 2007).

1.3.2.3.3. Réactions de Maillard

La réaction de Maillard est ainsi nommée en référence au chimiste français Louis Camille Maillard (1878-1936) qui l'a initialement décrite. Elle est souvent définie

comme une réaction de brunissement non enzymatique. En fonction des conditions de transformation des aliments, une réaction chimique génératrice de goûts, arômes et couleurs spécifiques peut en effet se produire entre les acides aminés et les sucres réducteurs. Connue pour se développer à haute température notamment lors d'opérations de cuisson et de séchage, les réactions de Maillard peuvent se développer pour des produits secs (a_w <0.65) au cours d'un stockage (T< 37°C) de quelques mois (Korbel, 2014) et dégrader la couleur qui vire au brun.

1.3.3. Facteurs influençant la qualité du poivre

Plusieurs facteurs peuvent influencer la qualité du poivre. C'est notamment le cas, comme nous l'avons plus haut, des procédés de transformation (Dhas*, et al.*, 2003; Orav*, et al.*, 2004; Suresh*, et al.*, 2007) et de la maturité (Jansz*, et al.*, 1984; Mathai, 1981; Rathnawathie*, et al.*, 1984). D'autres facteurs peuvent également influencer cette qualité. Il s'agit notamment de l'espèce, du terroir, du climat, des conditions de culture (Jirovetz*, et al.*, 2002; Sumathykutty*, et al.*, 1999) et des modes de consommation (Nisha*, et al.*, 2009).

1.4. Conclusion de l'étude bibliographique et objectifs de la thèse

L'analyse bibliographique montre que la qualité du poivre peut être évaluée à travers de nombreux attributs (taille des baies, densité, teneur en huile essentielle et en pipérine, couleur …) et influencée par plusieurs facteurs (espèce, terroir, climat, procédés ...). Nous avons également relevé que si certaines étapes et mécanismes influençant la qualité sont bien connus, l'influence d'une opération unitaire telle que le blanchiment par exemple, était moins évident.

Alors que plusieurs articles traitent du piquant, de l'arôme et de la couleur du *Piper nigrum* et quelques rares autres références de poivres domestiqués et commercialisés tels que le *Piper cubeba,* peu de travaux concernent les poivres sauvages et aucun travail publié ne décrit la qualité du *Piper borbonense*.

L'objectif de ce travail de thèse est d'évaluer l'impact des procédés, et notamment des étapes suivantes : blanchiment, étuvage (étape mise en œuvre à Madagascar mais non mentionné pour le poivre dans la littérature) et séchage sur la qualité du poivre *Piper borbonense* à travers trois composantes : le piquant, l'arôme et la couleur.

Pour atteindre notre objectif et *in fine* répondre à nos questions de recherche, la démarche expérimentale suivante (**figure 7**) a été mise en œuvre.

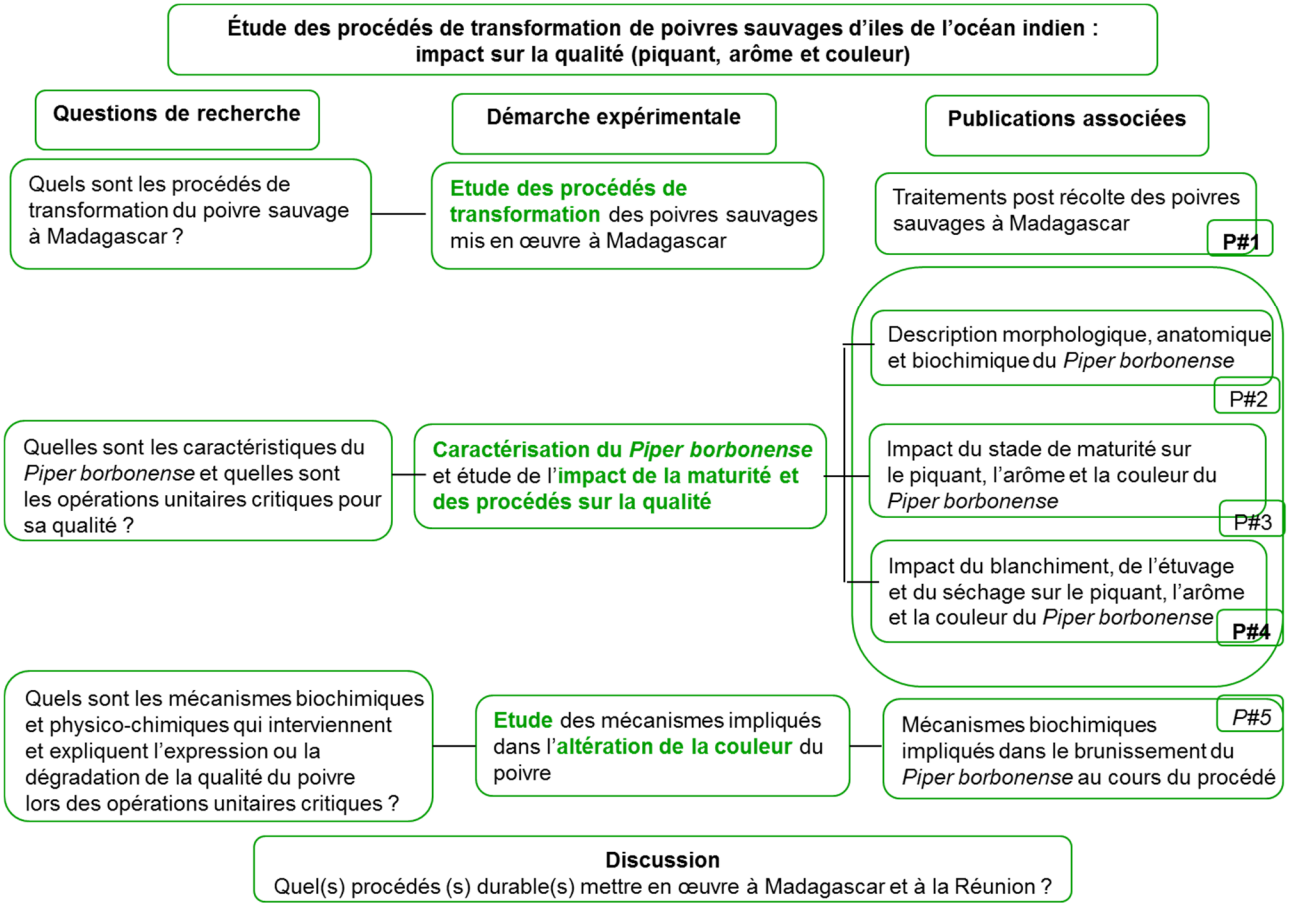


Figure 7. Démarche expérimentale

Ce travail de thèse propose dans un premier temps une étude des procédés traditionnels de transformation du poivre sauvage mis en œuvre à Madagascar (chapitre 1, publication #1). Cette étape préliminaire réalisée dans des conditions difficiles d'investigation en forêt primaire malgache, est suivie d'expérimentations menées en conditions maîtrisées à La Réunion, sur du *Piper borbonense* d'origine sauvage. Ainsi, la morphologie, l'anatomie et la composition biochimique de *Piper borbonense* réunionnais sont caractérisées (chapitre 2, publication #2) pour pouvoir ensuite étudier, l'impact de la maturité (chapitre 3, publication #3) et des procédés (chapitre 4, publication #4) sur son piquant, son arôme et sa couleur. Dans la mesure où c'est cette dernière qui est la plus affectée, les mécanismes impliqués dans l'altération de la couleur du poivre (chapitre 5, publication #5) sont enfin analysés. Les résultats obtenus sont présentés sous la forme de cinq articles scientifiques (publiés, soumis ou à soumettre).

Les résultats de nos travaux sont présentés dans ce mémoire sous forme d'articles scientifiques :

- Publication #1 : Traitements post récolte des poivres sauvages à Madagascar (parue)
- Publication #2 : Description morphologique, anatomique et biochimique du *Piper borbonense* (soumise)
- Publication #3 : Impact du stade de maturité sur le piquant, l'arôme et la couleur du poivre *Piper borbonense* (soumise)
- Publication #4 : Impact du blanchiment, de l'étuvage et du séchage sur le piquant, l'arôme et la couleur du *Piper borbonense* (parue)
- Publication #5 : Etude des mécanismes biochimiques impliqués dans le brunissement du *Piper borbonense* au cours du procédé (en préparation)

Matériel et Méthodes

2. Matériel et méthodes

Les différents matériels et méthodes rapportés dans cette partie constituent une synthèse de ceux décrits dans les différents articles constituant cette thèse. La plupart de ces matériels et méthodes sont plus précis et davantage détaillés dans les articles en question.

2.1. Matière première

Les travaux présentés dans cette thèse ont été réalisés à partir de poivres sauvages collectés à Madagascar (*Piper spp.)* et à la Réunion (*Piper borbonense*).

2.1.1. Poivre collecté à Madagascar

Les Tsiperifery, poivres sauvages de Madagascar, ont été collectés dans deux zones situées entre les hautes terres (à environ 100 km d'Antananarivo, la capitale) et les forêts primaires humides de la côte Est (au nord de Moramanga). Ces deux zones se trouvent à une altitude comprise entre 900 et 1300 m. La zone 1 correspond au corridor forestier de l'Angavo et la zone 2 au corridor forestier de l'Ankaï. L'enclavement et le réseau routier quasi inexistant ont rendu très laborieuses et chronophages les étapes de la prospection, d'enquête, puis de collecte des échantillons.

2.1.2. Poivre collecté à la Réunion

Le *Piper borbonense*, poivre sauvage de la Réunion, a été collecté sur une zone peu étendue (moins de 1500 m2) à la Rivière Langevin (21° 2' 04.49 S ; 55° 38' 33.07 E), tout au Sud de La Réunion. Selon les besoins, des poivres de maturités distinctes ont été collectés : poivre vert immature (maturité A), poivre orange de maturité intermédiaire (maturité B) et poivre rouge mature (maturité C) le plus souvent utilisé dans nos travaux.

2.2. Conservation des échantillons et préparation des lots

Le poivre était immédiatement congelé à -80 °C (Congélateur Froilabo - Bio Memory 690 litres) environ 2h après chaque collecte. Avant les essais, différentes collectes pouvaient être rassemblées et mélangées afin d'obtenir des lots homogènes de taille suffisante pour réaliser nos travaux. Notre objet d'étude étant le grain de poivre avec son pédicelle, les grains de poivre congelés en grappes étaient séparés manuellement

de la rafle avant préparation. Dans les paragraphes qui suivent, on appelle poivre frais un poivre congelé 2h après cueillette, conservé au froid (-80°C), puis décongelé 1h à température ambiante et qui n'a pas été soumis au procédé.

2.3. Procédés mis en œuvre

2.3.1. Blanchiment, étuvage et séchage

Les procédés consistaient en trois opérations unitaires – blanchiment, étuvage et séchage - appliquées, seules ou combinées, selon différents paramètres opératoires pour obtenir nos échantillons. Le blanchiment (B pour Blanching), parfois aussi appelé échaudage dans la filière, consistait à tremper selon un rapport 1 : 36 (p/p), les grains de poivre dans un bain d'eau chaude à une température donnée (Memmert Gmbh type WB 22 Schwabach, Allemagne). L'étuvage (S pour Sweating) consistait à placer les grains de poivre dans une enceinte climatique (BIA Climatic - Type CL 125, Conflans Sainte Honorine, France) à 35 ° C et 99% HR pendant 24 heures. Le séchage (D pour Drying) était réalisé en plaçant des plateaux en aluminium (300 cm2) contenant 250 g de grains de poivre disposés en couche compacte de 1 cm d'épaisseur jusqu'à ce que le poivre contienne 90% de MS. Pour le cas particulier des cinétiques de séchage, c'est une boucle de séchage et des dispositions spécifiques qui ont été mise en œuvre. Les conditions opératoires précises (durée, température, humidité relative …) propres à chaque opération unitaire sont précisées dans les matériels et méthodes de chacun des 5 articles constituant cette thèse.

2.3.2. Cinétique de séchage

Le séchage des échantillons de poivre a été réalisé sur un séchoir pilote (boucle de séchage), développé dans notre laboratoire. Dans la chambre de traitement (0,25 m de longueur x 0,25 m de largeur x 0,92 m de hauteur), 150 g de grains de poivre ont été placés sur un tamis (0,25 x 0,25 m^2) en une couche mince non compacte. L'air chaud (60 ± 1 °C, HR 20 ± 2 %) circulait de haut en bas à 2,7 ± 0,1 ms^{-1} à travers la couche de poivre. La perte de masse de la couche de poivre était mesurée en semi-continu (toutes les 15 min), avec une précision de ± 0,3 g. Lorsque le séchage était terminé, les grains de poivre étaient refroidis par ventilation à l'air à température ambiante et la teneur en matière sèche finale, était mesurée. La quantité de matière sèche était supposée constante et identique à celle initialement présente. La teneur en eau exprimée en base sèche et notée (X) a ainsi été calculée toutes les 15 min.

Ces valeurs discontinues *X* (t) ont été lissées en fonction du temps par interpolation de type spline cubique par morceaux (fonction « csaps » Matlab® Version 5.2, The Mathworks Inc., USA). La vitesse de séchage (d*X*/dt) a été calculée comme la dérivée analytique directe des fonctions spline de cubique.

2.4. Préparation des échantillons

Selon les besoins pour les procédés, mesures ou analyses à réaliser le poivre pouvait être décongelé pendant une heure à température ambiante ou moulu (encore congelé avec de l'azote liquide ajouté) pendant 10 secondes à 10 000 tr/min dans un broyeur à couteaux (Retsch - Grindomix GM200, Retsch Gmbh, Allemagne).

2.5. Méthodes d'analyses

Lorsque les méthodes d'analyses ont été employées à plusieurs reprises (dans différents articles), les coefficients de variation relatifs à ces méthodes donnés dans les lignes qui suivent, correspondent aux plus grands qui ont été obtenus.

2.5.1. Mesure de la masse des grains de poivre

Les masses ont été déterminées avec une balance de précision (Scaltec SBC 22 model, Scaltec Gmbh, Allemagne). La précision de l'équipement était de ± 0.1mg.

2.5.2. Mesure de la longueur et du diamètre des grains de poivre

Les longueurs et diamètres ont été déterminés avec un pied à coulisse (Absolute Digimatic, CD-15CPX model, Mitutuyo Corporation, Sakado, Japon). La précision de l'équipement était de ± 0.2mm

2.5.3. Mesure de la teneur en matière sèche

Les teneurs en matière sèche (comprendre matière sèche sans eau et sans huile essentielle) ont été déterminées par séchage de 5 g de mouture de poivre à 105°C pendant 30h (jusqu'à masse constante) dans une étuve de laboratoire (ULE 400, Memmert Gmbh, Allemagne). Les masses initiale et finale sont déterminées à l'aide d'une balance de précision (Scaltec SBC 22 model, Scaltec Gmbh, Allemagne). Le coefficient de variation est de 0,63% (n=3).

2.5.4. Mesure de la teneur en huile essentielle

La teneur en huile essentielle, exprimée en base sèche, a été déterminée selon une méthode adaptée de la norme standard ISO 6571 (International Standard Organization, 2008). La seule modification par rapport à la méthode standardisée réside dans le fait que n'avons pas employé de xylène dans notre protocole. Le coefficient de variation est de 2,08 % (n=3).

2.5.5. Mesure de la teneur en eau

Les teneurs en eau sont calculées à partir des teneurs en matière sèche (MS) et des teneurs en huile essentielle : T eau = 100 – MS – T huile essentielle.

2.5.6. Mesure de la teneur en piperine

La teneur en pipérine, exprimée en base sèche, a été déterminée, à une longueur d'onde de 343 nm, selon la méthode décrite dans la norme ISO-5564 (International Standard Organization, 1982). Le spectrophotomètre utilisé est un Thermospectronic Helios α v4.60 (Thermo Fisher Scientific, USA). Le coefficient de variation est de 3,32% (n=3).

2.5.7. Identification et quantification des composés de l'huile essentielle

2.5.7.1. Séparation sur colonne polaire

Les composés volatils ont été analysés sur une CPG (HP 6890, Hewlett Packard, Palo Alto, USA), équipée d'une colonne polaire Supelco-Wax (Supelco- 60m x 320 μm x 0,25 μm) couplée à un détecteur SM. Des aliquotes (0,1 μL) d'huile essentielle, obtenus comme décrit en 2.5.4., ont été injectés avec un split (1:30). La température de l'injecteur était de 250°C. La température de la ligne de transfert était de 250°C et le débit du gaz vecteur (hélium) était de 0,8 mL/min. La température du four était programmée comme suit : température initiale de 60°C, augmentation de 4°C/min jusqu'à atteindre une température finale de 230°C maintenue pendant 20 min. Les molécules ont été identifiées par CPG/SM avec une source d'ionisation électronique (70eV). Les masses des composés étaient déterminées par un balayage compris entre 25 et 350 m/z.

2.5.7.2. Séparation sur colonne apolaire

Les composés volatils ont été analysés sur une CPG (HP 6890 Hewlett Packard, Palo

Alto, USA), équipée d'une colonne apolaire SPB-5 (Supelco- 60m x 320 μm x 0,25 μm) couplée à un détecteur SM. Des aliquotes (0,2 μL) d'huile essentielle obtenue comme décrit en 2.5.4., ont été injectés avec un split (1:50). La température de l'injecteur était de 250°C. La température de la ligne de transfert était de 250°C et le débit du gaz vecteur (hélium) était de 0,7 mL/min. La température du four était programmée comme suit : température initiale de 60°C, augmentation de 4°C/min jusqu'à atteindre une température finale de 250°C maintenue pendant 50 min. Les molécules ont été identifiées par CPG/SM avec une source d'ionisation électronique (70eV). Les masses des composés étaient déterminées par un balayage compris entre 20 et 400 m/z.

2.5.7.3. Identification

Les composés aromatiques séparés sur les deux colonnes, ont été identifiés en comparant leurs spectres de masse à ceux disponibles dans les bases de données commerciales (NIST02, WILEY) ou constitués par nos soins et en comparant les indices de rétention calculés à ceux de la littérature (Adams, 1995; Jennings*, et al.*, 1980; Kondjoyan*, et al.*, 1996) et de bases internet (Internet databases, 2014).

2.5.7.4. Quantification sur colonne apolaire

Les composés aromatiques ont été quantifiés soit sur une CPG (HP 5890, Hewlett Packard, Palo Alto, USA), soit sur une Clarus 580 (Perkin Elmer, Villebon-sur-Yvette, France), équipées d'une colonne apolaire SPB-5 (Supelco- 60m x 320 μm x 0,25 μm) couplées à un détecteur FID. Des aliquotes (0,3 μL) d'huile essentielle de chaque échantillon (obtenus comme décrit en 2.5.4.), ont été injectés avec un split (1:33). Le débit du gaz vecteur (hélium) était de 0,7 mL/min. La température de l'injecteur était de 250°C. La température du four était programmée comme suit : température initiale de 60°C, augmentation de 4°C/min jusqu'à atteindre une température finale de 250°C maintenue pendant 20 min. Les quantifications ont été réalisées soit par rapport à un standard interne de terpinolène (2:22) injecté simultanément soit par rapport à la méthode de normalisation interne (Cachet*, et al.*, 2011). Le coefficient de variation est de 0 ,43% (n=3).

2.5.8. Mesure de la teneur en sucres solubles et en amidon

Les sucres solubles et insolubles ont été extraits selon la méthode décrite par Clegg (1956). Les séparations ont été réalisées par extraction à l'alcool ; l'amidon résiduel a ensuite été hydrolysé. 0,2 g de poivre moulu a été pesé dans de l'éthanol à 80% à chaud. Après 10 minutes, le mélange a été filtré sur verre fritté et extrait à nouveau à l'éthanol à chaud pour atteindre un volume final de 10 ml. Le réactif Anthrone a été fabriqué en diluant 1 g d'Anthrone dans 1 litre de solution d'acide sulfurique contenant 760 ml de H_2SO_4 concentré. La réaction a été conduite sur des extraits dilués pendant un temps de réaction de 12 minutes dans de l'eau bouillante et lue à température ambiante à 630 nm sur un spectrophotomètre Specord 600 (Analytiks Jena, Jena, Allemagne). L'hydrolyse de l'amidon a été réalisée à l'acide perchlorique pendant 20 minutes. Les réactions à l'anthrone ont été réalisées sur une solution diluée résultant de l'hydrolyse. Les teneurs en sucres solubles et insolubles sont exprimées en g.100g-1 de matière sèche. Les coefficients de variation sont de ± 12,73% pour l'amidon et de ± 7,36% pour les sucres solubles (n = 6).

2.5.9. Mesure de la teneur en glucose et en fructose

L'extraction aqueuse de sucres a été réalisée en ajoutant 100 ml d'eau milli-Q à 100 mg d'échantillon. Après 1 h d'homogénéisation, les échantillons ont été filtrés à travers un filtre de 0,45 µm (Millipore) et placés dans un flacon avant analyse. Le glucose restant et le fructose ont été contrôlés par HPLC Shimadzu équipée de pompes modèle LC-20AB et d'un passeur automatique SIL-20A (Shimadzu, Kyoto, Japon) couplé à un détecteur PAD Decade 2 (Antec Leyden, Pays-Bas). La séparation des sucres a été réalisée sur une colonne CarboPac MA1 de 4 x 250 mm (Dionex, Allemagne). L'éluant utilisé était une solution dégazée de NaOH 800 mM pompée à un débit de 0,4 ml / min. Des solutions fraîchement préparées de D-glucose et de D-fructose ont été utilisées pour calibrer le système. Les coefficients de variation sont de ± 10,72% pour le glucose (n = 4) et de ± 10,93% pour le fructose (n = 4).

2.5.10. Mesure de la teneur en lipides

La teneur en lipides a été déterminée sur du poivre moulu selon la méthode gravimétrique de Soxhlet. Le dispositif semi-automatique Soxtec-Avanti 250 (Foss, Hillerod, Danemark) a été utilisé pour extraire les graisses avec de l'éther de pétrole comme solvant. Le temps d'extraction était de 90 minutes à 110 ° C. Les extraits gras sont ensuite maintenus 16h à 110 ° C afin d'éliminer les traces de solvant. La teneur

en graisse a été exprimée en g.100 g-1 de matière sèche. Le coefficient de variation est de ± 3,39% (n = 6).

2.5.11. Mesure de la teneur en polyphénols

La teneur en polyphénols, exprimée en équivalent acide gallique, a été déterminée à une longueur d'onde de 760 nm selon la méthode colorimétrique inspirée de Folin-Ciocalteu, décrite dans l'ISO 14502-1 (International Standard Organization, 2005). Le spectrophotomètre utilisé était un Specord 600 (Analytik Jena AG, Lena, Allemagne). Le coefficient de variation est de ± 5,8% (n = 3)

2.5.12. Mesure de la teneur en caroténoïdes

Les caroténoïdes ont été extraits de 200 mg de poivre broyé mélangés dans un tube contenant 1 ml d'eau distillée pendant 2 minutes. Ensuite, 10 ml d'éthanol/hexane (4/3 v/v) ont été ajoutés avant homogénéisation pendant 60 secondes dans un Fastprep 24 (MpBiomedical, Santa Ana, USA) en utilisant du sable comme agent de lyse mécanique et une bille de céramique comme mortier. La phase hexanique a été récupérée et les résidus éthanoliques ont été mélangés à nouveau avec 5 ml d'hexane. Cette opération a été répétée trois fois. Toutes les phases organiques ont été recueillies ensemble et séchées avec du sulfate de sodium anhydre. Après évaporation sur Genevac HZ plus (Genevac, Warminster, USA), les extraits ont été récupérés dans 0,5 ml de dichlorométhane et 0,5 ml de méthanol/methyl tert-butyl ether (80/20 v/v). Les caroténoïdes ont ensuite été analysés selon la méthode décrite par Dhuique-Mayer *et al*. (2016). Le système HPLC utilisé était un détecteur à barrette de photodiodes Agilent 1100 (Agilent, Massy, France). La colonne était une colonne C30 (250 × 4,6 mm id, 5 µm: YMC Europ GmbH, [YMC, Dinslaken Allemagne].) La quantification des caroténoïdes a été réalisée par rapport à une courbe de calibration du β-carotène à 450 nm. Les coefficients de variation sont de ± 6,8% pour les caroténoïdes totaux (n = 9) et de ± 5.3% pour le β-carotène (n=9).

2.5.13. Mesure de la teneur en cellulose, hémicellulose et lignine

Les teneurs en fibres, exprimées en sec, ont été déterminées selon le principe de Van Soest, d'après la méthode décrite dans le fascicule de documentation FD U44-162 (AFNOR, 2016). Les coefficients de variation sont de ± 4,66% pour la cellulose, ± 18,44% pour l'hémicellulose et ± 7,65% pour la lignine (n = 4).

2.5.14. Mesure de la teneur en acides aminés

Les acides aminés libres ont été analysés selon la méthode utilisée par Moore (1958). L'analyse des acides aminés totaux a été réalisée en utilisant un analyseur d'acides aminés Biochrom 30 (Biochrom Ltd., Cambridge, Royaume-Uni). La séparation des acides aminés le long de la colonne cationique a été obtenue par une succession de quatre tampons de citrate de sodium ayant un pH croissant (2,6-8,6), une force ionique (0,2-0,5 M) et un gradient de température croissant (52-95 ° C). Les acides aminés ont été dérivatisés avec le réactif à la ninhydrine (135 °C) et détectés simultanément à 570 nm et 440 nm. L'ensemble du processus a duré 90 min par échantillon, incluant la phase de régénération de la résine. La quantification a été réalisée en comparant les aires des pics avec un étalon standard incluant 26 acides aminés acides, neutres et basiques (Sigma, St. Louis, Missouri, USA). La norleucine (250 nmol mL-1 dans du tampon citrate de sodium, 0,2 M, pH 2,2) a été utilisée comme étalon interne. L'écart relatif moyen de répétabilité de la méthode est d'environ ± 5%.

2.5.15. Mesure de la teneur en composes minéraux

500 mg de poivre sont minéralisés par deux calcinations successives de 1h30 et 30 min dans un four (Thermolyne Muffle Furnace 6000, Thermofisher, Waltham, USA) à 500 °C. Les cendres sont ensuite mises en solution avant d'être analysées par spectrométrie d'émission atomique à couplage inductif ICP- AES (Agilent 720 series, Agilent Technologies, Santa Clara, USA).

2.5.16. Mesure de la teneur en 5-Hydroxyméthylfurfural

La méthode est inspirée de celle décrite dans le Règlement CEE n°2676/90 de la Commission, du 17 septembre 1990 (JO, 1990). 150 mg d'échantillon sont agités pendant 1 h dans 2,25 ml d'eau Milli-Q. Les extraits sont ensuite centrifugés pendant 3 minutes à 5 000 g. Le surnageant est filtré à travers un filtre de 0,45 µm (Millipore) avant d'être placé dans des flacons pour analyse chromatographique. Les analyses sont effectuées sur une HPLC Dionex Ultimate 3000 (Dionex, Allemagne). Les échantillons ont été élués sur une colonne Gemini C18 110A 5 um 250 x 4,6 mm équipée d'une pré-colonne Gemini C18 4 x 3 mm (Phenomenex, USA). La phase mobile était une solution d'eau-méthanol-acide acétique [90/9/1% (v / v)] pompée à un débit de 1 ml / min. Les détections du 5-HMF ont été réalisées à 280 nm. Le calibrage du système a été effectué avec une solution fraîchement préparée de 5-HMF avec des

concentrations allant de 3 à 30 mg/l.

2.5.17. Mesure des activités enzymatiques

Les activités enzymatiques PPO et POD ont été évaluées par spectrophotométrie en utilisant un spectrophotomètre Thermospectronic Helios α v4.60 (Thermo Fisher Scientific, USA). Les enzymes ont été extraites par centrifugation (Centrifugeuse - Sigma 3-18K, rotor SIGMA 11135, Sigma Neustadt Gmbh Allemagne) de 0,2 g de poivre broyé (broyeur Retsch - Grindomix GM200, Retsch Gmbh, Allemagne) dans 1,6 ml de tampon Mac Laine pH 6,5 pendant 20 mn à 14 000 g et 4 ° C. Une solution de catéchol à 0,175 g/mol a été utilisée comme substrat phénolique pour la détermination de l'activité PPO. Du gaïacol 0,1 g/mol en présence de H_2O_2 0,05 g/mol a été utilisé comme substrat phénolique pour la détermination de l'activité de la POD. La densité optique a été mesurée à 420 nm à 30 °C à des intervalles de 5 secondes pendant 1 minute. Les activités de PPO ou POD ont été définies comme la variation de l'absorbance.min-1 .mg / g (db). Les activités résiduelles de PPO et de POD ont été exprimées en pourcentage de l'activité maximale mesurée dans le milieu réactionnel à partir d'échantillons de poivre frais non traités. Les coefficients de variation pour les activités enzymatiques sont de ± 17,5% pour la PPO (n = 9) et de ± 9,0 pour la POD (n = 9).

2.5.18. Mesure de la couleur

Les mesures de couleur (valeurs CIE L *, a * et b *, représentant respectivement la clarté, la nuance entre le rouge et le vert et la nuance entre le jaune et le bleu) ont été effectuées sur des grains de poivre entiers en utilisant un Minolta CR 410 et son logiciel associé. Dix mesures ont été effectuées sur chaque échantillon de grains de poivre étalé en une couche de 1 cm d'épaisseur dans une boîte de Pétri non couverte. Les coefficients de variation sont de 2,2%, 3,4% et 6,0% respectivement pour L *, a *, b * (n = 10). Des photographies de grains de poivre frais et transformés ont été faites à l'aide d'un appareil photo numérique hp d3500 (Hewlett-Packard, États-Unis).

2.6. Traitements statistiques

Les différences statistiques des moyennes ont été testées par analyse de variance (ANOVA); l'importance des différences entre les échantillons a été déterminée en utilisant le test de Tukey. Le niveau de signification était $P < 0,05$.

Résultats

3. Résultats

3.1. Chapitre 1. Traitements post récolte des poivres sauvages à Madagascar

3.1.1. Article paru

Postharvest treatments of wild pepper (*Piper* spp.) in Madagascar

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Abstract

Introduction. A study on postharvest treatments of wild peppers was carried out in Madagascar with the aim of describing the local practices and measuring their impacts on the quality of the products. **Materials and methods**. Four distinct pepper production systems (PPS) were observed, described and compared in two separate areas in East Madagascar. Major quality characteristics (piperine and essential oil) of the peppercorns were assessed in samples collected in the four systems. **Results and discussion**. Two main postharvest processes (dry and wet) were identified. The wet process differed from the dry one in that it involved two specific operations, blanching and sweating. The processes influenced the color of the pepper. Piperine contents were not affected by any of the pepper production systems, whereas essential oil contents were reduced by up to 27% by the wet process. After processing, piperine contents were up to eight times lower, whereas essential oil contents were up to six times higher than the specifications of the standard ISO 959-1 for black pepper ready for commercialization. **Conclusion**. Two main processes (dry and wet) for treatment of peppercorns in Madagascar were identified and described. The dry process, with two steps less, appeared to be easier to implement and more respectful to the product. Improving maturity control and processing according to the quality expected by the markets will be necessary to promote Malagasy peppers.

Key words
Madagascar / *Piper* / poivre / traitement / huile essentielle / couleur

1. Introduction

Since antiquity, spices and herbs have been used throughout the world to enhance flavor

and preserve food, as well as for medicinal and cosmetic purposes (Mamatha*, et al.*, 2008). They were highly sought after, much like gold. Today spices are no longer luxury items, but they are in high demand and their importance is still growing (Schweiggert, Carle*, et al.*, 2007). The genus *Piper* belongs to the family *Piperaceae* and comprises more than 700 species distributed throughout tropical and subtropical regions of the world (Sumathykutty*, et al.*, 1999). Among this huge diversity, one species, *Piper nigrum*, represents the vast majority of the 435,000 t of pepper (*Piper* spp.) produced in the world in 2011 for a value of 900 M$1. This black pepper (*Piper nigrum*) is used extensively; it is known as the king of spices as it is the most popular spice worldwide. It has been the subject of several studies showing, for instance, that it can be transformed by dry or wet processes. Dhas*, et al.* (2003) described the various types of operations such as blanching (wet process), cleaning or drying. The impacts of some of these operations on black pepper quality were assessed by Nisha*, et al.* (2009), who showed piperine stability after heat processing with only 5% loss after 20 min at 100 °C. Using the same process, essential oil was reduced by about 30%. Similarly, Suresh*, et al.* (2007) observed a maximum piperine loss of 34% in black pepper cooked under pressure for 10 min. However, most peppers remain non-cultivated wild species, mostly handpicked in limited quantities and consumed locally. To our knowledge, no scientific studies have been published on wild peppers. One or several wild pepper species that do not belong to *P. nigrum* (genetic determination is ongoing), locally named Tsiperifery, grow in Madagascar's primary rainforests. Part of the Tsiperifery production, estimated at (30 to 50) t of dry product per year (unpubl. results) is collected and transformed for local consumption or export. These Malagasy wild peppers, little known compared with *P. nigrum*, have started to gain fame in French gastronomy. The literature is thus very scarce but there is a need to acquire knowledge about the transformation processes. In our study we describe the main local postharvest treatments of wild peppers (*Piper* spp.) in the East coast forest corridors of Madagascar and assess the impacts of these processes on some main quality characteristics, *i.e.*, essential oil and piperine contents, and visual aspect.

2. Materials and methods

2.1. Wild pepper production systems

In Madagascar, although September to December is the most suitable period, it is

1 FAOSTAT,http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor, 30 Oct. 2013.

possible to find mature wild pepper almost throughout the year, *i.e.*, from April to January. Our study (sampling included) was carried out from July to November 2012 in two different zones (zones 1 and 2), located between the Madagascar highlands (≈ 100 km from Antananarivo, the capital) and the primary rainforests of the East coast (north of Moramanga), both at an altitude between (900 and 1300) m (**figure 8**). These zones were selected because chief local traders described them as being the main locations from which most Malagasy wild pepper is collected. Our study was not easy to carry out because the actors were difficult to reach most of the time and several hours' walking was needed to reach picking and collecting sites in both areas. Nevertheless, four distinct pepper production systems were selected as study cases in the two zones (**table 3**). One pepper production system (PPS1) was located in zone 1 (Angavo forest corridor zone), and the other three pepper production systems (PPS2, PPS3 and PPS4) were in zone 2 (Ankaï forest corridor zone). The pepper production systems PPS1 and PPS2 were operated by Madépices Company (Anatananarivo). The pepper production systems PPS3 and PPS4 were operated by Cent. Techn. Hortic. Tamatave (CTHT) and SOPRAL Co. (Tamatave), respectively. These actors produce annually about 15 t of dry wild pepper of a total estimated to be between (30 and 50) t; this represents between 30% and 50% of the total Malagasy production. A checklist was used for interviewing 28 actors or groups of actors (four groups of pickers, eighteen collectors and six processors- exporters) in order to characterize the pepper production systems. The checklist included the following: (i) description of activities (history, motivation, organization); (ii) pepper quality perception, evaluation and control; (iii) process description; (iv) commercialization (volumes collected or purchased, sales, prices); and (v) relations with other actors of the chain. For the process description, we used the 5M methodology – a widely utilized tool in developing hazard analysis critical control point (HACCP) systems (Trienekens*, et al.*, 2008) – to describe each step of the four studied pepper production systems accurately and exhaustively through five dimensions: men, materials, machines, methods and the environment (“mother nature”). At least three visits per actor (pickers, collectors, processors and exporters) were made to achieve process descriptions.

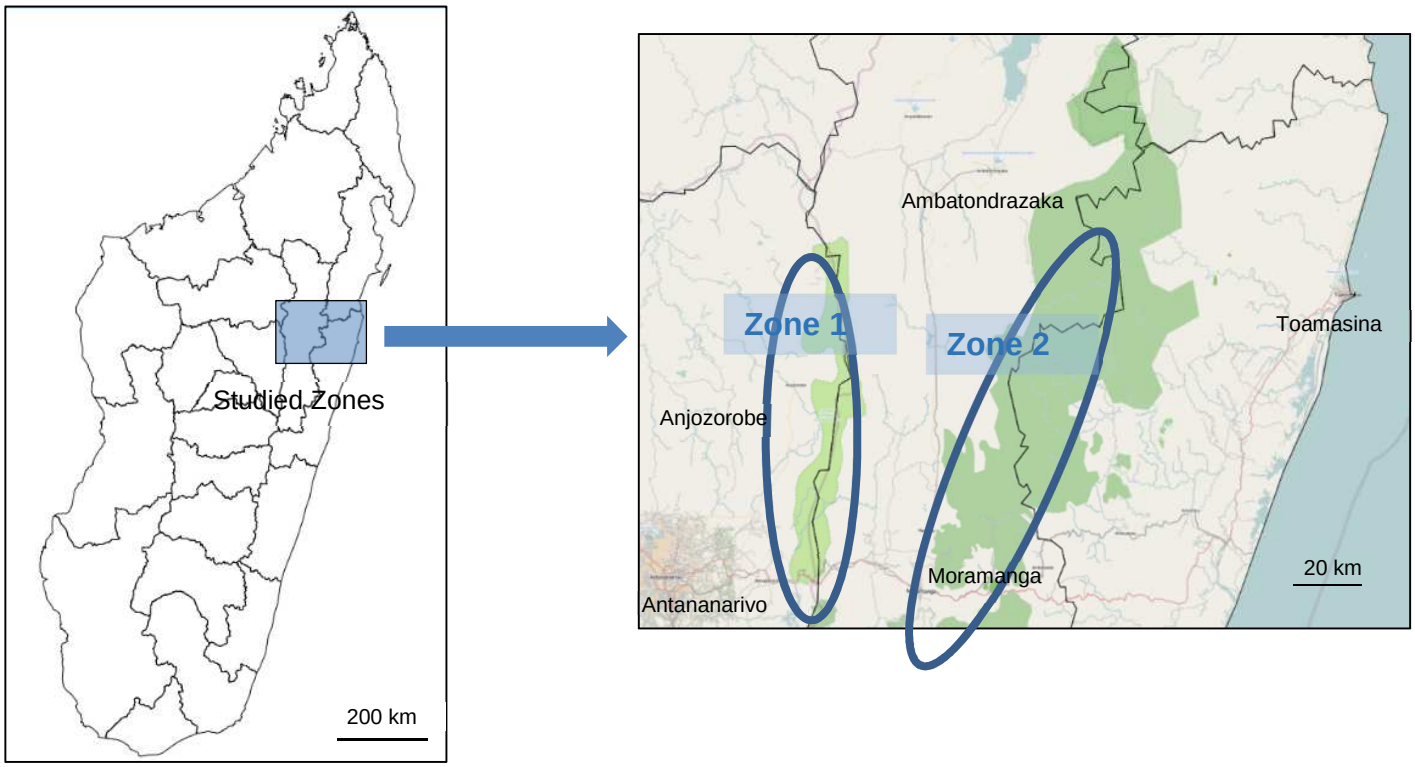

Figure 8. Zones selected for studying postharvest treatments of wild pepper (*Piper* spp.) in Madagascar: Zone 1, Angavo forest corridor; Zone 2, Ankaï forest corridor

Table 3. Mean values of piperine (*n* =3) and essential oil (*n* =2) contents at different steps (t_0 and t_1) of the postharvest processes (DP: dry process; WP: wet process) with samples collected in two different zones (Zone 1: from 40 to 100 km northeast of Antananarivo; Zone 2: from 0 to 100 km north of Moramanga) in four distinct pepper production systems (PPS1, PPS2, PPS3 and PPS4; see figure 9).
(): standard deviation. In columns values (considered 2 by 2) with different letters are significantly different (P<0.05).

Postharvest Process	**Zone**	**Reference**	**sample**	**content (g/ 100 g dry matter)**	
				piperine	**essential oil**
DP	1	PPS1	1rst	2,3 (0,4) a	12,4 (0,2) a
DP	1	PPS1	2nd	2,8 (0,2) a	11,7 (0,3) a
DP	2	PPS2	1rst	3,4 (0,1) b	13,1 - b
DP	2	PPS2	2nd	3,1 (0,2) b	13,4 (0,3) b
WP	2	PPS3	1rst	0,5 (0,1) c	6,9 (0,2) c
WP	2	PPS3	2nd	0,5 (0,1) c	5,6 (0,2) d
WP	2	PPS4	1rst	1,2 (0,2) d	2,8 - e
WP	2	PPS4	2nd	1,2 (0,1) d	2,0 - f

2.2. Determination of peppercorn quality

Pepper samples were collected at different steps (**figure 9**) of the four pepper production systems for quality analysis.

2.2.1. Sampling procedure

Eight samples (two per pepper production system) of about 400 g each were collected at two steps (*t0* and *t1*) of each of the four systems studied in zone 1 (PPS1) and zone 2 (PPS2, PPS3 and PPS4). In PPS1, PPS2 and PPS3, the two samples corresponded to the beginning (*t0*) and the end (*t1*) of the processes. In PPS4, as the objective was to determine the impact of blanching, *t0* and *t1* corresponded to the steps just before and just after the critical step, respectively. All samples were carried to the laboratory and stored at –80 °C before analysis. The practices observed in the field consisted of collectors gathering lots picked by different pickers in various places (in our defined zones). The samples thus collected in each zone were a mixture of peppers (various species) from different plots (with possibly different climates and soils) at various stages of maturity. We ensured, however, that peppers had not been mixed between *t0* and *t1* in the four pepper production systems.

2.2.2. Sample preparation

Each sample was thawed for 24 h at 50 °C in an oven (Memmert ULE 400, Memmert Gmbh, Germany). Peppercorns with peduncles were then manually separated from fruit stems before being ground for 10 s at 10,000 rpm with a cutting mill (Retsch – Grindomix GM200, Retsch Gmbh, Germany).

2.2.3. Analytical methods

2.2.3.1. Dry matter content

The dry matter content was obtained by drying 5 g of ground pepper in an aluminum cup in the oven at 105 °C for 30 h (*i.e.*, until constant weight). Initial and final masses were determined with a precision balance (Scaltec SBC 22 model, Scaltec Gmbh, Germany). The maximum standard deviation of repeatability was ± 0.1% with $n = 3$.

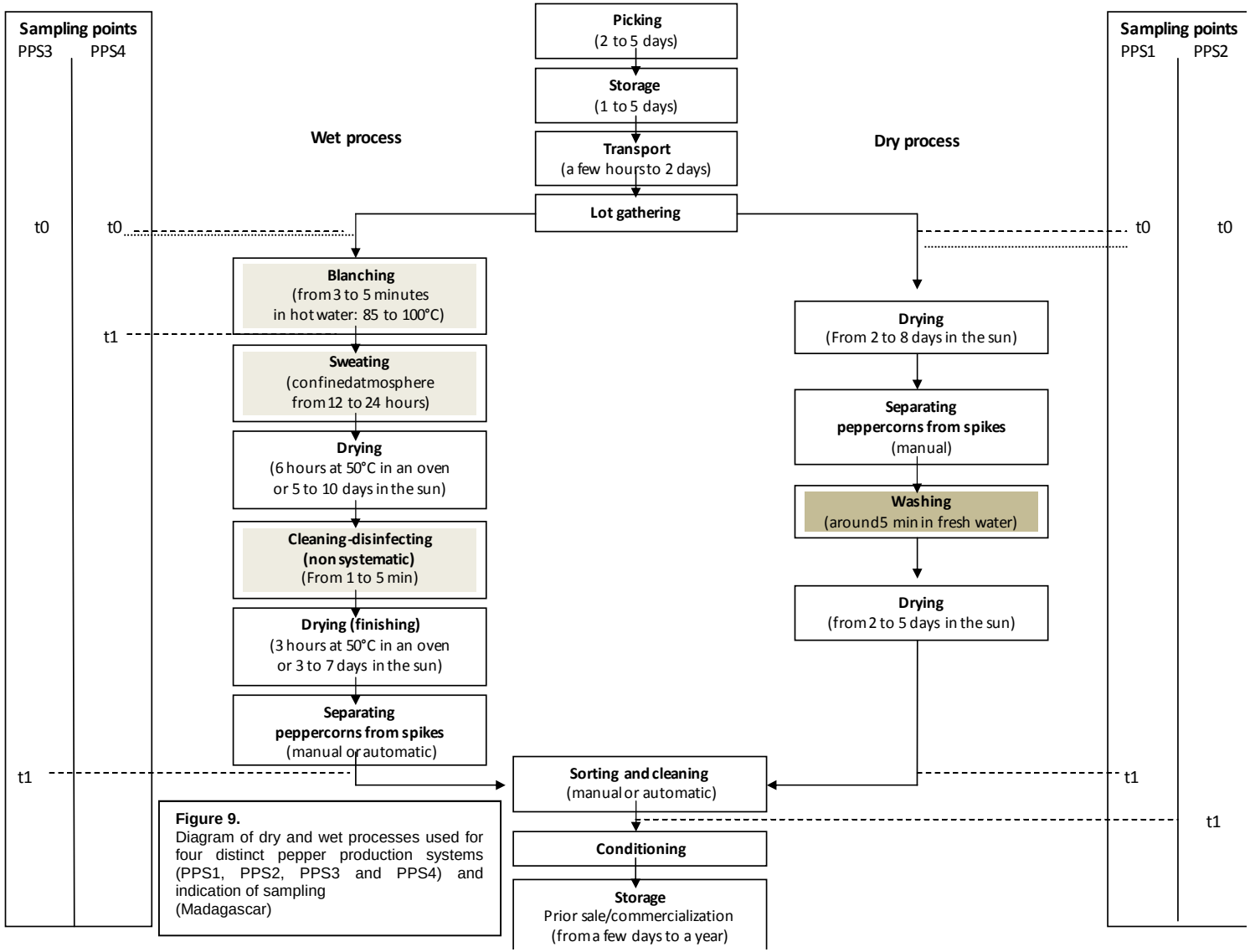

Figure 9.
Diagram of dry and wet processes used for four distinct pepper production systems (PPS1, PPS2, PPS3 and PPS4) and indication of sampling
(Madagascar)

2.2.3.2. Piperine content

The piperine content, expressed on a dry basis, was determined according to the spectrophotometric global method described in the standard ISO 5564 (International Standard Organization, 1982). The spectrophotometer used was a Thermospectronic Helios α v4.60 (Thermo Fisher Scientific, USA). The maximum relative deviation of repeatability was ± 12% with n = 3.

2.2.3.3. Essential oil content

The essential oil content, expressed on a dry basis, was determined using a method adapted from the standard ISO 6571(International Standard Organization, 2008). One modification in the applied method was the elimination of xylene. The maximum relative deviation of repeatability was ± 11% with n = 8.

3. Results and discussion

3.1. Description of pepper postharvest treatments

Two different types of processes, one dry and one wet process, were identified in the studied zones. The dry process appeared to be easier to implement. The main difference between the dry and wet processes was that the wet process included two successive steps called blanching and sweating (**figure 9**). The 5M methodology provided information on: (i) the material, *i.e.*, maturity, size, color and state of the peppercorns (fresh, wet, dry); (ii) objects, tools or equipment, *e.g.,* bags, winnows, separators; (iii) conditions, *e.g.*, inside or outside, temperature and humidity, cleanliness; (iv) the method, *i.e.,* the way each step is handled, what method was used; and (v) the persons involved in processing pepper. The observed processes are precisely detailed hereafter. Some process steps were common to both processes, whereas others were not.

3.1.1. Picking

Picking could last two to five days depending on the time pickers spent in the forest. The methods used consisted of (i) tree climbing up to 20 m to pick fruits directly, and, more often, (ii) uprooting vines or even (iii) cutting off live supports with machetes and axes. The last two methods are considered as having a negative impact on the pepper resource, and sometimes even on the forest after the trees have been logged. The maturity of the picked peppercorns was very heterogeneous (**figure 10**, fresh wild

peppercorns at *t0*) for the following reasons: vine fructification within the same area could last several months, spike maturity varied on the same vine, and fruit maturity also varied on a given spike. This heterogeneity of maturity affects the size and color of the peppercorns. After picking, the gatherers separated the spikes from the vine and leaves. Sometimes pickers kept the spikes (covered with fruits) in their hats and pockets before putting them into plastic bags. The quantity of pepper picked by one picker varied from (1 to 20) kg per day.

3.1.2. Storage

Storage was repeated between several steps of the production systems. After picking, transport or gathering, intermediary storage consisted of a period that could last of from one to five days depending on the practices, the time spent by the gatherers in the forest, and the distance between the forest and village markets or collecting points. Pepper spikes were kept in plastic bags that were sometimes hung above ground to protect them from animals. At night, the pepper was sometimes spread on the plastic bags or on banana leaves. In their final storage phase before conditioning or before commercialization, peppercorns could be kept for more than a year in baskets made of natural local fibers, in plastic buckets or in individual conditioning polyethylene or polypropylene bags.

3.1.3. Transport

Transport could take from a couple of hours to two days depending on the distances and means used: by foot, bicycle, motorbike, bus and, less frequently, car. It was repeated each time pepper was traded from one actor to another as gatherers, collectors and distributors were generally located in different places. Peppers, which were only partially dried, were usually kept in plastic bags during transport.

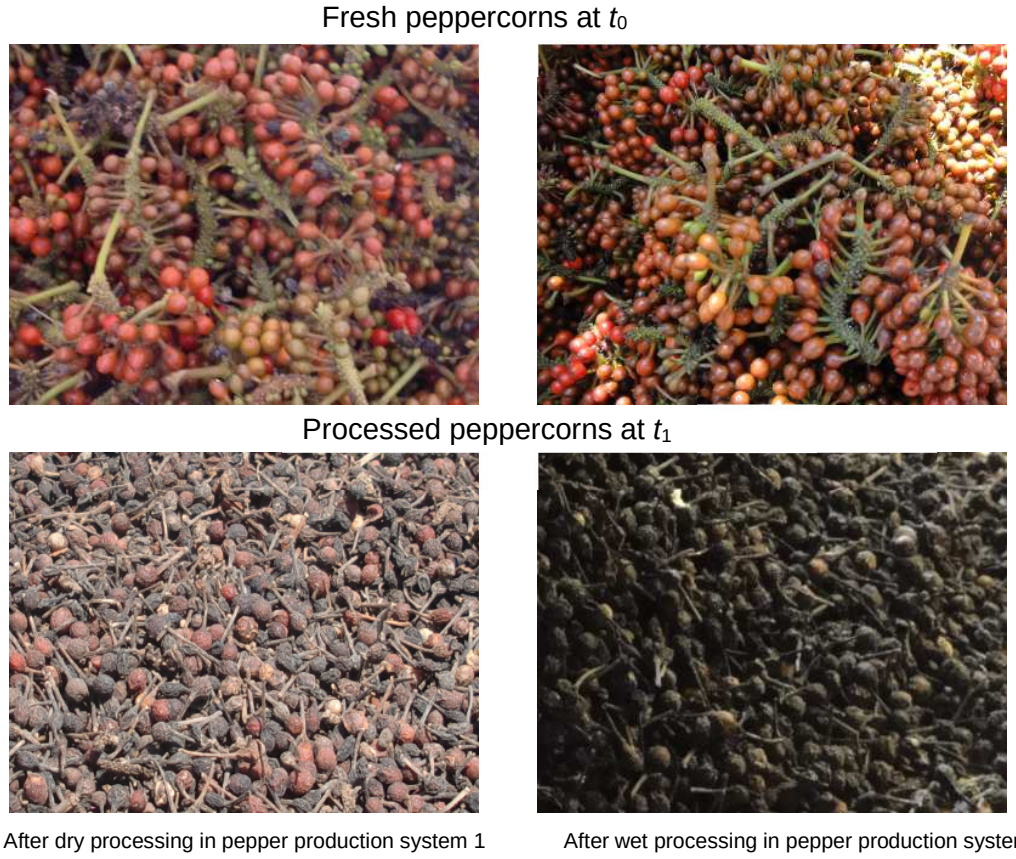

Figure 10. Fresh wild peppercorns at t0, and processed peppercorns at t1 either after dry processing in pepper production system 1 or after wet processing in pepper production system 3 (Madagascar).

3.1.4. Gathering the lots

Gathering could be performed by primary and secondary collectors (who collected from a few to one hundred kilograms a week) as well as by distributors (up to one ton). It consisted of transferring pepper from one container to another (usually larger) without consideration of quality except at the distributors' level. The containers, usually plastic bags of different sizes, were sometimes made of natural fibers such as burlap.

3.1.5. Drying

Drying was carried out by various actors and repeated at different steps during the process: reduction in water content ensured peppercorn preservation essentially by limiting microbial growth. The dry matter content measured in fresh peppercorns at the beginning of the drying step ranged from 26% to 30%. In PPS3, drying was conducted

in thin layers on racks made of synthetic fiber in an oven at 50 °C for (6 to 12) h. In PPS1, PPS2 and PPS4 it was conducted in the sun on or above ground, and the pepper was kept inside (spread out or not) during rainy periods and at night. The support types (palm mats, drying racks in natural or synthetic fibers, or plastic sheets) and the height of the layers, that were or were not regularly turned, changed according to the actors in the various systems. Sun-drying periods, continuous or not, lasted from two to almost fifteen days depending on the pepper lots, the actors' know-how and availability, and the climate. It must be emphasized that, depending on the time of the day, the season and the zone, the temperature and humidity varied from 15°C to 35°C and from about 40% to 90%, respectively. At the end of drying, the dry matter measured (*e.g.,* 95% in PPS1 and 93% in PPS2) was all above the 87% minimum value specified in the standard ISO 959-1 for black pepper ready for commercialization. Partial stalking and sorting (*e.g.*, removal of foreign matter, leaves and spikes) were also routinely performed during drying.

3.1.6. Separating peppercorns from spikes

This step consisted of separating berries from spikes. It was easier to perform after drying but was sometimes carried out during drying. It was usually done manually by rubbing spikes against one another, by threshing, by trampling, or by rubbing spikes on an abrasive object such as a metal colander to facilitate separation, but this last method damages peppercorns. An automatic separator (a pilot made by Sunthesis, Antananarivo, Madagascar) was used in PPS3 and PPS4.

3.1.7. Sorting and cleaning

The various actors partially sorted and cleaned the peppercorns throughout the process, activities which were usually finalized by the distributor or the exporter before conditioning and storage. Sorting served to eliminate foreign matter, dirt, and immature and lightweight aborted or broken berries. It was usually carried out manually or with a winnow and, more rarely, with a densitometric separator (such as the cyclone separator, a pilot made by Sunthesis and used in PPS3). Separate batches could then be classified into different pepper commercial categories.

3.1.8. Conditioning

The distributors conditioned peppercorns by packing them into sale units. The materials used were in direct contact with the product and were made of one or several layers of polyethylene or polypropylene. In PPS3 and PPS4 peppercorns could be packed under partial vacuum.

3.1.9. Washing (dry process only)

The washing phase, observed in PPS1 and PPS2, was carried out with fresh tap water on dry peppercorns when their dry matter content was about 93%. The peppercorns were hand-washed in a colander set inside a plastic bowl for around five minutes. Floating impurities and dust were removed with a small steel strainer. The operation was usually repeated twice before the peppercorns, whose dry matter content decreased from 93% to 61% in PPS1 and from 93% to 74% in PPS2, were set to dry again.

3.1.10. Blanching (wet process only)

Blanching (sometimes called bleaching or scalding) consisted of dipping peppercorns either directly or inside a net (mosquito-net type) or in a basket made of natural fibers or in a metal colander into simmering or boiling water (100 °C) for (3 to 5) min. The pepper was then drained. Blanching is used, according to (Dhas*, et al.*, 2003), not only to remove impurities (dust and foreign matter) and decrease the microbial load but also to increase the speed of drying that follows. Blanching also allows the development of a uniform browning by promoting oxidation of phenols by phenolase enzymes (Mangalakumari*, et al.*, 1983) or by other browning mechanisms that have not yet been determined (F. Gu*, et al.*, 2013).

3.1.11. Sweating (wet process only)

Sweating was performed immediately after blanching and consisted of storing pepper inside or in the shade and above ground, in a confined atmosphere, *i.e.*, burlap, fabric or plastic bag for (12 to 24) h. According to some PPS3 and PPS4 actors, the practice of combining blanching and sweating had been implemented according to the method used for traditional vanilla bean curing, which was described by Odoux*, et al.* (2006). Indeed, in vanilla processing, the curing step triggers enzymatic reactions that contribute to aroma development.

3.1.12. Cleaning and disinfecting (wet process only)

Cleaning and disinfecting were not systematic. They were sometimes observed before blanching in PPS3, but were more often carried out in PPS4 after a first drying operation. In addition to washing the product, this step aimed at reducing microbiological contamination when there was presumption or proof of microorganism development (*e.g.*, presence of white mold on the surface). To do so, pepper was soaked in chlorine water in plastic bowls for (1 to 5) min. The available chlorine ranged between (6 and 50) µL·L–1. These concentrations are much below those proposed in the European standard EN 13697 (European Commission, 2001), that recommends 260 µL·L–1 with a contact time of 15 min for efficient disinfection.

3.2. Determination of some quality characteristics of the peppercorns

The visual aspect (color and size), pungency, aroma of the peppercorns and homogeneity of the batches were the quality criteria that were the most cited by the various actors. We decided to consider the visual aspect, and essential oil and piperine contents, as all three are cited in the standard ISO-959-1 (International Standard Organization, 1998). There are more piperine and essential oil in the pepper used as a raw material in PPS1 and PPS2 than in PPS3 and PPS4. These differences could be due to the origins (climate and soils, for instance), maturity and species of the different lots of wild pepper.

3.2.1. Piperine content

At the beginning of the process (*t0*), the piperine contents were measured in the four samples collected after gathering the lots. They ranged from 0.5% to 3.4% (dry basis) (**table 3**). The rates of 0.5% to 3.1% obtained in samples after treatments (*t1*) were all below (and up to eight times lower than) the 4% content recommended by the standard ISO 959-1(International Standard Organization, 1998). Our analysis also revealed that the processes, whether wet or dry, had no impact on the piperine contents of the peppercorns. This result agreed with those of Nisha *et al.* regarding the kinetic reaction rates of piperine degradation during heat treatment (Nisha*, et al.*, 2009). However, it differed from that reported by Suresh*, et al.* (2007) who obtained about 25% loss in peppercorns after heat processing.

3.2.2. Essential oil content

At the beginning of the process (*t0*), the contents of essential oil measured in samples collected after gathering the lots (**table 3**) ranged from 2.8% to 13.1% (dry basis). The 2.0% to 13.4% rates found in the samples after treatments (*t1*) were all higher (and up to six times more than) the 2% rate indicated in the standard ISO 959-1(International Standard Organization, 1998). The dry process did not impact the essential oil content, whereas the wet process reduced the essential oil content of peppercorns by up to 28%.

In a study on rosemary, Szumny*, et al.* (2010) reported a reduction of around 40% in the essential oil content when they treated the leaves for 30 min at 60 °C.

3.2.3. Visual aspect

Considering the evolution of wild peppercorns at the beginning (*t0*) and at the end (*t1*) of PPS1 and PPS3, in both processes, the fresh peppercorns (*t0*) used as raw material appeared to be heterogeneous in size and color (**figure 10**). Their lengths varied from (0.2 to 0.6) cm and their sections from (0.2 to 0.5) cm. Color ranged from green to deep purple, with red dominant. This heterogeneity reflected the many differences in maturity. After treatment (*t1*), the lengths and sections of the peppercorns were all reduced to values between (0.1 and 0.4) cm. The color of peppercorns dry-processed in PPS1 appeared lighter with a majority of gray and some light purple, whereas the peppercorns wet-processed in PPS3 appeared black and dark gray (**figure 10**). In both cases, the heterogeneity of colors was reduced by the processes, especially in the wet process.

4. Conclusion

We described the local processing practices of Malagasy wild pepper in detail through the study of four pepper production systems located in two separate areas, known as the main picking and processing zones for this product in Madagascar. Observing and describing the four pepper production systems in these areas has been quite a challenge because the systems were informal, and it was difficult to reach the locations and schedule meetings with the various actors. Despite the lack of structure for this wild pepper commodity, two main processes (dry and wet) were identified and analyzed. The dry process appeared to be more respectful to the product and easier to implement; indeed, the wet process differed from the dry one in that it included two

additional operations: blanching and sweating. Piperine was not affected by the type of production system, whereas essential oil was reduced by the wet process. After processing, piperine was up to eight times lower and essential oil up to six times higher than the specifications of the standard ISO 959- 1 (International Standard Organization, 1998) for black pepper ready for commercialization. Improving maturity control and processing according to the quality expected by the markets will be necessary to promote Malagasy peppers.

3.1.2. Synthèse du Chapitre 1 et perspectives

Deux procédés de transformation distincts sont mis en œuvre à Madagascar : une voie sèche consistant en un simple séchage et une voie humide incluant blanchiment et étuvage avant séchage. La couleur est fortement influencée (le poivre brunit) par les procédés quels qu'ils soient alors la teneur en huile essentielle est peu impactée et la teneur en pipérine pas du tout. Les poivres malgaches se distinguent du poivre noir tel qu'il est décrit dans la norme internationale ISO 959-1 (International Standard Organization, 1998) par une teneur en huile essentielle six fois plus importante et une teneur en pipérine huit fois plus faible.

Nous nous sommes ici intéressés aux procédés de transformation, méconnus et jamais décrits, de poivres sauvages malgaches. Rappelons qu'il n'existe pas de procédés de transformation du *Piper borbonense* à la Réunion. La principale limite de notre approche réside dans le fait que les procédés sont mal maitrisés à Madagascar. En effet, le mode de cueillette puis d'échange entre acteurs conduit à la constitution de lots composés de diverses espèces de poivre de maturité hétérogène. En outre, les étapes de transformation, souvent mal contrôlées du fait du manque d'équipements et/ou de connaissance génèrent des grains dont la qualité, variable, n'est pas toujours celle attendue. Enfin, l'enclavement des zones d'études et le caractère informel de la filière ont rendu délicats la collecte des informations.

Dans les travaux qui suivront nous nous intéresserons au *Piper borbonense* dont nous organiserons la cueillette et qui sera transformé, par nos soins, en conditions maîtrisées. Avant de pouvoir étudier l'impact des procédés et de la maturité sur sa qualité, nous nous sommes logiquement, et en préalable, attachés à connaitre la qualité de ce poivre sauvage à travers une description morphologique, anatomique et biochimique.

3.2. Chapitre 2. Morphologie, anatomie et caractérisation biochimique du *Piper borbonense*

3.2.1. Article soumis

Morphological, anatomical and biochemical description of *Piper borbonense*

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Article soumis à Scientia Horticulturae

Abstract

Piper borbonense is an overlooked wild pepper that remains unutilized today. The purpose of this work was to study its anatomy, morphology and biochemical composition with a view to its possible commercial development. This pepper differs from *Piper nigrum* through the peduncle which forms an integral part of the peppercorn and from *Piper cubeba* through its ovoid shape, though it resembles the wild peppers of Madagascar. 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%) and high aromatic potential (essential oil content: 9.8%), distinguishing it from *Piper nigrum* and bringing it closer to the tailed peppers, such as *Piper cubeba* and the wild peppers of Madagascar. Its aromatic composition can be considered as that of a good quality pepper.

Keywords
Pepper, morphology, composition, starch, piperine, essential oil

Introduction

There are around 700 species of pepper worldwide (Sumathykutty*, et al.*, 1999). In 2014, production of this spice amounted to 463,000 tonnes, with 32% supplied by Vietnam, the leading producer ahead of Indonesia and India (FAO Statistics Division, 2017). 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*, et al.*, 2003; Schweiggert, Carle*, 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 virtues of pepper (anti-microbial and antioxidant) 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*, et al.*, 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 aromatic composition. The wild peppers of Madagascar *(Piper* spp.*),* known locally as Tsiperifery, are used and consumed locally and exported, notably to Europe. The processing methods for these Malagasy peppers have been described (Weil*, et al.*, 2014). *Piper borbonense*, which is also a wild pepper, is common in the low- and medium-altitude rainforests of the Island of Reunion (Inventaire National du Patrimoine Naturel, 2017); work is under way to prove its endemic nature. Although it 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*.

1. Materials and methods

1.1. Plant material

Wild mature pepper spikes were picked in southern Reunion Island. Spikes collected on different occasions in 2015 were frozen at –80°C (Froilabo freezer - Bio Memory, 690 litres) before being pooled and mixed to form a single homogenous batch. The frozen peppercorns with their peduncles were separated from the spikes by hand prior to preparation.

1.2. 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.

1.3. Analytical methods

1.3.1. Peppercorn mass

The mass was determined with a precision balance (Scaltec SBC 22 model, Scaltec Gmbh, Germany). The precision of the balance was ± 0.1mg.

1.3.2. Peppercorn length and diameter

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

1.3.3. 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 mass was 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.

1.3.4. 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).

1.3.5. 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 modification made to the applied method was the elimination of xylene. The mean relative standard deviation of repeatability was ± 2.2% (*n* = 3).

1.3.6. Identification and quantification of essential oil compounds

1.3.6.1. 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 essential oil (obtained as described in section 1.3.5. below) 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. The temperature programme was as follows: initial temperature 60°C, heating rate of 4°C/min 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.

1.3.6.2. 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 in section 2.4.3 below) 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. The temperature programme was as follows: initial temperature 60°C, heating rate of 4°C/min 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.

1.3.6.3. Identification

The aromatic compounds separated on the two columns were identified by comparing their mass spectra to those available in commercial libraries (NIST02, WILEY) or constituted by us and by comparison of their retention indexes calculated relative to those available in the literature (Adams, 1995; Jennings*, et al.*, 1980; Kondjoyan*, et al.*, 1996) and Internet databases (2014).

1.3.6.4. Quantification on a non-polar column

The aromatic 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 in section 2.4.3. below) and an internal standard terpinolene (2:22; 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. The oven temperature programme was as follows: initial temperature 60°C, rate of 4°C/min 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).

1.3.7. 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. Next, 0.2 g of ground pepper was weighed in 80% hot ethanol. 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 litre 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 $g.100g^{-1}$ of dried matter. The mean relative standard deviations of repeatability were ± 12.73% for starch and ± 7.36% for soluble carbohydrates (n = 6).

1.3.8. 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, 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. 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).

1.3.9. Lipid content

The lipid content was determined on ground pepper according to the Soxhlet gravimetric method. A Soxtec-Avanti 250 semi-automatic device (Foss, Hillerod, 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 $g.100g^{-1}$ of dry matter. The mean relative standard deviation of repeatability was ± 3.39% (n = 6).

1.3.10. Polyphenol content

The polyphenol content, expressed on a dry basis, in Gallic Acid Equivalent, was determined according to the colorimetric method 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).

1.3.11. 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 Europ 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).

1.3.12. 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, ± 18.44% for hemicellulose and ± 7.65% for lignin (n=4).

1.3.13. 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%.

1.3.14. 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).

2. Results

2.1. Description of the plant and its fruit

2.1.1. Plant morphology

Piper borbonense was authenticated by the *Conservatoire Botanique National de Mascarin*. 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 (**Figure 11**).

Figure 11. Vine of *Piper borbonense* bearing fruits on spikes (Descroix, 2014)

The fruiting of *Piper borbonense* plants observed in Reunion takes place from July to November, depending on the years and the places where the plant is growing. Fruiting on the same vine is staggered over several weeks, up to two months. Consequently, all the spikes on a given vine never reach their ripe stage at the same time; the ripening of peppercorns on the same spike is also staggered. Fully ripe pepper fruits are red (**Figure 12**).

Figure 12. Spikes of mature *Piper borbonense* (Weil, 2016)

2.1.2. Fruit morphology and anatomy

The fruit is ovoid in shape (**figure 13**); 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 4**).

Table 4. Main characteristics of a fresh mature *Piper borbonense* corn (mean values ± 95 % confidence interval with *n* noted in brackets)

Part of pepper	Weight × 10^{6} (kg)	Length × 10^{3} (m)	Diameter × 10^{3} (m)
Whole pepper corn	47.3[a] ± 0.9 (429)	10.69[a] ± 0.66 (10)	3.63 ± 0.24 (10)
Perispem (almond)	17.1 ± 0.9 (25)	3.87 ± 0.21 (10)	2.16 ± 0.15 (10)
Mesocarp (pulp)	30.2[b] ± 1.8	NA	NA

[a] including tail 5.09 ± 0.43 × 10^{3} (m)

[b] estimated from whole peppercorn minus kernel weight data

NA: Not Applicable

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 13)**.

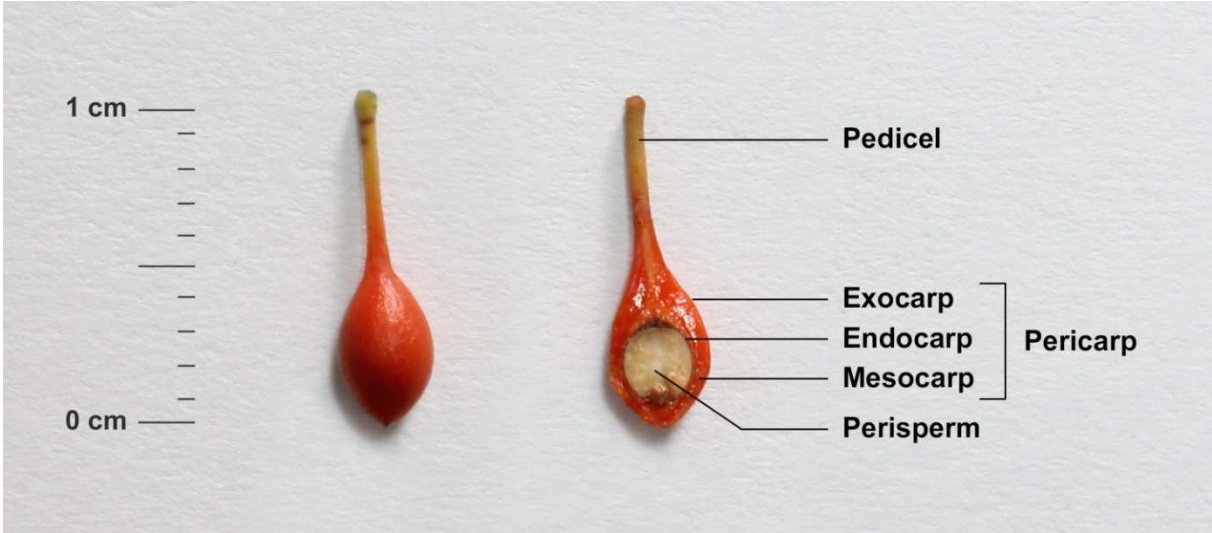

Figure 13. Whole mature peppercorn and longitudinal section of a corn from *Piper borbonense* (Chevassus, 2017)

2.2. 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 5**).

Table 5. Dry matter, water, essential oil and piperine contents in different parts (whole peppercorn, kernel and pulp) of the fresh mature *Piper borbonense* corn

			Essential oil		Piperine [a]	
	mass (mg)	content (%)	mass (mg)	content (%)	mass (mg)	content (%)
Whole pepper corn	47,300	100	1,437	100	0,029	100
Perisperm (kernel)	17,100	36,152	1,329	92,436	0,014	48,654
Mesocarp (pilp) [b]	30,200	63,848	0,109	7,564	0,015	51,346

[a] included in dry matter
[b] estimated from quantities in whole peppercorn minus kernel

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 **table 5** and **figure 14**

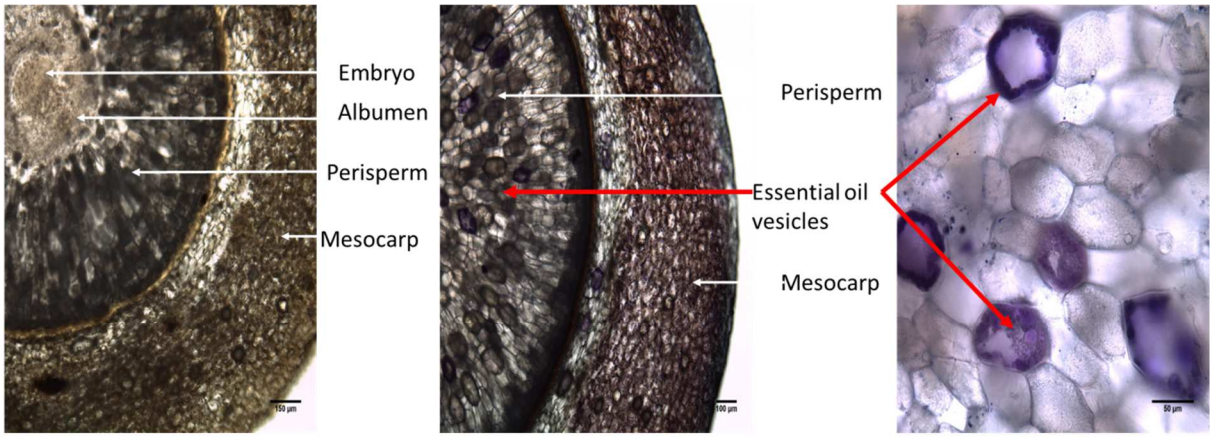

Figure 14 a) Figure 14 b) Figure 14 c)

Figure 14. Histological sections of *Piper borbonense* showing the essential oil vesicles (stained with Nadi reagent - figure 14c) (Sanier, 2016)

2.3. Peppercorn composition (db)

According to **table 6,** carbohydrates, with 41% starch and 7% soluble sugars (of which 2.50% glucose and 3.32% fructose), were the main compounds of the peppercorns. Fibres (cellulose 9%, lignin 4% and hemicellulose 3%) accounted for 16% while proteins accounted for 12% and lipids 9.5% of the total. The essential oil content was close to 10%. Mineral salts accounted for a little over 3% and antioxidant compounds (polyphenols and carotenoids) around 2% of the peppercorn. The piperine content was 0.2%.

Table 6. Composition of fresh mature *Piper borbonense* (mean values ± 95 % confidence interval with *n* noted in brackets)

Component	**Content (g/100 g 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 [b]	1.56 ± 0.08	(3)
Piperine	0.20 ± 0.05	(3)
Carotenoids [c]	0.031 ± 0.004	(9)

[b]g eq gallic acid/100 g

[c]g eq β-caroten/100 g

2.4. Detailed composition of a few compounds

2.4.1. Volatile compounds

Twenty-four aromatic compounds were identified amounting in all to almost 97% of the essential oil of *Piper borbonense* **(table 7)**. 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 3 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.

Table 7. Aromatic compounds in essential oil of fresh mature *Piper borbonense* (mean values ± 95 % confidence interval with *n* = 3)

Compound	**KI (measured) (supelcowax)**	**KI (spb5)**	**% (v/v) in essential oil (spb5)**
Limonene	1180	1034	27.31 ± nd
Alpha-phellandrene	1140	1010	14.47 ± 0.20
Beta-pinene	1082	982	6.81 ± 1.07
Alpha-pinene	1015	931	6.78 ± 2.46
Delta-3-Carene	1120	1016	3.42 ± 0.07
Eucalyptol	1192	1037	2.77 ± nd
Para-cymene	1245	1028	1.92 ± 0.94
Beta-myrcene	1130	990	1.72 ± 0.07
Camphene	1047	949	1.60 ± 0.50
Sabinene	1092	975	1.43 ± 0.01
Alpha-Terpineol	1676	1197	0.59 ± 0.19
total monoterpens	-	-	68.82 ± 5.50
Asaricin	2180	1508	13.47 ± 1.50
Dillapiole	2354	1636	4.12 ± 0.57
Safrole	1855	1298	3.55 ± 1.27
Elemicin	2205	1559	1.89 ± 0.14
Myristicin	2246	1535	1.39 ± 0.14
Methyl-eugenol	1522	1413	0.46 ± 0.07
total phénnylpropanoïds	-	-	24.88 ± 3.69
Delta-elemene	1447	1352	1.32 ± 0.03
Germacrene D	1690	1500	0.79 ± 0.10
Caryophyllene	1577	1437	0.56 ± 0.04
Delta cadinene	1733	1546	0.44 ± 0.47
Ylangene	1461	1385	0.32 ± 0.06
Alpha-cubebene	1470	1366	0.19 ± 0.03
Alpha-humulene	1634	1469	0.12 ± 0.01
total sesquiterpens	-	-	3.74 ± 0.73
Undetermined compounds	-	-	3.48 ± 0.93

nd means not determined

2.4.2. Antioxidant compounds (polyphenols and carotenoids)

The respective contents of polyphenols and carotenoids in *Piper borbonense* were found to be 1.56 g/eq gallic acid and 0.031 g eq β-carotene for 100g (db) of pepper **(table 6)**.

2.4.3. Amino acids

The amino acid content (**table 8**) of *Piper borbonense* was around 10 g for 100 g of pepper, dry basis, of which 0.4 g/100 g of free amino acids. The three amino acids that were most present were glutamic acid (1.5 g/100 g db), leucine (1 g/100 g db) and aspartic acid (1 g/100 g db).

Table 8. Amino acid contents in fresh mature *Piper borbonense*

	Concentrations (g/100g db)	
Amino acid	**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

2.4.4. Mineral elements

The mineral salts in fully ripe fresh pepper amounted to a total of around 3% (db). Potassium, at almost 2.5%, was the most abundant compound (**table 9**).

Table 9. Mineral contents in fresh mature *Piper borbonense*

Mineral compounds	Concentration	
P	0.24	g/100 g 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	

3. Discussion

3.1. Peppercorn morphology

Piper borbonense is easily distinguished from *Piper nigrum* which is spherical and does not have a tail in peppercorn form, as it remains on the spike when black pepper is threshed. *Piper borbonense* is also easily distinguished from *Piper cubeba* which is also a tailed pepper but which is spherical in shape and not ovoid, as shown in the photos proposed by Khan (2015). On the other hand, the morphology of *Piper borbonense* is quite similar to that of the wild Malagasy peppers (Weil*, et al.*, 2014).

3.2. Carbohydrate, protein and lipid contents

The starch content (41%) of *Piper borbonense* was 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. The protein content (12%) was 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 the contents (1.9 to 9%) reported by Ravindran (2000) for black pepper.

3.3. Piperine and essential oil contents

3.3.1. 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%.

3.3.2. 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 aromatic 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.*

3.4. Composition, specificity and quality of the essential oil

The same major families of aromatic compounds (monoterpenes, sesquiterpenes and phenylpropanoids) were found in the essential oils of *Piper borbonense* and *Piper nigrum* (Jagella*, et al.*, 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*, et al.*, 2005b; Zachariah*, et al.*, 2010). Likewise, pinenes (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* caryophillene 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*, et al.* (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, Buchbauer *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*, et al.* (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, 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.

3.5. Antioxidant compounds (polyphenols and carotenoids)

3.5.1. Polyphenols

The polyphenol content of *Piper borbonense*, 1.56 g GAE/100 g (db), was slightly higher than that (1.2 g/100 g) measured by Agbor*, et al.* (2006) or that (1.3g/100g) 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).

3.5.2. Carotenoids

The total carotenoid content of *Piper borbonense* was 31 mg/100g (db) while 9.5 mg/100 g is found in *Piper longum* (Veeru*, et al.*, 2009) and 500 mg/100 g in chilli pepper, which is known to be particularly rich in carotenoids (Schweiggert, Kurz*, 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 luteine (Variyar*, et al.*, 1990). These carotenoids could be responsible for the red colour of *Piper borbonense*.

3.6. Amino acids

Although pepper is not especially consumed for tis 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 (0.4 g/100 g db), 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.

4. Conclusion

Piper borbonense differs from *Piper nigrum* through its morphological characteristics and through its high essential oil content and very low piperine content. *Piper borbonense* also differs from *Piper cubeba*, which is also a tailed pepper, through its ovoid shape. The aromatic composition of *Piper borbonense* suggests a pepper of good quality which, associated with its typicity, affords it interesting potential for its domestication. While the presence and/or proportion of certain volatile compounds of the essential oil seem to differentiate it from black pepper and from some other more marginal peppers such as *Piper cubeba* and *Piper longum*, we currently lack data to be able to state that such differences are effectively due to the species rather than to abiotic factors such as the 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 be able to make use of and distinguish the *Piper borbonense* of Reunion from other peppers, either domesticated or wild, notably from the Indian Ocean.

3.2.2. Synthèse du Chapitre 2 et perspectives

Les grains de *Piper borbonense* sont de forme ovoïde et portent un pédicelle ; l'amidon en est le constituant principal. L'huile essentielle et la pipérine sont essentiellement concentrées dans le périsperme. Limonène, asaricin, et phellandrène qui représentent à eux trois, 50% du total de l'HE, sont les composés aromatiques majoritaires. Certaines caractéristiques morphologiques, anatomiques et biochimiques du *Piper borbonense* le distinguent de ces cousins malgaches - notamment une teneur en pipérine deux fois plus faible - et du *Piper cubeba*. Sa teneur en huile essentielle est 5 fois plus importante et sa teneur en pipérine 20 fois plus faible que celle du *Piper nigrum* tel qu'il est décrit dans l'ISO 959-1 (International Standard Organization, 1998). Bien que certains travaux restent à mettre en œuvre pour s'assurer que les critères typiques observés sont bien liés à l'espèce, cette étude nous a permis de déterminer la qualité spécifique du *Piper borbonense* et son potentiel de valorisation.

La suite de nos travaux concernera l'étude de l'impact des procédés post récolte. Comme son nom l'indique le post récolte, ou plutôt le post cueillette dans le cas d'une espèce sauvage, démarre juste après la cueillette. Il est communément admis que la qualité se fait en grande partie aux champs – ou dans la forêt dans notre cas – et que les procédés qui suivent, permettent d'exprimer et de conserver cette qualité mais ne permettent pas, ou alors très rarement, de l'améliorer. C'est pourquoi il est fondamental de collecter les denrées alimentaires à un stade optimal. Quelques enseignes bien connues de la grande distribution française mettent d'ailleurs en avant ce concept - argument marketing de « fruits mûrs à points ». Pour un produit qui n'est pas consommé à l'état frais, mais qui est transformé, tel que le poivre, ce stade de maturité optimal correspond tout d'abord au stade où les attributs de qualité recherchés sont présents en quantité suffisante dans le poivre frais mais aussi au stade où ces attributs de qualité s'exprimeront et/ou résisteront le mieux après procédés. Ainsi, dans le but d'identifier le bon stade de cueillette pour le poivre sauvage de la Réunion, les travaux qui suivent visent non seulement à étudier l'impact de la maturité sur la qualité du *Piper borbonense* frais mais également de soumettre les stades de maturités étudiés aux procédés afin d'évaluer la qualité des produits finis issus de différentes maturités.

3.3. Chapitre 3. Impact du stade de maturité sur le piquant, l'arôme et la couleur du *Piper borbonense*

3.3.1. Article soumis

Impact of *Piper borbonense* maturity stage on pungency, aroma and colour

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Abstract

The impact of maturity on the quality of *Piper borbonense,* a little known wild pepper from Reunion Island, was studied. Piperine and essential oil contents, aromatic composition and colour were measured at 3 maturity stages - A (immature - green), B (intermediate maturity - orange) and C (mature - red) – in fresh and processed pepper. The quantities of essential oil (+ 100% w/w) and piperine (+ 67% w/w) in a peppercorn increased during maturation (from A to C) but less than the simultaneous increase in dry matter (+160% w/w), leading to a reduction in piperine and essential oil contents (db). The aromatic profiles remained similar whatever the maturity. The L*, a* and b* colour values increased during pepper maturation and decreased, for all maturities, after processing. The pepper at maturity stage C, whose quality was the most resistant to processing, is recommended in the context of a high value supply chain.

Keywords

Pepper, maturity, piperine, essential oil, aromatic compounds, colour

1. Introduction

Of the 700 species of pepper (Sumathykutty*, et al.*, 1999) inventoried worldwide, the cultivated species *Piper nigrum*, with over 450,000 tons per year (FAO Statistics Division, 2017), is by far the most produced pepper in the world. However, most of the species remain wild, hence uncultivated. This is the case of a species in Reunion Island, *Piper borbonense* (Miq.) C. DC*.* This pepper, which has yet to be exploited, was studied to assess its commercial development potential and control the mechanisms that give it its typical quality. Indeed, this wild pepper is 5 times richer in essential oil and 20 times poorer in piperine than black pepper (Weil*, et al.*, 2017). Several parameters can affect pepper characteristics, including the species, *terroir,* climate and growing conditions (Jirovetz*, et al.*, 2002; Sumathykutty*, et al.*, 1999), or even post-harvest processes (Dhas*, et al.*, 2003; Suresh*, et al.*, 2007; Weil*, et al.*, 2014), storage (Orav*, et al.*, 2004) and its culinary preparation method (Nisha*, et al.*, 2009). The degree of maturity when harvested can also affect pepper quality. *Piper nigrum* is usually gathered before full maturity to avoid theft (Jansz*, et al.*, 1984), consumption by birds and peppercorn rot (Dhas*, et al.*, 2003). According to the last authors, the maturity stage on harvesting is also chosen according to the desired end-product. Some work on *Piper nigrum* (Jansz*, et al.*, 1984; Mathai, 1981; Rathnawathie*, et al.*, 1984) showed two phases during maturation. An initial phase during which the quantities of essential oil and piperine accumulated faster than the dry matter in the peppercorn, leading to a larger amount of compounds of interest expressed on a dry basis. A second phase where the accumulation of dry matter, primarily starch in this case, was such that it led to a decrease in the essential oil and piperine contents expressed on a dry basis. This study set out to determine how maturity affects the quality of *Piper borbonense*. In our work, quality was assessed in fresh and processed peppers through pungency (piperine content), aroma (essential oil content and composition) and colour (measured with a colorimeter).

2. Materials and methods

2.1. Plant material

The pepper was gathered from 3 vines identified in a small area (less than 1500 square meters) on the River Langevin (21° 2' 04.49 S ; 55° 38' 33.07 E), in the far South of Reunion Island. Three successive harvests around one month apart (01/07/2015, 12/08/2015, 02/09/2015) enabled to compose 3 batches with distinct maturity. Each batch (3 x 130 g) was the assembly of equal shares of pepper from each of the 3 vines. The three stages of maturity corresponded visually to an immature green pepper (called A), an orange pepper with intermediate maturity (called B) and a mature red pepper (called C). The pepper was immediately frozen at -80°C (Froilabo freezer - Bio Memory 690 litres) after each harvest. In the following pages, we considered “fresh pepper” as unprocessed pepper frozen within 2 hours after being picked, stored chilled, then thawed out for 1 hour at room temperature.

2.2. Processing conditions

As pepper is generally consumed dried, the impact of maturity on pepper quality was measured on both fresh and dried samples. The fresh samples (FA, FB and FC) were thawed out beforehand for 1 hour at room temperature. 300g of each of these 3 fresh samples was processed by a standard process into dried samples (PA, PB and PC). The process used comprised two separate operations: blanching at 100°C for 3 min in a water bath (Memmert Gmbh type WB 22 Schwabach, Germany) followed by drying at 60°C for 24 h at 20% RH in a climatic chamber (BIA Climatic – Type CL 125, Conflans Sainte Honorine, France).

2.3. Sample preparation

The fresh pepper samples (FA, FB, FC) and processed samples (PA, PB, PC) were ground (Retsch– Grindomix GM200, Retsch Gmbh, Germany) in liquid nitrogen at 10,000 rpm for 10 s then stored in the freezer at – 80°C prior to chemical analysis.

2.4. Analytical methods

2.4.1. Dry matter content

The dry matter content (meaning “essential oil-free dry matter”) was obtained by drying 3 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). Initial and final masses were

determined with a precision balance (Scaltec SBC 22 model, Scaltec Gmbh, Germany). The mean relative deviation of repeatability was ± 0.63% (n = 3). The water content expressed on a dry basis was deduced from the essential oil and dry matter contents.

2.4.2. Piperine content

The piperine content, expressed on a dry basis, was determined according to 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 deviation of repeatability was ± 3.32% (n = 3).

2.4.3. 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 modification to the applied method was the elimination of xylene. The mean relative deviation of repeatability was ± 2.08% (*n* = 3).

2.4.4. Colour measurement

Colour measurements (CIE *L**, *a** and *b** values, representing lightness, redness and yellowness, respectively) were made on whole peppercorns using a Minolta CR 400 and utility software; h° (representing the hue) was calculated from *L**, *a** and *b** values. Ten measurements were made on each sample of peppercorns spread in a 1-cm layer in an uncovered Petri dish. The mean relative deviation of repeatability was 0.93%, 2.68%, 4.79% and 2.72% respectively for L*, a*, b*, h° (n = 10). Photographs of fresh and processed peppercorns were taken using an HP - digital camera d3500 (Hewlett-Packard, USA).

2.4.5. Identification and quantification of essential oil compounds

2.4.5.1. 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 in section 2.4.3. above) were injected 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. The temperature programme was as follows: initial temperature 60°C, heating rate of 4°C/min 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.

2.4.5.2. Separation on a non-polar column

Volatile compounds were analysed with a GC (HP 6890), equipped with a 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 in section 2.4.3 above) were injected 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. The temperature programme was as follows: initial temperature 60°C, heating rate of 4°C/min 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.

2.4.5.3. Identification

The aromatic compounds separated on the two columns were identified by comparing their mass spectrum to those available in commercial libraries (NIST02, WILEY) or composed by us and by comparison of their calculated retention indexes with those available in the literature (Adams, 1995; Jennings*, et al.*, 1980; Kondjoyan*, et al.*, 1996) and Internet databases (2014).

2.4.5.6. Quantification on a non-polar column

The aromatic compounds were quantified by a GC (HP 5890), equipped with a 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 in section 2.4.3.above) were injected into the GC–FID in split mode (1:33). The flow rate of the gas carrier (Helium) was 0.7 mL/min. The oven temperature programme was as follows: initial temperature 60°C, rate of 4°C/min until a final temperature of 250°C was reached then maintained constant for 20 min. Quantifications were made according to internal normalization (Cachet*, et al.*, 2011).The mean relative deviation of repeatability was ± 0.49% (*n* = 3).

2.5. Statistical analysis

Differences in the mean values of piperine content, essential oil content, essential oil composition and L*, a* b* and h° values were tested by an analysis of variance (ANOVA); the significance of differences between samples was determined using the Tukey test. The level of significance was $P < 0.05$.

3. Results

In the following results and coming discussion, the impact of maturity on pepper quality is presented and discussed both for fresh and processed samples.

3.1. Increase in compounds of interest in the peppercorn during maturation

The mass of a peppercorn increased during maturation (**table 10**) by 40% from FA (30.81 mg) to FB (43.12 mg) and by 53% from FA to FC (47.30 mg). While the quantity of water, the major component, increased little (+32%) during maturation from FA (25.3 mg) to FC (33.3 mg), the mass rise was mostly due to the accumulation of dry matter: 161% from FA (4.82 mg) to FC (12.58 mg). In a peppercorn, the quantity of essential oil increased (+ 100%) during maturation from FA (0.687 mg) to FC (1.399 mg); the quantities of piperine also increased (+ 67%) from FA (0.0156 mg) to FC (0.0260 mg) but less so than the dry matter. Consequently, the piperine and essential oil contents (db) decreased during maturation: indeed, reduction of 35% from FA (0.32%) to FC (0.21%) for piperine and reduction of 22% for essential oil from FA (14.26%) to FC (11.10%) were observed.

Table 10. Impact of maturity stage (A: immature; B: intermediate maturity; C: mature) on the weight of a corn, dry matter, piperine and essential oil (weight and contents). F: fresh pepper; P: processed (blanched and dried).

Maturity stage	sample	mass (mg) per one pepper corn				content (g/100g db)	
		one corn [$]	dry matter [#]	piperine [#]	essentiall oïl [#]	piperine [#]	essential oil [#]
A	Fresh (FA)	30.81 ± 0.58 *c*	4.82 ± 0.09 *c*	0.0156 ± 0.0003 *c*	0.687 ± 0.013 *e*	0.32 ± 0.03 *a*	14.26 ± 0.84 *a*
B	Fresh (FB)	43.12 ± 0.81 *b*	11.19 ± 0.20 *b*	0.0257 ± 0.0005 *a*	1.305 ± 0.025 *b*	0.23 ± 0.03 *c*	11.67 ± 0.43 *b*
C	Fresh (FC)	47.30 ± 0.89 *a*	12.58 ± 0.23 *a*	0.0260 ± 0.0005 *a*	1.399 ± 0.026 *a*	0.21 ± 0.01 *d*	11.10 ± 0.40 *bc*
A	Processed (PA)	5.90 ± 0.11 *f*	4.82 ± 0.09 *c*	0.0125 ± 0.0002 *d*	0.526 ± 0.010 *f*	0.26 ± 0.01 *b*	10.92 ± 1.11 *c*
B	Processed (PB)	13.30 ± 0.25 *e*	11.19 ± 0.20 *b*	0.0226 ± 0.0004 *b*	1.063 ± 0.020 *d*	0.20 ± 0.03 *de*	9.50 ± 0.36 *d*
C	Processed (PC)	14.83 ± 0.28 *d*	12.58 ± 0.23 *a*	0.0228 ± 0.0004 *b*	1.133 ± 0.021 *c*	0.18 ± 0.01 *e*	9.00 ± 0.78 *d*

[$] Mean values (n = 429) ± confident interval (P=0.05)

[#] Mean values (n = 3) ± confident interval (P=0.05)

In the columns, values with the same letters are not significantly different (P<0.05)

3.2. Impact of maturity stage on piperine content

For fresh pepper (F), the piperine content (**table 10**) fell by 28% from maturity A (0.32%, db) to B (0.23% db) and by 10% from maturity B to C (0.21%, db). Between A and C, the reduction was 35%. Processing led to maximum piperine losses of 20% for maturity A pepper (FA/PA) and around 13% for maturity B and C peppers (FB/PB, FC/PC) which were therefore less impacted by processing. The maturity A dry pepper (PA) had 25% more piperine than the maturity B dry pepper (PB). PB and PC were not significantly different from each other.

3.3. Impact of maturity stage on essential oil content

For fresh pepper (F), the essential oil content fell by 18% between maturity A (14.26%, db) and maturity B (11.67%, db). The decrease between maturity B and maturity C (11.10%, db) was not significant. Between FA and FC, the decrease was 22%. Processing led to losses of 23% for maturity A pepper (FA/PA) and 19% for maturity B and C peppers (FB/PB, FC/PC), which were therefore slightly less impacted by processing. The dry pepper PA, with10,92% dry base, had 13% more essential oil than the maturity B dry pepper PB (9.50%, db). PB and PC (9.00%, db) were not significantly different from each other **(table 11).**

Table 11. Major aromatic compounds and their characteristic odors[#] in *Piper borbonense* essential oil (determined from fresh mature sample (FC))

Aromatic coumpounds	KI (supelcowax)	KI (spb5)	Characteristic odor[#]	% (v/v) in essential oil (spb5)
Limonene	1200	1039	citrus, sweet	22.63 ± 0.01
Asaricin	2207	1511	spicy, peppery	11.87 ± 0.01
α-phellandrene	1163	1012	terpenic, citrus, herbal	10.63 ± 0.01
đ-3-carene	1146	1019	citrus, sweet, terpenic	8.51 ± 0.01
β-pinene	1110	985	herbal, woody, green	5.33 ± 0.01
Dillapiole	2382	1656	woody, spicy	4.81 ± 0.01
α-pinene	1023	940	herbal, fresh, camphor	4.03 ± 0.01
Safrole	1867	1296	spicy, sweet	3.27 ± 0.01
α-guaiene	nd	1481	woody, sweet	2.95 ± 0.01
Elemicin	2234	1557	spicy, floral	2.02 ± 0.01
đ-elemene	1463	1345	spicy, sweety, fruity	2.01 ± 0.01
β-myrcene	1152	992	terpenic, herbal, woody	1.91 ± 0.01
Camphene	1066	955	woody, herbal	1.24 ± 0.01
Methyl-eugenol	nd	1402	spicy, sweet, fresh	0.71 ± 0.01
Germacrene-D	1723	1493	spicy, woody	0.58 ± 0.01
α-cubebene	1451	1357	spicy	0.55 ± 0.01
Sabinene	1120	979	woody, spicy, sweet	0.50 ± 0.01
Terpinolene	1278	1093	herbal, fresh, woody	0.45 ± 0.01
Total				84.00 ± 0.13

Mean values (n=3) ± confident interval (P=0.05)

[#]Characteristic odors were determined according to http://www.thegoodscentscompany.com/

nd means not determined

3.4. Impact of maturity stage on essential oil composition

CG-MS led to the identification of 24 aromatic compounds present at over 0.45% (v/v), representing 91% (v/v) of the total essential oil. Eighteen of them, present in each of the 6 samples, amounted to 84% (v/v) of the essential oil **(table 11).** The aromatic compounds identified belonged to 3 distinct families: monoterpenes (66% of the compounds identified), phenylpropanoids (27% of the compounds identified) and sesquiterpenes (7% of the compounds identified). These three families of compounds were equally affected by maturity with a reduction of around 25% between FA and FC. Regarding process impact, phenylpropanoids (with 35% FC/PC losses) were the most sensitive and sesquiterpenes (5% losses) were the most resistant. Four compounds alone amounted to 54% of the total essential oil (**table 11**): limonene (23%), asaricin (12%), alpha-phellandrene (11%) and delta-3-carene (8%) were the major compounds

whatever the maturity stage considered, whether for fresh pepper or after processing. From maturity A to B (FA/FB), these 4 compounds decreased (**figure 15**) and, given their large proportion, they largely explained the overall drop. For instance, the reduction in limonene alone accounted for 21% of the overall reduction. Safrole, alpha-guaiene, delta-elemene, alpha-cubebene, and terpinolene, were the most impacted (downwards) by the switch from maturity A to maturity B. Conversely, asaricin, alpha-phellandrene, and germacrene-D were the least affected compounds. From maturity B to maturity C (FB/FC), asaricin, safrole, dillapiole, elemecine and sabinene suffered the greatest decreases. The decrease in asaricin alone amounted to 33% of the overall decrease. On the other hand, beta-pinene, delta-elemene, camphene, alpha-cubene and germacrene-D were not impacted. From maturity A to maturity C, safrole with a 57% reduction was particularly impacted (FA/FC) while germacrene-D was little affected (loss of 4%).

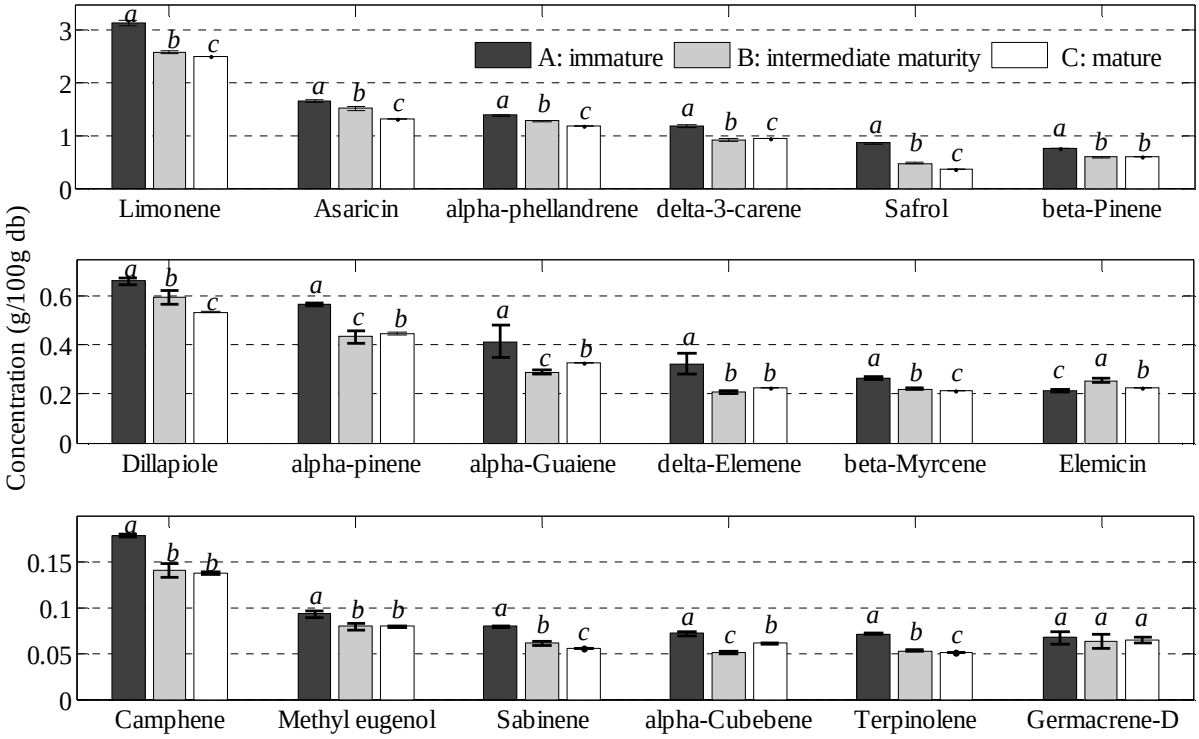

Figure 15. Impact of 3 maturity stages (A, B, C) on the composition of fresh pepper essential oil. Bars are means (n = 3) ± confidence interval (P = 0.05). Different letters (a–c) at the top of the bars mean significantly different (P < 0.05)

After processing (**figure 16**), i.e. after blanching and drying, limonene losses amounted to 25% of the overall losses for maturity A. For this maturity A, safrole, elemicin, sabinene and terpinolene were particularly affected by processing with losses of 47%, 28%, 34% and 33% respectively (FA/PA). For maturity B, losses in asaricin during

processing amounted to 32% of the overall losses (FB/PB). For this maturity B, asaricin, dillapiole and elemicin were particularly affected by processing with losses of 46%, 70% and 76%, respectively. For maturity C, the losses in asaricin after processing amounted to 16% of the overall losses, while safrole, dillapiole and elimicine suffered losses of 28%, 55% and 57%, respectively (FC/PC). Alpha-guaiene, methyl eugenol alpha-cubebene, and germacrene-D (which did not lose more than 15%) were the compounds that resisted processing best, whatever the maturity stage considered **(figure 16)**.

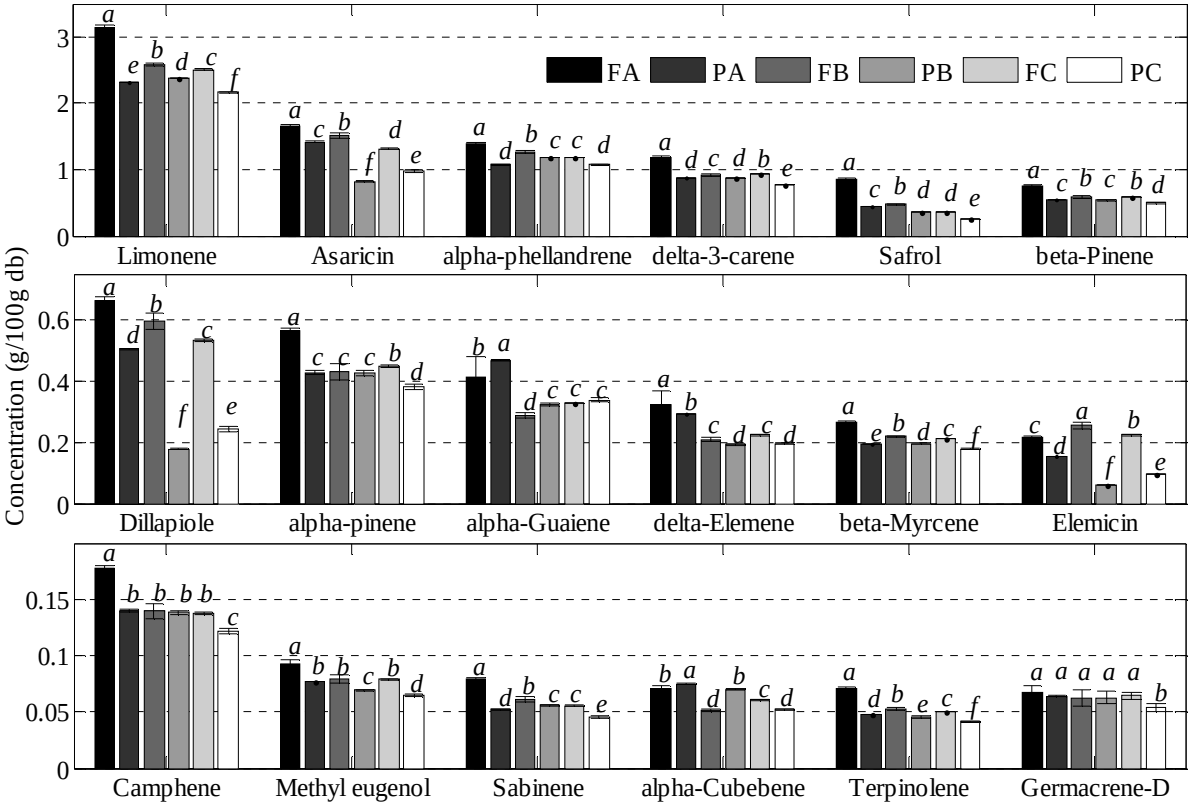

Figure 16. Impact of processing (F: fresh; P: processed) on essential oil composition at three different pepper maturity stages (noted: A for immature, B for intermediate maturity and C for mature). Bars are means (n = 3) ± confidence interval (P = 0.05). Different letters (a–f) at the top of the bars mean significantly different (P < 0.05)

The characteristic notes of the compounds composing pepper are notably fruity, spicy, green and woody (**table 11**). Despite some substantial drops in concentration identified for some compounds, the aroma profiles for *Piper borbonense* remained similar for the three maturity stages considered, for both fresh and dried.

3.5. Impact of maturity stage on colour

All the colour values L*, a*, b* increased during maturation (**figure 17**), with the pepper turning from green (FA) to orange (FB) then to red (FC) (**figure 18**). It was a* (characteristic of red) that was most impacted; its value was multiplied by 4.5 between maturity A and maturity C for fresh pepper.

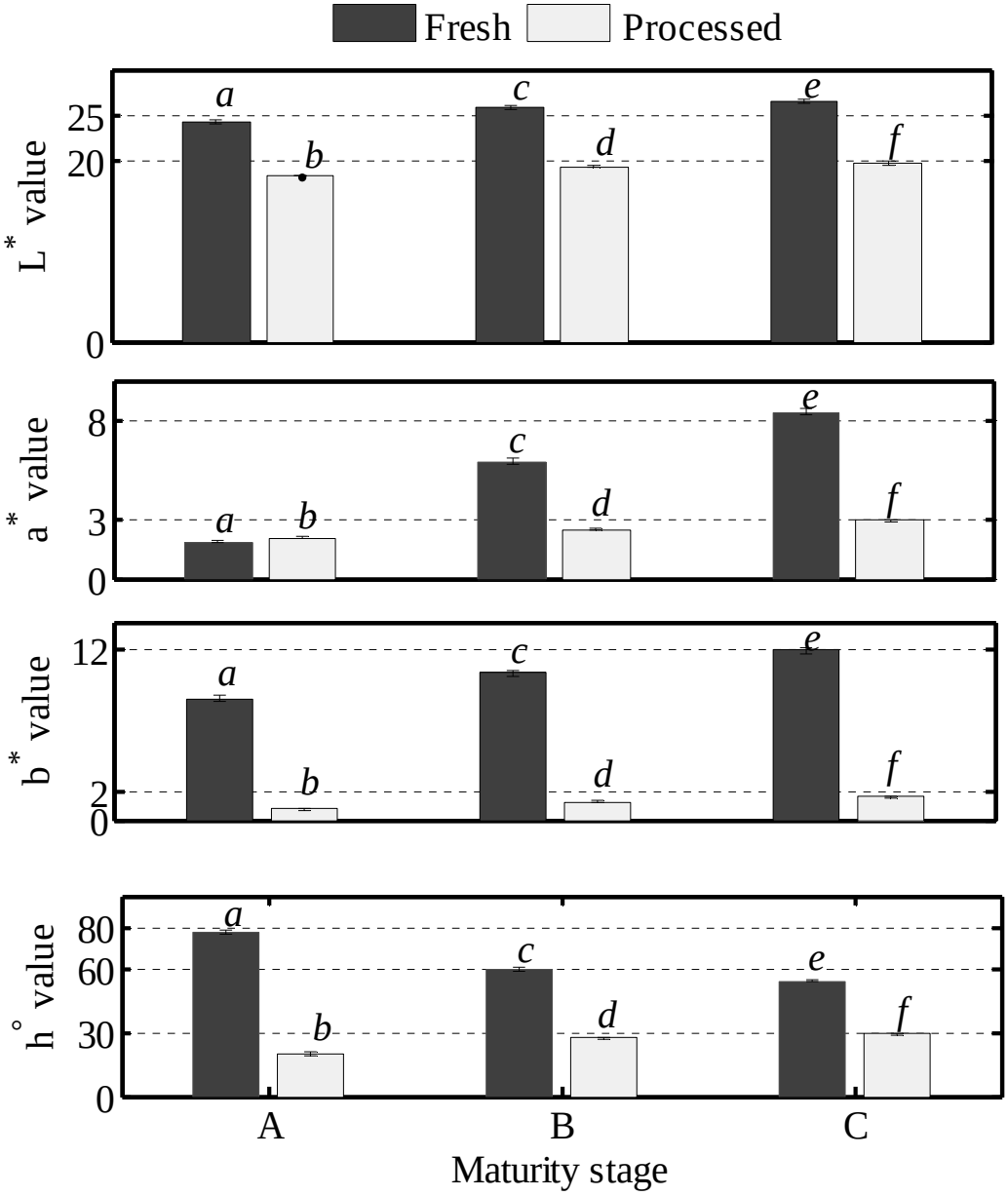

Figure 17. Impact of 3 maturity stages (A for immature, B for intermediate maturity and C for mature) on the L* a* b* and h° values of fresh (F) and processed (P) peppercorns. Bars are means (n = 3) ± confidence interval (P = 0.05). Different letters (*a*–*f*) at the top of the bars mean significantly different (P < 0.05)

Whatever the maturity stage considered, processing had a downward impact on all the colour values: b* divided by 11 between FA and PA was the most affected. The processed maturity C pepper (PC) had the highest colour values after processing, with

its colour turning from red to deep purple, whilst a brown colour was seen after processing for the maturity B pepper (PB) and black for the maturity A pepper (PA).
As regards the hue value, h° decreased during maturation (-23° between FA and FC) and also decreased after processing (-25° between FC and PC).

Figure 18. Pictures of fresh (F) and processed (P) peppers of different maturity stages (A, B, C)

4. Discussion

4.1. Three batches with distinct maturity

The way our batches were picked and composed enabled us to have three batches of different maturity. A, B and C differed significantly in terms of mass, colour and chemical composition of the peppercorns. Batches B and C were similar to each other but distant from A.
These results were obtained for degrees of maturity determined using visual colour criteria (green – orange – red) without knowing the flowering dates generally used as an initial monitoring criterion (Jansz*, et al.*, 1984; Mathai, 1981; Rathnawathie*, et al.*, 1984). In fact, the phenology of *Piper borbonense,* a wild pepper, has yet to be studied.

4.2. Impact of maturity stage on the piperine and essential oil contents of fresh and dried peppers

Peppercorns expand during maturation partly due to the increase in moisture content, but especially to the increase in dry matter content, primarily consisting of starch (Zachariah*, et al.*, 2010). Indeed, starch accounts for 40 to 50% of the dry matter in pepper (Jayashree*, et al.*, 2009). In our study, while the piperine and essential oil contents were multiplied by 2 and 1.6, respectively, between maturity A and maturity C, the quantity of dry matter increased by 2.6 over the same period. It is therefore the fact that those two compounds are much less abundant than dry matter (primarily starch) which results in a reduction in piperine and essential oil contents in relation to dry matter. The previously mentioned authors (Jansz*, et al.*, 1984; Mathai, 1981; Rathnawathie*, et al.*, 1984) had also found, studying *Piper nigrum,* that piperine and essential oil contents increased during maturation up to a maximum, then decreased[11]. In our case, we only found a reduction in those contents during maturation. It is likely that earlier harvests (before our maturity A) would have enabled us to see that increase in piperine and essential oil contents.

According to Schweiggert, Mix*, et al.* (2005), the aroma is more important than pungency when judging the quality of spices. In their work, the essential oil content of pepper was affected more by maturity than by processing. In our study, the essential oil content was affected as much by maturity as by processing, since (for fresh pepper) we found a 22% decrease between maturity A and maturity C and a 19% decrease between fresh pepper and dry pepper (for maturity C). On the other hand, the piperine content was affected more by maturity than by processing, since for fresh pepper we found a 37% decrease between maturity A and maturity C, and only 19% between fresh pepper and dry pepper (maturity C). For the maturity A pepper, we found a larger drop in piperine and essential oil contents after processing than for the maturity B and C peppers. The thinner and more permeable pericarp of the immature pepper compared to the more mature peppers could explain this greater sensitivity to processing. These results tally with those of Nisha*, et al.* (2009), who worked on black pepper and reported a 38% loss of black pepper essential oil after 20 min at 100°C, and with those of Suresh*, et al.* (2007) who found 28% piperine losses under the same conditions.

4.3. Impact of maturity on essential oil composition

Three families of aromatic compounds - monoterpenes, sesquiterpenes and

phenylpropanoids – also found in black pepper (Jagella*, et al.*, 1999; Menon*, et al.*, 2005a; Pino*, et al.*, 1990) were identified in *Piper borbonense*. Their contents decreased in the same proportions during maturation (around 25% decrease between A and C). Thus, the composition (aroma profile) was little affected overall by maturation, in line with what was established by (Jansz*, et al.*, 1984). However, a few quantitative differences were found when the compounds were taken individually. For instance, safrole and asaricin underwent a very significant downward impact during maturation, while germacrene-D remained stable.

While the general composition remained unchanged in fresh and processed pepper, as already shown in an earlier article (Weil*, et al.*, 2017), the concentrations of most of the compounds (as the overall essential oil content) underwent a downward impact during processing for all the maturity stages. For instance, only germacrene-D remained highly stable and was an exception, while safrole, asaricin and elimicine quite largely underwent a downward impact during processing whatever the maturity stage considered. This great sensitivity of safrole was also found by Farag*, et al.* (1997).

The aromatic profile of this wild pepper, characterized by fruity, spicy, green and woody notes, was quite well conserved during maturation and after processing. As already discussed for the global essential oil content, it appeared that the aromatic quality of maturities B and C, expressed by all the major compounds (limonene, alpha phellandrène, delta-3-carene, safrole and beta-pinene) except asaricin, was less affected by the process than maturity A.

Some compounds (limonene, asaricin, alpha-phellandrene, delta-3-carene, beta-pinene and elimicine) which were largely present in *Piper borbonense* were also considered by Jirovetz*, et al.* (2002) as typical compounds of black pepper.

4.4. Impact of maturity on colour

During maturation, the colour values L*, a*, b* increased while the hue, h°, decreased, with the colour of the peppercorns turning from green to red. According to Variyar*, et al.* (1990) this red colour is directly linked to the presence of carotenoids (notably beta-carotene, lycopene, leutine) in the pericarp of *Piper nigrum* pepper. Starting from a given stage of maturation, the carotenoids (tetraterpenes) responsible for the red colour of our pepper were synthesized in abundance according to the scheme proposed by Bohlmann*, et al.* (2008).

Whatever the maturity stage, the colour was greatly affected by processing and the

colour profiles obtained on dry peppers differed considerably from the profiles obtained on fresh peppers: the colour values L*, a*, b* and hue value h° decreased. After processing, the C maturity pepper had a deep purple colour, while the less mature peppers were brown (B) and black (A). Some authors (Dhas*, et al.*, 2003; Mangalakumari*, et al.*, 1983; Variyar*, et al.*, 1988) showed that pepper without prior treatment to deactivate enzymes turned brown during drying. The drastic blanching conditions applied during our study (100°C for 3 minutes) deactivated the enzymes (F. Gu*, et al.*, 2013) and thereby prevented browning of enzymatic origin in our pepper. The fact that the pepper nonetheless turned brown during drying (following blanching) revealed the existence of non-enzymatic browning of pepper as described by F. Gu*, et al.* (2013). This browning may have been due to (non-enzymatic) oxidation of polyphenols and/or Maillard reactions occurring during drying.

4.5. Suitability of the different maturity stages for processing

We showed that the quantities of compounds of interest (piperine and essential oil) were lower in the fresh maturity B and C peppers than in the immature A pepper. However, these compounds of interest underwent less of a downward impact during processing for the B and C peppers than for the A pepper. Given this greater resistance to processing of the maturity B and C peppers, no doubt linked to their structure, they would be of interest in the development of a supply chain. In addition, the colour obtained after processing was less dull for the maturity C pepper than for the maturity A and B peppers. Lastly, the moisture content of the maturity B and C fresh peppers was half that of the maturity A pepper. The energy needed to dry them would therefore be less, making the maturity B and C stages more interesting for supply chain purposes.

5. Conclusion

The originality of dry *Piper borbonense* is based on its high aroma potential, low pungency and red colour. Piperine and essential oil biosynthesis and the appearance of the red colour occur during maturation. Although the quantities of compounds of interest increase during pepper maturation, the contents expressed in relation to dry matter decrease due to the concomitant substantial accumulation of starch. Pepper at its most advanced stage of maturity (red) should be recommended as it offers better picking yields (fuller clusters and larger peppercorns), remains redder and because the

compounds of interest (piperine and essential oil) resist processing better. As for pepper of intermediate maturity (orange), it offers the advantage of being less subject to harvest losses (theft, birds, fallen fruits on the ground) and of being less fragile to handle than mature or overmature pepper. Before consideration can be given to developing a supply chain to commercially develop this original pepper, it proves necessary to be able to domesticate it and, notably, to achieve optimum maturity.

3.3.2. Synthèse du Chapitre 3 et perspectives

Au cours de la maturité le poivre passe de la couleur verte (pour le poivre immature) à une couleur orange (maturité intermédiaire) puis à une couleur rouge (poivre mature). Les quantités d'huile essentielle et de pipérine augmentent au cours de la maturité du poivre mais moins que l'amidon, ce qui conduit à une diminution relative des teneurs en huile essentielle et en pipérine dans le grain au cours de la maturité.

Les composés d'intérêts pipérine et huile essentielle sont davantage impactés par le process pour le poivre immature alors que la couleur est mieux préservée après process pour les grains matures. Ainsi, *le Piper borbonense* à pleine maturité est à privilégier pour la mise en place d'une filière maîtrisée. En effet son rendement à la cueillette est plus intéressant et ses grains restent plus rouges et conservent des teneurs élevées en huile essentielle et en pipérine après procédés.

Nous avons testé ici l'impact d'un procédé défini sur la qualité du *Piper borbonense*, en fonction de la maturité ; ce procédé consistant en un blanchiment à 100°C pendant 3 minutes suivi d'un séchage à 60°C et 20%HR. Nous pouvons maintenant envisager, pour la maturité déterminée comme idéale (le poivre rouge mature), d'aller plus loin dans l'étude des procédés et donc de tester l'impact, sur le piquant, l'arôme et la couleur du poivre, des principales opérations unitaires que sont le blanchiment, l'étuvage et le séchage.

3.4. Chapitre 4. Impact du blanchiment, de l'étuvage et du séchage sur le piquant, l'arôme et la couleur du *Piper borbonense*

3.4.1. Article paru

Impact of blanching, sweating and drying operations on pungency, aroma and color of Piper borbonense

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Abstract

Low pungency, high aromatic potential and red color, give to Piper borbonense its originality when compared to Piper nigrum. Effects of blanching, sweating and drying on these characteristics were assessed. The three operations had no impact on the concentration of piperine and essential oil but affected the composition of essential oil slightly and considerably affected the color of the pepper. The "wet process", including blanching, sweating and drying, had the largest impact on the composition of aroma, increasing para-cymene content by 89% and reducing safrole content by 33% in dried pepper compared to fresh. Blanching increased the drying rate thus reducing drying time. Drying had a major impact on color, which changed from red to brown. The biggest differences observed led to reductions of 2.2, 7.9 and 8.4 units in L*, a* and b* values, when chromatic values measured in fresh pepper were compared to those of dried pepper.

Keywords

Processing; pepper; piperine; essential oil; color

1. Introduction

Pepper (*Piper* spp.) is the most common spice worldwide; 472 500 tons were produced in 2013 (FAO Statistics Division, 2015). Although, also known for its medicinal properties (Ahmad*, et al.*, 2012), pepper is mainly used to enhance the taste and flavor of food. The quality of pepper as a spice is measured throughout pungency, aroma and color (F. Gu*, et al.*, 2013). Although more than 700 species grow in tropical and subtropical regions, most of which are wild (Sumathykutty*, et al.*, 1999), one single domesticated species – *Piper nigrum* is by far the most widely consumed. A wild pepper, named *Piper borbonense* grows in Reunion Island but has not been collected until now. Some very closely related wild species of pepper, local name Tsiperifery, grow in Madagascar and are picked for both local consumption and for sale, including for export. These wild peppers differ from domesticated *Piper nigrum* in their low piperine content, high essential oil content and particular red color (Weil*, et al.*, 2014). Although they are sold at high prices in Europe, these Malagasy peppers are of heterogeneous quality which could affect their reputation and valorization. As pepper quality varies with the species, origin, agricultural system (when domesticated), climate, or maturity, it may also be influenced by postharvest treatments. Dhas and Korikanthimath (2003) described the different types of processing of pepper and the advantages of each, but few studies have focused on the impacts of processing on pepper quality. Existing studies generally tested domestic cooking, and reported contradictory results. Wild pepper is currently not processed in Reunion Island. In Madagascar, wild peppers are processed according to “dry” and “wet” processes (Weil*, et al.*, 2014). The “dry” process only consists in drying, whereas the “wet” process includes blanching and sweating prior to drying. Traditionally, sweating, i.e. keeping the hot blanched product in a blanket for 24h is widely used in the treatment of Malagasy vanilla beans. However, it is not used elsewhere on pepper and not described either in the literature. The objective of our study was thus to assess the impact of blanching, sweating and drying in controlled conditions on the quality of wild *Piper borbonense* pepper originating from Reunion Island. The quality characteristics considered in this study were pungency (piperine content), aroma (essential oil content and composition) and color (judged by eye and through L*, a*, b* chromatic values). The influence of blanching and sweating on drying kinetics was also assessed.

2. Materials and methods

2.1. Plant material

We defined three maturity stages according to pepper color: A (immature green pepper), B (orange pepper – intermediate maturity) and C (red mature pepper). Wild mature (C) pepper spikes were picked in the south of Reunion Island. Spikes picked on different occasions were frozen at - 80°C (freezer Froilabo - Bio Memory, 690 liters) before being pooled and mixed to form a single homogenous batch. Before processing, the peppercorns with their peduncles were separated from the fruit stems by hand and defrosted for two hours at room temperature. The defrosted pepper is called “fresh” pepper in the rest of this article.

2.2. Processing experiments

2.2.1. Blanching, sweating and drying

The processes consisted in three unit operations that were applied (alone or combined) to obtain different samples (**figure 19**). F: fresh pepper; B: blanched (B1: 60 °C/30s; B2: 75 °C/180 s; B3: 100 °C/300 s); S: sweated (35 °C, 99 % RH, 24 h); D: dried (60 °C, 20 % RH, 39 h). Blanching consisted in soaking the peppercorns in a hot water bath (Memmert Gmbh type WB 22 Schwabach, Germany) at a ratio of 1:36 peppercorns to water in three different conditions: at 60 °C for 30 s; 75 °C/180 s; and 100 °C/300 s. Sweating consisted of storing the peppercorns in a climatic chamber (BIA Climatic – Type CL 125, Conflans Sainte Honorine, France) at 35 °C and 99% RH for 24 hours. Drying was performed by placing aluminum trays (300 cm^2) containing 250 g of peppercorns arranged in a compact 1 cm thick layer for 39 hours at 60 °C ± 1°C, RH 20% ± 2% in the same climatic chamber. Hot air (60 ± 1 °C, RH 20 ± 2%) was circulated over the surface of the layer.

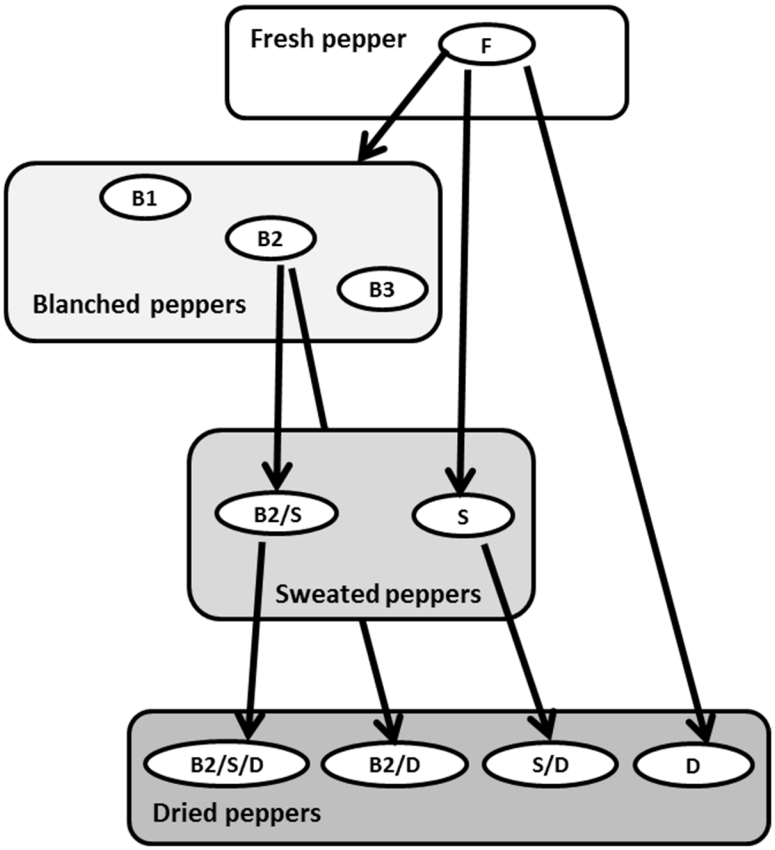


Figure 19. Processes applied to pepper
F: fresh pepper; B: blanched (B1: 60 °C/30s; B2: 75 °C/180 s; B3: 100 °C/300 s); S: sweated (35 °C, 99 % RH, 24 h); D: dried (60 °C, 20 % RH, 39 h)

2.2.2. Drying kinetics

Drying of peppercorn samples used a cross flow pilot dryer, developed in our laboratory. In the treatment chamber (0.25 m long × 0.25 m wide × 0.92 m high), 150g of peppercorns were placed on a sieve (0.25 × 0.25 m^2) in a single thin non-compact layer. Hot air (60 ± 1 °C, RH 20 ± 2%) was circulated downwards at 2.7 ± 0.1 ms^{-1} through the layer of peppercorns by a high-capacity fan. The air velocity was just high enough to have no significant effect on temperature when passing through the layer of peppercorns to ensure a proper treatment, and to enable statistical analyses. When the heat treatment was complete, the peppercorns were cooled by ventilation with air at ambient temperature. The water content, which was measured on a dry basis (noted *X*) as a function of time, was estimated in line, using the mass reading of the sieve. Water content kinetics $X^{(t)}$ were fitted with a cubic smoothing spline (Matlab® Version 5.2, The Mathworks Inc., USA). The drying rate (d *X* /dt) was calculated as the direct analytical derivative of the cubic smoothing spline function on $X^{(t)}$.

2.3. Sample preparation

The samples resulting from the different processing operations were frozen at - 80°C for further preparation and analysis. The pepper samples were ground for 10 seconds at 10 000 rpm in mill (Retsch – Grindomix GM200, Retsch Gmbh, Germany) for all analyses except color which was measured on whole peppercorns.

2.4. Analytical methods

2.4.1. Dry matter content

The dry matter content (mean “essential oil free dry matter”) was obtained by drying 5 g of ground pepper in an aluminum cup in the oven (ULE 400, Memmert Gmbh, Germany) at 105 °C for 30 h (i.e., until constant weight). Initial and final mass was determined with a precision balance (Scaltec SBC 22 model, Scaltec Gmbh, Germany). The mean standard deviation of repeatability was ± 0.6% (n = 3). Water content expressed on a dry basis was deduced from essential oil and dry matter content.

2.4.2. Piperine content

The piperine content, expressed on a dry basis, was determined according to 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 deviation of repeatability was ± 7.3% (n = 3).

2.4.3. Essential oil content

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

2.4.4. Color measurements

Color measurements (CIE *L**, *a** and *b** values, representing lightness, redness and yellowness, respectively) were made on whole peppercorns using a Minolta CR 400 and utility software. Ten measurements were made on each sample of peppercorns spread in a 1-cm layer in an uncovered Petri dish. The mean relative deviation of repeatability was 1.2 %, 2.3 % and 3.6 % respectively for L*, a*, b* (n = 10).

2.4.5. Identification and quantification of essential oil compounds

2.4.5.1. Separation on a polar column

Volatile compounds were analyzed 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 in section 2.4.3. above) were injected into the GC–MS in split mode (1:30). The injector's temperature was 250°C. The temperature of the transfer line was 250° C and the flow rate of the gas carrier (Helium) was 0.8 mL/min. The temperature program was as follows: initial temperature 60 °C, heating rate of 4 °C/min 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) which functions in electron impact (70 eV) mode. The mass range was between 25 and 350 m/z.

2.4.5.2. Separation on a non-polar column

Volatile compounds were analyzed with a GC (HP 6890), equipped with a 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 in section 2.4.3 above) were injected into the GC–MS in split mode (1:50). The injector's temperature was 250°C. The temperature of the transfer line was 250 °C and the flow rate of the gas carrier (Helium) was 0.7 mL/min. The temperature program was as follows: initial temperature 60 °C, heating rate of 4 °C/min until final temperature of 250 °C was reached then maintained constant for 50 min. The molecules were identified using a GC/MS (HP 6890) which functions in electron impact (70 eV) mode. The mass range was between 20 and 400 m/z.

2.4.5.3. Identification

The aromatic compounds separated on the two columns, were identified by comparing their mass spectrum to those available in commercial libraries (NIST02, WILEY) or constituted under our care and by comparison of their retention indexes calculated relative to those available in the literature (Adams, 1995; Jennings*, et al.*, 1980; Kondjoyan*, et al.*, 1996) and Internet databases (2014).

2.4.5.4. Quantification on non-polar column

The aromatic compounds were quantified by a GC (HP 5890), equipped with a 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 in section 2.4.3. above) and internal standard terpinolene (20:2; v/v) were injected into the GC–FID in split mode (1:33). The injector's temperature was 250°C. The flow rate of the gas carrier (Helium) was 0.7 mL/min. The oven temperature program was as follows: initial temperature 60 °C, rate of 4 °C/min until a final temperature of 250 °C was reached then maintained constant for 20 min. The mean relative deviation of repeatability was ± 2.5% (n = 3).

2.5. Statistical analysis

Differences in the mean values of piperine content, essential oil content, essential oil composition and L*, a* and b* values were tested by analysis of variance (ANOVA); the significance of differences between samples was determined using Fisher's test. The level of significance was $P < 0.05$.

3. Results

Two levels of observation were considered: unit operations and full processes. In this paper, we considered four full processes: three "wet processes" including blanching and/or sweating and drying and a "dry process" consisting in one drying single operation.

3.1. Impacts of the unit processing operations

Here we describe the impacts of blanching, sweating and drying operations (**Figure 19**) on piperine and essential oil contents (**Figure 20**), color (**Figure 21**) and on drying kinetics (**Figure 22**).

3.1.1. Impact of blanching

Blanching had no impact on piperine content **(Figure 20)**. There was no significant difference in the results obtained (ranging from 0.18% to 0.20%, dry basis) in samples F, B1, B2, and B3. Blanching had no impact on essential oil content (**Figure 20**) as evidenced by the absence of a significant difference in the results obtained for essential oil (ranging from 11.31% to 12.09%, dry basis) in the same samples. Blanching did have a slight impact on color (**Figure 21**) as some slight yet significant differences were observed. The L* value of sample B3, which was subjected to the most drastic treatment, differed from all the other samples: + 2.1 units compared to sample F. The

biggest differences in a* and b* values were respectively lower than 0.5 and 1 in samples F, B1, B2, and B3. **Figure 22** shows the impact of blanching on the drying curves. Two hours were required to obtain a 50% reduction in the initial water content of fresh pepper and 1h20min for blanched pepper. The comparison of the drying curves showed a much higher initial drying rate (1.44 ± 0.10 kg. $kg^{-1}.h^{-1}$) in blanched pepper than in fresh pepper (0.84 ± 0.02kg.$kg^{-1}.h^{-1}$). The differences between the drying rates were no longer significant when the water content was below 0.5kg.$kg^{-1}.h^{-1}$. This water content was obtained after 4 hours in fresh pepper and 3h15 in blanched pepper. Blanching greatly increased the drying rate and consequently reduced total drying time.

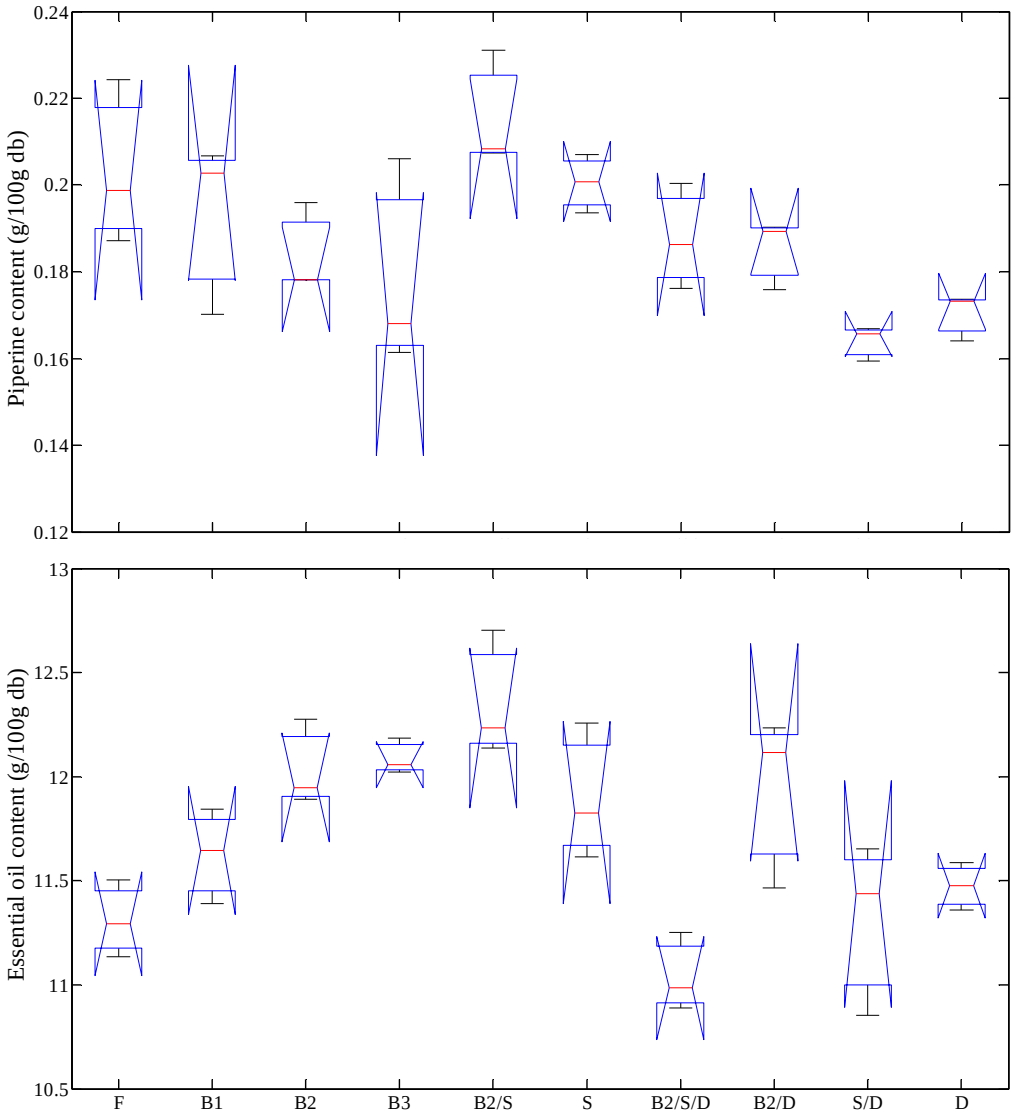


Figure 20. Impact of processing on piperine and essential oil contents
F: fresh pepper; B: blanched (B1: 60 °C/30 s; B2: 75 °C/180 s; B3: 100 °C/300 s); S: sweated (35 °C, 99 % RH, 24 h); D: dried (60 °C, 20 % RH, 39 h). Boxplots provide a statistic test of group medians: the line in the middle of each box is the sample median (n = 3); the tops and bottoms of each box are the 25th and 75th percentiles of the samples.

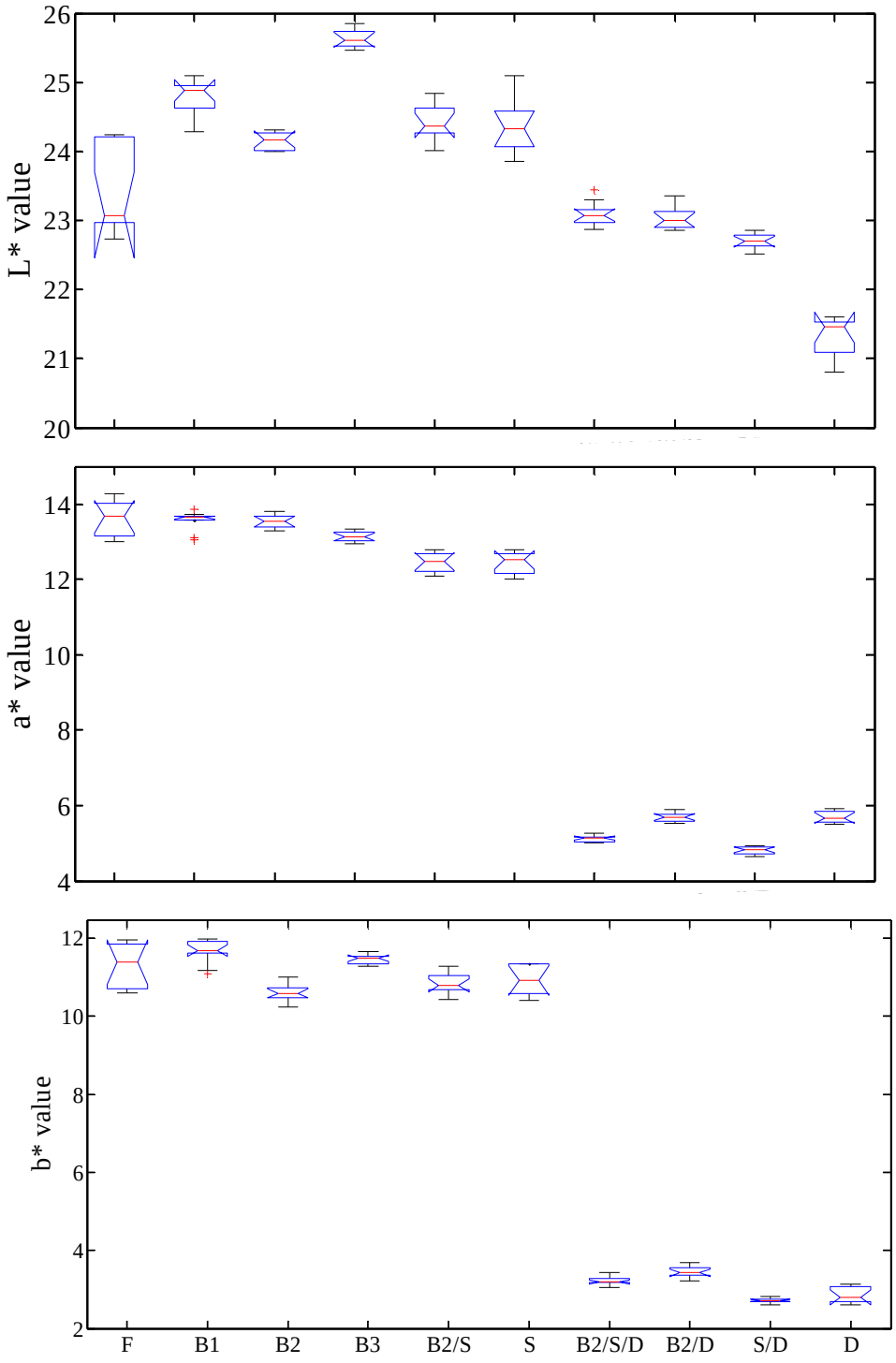


Figure 21. Impact of processes on L*a*b* chromatic values
F: fresh pepper; B: blanched (B1: 60 °C/30 s; B2: 75 °C/180 s; B3: 100 °C/300 s); S: sweated (35 °C, 99 % RH, 24 h); D: dried (60 °C, 20 % RH, 39 h). Boxplots provide a statistic test of group medians: the line in the middle of each box is the sample median (n = 10); tops and bottoms of each box are the 25th and 75th percentiles of the samples.

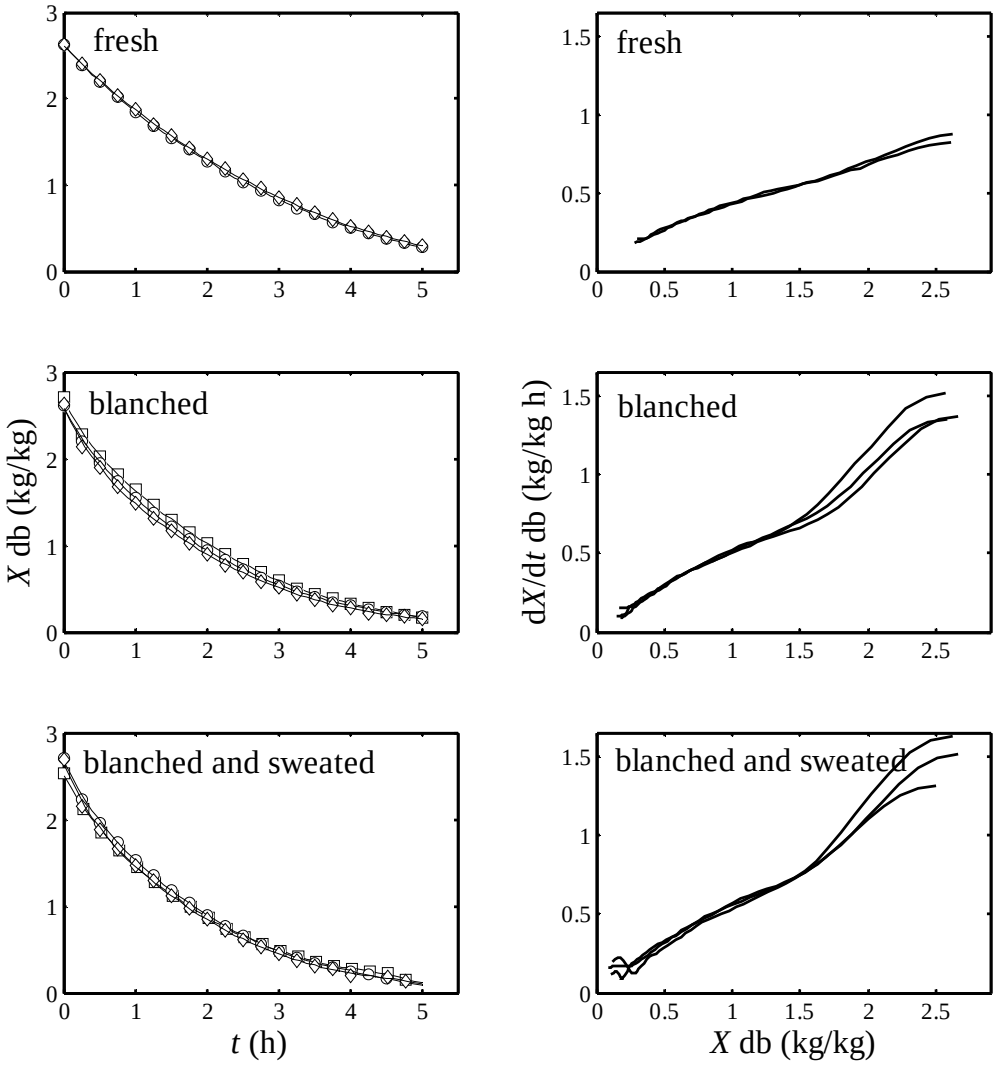


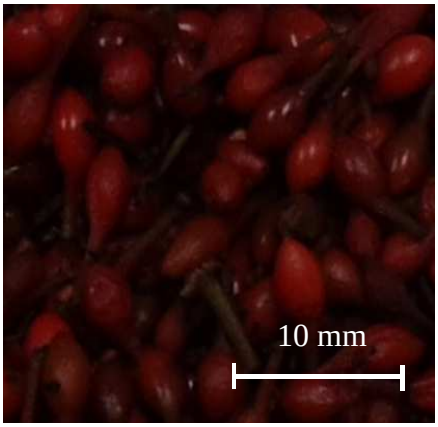
Figure 22. Drying curves of fresh, blanched and blanched/sweated pepper.
Water content (*X*) on a dry basis as a function of time (*t*) and drying rate (d*X* / dt) as a function of *X*. Drying curves of a "single thin non-compact layer" recorded by an air dryer (60 °C, RH 20 %, air velocity 2.7 ± 0.1 ms^{-1}). Blanching (75 °C/180 s); Sweating (35 °C, 99 % RH, 24 h). The different symbols on curves correspond to different trials.

3.1.2. Impact of sweating

Sweating had no impact on piperine content (**Figure 20**). There was no significant difference between sample B2 and sample B2/S on the one hand, and between sample F and sample S on the other hand. Sweating had no impact on essential oil content (**Figure 20**) as there was no significant difference between the same samples. However, sweating did have an impact on color (**Figure 21**) as sample B2 differed (+ 1 unit) from sample B2/S in a* value, and sample F differed from sample S in L* (- 0.9 units) and a* (+ 1.2 units) values. **Figure 22** shows the impact of sweating after blanching on the drying curves. One hour twenty minutes was required to obtain a 50% reduction in the initial pepper water content of blanched pepper and 1h15 min for "blanched plus sweated" pepper. Comparison of the drying curves revealed very similar drying rates in blanched plus sweated pepper (1.47 ± 0.16 kg. $kg^{-1}.h^{-1}$) and in blanched pepper (1.44 ± 0.10kg.$kg^{-1}.h^{-1}$). Sweating after blanching did not increase the drying rate; consequently the combined operation did not significantly reduce drying time compared to blanching alone.

3.1.3. Impact of drying

Drying had no impact on piperine content (**Figure 20**), as there was no significant difference between samples B2/S and B2/S/D, B2 and B2/D, S and S/D, F and D considered in pairs. Drying had no impact on essential oil content (**Figure 20**) as no significant visible difference was observed between the different samples except a slight difference between sample B2/S and sample B2/S/D (relative loss of 11%). Drying did have a marked impact on color (**Figure 21**) as there were significant differences in all values (L*, a*, b*) in all dried samples. The greatest differences were observed between samples F and D (**figure 23**) with reductions of 2.2, 7.9 and 8.4 units for L*, a* and b* values respectively (**figure 21**).



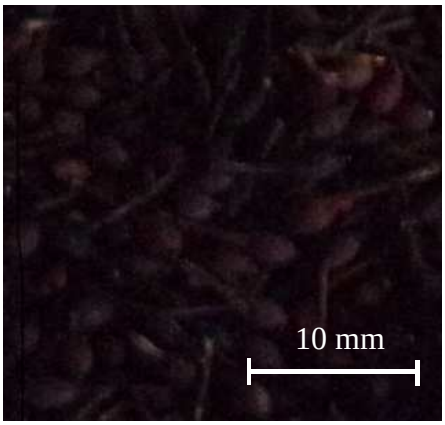


Figure 23. . Impact of drying on the color of the pepper
Sample F (fresh pepper); Sample D (dried pepper).

3.2. Impacts of "full" processes

In this study a 'full' process referred either to drying alone (dry process) or a succession of processing operations (wet processes) including blanching and/or sweating before drying (**Figure 19**). Here we describe the impacts of these 'full' processes on piperine and essential oil contents (**Figure 20**), essential oil composition (**table 12** and **figure 24**), and pepper color (**figures 21** and **23**).

Table 12: Major volatile compounds in *Piper borbonense* fresh pepper (F) essential oil

Aromatic compounds	Concentrations (g/100g)
Limonene + Eucalyptol*	29.54 ± 0.05
Alpha-phellandrene	14.38 ± 0.09
Asaricin	13.94 ± 0.33
Beta-pinene	6.46 ± 0.17
Alpha-pinene	6.00 ± 0.36
Dillapiole	4.32 ± 0.13
Safrole	3.89 ± 0.09
Delta-3-Carene	3.39 ± 0.03
Elemicin	1.95 ± 0.05
Myristicin	1.49 ± 0.24
Para-cymene	1.70 ± 0.01
Myrcene	1.70 ± 0.01
Delta-elemene	1.35 ± 0.04
Sabinene	1.41 ± 0.03
Camphene	1.45 ± 0.07
Total	92.98 ± 0.12

Mean values (n=3); *Eucalyptol represents around 2.5g/100g of essential oil

3.2.1. Impact of the "full" processes on piperine and essential oil contents, and on color

None of the "full" processes had an impact on piperine content or on essential oil content (**figure 20**). There was no significant difference between samples F, B2/S/D, B2/D, S/D and D. However, "full" processes did have a marked impact on color, confirming the major impact of the drying operation on all chromatic values (**figure 21**)**.** The chromatic dimensions L*, a* and b* were impacted irrespective of the operation considered concerned, as clearly shown by comparing the values obtained for fresh pepper (sample F) to values obtained for processed peppers (samples B2/S/D, B2/D, S/D and D) or by comparing the values obtained for processed-peppers among themselves. When drying was preceded by blanching and or/sweating, the L* value decreased less than during drying alone (≤ 0.8 compared to 2.2 units). The greatest differences (detailed in section 3.1.3.), mostly due to drying, were observed between sample F and sample D (**figure 23**) and between F and S/D while small but significant differences (all < 1 unit) were observed for a* and b* values between samples B2/S/D, B/2/D, S/D and D.

3.2.2. Impact of the "full" processes on aromatic composition

Taking sample F (fresh pepper) as a reference, we were able to identify 25 aromatic compounds representing 97% (m/m) of the total essential oil. Among these, 15 major compounds, all of which were present at a rate of more than 1%, represented 93% (**Table 12**) of the total essential oil. The monoterpenoïd family represented 55% of the total aromatic compounds. Limonene (27% of the total), was the most abundant compound followed by alpha phellandrene and asaricin (14% each). As the amounts of the compounds in sample F (fresh pepper) were very close to the amounts of compounds in samples B2/S/D, B2/D, S/D or D (processed peppers), we can conclude that globally, the composition of the flavor of the pepper was little affected by any of the processes (**figure 24**). Nevertheless, two monoterpens (para-cymene and camphene) were found at higher concentrations in processed peppers (samples B2/S/D, B2/D, S/D and D) than in fresh pepper (sample F). The most remarkable increase (89%) was obtained for para-cymene between samples F and B2/S/D. Conversely, the concentration of safrole, a non-monoterpenic compound, was lower in processed peppers (samples B2/S/D, B2/D, S/D and D) than in fresh pepper (sample F). The most remarkable decrease (33%) for safrole was observed in sample F versus

sample B2/S/D. The least affected compounds were delta 3-carene, myrcene and sabinene; no differences of more than 9% in these compounds were found between fresh (sample F) and processed peppers (samples B2/S/D, B2/D, S/D and D). The impacts of single processing operations could be deduced from “full” processes comparison. For example, the concentrations of some monoterpens (alpha phellandrene, beta pinene and delta-elemene) were reduced by sweating, as shown by the values obtained for samples B2/D and B2/S/D (this sample including sweating) on the one hand and for samples D and S/D (this sample including sweating) on the other hand. The most remarkable significant difference (a drop of 13%) was observed for delta-elemene between samples B2/D and B2/S/D. The aromatic profiles of sample B2/S/D, which included blanching, sweating and drying (full wet process), and sample D (dry process), which only underwent single drying, were very similar. No significant differences were found in 13 out of 15 compounds, while significant differences were found for two compounds, alpha phellandrene and para-cymene, which was 25% higher in sample B2/S/D than in sample D.

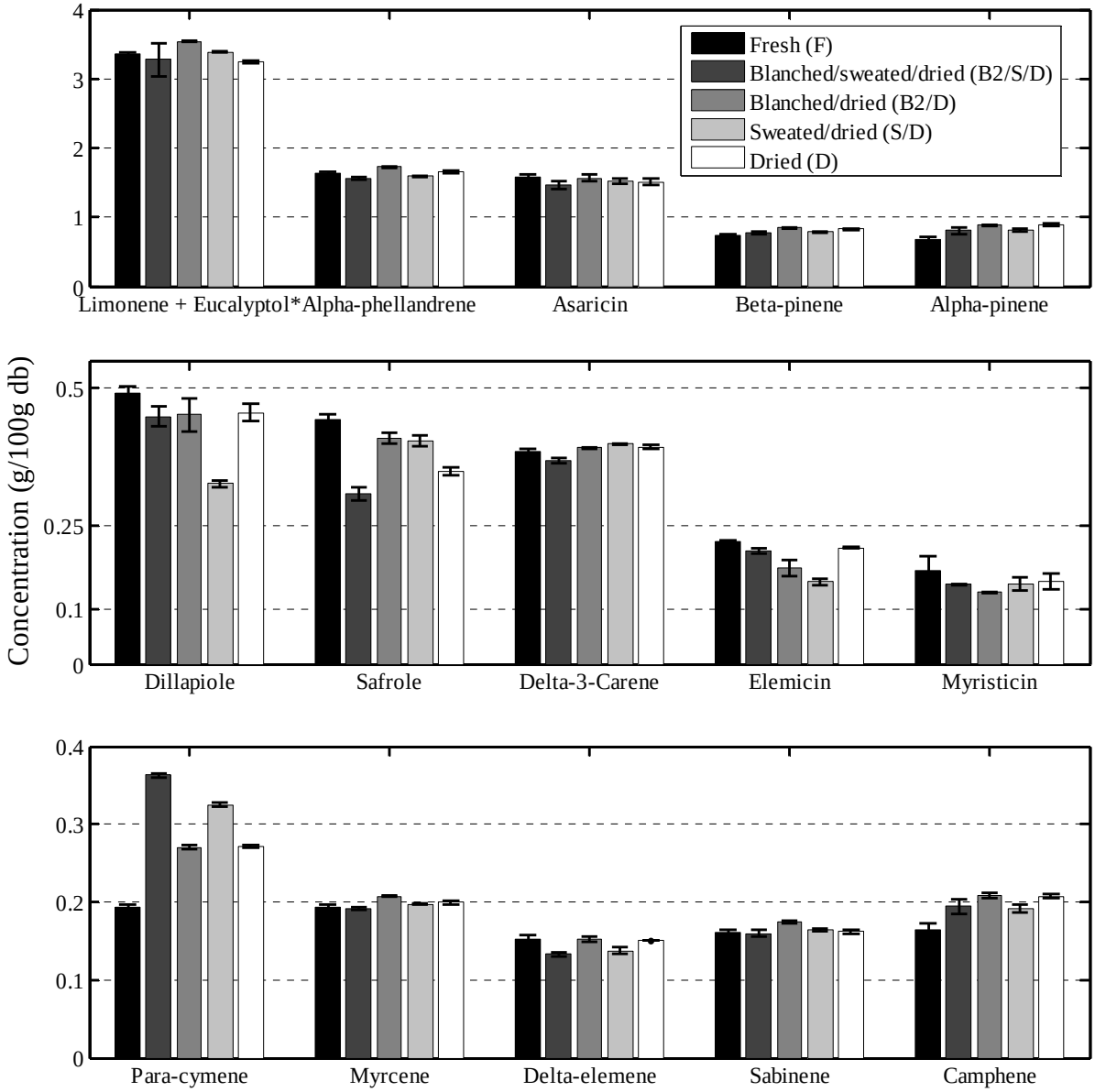


Figure 24. Composition of essential oil of fresh and processed pepper corns
The process conditions were: blanching (75 °C/3 min), sweating (35 °C, 90 % RH, 24 h) and drying (60 °C, 20 % RH, 39 h). The error bars represent the standard error (*n* = 3)
* Eucalyptol represents 0.2 to 0.3 g/100 g (db) of pepper.

4. Discussion

4.1. Originality of *Piper borbonense* composition

The 11.3% (db) rate of essential oil in fresh *Piper borbonense* is more than 5 times higher than the 2% content recommended by standard ISO 959-1 (International Standard Organization, 1998) for black pepper. It is also very close to the highest concentration of 13.1% (db) found in the richest Malagasy wild pepper species, local name Tsiperifery (Weil*, et al.*, 2014). The rate of 0.20% (db) piperine obtained

in fresh *Piper borbonense* is 20 times lower than the value of 4% indicated by the same standard. It is also 2.5 times lower than the lowest concentration of 0.5% found in Malagasy wild pepper species. These low pungency and high aroma values give *Piper borbonense* its originality compared to cultivated black pepper (*Piper nigrum*) and Malagasy wild peppers.

4.2. Impact of the processes on piperine and essential oil contents, essential oil composition and color

The different unit operations and 'full' processes tested had no impact on piperine, nor on essential oil contents of the pepper. The results we obtained for piperine are in agreement with those of Nisha, *et al.* (2009) but not with those reported by Suresh, *et al.* (2007). Nisha, *et al.* (2009) reported piperine to be stable to heat processing, with only 2.5% loss after 20 min at 100 °C, whereas Suresh, *et al.* (2007) reported 28% losses of piperine in black pepper in the same conditions. Our results concerning essential oil differ from those of Nisha, *et al.* (2009) who reported a 38% reduction in the essential oil content after 20 min at 100 °C, and from those of Schweiggert, Mix*, et al.* (2005) who reported a 75% loss after 10 minutes at 90 °C. These differences could be explained by the fact that both Nisha and Suresh used ground pepper and applied blanching conditions that were more drastic than ours. The fact that we observed no drop in piperine and essential oil contents in our study, even after 5 min at 100 °C, could be because the pericarp of the peppercorn acts as a barrier against mass transfer.

In our study, Limonene, alpha phellandrene and asaricin were shown to be the most abundant compounds in *Piper borbonense*. These compounds are also present in cultivated black pepper and play a role in the appreciation of its quality. 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 analyzed in our study contained 55% of monoterpenoïds excluding pinenes, which represented only 13% of the total, we can conclude that the aroma of wild pepper *Piper borbonense* is of good quality and is preserved even after heat treatment. According to Jirovetz, Buchbauer *et al*. (2002), limonene, β-pinene, α-phellandrene, δ-carène, asaricin and elemicin give black pepper its characteristic aroma. In our study, each of these compounds represented more than 1% of the total essential oil; together they

represented over 65% of the essential oil of wild pepper *Piper borbonense*. According to Jagella*, et al.* (1999), α-pinene, α-phellandrene, myrcene, and limonene are key odorants in *Piper nigrum*. These four compounds represented 48% of essential oil in *Piper borbonense* in our study**.** The composition of essential oil as expressed by the relative amounts of the 15 major compounds in the different samples was almost not affected by any of the processing operations tested. The biggest decrease (33%) we observed for safrole after the 'full' wet process is in agreement with the results of Farag*, et al.* (1997) who reported a loss of more than 90% after boiling the seeds for 30 min or drying the seeds for 30 min at 70 °C. Safrol, which is known to be carcinogenic, could have been degraded during blanching by hydroxylation of the dioxolane ring. Our results which unexpectedly showed that, the volatile compounds remained stable during processing, differ from those of Asekun*, et al.* (2007) who analyzed essential oil extracted from *Mentha longifolia* leaves. These authors observed significant differences in the chemical composition of the essential oils obtained using different drying methods. Four monoterpenoïd compounds (alpha pinene, beta pinene, limonene, 1,8-cineole), stable in our study, were all widely affected (increased or reduced) by the three drying methods tested by these authors. In their case, the fact that the essential oil is known to be stored on or near the surface of the leaf may explain this sensitivity. Argyropoulos*, et al.* (2014), who studied lemon balm (*Melissa Officinalis L*.) reported pronounced changes in the relative composition of essential oil when the leaves were dried at 60 °C. Most of these changes occurred during the initial period of drying. Apart from the sensitivity of the essential oil constituents to temperature, these authors also attributed these losses to the structure of the leaves. In our case the structure of the peppercorn, and in particular the pericarp, may protect the volatile compounds from transfer during processing.

The three different unit operations tested, especially drying, impacted pepper color, reducing, in particular, a* and b* chromatic values leading to browning that was visible to the naked eye. Even blanching, which should reduce enzyme activity and hence limit browning, had a slight but significant impact on color. As suggested by F. Gu*, et al.* (2013), aside from enzymatic browning described by several authors (Dhas*, et al.*, 2003; Mangalakumari*, et al.*, 1983; Variyar*, et al.*, 1988), other browning mechanisms may be involved in the change in the color of the pepper.

4.3. Role and interest of each unit processing operation

Blanching significantly increased the drying rate of the pepper. By itself, this result, which can be explained by the partial destruction of the pepper cell walls, thus facilitating water transfer (Kaymak-Ertekin, 2002), justifies this step. Indeed, a blanching step, which reduces drying time, could save energy or limit climate-dependence in the case of sun drying. Blanching is also useful as it removes any dust from the pepper. Dhas*, et al.* (2003), suggested that moderate blanching could contribute to uniform browning by promoting the oxidation of phenols by phenolase enzymes. Depending on the length and temperature applied, as a thermal treatment, blanching could be a critical sanitary step as it reduces the microbial load as well as the safrole content of the pepper. Sweating affected the color of the pepper, systematically reducing the a* value, which corresponds to red, validating the hypothesis that the conditions (24 hours at 35 °C in a water saturated atmosphere) used for sweating favor enzymatic browning, as described by Mangalakumari (1983). The favorable humidity and temperature conditions could also stimulate the growth of microorganisms. Considering these results, we question the interest of this operation, which is used in Madagascar (Weil*, et al.*, 2014) for wild pepper species that are close to our *Piper borbonense,* as well as for *Piper nigrum*. Drying is crucial; it not only stabilizes the product but is also critical for the sanitary and sensorial quality of the pepper. Drying had a major influence on color as all values (L*, a*, b*) were affected, and the color turned from red to brown. Referring to Agudelo-Laverde*, et al.* (2013) who demonstrated that browning increased with an increase in water content on strawberry slices, we hypothesize that accelerating drying, by rapidly reducing water activity, could help preserve the red color.

5. Conclusion

The originality of dry *Piper borbonense* is based on its high aromatic potential, low pungency and red color. Separate or combined, the blanching, sweating and drying operations had no impact on piperine and essential oil content and only a slight impact on essential oil composition. The three unit operations influenced color, drying having the most impact. To preserve the color, sweating should be avoided, while blanching and drying could be optimized. Indeed, as demonstrated in this study, right blanching parameters could reduce drying time as well as limit enzymatic browning. Enhancing and innovating drying conditions would also help reduce both enzymatic and non-enzymatic oxidative reactions.

3.4.2. Synthèse du Chapitre 4 et perspectives

Les différentes opérations unitaires, notamment le séchage mais aussi le blanchiment et dans une moindre mesure l'étuvage, impactent largement la couleur du poivre en le faisant brunir. Les teneurs en huile essentielle et en pipérine ne sont pas affectées par les différents traitements testés : opérations unitaires ou combinées. En revanche, la composition en huile essentielle l'est un petit peu. Le blanchiment améliore l'efficacité du séchage. Il ressort des travaux réalisés jusqu'ici que l'étuvage ne présente pas d'intérêt et est même néfaste en ce qui concerne la couleur, pour la qualité du poivre. En revanche si le blanchiment s'avère intéressant et le séchage indispensable, ces deux étapes doivent être étudiées et optimisées si l'on souhaite améliorer la qualité du poivre, et conserver notamment une teinte rouge en fin de procédé.

Les travaux suivants visent, à travers l'application de différentes conditions d'échaudage et de séchage, à mieux comprendre les mécanismes impliqués dans le brunissement du poivre.

3.5. Chapitre 5. Etude des mécanismes biochimiques impliqués dans le brunissement du Piper borbonense au cours du procédé

3.5.1. Article en préparation

Study of the biochemical mechanisms involved in the browning of *Piper borbonense* during the process

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Abstract

Different processes, combining unit blanching and/or drying operations, have been tested in order to understand the mechanisms involved in the browning of the *Piper borbonense* wild pepper, which is initially red. An intense blanching (100°C/3 min) allows for better preservation of the chromatic values and has a positive impact on the carotenoid content, which is less negatively affected at the end of the process than with drying alone. For all the processes tested, similar and substantial losses in polyphenols (around 80%) were obtained, and the pepper was brown in colour after drying. The conditions for the inactivation of the enzymes polyphenol oxidase (PPO) and peroxidase (POD), and Maillard reactions, were also measured. POD is more temperature-sensitive that PPO and Maillard reactions were negligible in standard drying conditions. Non-enzymatic browning mechanisms, such as chemical oxidation of polyphenols, are predominant and remain difficult to control by traditional transformation processes.

Keywords

Pepper, colour, carotenoids, polyphenols, oxidation, enzymatic browning

1. Introduction

Pungency, aroma and colour are some of the common characteristics noted in pepper. While the pungency and aroma of the *Piper borbonense* are quite resistant to the processes (Weil*, et al.*, 2017), this is not the case for the colour, which changes from red to brown during the transformation of this spice. However, colour is a fundamental element in the evaluation of quality of food products, particularly dried products; it influences appreciation, prices and, in the end, acceptance by consumers (Sahin*, et al.*, 2011).

Polyphenols and carotenoids are phytomicronutriments naturally present in the majority of fruits, vegetables and spices, and they share certain characteristics, including anti-oxidant properties, and are involved in colour (Renard C., 2014). Some carotenoids (for example, lycopene) and polyphenols (including anthocyanins), depending on their concentration, give foods a red colour. Their degradation can be enzymatic or non-enzymatic. The degradation of carotenoids leads to a dulling, while the degradation of polyphenols generally leads to a browning (Guyot S., 2014). In the case of enzymatic browning, phenolic compounds are oxidised in quinones, very reactive compounds, which then quickly polymerise in melanins, brown and black compounds, with high molecular mass.

Variyar*, et al.* (1990) and Deli*, et al.* (2001) described several carotenoids, such as lycopene, beta-carotene and capsorubin, which give mature *Piper nigrum* its red colour. Other authors (Dhas*, et al.*, 2003; Mangalakumari*, et al.*, 1983) have demonstrated that *Piper nigrum*, without prior enzyme-inactivation treatment, underwent an enzymatic browning during drying.

F. Gu*, et al.* (2013) have demonstrated that black pepper could brown despite a blanching that is drastic enough to denature the enzymes, thus proving the existence of non-enzymatic browning mechanisms. This non-enzymatic browning could be due to thermal degradation and/or chemical oxidations (Jacquot*, et al.*, 2011) of the phytomicronutriments (Renard C., 2014), or to mechanisms that do not even involve polyphenols or carotenoids, such as the Maillard reaction (Horvathova*, et al.*, 2007) or caramelisation (Oliveira*, et al.*, 2011), which appear during drying.

While there is some scientific research concerning the colour of *Piper nigrum*, there is not any, by contrast, on the *Piper borbonense* pepper. This study seeks to understand the colour degradation mechanisms of *Piper borbonense* during the process.

The impacts of transformation operations (blanching and drying) on the colour, and

polyphenol and carotenoid content, of pepper from Réunion were measured. Enzymatic degradations (PPO and POD) have been determined and the dosage of 5-hydroxymethylfurfural (HMF) makes it possible to measure the effect of Maillard reactions on non-enzymatic browning.

2. Material and methods

2.1. Plant material

Wild mature pepper spikes were picked in the south of Réunion Island in a place called Rivère Langevin (21°2'04.49"S; 55°38'33.07"E). Spikes picked on different occasions in October 2015 were frozen at -80°C (freezer Froilabo - Bio Memory, 690 litres) before being pooled and mixed to form a single homogenous batch. Before processing, the peppercorns with their pedicels were separated from the fruit stems by hand and defrosted for two hours at room temperature. The defrosted pepper is called “fresh” pepper in the rest of this article.

2.2. Processing conditions: blanching and drying

The processes we tested consisted in two unit operations (blanching and drying) that were applied (alone or combined) to obtain different samples (**Table 13**). Blanching consisted in soaking the peppercorns in a hot water bath (Memmert Gmbh type WB 22 Schwabach, Germany) at a ratio of 1:36 peppercorns to water, in two different conditions: 75°C for 3 min; and 100°C for 3 min. Drying was performed by placing aluminium trays (300cm^2) containing 250g of peppercorns arranged in a compact 1cm-thick layer in a climatic chamber (BIA Climatic – Type CL 125, Conflans Sainte Honorine, France) at different temperatures (±1°C) and relative humidity (±2%) until samples reached 90% dry matter. Hot air was circulated over the surface.

Table 13. Process conditions applied to pepper

F: fresh pepper; B: blanched pepper; D: dried pepper; BD blanched and dried pepper

Sample names	Treatments	
	Blanching	**Drying**
	Fresh pepper (F)	
F	no	no
	Blanched pepper (B)	
B1	75°C/3min	no
B2	100°C/3min	no
	Dried pepper (D)	
D1	no	60°C/20%HR
D2	no	70°C/50%HR
D3	no	30°C/50%HR
D4	no	60°C/40%HR
D5	no	100°C/10%HR
	Blanched and dried pepper (BD)	
B1D1	75°C/3min	60°C/20%HR
B2D1	100°C/3min	60°C/20%HR
B2D3	100°C/3min	30°C/50%HR
B2D4	100°C/3min	60°C/40%HR
B2D5	100°C/3min	100°C/10%HR

2.3. Sample preparation

The pepper samples resulting from the different processing operations were frozen at -80°C for further preparation and analysis. These samples were ground in liquid nitrogen for 10 seconds at 10 000 rpm using a mill (Retsch – Grindomix GM200, Retsch Gmbh, Germany) for all chemical analyses. Colour measurements were made on whole peppercorns.

2.4. Analytical methods

2.4.1. Dry matter content

The dry matter content (mean “essential oil free dry matter”) was obtained by drying 5g 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). Initial and final masses were determined with a precision balance (Scaltec SBC 22 model, Scaltec Gmbh, Germany). The mean relative deviation of repeatability was ±0.8% (n = 3).

2.4.2. Colour measurements

Color measurements (CIE *L**, *a** and *b** values, representing lightness, redness and yellowness, respectively) were made on whole peppercorns using a Minolta CR 410 and utility software. Ten measurements were made on each sample of peppercorns spread in a 1-cm layer in an uncovered Petri dish. The mean relative deviation of repeatability was 2.2%, 3.4% and 6.0% respectively for L*, a*, b* (n = 30).
Photographs of fresh and processed peppercorns were made using an HP d3500 digital camera (Hewlett-Packard, USA).

2.4.3. Total polyphenol content

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

2.4.4. Total carotenoid content and β-carotene content

Carotenoids were extracted from 200mg of ground pepper mixed in a tube containing 1ml of distilled water for 2min. Then 10ml of ethanol/hexane (4/3 v/v) were added before homogenization for 60 seconds in a Fastprep 24 (MpBiomedical, Santa Ana, USA) using sand as a lising matrix and ceramic ball as a mortar. The hexanic phase was recovered and ethanolic residues were mixed again with 5ml of hexane. This operation was repeated three times. All organic phases were collected together and dried with anhydrous sulphate sodium. After evaporation on a Genevac HZ plus (Genevac, Warminster, USA), extracts were recovered in 0.5ml dichloromethane and 0.5ml methanol/methyl tert-butyl ether (80/20 v/v) and analysed though HPLC. Carotenoids were then analyzed 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 × 4.6mm i.d., 5µm: YMC Europ GmbH, [YMC, Dinslaken Germany]. Carotenoids quantification was achieved by calibrating β-carotene at 450nm. The mean relative deviation of repeatability was ±8.4% (*n* = 9) for total carotenoids and ±5.3% for β-carotens (n=9).

2.4.5. Glucose and fructose content

The aqueous extraction of sugars was performed by adding 100ml of milli-Q water to 100mg of sample. After 1h of shaking, samples were filtered through a 0.45-µm filter (Millipore) and placed in vial before analysis. The remaining glucose and fructose were monitored by Shimadzu HPLC equipped with model LC-20AB pumps and an SIL-20A autosampler (Shimadzu, Kyoto, Japan), coupled with a PDA Decade 2 detector (Antec Leyden, the Netherlands). Separation of the sugars was performed in a 4 × 250mm CarboPac MA1 Column (Dionex, Germany). The eluent used was a degassed NaOH 800mM solution pumped at a flow rate of 0.4ml/min. A freshly prepared solution of D-glucose and D-fructose was used to calibrate the system. The mean relative deviation of repeatability was ±7.4% for soluble carbohydrates (n = 6), ±10.7% for glucose (n=4) and ±10.9% for fructose (*n* = 4).

2.4.6. Amino acids 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 analyser (Biochrom Ltd., Cambridge, UK). This system uses ion exchange chromatography with post-column ninhydrin derivatization and photometric detection with dual-wavelength measurements. The 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 (0.2–0.5M) and with an increasing temperature gradient (52–95°C). Amino acids were derivatized with the ninhydrin reagent (135°C) and detected simultaneously at 570nm and 440nm. The entire process lasted 90 min per sample, including the resin regeneration phase. Quantification was performed by comparing peaks areas with a complete standard including 26 acidic, neutral and basic amino acids (Sigma, St. Louis, Missouri, USA). Norleucine (250 nmol mL−1 in sodium citrate buffer, 0.2M, pH 2.2) was also used as an internal standard. The mean relative deviation of repeatability was ±5.0%.

2.4.7. 5-Hydroxymethylfurfural content

The method is inspired by the one described in Regulation (EEC) No 2676/90 of the Commission, of 17 September 1990, determining methods for the analysis of wines (1990) (JO, 1990). 150mg of sample was shaken for 1h in 2.25ml of Milli-Q water. Extracts were then centrifuged for 3 min at 5,000*g*. The supernatant was filtered through 0.45µm filter (Millipore) and then placed in vials before chromatographic

analysis. Analyses were carried out using a Dionex HPLC Ultimate 3000 (Dionex, Germany). Samples were eluted on a Gemini C18 110A 5µm 250 x 4.6mm column equipped with a Gemini C18 4 x 3mm pre-column (Phenomenex, USA). The mobile phase was a solution of water-methanol-acetic acid [90/9/1% (v/v)] pumped at a flow rate of 1 ml/min. Detections of 5-HMF was performed at 280nm. Calibration of the system was performed with freshly prepared solution of 5-HMF with concentrations ranging from 3 to 30mg/l.

2.4.8. Enzymes activities

PPO and POD activities were assessed by spectrophotometry in triplicate using a spectrophotometer Thermospectronic Helios α v4.60 (Thermo Fisher Scientific, USA). Enzymes were extracted by centrifuging (Centrifuge - Sigma 3-18k; rotor SIGMA 11135, Sigma Neustadt Gmbh Germany) 0.2g of ground (grinder Retsch – Grindomix GM200, Retsch Gmbh, Germany) pepper in 1.6ml of Mac Laine Buffer pH 6.5 for 20mn at 14 000g and 4°C. Catechol solution at 0.175M was used as a phenolic substrate for the determination of PPO activity. Guaiacol 0.1M in the presence of H_2O_2 0.05M was used as a phenolic substrate for the determination of POD activity. Optical density was measured at 420nm at 30°C at 5-second intervals for 1 min. The activities of PPO or POD were defined as the change in absorbance. min-1.mg/g (db). Residual PPO and POD activities were expressed as a percentage of maximum activity measured in the reaction medium from non-treated pepper samples. The mean relative deviation of repeatability for enzyme activities was ±17.5% for PPO (n=9) and ±9.0 for POD (*n* = 9).

2.5. Statistical analysis

Differences in the mean values of chemical contents and L*, a* and b* values were tested by analysis of variance (ANOVA); the significance of differences between samples was determined using Tukey's test. The level of significance was $P<0.05$.

3. Results

3.1. Impact of the processes on the colour of the pepper

Figure 1 shows that the 3 processes tested affect the colour of the pepper. For each case a significant decrease of the three chromatic values (L*, a* and b*) was found between fresh F pepper and dried pepper at the end of the process (B1D1, B2D1 and D1). Direct drying (D1) has a greater impact on the colour of the pepper than when it

is combined with preliminary blanching at 100°C for 3 minutes (B2D1). However, if the blanching is less drastic (75°C for 3 minutes - B1D1), the chromatic values decrease more than with a single drying (D1).

During the process, the chromatic values (L*, a* and b*) decreased significantly, firstly through blanching (F vs B1), with the pepper changing from bright red to dark red; then through drying, with the pepper changing from dark red to brown (B1 vs B1D1). The lightness L* value, only divided by 1.7 after process B1D1 (which has the greatest impact) resists the process quite well. For the same time and the same process, the value of a* is divided by 3.7 and the value of b* by 8.1. For the B1D1 process, it is always the unit drying stage that has the greatest impact on colour; the three chromatic values are effectively more affected by this stage than by blanching; thus, between blanching (B1) and drying (B1D1), the value of the b*, the most affected, is divided by 5.4.

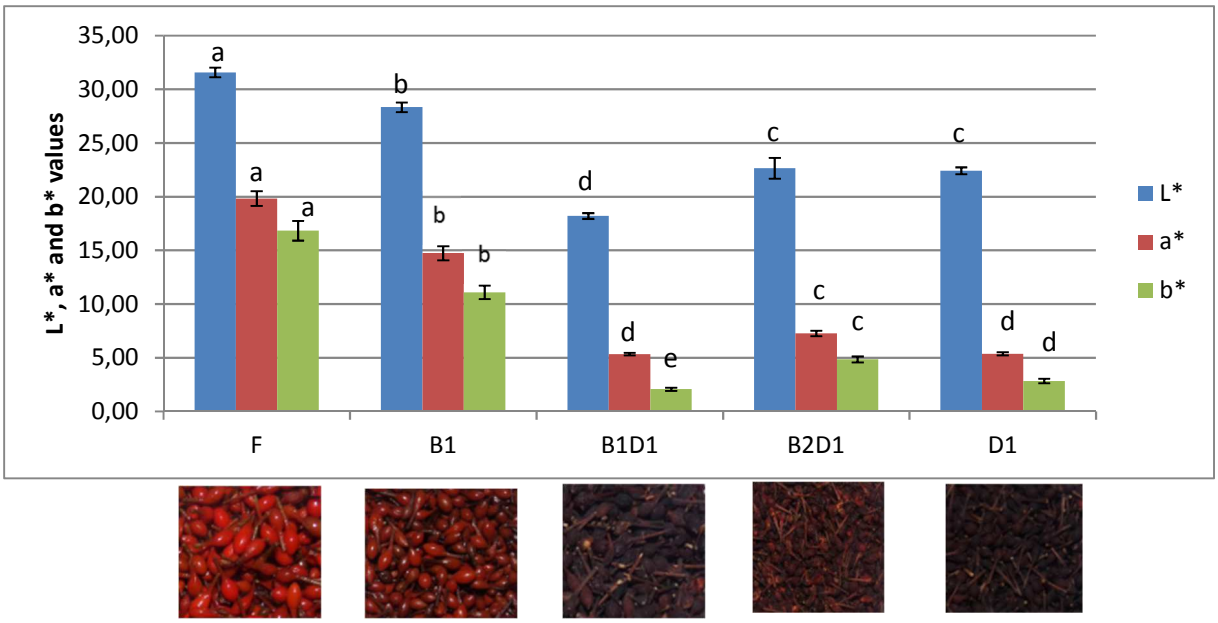

Figure 25. Impact of processes on L*, a* and b* chromatic values of pepper corns
F: fresh pepper; B: blanched (B1: 75°C / 3 min, B2: 100°C / 3 min); D1: dried (60°C / 20% HR). Bars are mean (n = 30) ± confidence interval (P = 0.05). Different letters (*a*–*e*) at the top of the bars means significantly different (P < 0.05) for each chromatic value (L*, a* and b*) considered separately.

3.2. Impact of the processes on polyphenol content and carotenoid content

3.2.1. Impact of the processes on polyphenol content

The total polyphenol content of fresh F *Piper borbonense* is 1.56g equivalent gallic acid for 100g of pepper (db). For the three processes tested, D1, B1D1 and B2D1 (**Figure 26**), all recorded a significant decrease in the polyphenol content of the pepper. Losses are between 75% and 80%. There are not significant differences between the losses recorded for drying directly (D1) and drying preceded by blanching (B1D1 and B2D1). Nor are the differences significant, in terms of losses, between the two blanching conditions (B1D1 and B2D1).

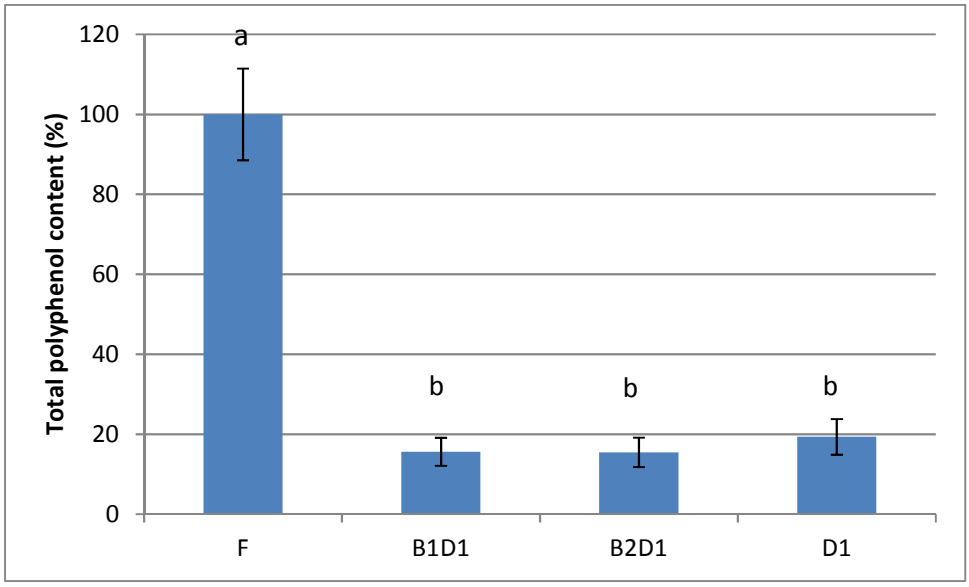

Figure 26. Levels of total phenolic content in pepper compared to the initial content (1.56g eq. gallic acid/100g db) expressed in percentage
F: fresh pepper; B: blanched (B1: 75°C / 3 min, B2: 100°C / 3 min); D1: dried (60°C / 20% HR).
Bars are mean (n = 3) ± confidence interval (P = 0.05). Different letters (a–b) at the top of the bars means significantly different (P < 0.05).

3.2.2. Impact of the processes on carotenoid content

The total carotenoid content of fresh *Piper borbonense* is 315 µg equivalent β-carotene/g of pepper (db) including 104 µg/g (db) of β-carotene. For the three processes tested, D1, B1D1 and B2D1 (**Figure 27**), all recorded a significant decrease in the carotenoid content of the pepper. The beta-carotene is affected in the same proportions as the total carotenoids, regardless of the process tested. It is direct drying D1 which most affects carotenoids, with a reduction of 50% compared to fresh (F). Drying preceded by blanching has less impact on the carotenoid content since B1D1 induces approximately 40% of the losses and B2D1 only 15%, with respect to the content of fresh pepper F. Thus, carotenoids are better preserved at the end of the process when the drying is preceded by blanching and more so when the blanching is

drastic.

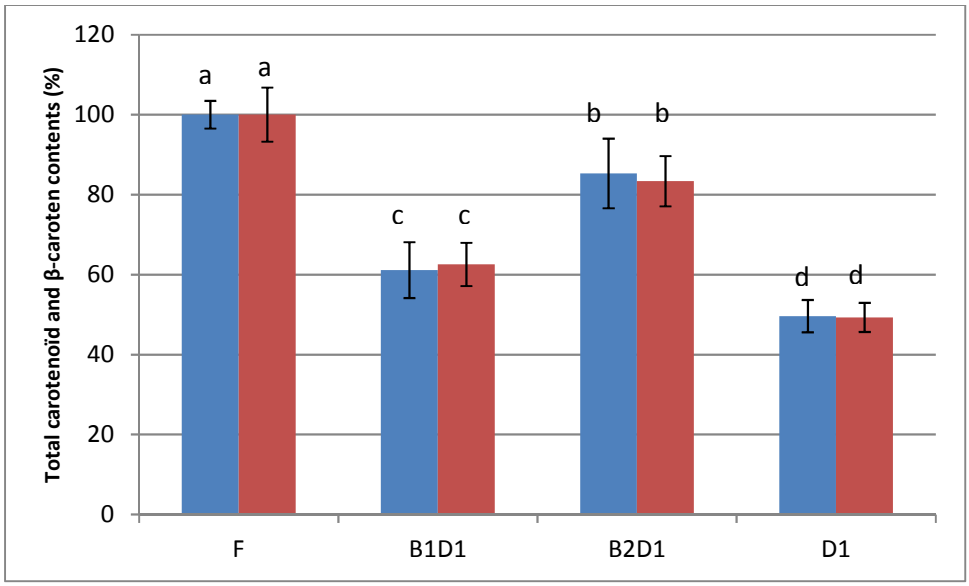

Figure 27. Levels of **total carotenoid** and **β-carotenoid** content in pepper compared to the initial contents (total carotenoids: 315 µg eq. β-carotens/g db; β-carotens: 104 µg eq. β-carotens/g db) expressed in percentage
F: fresh pepper; B: blanched (B1: 75°C / 3 min, B2: 100°C / 3 min); D1: dried (60°C / 20% HR).
Bars are mean (n = 9) ± confidence interval (P = 0.05). Different letters (a–d) at the top of the bars means significantly different (P < 0.05) for carotenoids and β-carotens considered separately.

3.3 Study of the browning mechanisms of pepper

3.3.1. Enzymatic browning

This research seeks to identify the blanching conditions necessary for limiting the phenomenon of enzymatic browning in *Piper borbonense*. **Figure 28** demonstrates that a type B1 blanching (75°C for 3 minutes) reduces the activity of PPO by 36% and of POD by 50%. The total inactivation (reduction of 100%) of the two enzyme activities is obtained by the B2 conditions of blanching (100°C for 3 minutes).

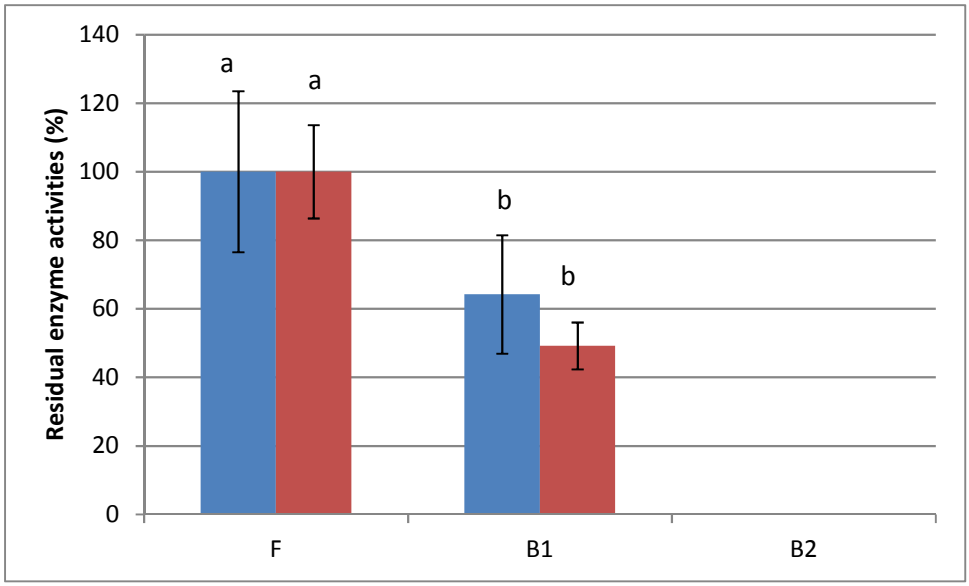

Figure 28. Residual enzyme activities according to processes
F: fresh pepper; B: blanched (B1: 75°C / 3 min, B2: 100°C / 3 min)
Bars are mean (n = 9) ± confidence interval (P = 0.05). Different letters (a–b) at the top of the bars means significantly different (P < 0.05) for **PPO** and **POD** enzymes considered separately

3.3.2. Non-enzymatic browning

3.3.2.1. Elements concerning the Maillard reaction

Table 15 and **Table 14** illustrate, respectively, the presence of reducing sugars, such as glucose (2.5% db) and fructose (3.2% db) and amino acids (in particular, lysine 0.345% db and methionine 0.225% db) which are likely to be involved in Maillard reactions. Indeed, according to **Table 16**, a drastic drying of type D2 (70°C, 50%HR) generates products of the Maillard reaction, in this case HMF (7.36mg/kg db), while moderate drying of type D1 (60°C, 20%HR) does not significantly (1.47mg HMF/kg pepper db), if one compares it to the 1.29mg HMF/kg (db) initially measured in fresh (F) pepper.

Table 14. Amino acid content in fresh mature *Piper borbonense*

	Concentrations (g/100g db)	
Amino acid	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

Table 15. Sugar content in fresh mature *Piper borbonense*

	Sugar content (g/100g db)	
Soluble carbohydrates (sugars)	7.35 ± 0.57	(6)
Glucose	2.50 ± 0.43	(4)
Fructose	3.32 ± 0.58	(4)

(Mean values ± 95 %; confidence interval with *n* noted in brackets)

Table 16. HMF content in fresh mature *Piper borbonense*

Sample	HMF content mg/kg (db)
F	1.29
D1	1.47
D2	7.36

3.3.2.2. Other non-enzymatic browning mechanisms

Figure 29 shows us that, despite enzyme inactivation through blanching B2 (100°C, 3 min) followed by drying in conditions that are not very conducive to the development of Maillard reactions (30°C / HR 50% and 100°C / HR 10%), the colour of the pepper is nevertheless strongly degraded. In the 3 processes (blanching + drying) tested (B2D3, B2D4, B2D5), a significant browning decreases the 3 chromatic values L*, a* and b*, when compared to the values of the fresh (F) pepper.

Drastic drying D5 (100°C / HR 10%) enables, after the application of the same blanching B2 in all cases, better preservation of colour than moderate drying D3 (30°C / HR 50%) and D4 (60°C / HR 40%). Chromatic value L* is the most resistant, followed by the values of a* and b* (the most reduced). For the process that has the greatest impact, B2D3 (temperature 30°C, HR 50%), the value of L* is divided by 1.5, the value of a* by 3 and of b* by 5.

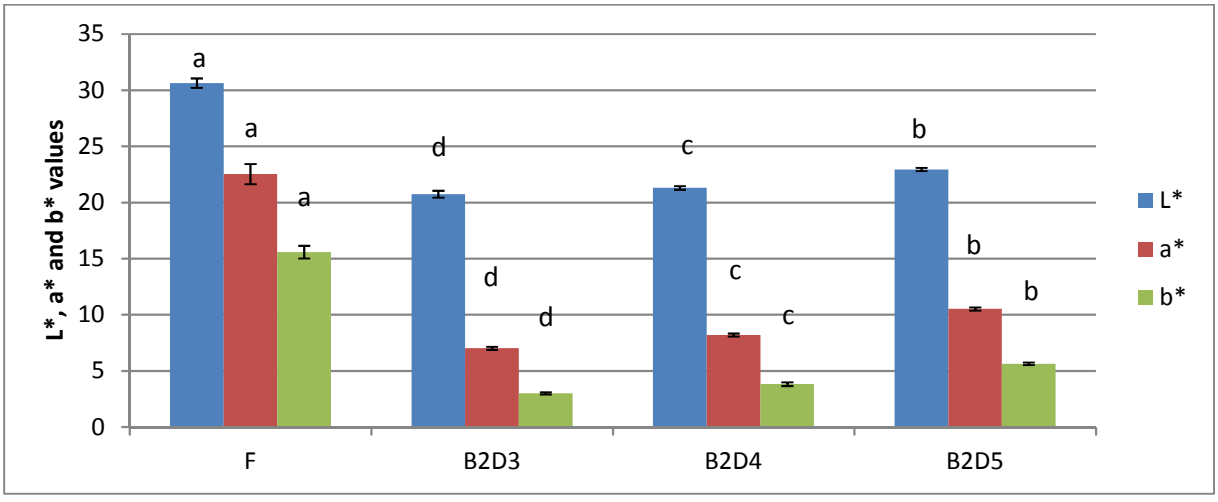

Figure 29. Impact of processes on L*, a* and b* chromatic values of pepper corns
F: fresh pepper; B2: blanched (100°C / 3 min); D: dried (D3: 30°C / 50% HR, D4: 60°C / 40%HR, D5: 100°C / 10%HR).
Bars are mean (n = 10) ± confidence interval (P = 0.05). Different letters (a–d) at the top of the bars means significantly different (P < 0.05) for each chromatic value (L*, a* and b*) considered separately.

4. Discussion.

4.1. Impact of the processes on the colour of *Piper borbonense*

The fact that the colour (**Figure 25**) is better preserved at the end of the drying process for pepper blanched at 100°C/3min (B2D1) than for pepper blanched at 75°C/3min (B1D1) and unblanched pepper (D1) demonstrates that the enzymes are involved in the degradation of colour. The 100°C/3 min conditions of blanching (B2) totally inactivate the endogenous enzymes, limiting colour degradation. A partial inactivation of the enzymes 75°C/3min (B1D1) will, on the contrary, favour the degradation of colour and the browning of the pepper. According to Renard C. (2014), a thermal treatment like this could induce the destruction of the plasma membranes and then of the parietal structures, thus enabling oxygen and enzymes to access their substrates; the carotenoids and polyphenols could then be exposed to these reactions; the main reaction would be oxidation, either catalysed by enzymes or purely chemical.

4.2. Impact of the processes on polyphenols and carotenoids

4.2.1. Impact of the processes on polyphenol content

The polyphenol content of fresh *Piper borbonense*, 1.56g GAE/100g (db), is very similar to that measured by Agbor*, et al.* (2006) (1.2g/100g) and to that measured by Cheng (2015) (1.3 g/100 g) in *Piper nigrum*. This level of 1.56 GAE/100g (db) is close to that of fruits considered to be rich in polyphenols, such as strawberry (Brat*, et al.*, 2006) and mango (Murillo*, et al.*, 2012). The significant degradation of polyphenols during thermal treatments at 100°C has been demonstrated by Jimenez*, et al.* (2010). The losses of 75 to 80% of polyphenols found in the *Piper borbonense* after drying, regardless of the process used (D1, B1D1 or B2D1), are higher than the values of 40 to 50% measured by Wojdyo*, et al.* (2014) for different parameters of cherry drying. In their research on black pepper, Horvathova*, et al.* (2007) did not find an immediate positive effect of heat treatment on the content of polyphenols; however, the polyphenols were better preserved during subsequent storage. In our study, regardless of the conditions applied, blanching did not impact the losses in polyphenols, which are identical after drying for D1, B1D1 and B2D1. This may be explained, on the one hand, by the fact that a few hours of drying are insufficient for observing the differences that appear after several months of storage and, on the other hand, by the fact that the degradation of polyphenols and browning are essentially non-enzymatic. Indeed, according to Renard C. (2014), in treatments at 50-60°C, during industrial

transformations, the phenolic structure of polyphenols may be degraded and these thermal treatments may lead to the formation of o-quinones and o-semiquinones, highly reactive molecules which may react with nucleophilic groups of proteins and/or polysaccharides, giving brown compounds. Lastly, polyphenols can play the role of antioxidant for carotenoids (Schweiggert, Kurz*, et al.*, 2007; Schweiggert, Schieber*, et al.*, 2005), which would explain greater losses in polyphenols than in carotenoids during browning.

4.2.2. Impact of the processes on carotenoid content

The total carotenoid content of 315 µg equivalent β-carotene/g of pepper (db) including 104 µg/g (db) beta-carotene, found in *Piper borbonense* can be considered to be relatively high for fruit, since Deli*, et al.* (2001) found 1,300 µg/g total carotenoids in sweet pepper, which is known for being particularly rich in carotenoids, and since Penicaud*, et al.* (2011) describe the fruit of oil palm as one of the richest in beta-carotenes with a content of 200 µg/g (db). This value of 315 µg/g (db) total carotenoids in *Piper borbonense* is particularly high for a pepper. The total carotenoid concentrations found by Veeru*, et al.* (2009) in *Piper longum* and by Variyar*, et al.* (1990) in *Piper nigrum,* were only 9.5 and 45 µg/g (db), respectively. The significant differences in the carotenoid content of these different peppers could be due to the species, but also to the level of maturity (during harvesting) which greatly influences the carotenoid content of fruit. This has been demonstrated for paprika with a ratio of 1 to 60 for total carotenoid content between immature green fruit and mature red fruit (Deli*, et al.*, 2001). Note that, in general, in the case of black pepper, the fruit is not harvested at full maturity, meaning it is not yet red, in order to prevent theft (Jansz*, et al.*, 1984), or the fruit being removed by birds, or even rotting (Dhas*, et al.*, 2003), thus increasing production yields.

50% of total carotenoids, as well as beta-carotenoids, are lost in the event of a simple drying, while Sahin*, et al.* (2011) have found between 70 and 85% lycopene losses in tomatoes dried at temperatures between 65°C and 85°C. Only 15% of losses are observed in processes including a blanching that totally inactivates enzymes (100°C/3min). Schweiggert, Kurz*, et al.* (2007) have also shown, with paprika, that a process including a blanching of 90°C/10 minutes, prior to drying, had less impact (20% losses instead of 45%) on carotenoids than direct drying. Nevertheless, in their studies and ours alike, losses remain consistent despite blanching. The explanations given by

these authors and by Baldermann*, et al.* (2005) are that the degradation of carotenoids is not due solely to the enzymes, but equally to non-enzymatic mechanisms such as thermal degradation, photo-oxidation and auto-oxidation.
According to Variyar*, et al.* (1990) and Deli*, et al.* (2001), who have shown that the red colour of mature *Piper nigrum* could be attributed to different carotenoids such as lycopene, beta-carotene or even capsorubine, we are able to estimate that the high carotenoid content, including that of beta-carotene, give the *Piper borbonense* its red colour. These carotenoids are subject to significant degradations of various natures (enzymatic and non-enzymatic) during the process. However, to the extent where the degradation of carotenoids leads to a dulling of the colour and not to browning (Cinar, 2004), other mechanisms are likely to be involved in the browning of the pepper.

4.3. Browning mechanisms of the pepper

The complete inactivation of the PPO and POD enzymes, which are responsible for the enzymatic degradation of polyphenols, is obtained by treatment at 100°C for 3 min. If these conditions do not eliminate the phenomenon of browning, they partially limit it, meaning that a part of the browning is of enzymatic origin but that another part of the browning observed, the most important part, is not of enzymatic origin.

4.3.1. Enzymatic mechanisms

For *Piper borbonense*, POD, which maintains only 50% activity after blanching B1 (3min at 75°C), is more heat sensitive than PPO, which maintains 65% activity after the same treatment. POD, on the contrary, proved more resistant than PPO (completely denatured after 10 minutes at 80°C) in the research of Schweiggert, Schieber*, et al.* (2005) on paprika and chili pepper. F. Gu*, et al.* (2013) Observed a loss of 97% in the PPO activity of black pepper after a blanching of one minute at 80°C. The degradation of carotenoids, on the other hand, leads to a loss of pigmentation, or to dulling, (Cinar, 2004) which we have not seen in our pepper. In addition, we have not observed a correlation between carotenoid content and colour intensity; D1 (**Fig. 25**) shows higher chromametric values than B1D1, but a lower carotenoid content (**Fig.27**). This suggests that if carotenoid degradation mechanisms (enzymatic and also non-enzymatic) are present during the transformation processes of pepper, they are less significant with regard to the colour than the polyphenol degradation mechanisms (also enzymatic and non-enzymatic) that lead to browning.

Although thermal treatments such as blanching may, in the first stage, negatively influence the colouration of the pepper, in the end they prove to be significant, in the second stage, for the preservation of the colour. In the first instance, blanching may generate losses in phytomicronutrients by leaching and diffusion into the environment (Hong-Wei Xiao*, et al.*, 2017), as well as losses through the thermal degradation of polyphenols and carotenoids and the destructuring of cell walls (Renard C., 2014), favouring contact between enzymes and substrates. However, in the case of black pepper, as demonstrated by Horvathova*, et al.* (2007), thermal treatments allow better preservation of antioxidants, in particular polyphenols, during subsequent storage, because they enable the degradation of the enzymes responsible for enzymatic browning.

4.3.2. Non-enzymatic mechanisms

4.3.2.1. Maillard reactions

The most favourable drying conditions for Maillard reactions were generated by HMF, in a very low quantity. As a comparison, the levels of HMF measured in the mango during drying by Korbel*, et al.* (2013) were 1000 times higher than those measured for *Piper borbonense*. Also, one can consider that the Maillard reactions are not, in a standard transformation process of pepper, responsible for browning. However, given the concentrations of amino acids and reducing sugars in *Piper borbonense*, one can envisage that storage (even at ambient temperature) that is long-term (a few months) could generate Maillard reactions, as shown by Korbel (2014).

4.3.2.2. Other non-enzymatic browning mechanisms

The fact that significant browning is generated despite the application of blanching that denatures enzymes, followed by a drying that is not very favourable for caramelisation or Maillard reactions (process B2D3: blanching 100°C / 3 minutes followed by drying at 30°C / 50%HR) proves the existence of other phenomena involved in the degradation of the colour of the pepper. These could be thermal degradations or chemical oxidations of the polyphenols and carotenoids (Renard C., 2014). The dulling of the colour linked to losses in carotenoids (particularly of chemical origin) is, according to our findings and assumptions, marginal since it is masked by the browning of the polyphenols, which determine the colour on the whole.

4.4. Impact, and advantage for colour, of implemented unit operations

Although we have not observed this for polyphenols, Horvathova*, et al.* (2007) suggest that blanching, as a result of the inactivation of the enzymes, despite an immediate loss, subsequently allows the better preservation of polyphenols and carotenoids, in this case during storage. The limitation of subsequent oxidation phenomena will also be linked to the fact that blanching makes it possible to remove the air, and therefore the oxygen, from the materials (Hong-Wei Xiao*, et al.*, 2017). The interest in blanching is also related to increasing the speed of subsequent drying (Weil*, et al.*, 2017). Although it has a great impact on colour, drying is essential. All the different drying conditions tested generated significant browning, including those for which the phenomena of enzymatic browning and Maillard reactions, or even caramelisation, were controlled.

5. Conclusion

A process including blanching that denatures enzymes, followed by rapid drying that is not very favourable for Maillard reactions or caramelisation, makes it possible to limit the browning of *Piper borbonense*, but not in any case eliminate it: indeed, the browning observed remains significant effect. This confirms the hypothesis that non-enzymatic mechanisms, which are not Maillard reactions or caramelisation, are involved in the degradation of the colour of the pepper. To the extent where substantial losses in polyphenols and carotenoids were observed in parallel to the degradation of the colour, it is highly probable that the pepper is subject to chemical oxidation reactions of the polyphenols and carotenoids. The brown colour of the set is attributed to the degradation of polyphenols since the degradation of carotenoids led instead to dulling. Reducing to a minimum the time between harvesting and processing, and respecting the integrity of the product during trials will limit damage to the surface and therefore the penetration of oxygen, necessary for chemical (and enzymatic) oxidation. However, different and / or innovative processes seem necessary to retain the red color.

3.5.2. Synthèse du chapitre 5 et perspectives

Pour tous les procédés testés, le poivre est de couleur brune en sortie de séchage et les pertes en polyphénols sont conséquentes. Un blanchiment à 100°C pendant 3 minutes permet d'inactiver complètement la peroxydase (POD) et la polyphénol

oxydase (PPO). Le blanchiment, d'autant plus qu'il est drastique, a un impact positif sur la teneur en caroténoïdes du poivre en fin de procédé. Par ailleurs, les réactions de Maillard sont négligeables dans des conditions de séchage standard. Aussi, il est possible de conclure que le brunissement non enzymatique lié aux oxydations chimiques des polyphénols est prépondérant dans la dégradation de la couleur du poivre.

Discussion Générale

4. Discussion générale

L'objectif de cette thèse était d'acquérir des connaissances nouvelles sur les caractéristiques des poivres sauvages malgaches et réunionnais et d'étudier l'impact des procédés de transformation sur leur qualité, évaluée à travers le piquant, l'arôme et la couleur.

Les questions de recherche posées étaient les suivantes :

- Quels sont les procédés de transformation des poivres sauvages mis en œuvre à Madagascar ?
- Quelles sont les caractéristiques du *Piper borbonense* et quelles sont les opérations unitaires critiques pour la maîtrise de sa qualité ?
- Quels sont les mécanismes biochimiques et physico-chimiques qui interviennent et expliquent l'expression ou la dégradation de la qualité du poivre lors des opérations de transformation ?

L'enjeu de ce travail était, grâce aux résultats obtenus, de pouvoir proposer un ou plusieurs procédés de transformation valorisant la qualité des poivres sauvages malgaches et réunionnais. Dans les paragraphes qui suivent, sont discutés les principaux résultats qui tentent de répondre aux questions de recherche posées.

4.1. Deux procédés traditionnels mis en œuvre à Madagascar

Deux procédés de transformation distincts ont été identifiés à Madagascar : une voie dite « sèche » consistant en un simple séchage et une voie dite « humide » incluant un blanchiment et un étuvage avant le séchage. Si le blanchiment est largement décrit dans la littérature comme opération unitaire d'intérêt sur fruits et légumes en général (Hong-Wei Xiao*, et al.*, 2017) ou plus spécifiquement sur poivre (Dhas*, et al.*, 2003; F. Gu*, et al.*, 2013), la littérature scientifique concernant le poivre n'évoque jamais l'étuvage. Le fait, qu'à Madagascar, l'étuvage soit fréquemment appliqué immédiatement après le blanchiment, pourrait être dû au fait que de nombreux acteurs (cueilleurs, collecteurs, transformateurs, exportateurs …) impliqués dans la filière poivre sauvage sont également impliqués dans la filière vanille. Dans le cas de la vanille, l'étuvage est systématiquement appliqué pour permettre le développement de réactions enzymatiques nécessaires à la synthèse de l'arôme vanilline (Odoux*, et al.*, 2006). Dans le cas du poivre sauvage un tel effet n'a pas été constaté ; au contraire, la voie humide impacte la teneur en huile essentielle légèrement à la baisse et

n'affectait pas ou très peu sa composition. Dans la mesure où la voie humide dégrade en outre d'avantage la couleur, la voie sèche est à privilégier à Madagascar. Cette voie est simple à mettre en œuvre, moins couteuse en matériel et en temps et nécessite moins de contrôles des opérations. En outre, des travaux menés par ailleurs mais non présentés dans cette thèse nous ont permis de constater que la voie humide dégradait le poivre d'un point de vue microbiologique. En effet alors que le blanchiment permet de diminuer les taux de contaminations, l'étuvage qui suit favorise au contraire le développement des flores, qui se retrouvent, à la suite de cette étape, en quantités supérieures à ce qu'elles étaient dans le poivre frais.

Les travaux réalisés à Madagascar ne constituent en réalité qu'un sondage. En effet seuls deux bassins de collectes ont été observés. Si ce sont les principaux en termes d'acteurs impliqués et de volumes produits, ils ne représentent que quelques centaines ou milliers de kilomètres carrés alors que le corridor forestier présent du Sud-Est au Nord-Ouest du pays, dans lequel se trouve du poivre sauvage, s'étend sur plusieurs dizaines de milliers de kilomètres carrés. Rappelons que l'enclavement géographique, la barrière de la langue, la volonté des acteurs locaux de préserver une filière rémunératrice et encore informelle, ou encore la méfiance vis-à-vis du Vazaha[2] ont rendu difficiles l'obtention d'informations et la collecte d'échantillons. En plus de la description de deux procédés de transformation résolument distincts, il a été possible, dans ces conditions délicates, de caractériser une qualité moyenne représentant vraisemblablement plusieurs espèces de poivres sauvages. Ainsi, les Tsiperifery, poivres sauvages de Madagascar, se distinguent du *Piper nigrum* par une teneur en huile essentielle six fois plus importante et une teneur en pipérine huit fois plus faible. Bien que cela reste à confirmer, rappelons l'immensité de l'île (plus de 600 000 kilomètres carrés) et l'enclavement géographique de certaines zones non encore prospectées, le *Piper borbonense* ne semble pas exister à Madagascar. Des travaux en botanique et génétique menés à Madagascar, des travaux en chimie et en analyse sensorielle menés à Madagascar et à la Réunion et des travaux en biologie moléculaire menés à la Réunion devraient prochainement nous permettre d'en savoir d'avantage (nombre et caractéristiques) sur les différentes espèces de poivres sauvages existant à Madagascar.

[2] Nom vernaculaire malgache désignant le « blanc », « l'étranger »

4.2. Le *Piper borbonense*, un poivre original dont la qualité est influencée par la maturité et les procédés

Afin de pouvoir étudier l'impact des procédés sur sa qualité, il était nécessaire de connaître et donc de caractériser la qualité du *Piper borbonense*. En outre, considérant que « la qualité se fait essentiellement aux champs », et que les procédés post récolte démarrent à la cueillette nous avons également étudié l'impact de la maturité sur la qualité du poivre frais et transformé.

4.2.1. La typicité du Piper borbonense et des poivres sauvages malgaches

Les poivres sauvages malgaches et réunionnais se distinguent du *Piper nigrum* par la présence d'un pédicelle, d'une plus forte teneur en huile essentielle et d'une plus faible teneur en pipérine. La morphologie, l'anatomie et la biochimie du poivre sauvage réunionnais n'avaient jamais été décrits auparavant. Le *Piper borbonense* est un poivre à queue qui se différentie non seulement du *Piper nigrum* mais également d'un poivre à queue réputé : le *Piper cubeba* (Bos*, et al.*, 2007) et des poivres à queues sauvages malgaches (Weil*, et al.*, 2014). Parmi ses critères distinctifs et typiques on retiendra notamment sa forme ovoïde spécifique, sa teneur particulièrement faible en pipérine (encore deux fois plus faible que celle des poivres malgaches), sa teneur particulièrement élevée en huile essentielle (équivalente à celle des poivres malgaches) et la présence et/ou la concentration plus ou moins élevée de certains composés d'arômes par rapport à d'autres poivres. Par exemple le caryophylène qui est présent en très faible quantité dans le *Piper borbonense* est abondant dans le poivre noir alors que c'est exactement l'inverse pour l'asaricin.

4.2.2. Cueillir à maturité pour avoir le meilleur potentiel de qualité

Notre étude a révélé que les composés d'intérêts pipérine et huile essentielle sont présents en quantité plus importantes dans le poivre mature. Cependant ces composés sont, en teneur relative, davantage impactés à la suite du process pour les grains immatures ; sans doute parce que l'épaisseur du péricarpe et la teneur plus importante en amidon les protègent mieux dans le grain mature. Ainsi, *le Piper borbonense* à pleine maturité est à privilégier pour la mise en place d'une filière maîtrisée pour plusieurs raisons : à cette maturité, le poivre présente les rendements massiques de cueillette les plus élevés ; les grains restent plus rouges après process ; et enfin, les teneurs en huile essentielle et en pipérine restent élevées. Une des limites

de notre travail réside dans la détermination des stades de maturité de poivres étudiées. En effet, par manque de connaissances sur la phénologie du *Piper borbonense*, la sélection des trois différents stades de maturité a été réalisée sur des critères visuels de couleur (vert – orange – rouge) sans connaître les dates de floraison généralement utilisées comme critère initial de suivi (Jansz*, et al.*, 1984; Mathai, 1981). Au vu des résultats obtenus, les maturités moyenne et pleine se sont ainsi révélées très proches en termes de qualité. Pour les stades de maturité étudiés, nous avons observé que l'amidon s'accumulait davantage que la pipérine et l'huile essentielle. Ainsi les teneurs en pipérine et en huile essentielle diminuent lorsque la maturité évolue. Pour les stades de maturité très précoces, la littérature relate que l'accumulation des composés d'intérêts est plus rapide que celle de l'amidon (Rathnawathie*, et al.*, 1984). Ces stades de « prématurité » n'ont pas été étudiés dans ce travail car ces poivres ne présentent aucun intérêt à être transformés.

4.2.3. Le procédé et ses impacts : bonne conservation du piquant et de l'arôme mais dégradation de la couleur

Les différentes opérations unitaires, notamment le séchage mais aussi le blanchiment et l'étuvage dans une moindre mesure, impactent largement la couleur du poivre qui brunit au cours du procédé. Les teneurs en huile essentielle et en pipérine ne sont pas affectées par les opérations unitaires ou combinées en procédés ; la composition en huile essentielle l'est peu.

Si nous nous attendions à ce que la pipérine, selon la littérature (Nisha*, et al.*, 2009) résiste bien aux procédés, les résultats obtenus concernant l'huile essentielle et les composés d'arômes sont plus étonnants et en désaccord avec ceux trouvés par certains auteurs (Asekun*, et al.*, 2007; Schweiggert, Mix*, et al.*, 2005). Dans la mesure où ces auteurs avaient réalisé leurs travaux sur mouture de poivre et sur d'autres matrices, on peut penser que c'est la structure du péricarpe du *Piper borbonense* qui limite les pertes en huile essentielle et l'impact sur sa composition. Il est possible que les différences néanmoins perçues pour quelques composés d'arômes (para-cymène, camphène, safrol …), génèrent des différences organoleptiques significatives. Quoi qu'il en soit, c'est bien la couleur rouge, caractéristique intéressante du *Piper borbonense*, qui est la plus affectée d'abord par la maturité et ensuite par les procédés. C'est pourquoi nous nous sommes attachés à étudier les mécanismes de sa dégradation.

4.3. Mécanismes impliqués dans le brunissement du *Piper borbonense* au cours du procédé

La couleur rouge du *Piper borbonense* provient des caroténoïdes (315 µg/g bs). Ces composés, tout comme les polyphénols (1,56 g/100g bs), sont susceptibles de subir des dégradations lors des opérations de blanchiment et de séchage. La dégradation des caroténoïdes conduit plutôt à un ternissement tandis que celle des polyphénols conduit généralement à un brunissement. Le brunissement est susceptible d'être généré par d'autres mécanismes que ceux impliquant les polyphénols. Ainsi, plusieurs traitements ont été réalisés pour mettre en évidence (inhiber ou exacerber) les réactions biochimiques possiblement impliquées dans la dégradation de la couleur du poivre (**figure 30**). Les caroténoïdes subissent des dégradations enzymatiques et non enzymatiques. Les pertes les plus importantes (50%) sont observées dans le cas d'un séchage direct sans blanchiment. Cette dégradation peut conduire à un ternissement mais pas à un brunissement. Les polyphénols subissent des dégradations enzymatiques et non enzymatiques ; ces dernières semblent prédominer puisque les pertes importantes (75 à 80%) constatées pour les polyphénols sont les mêmes après séchage, que celui-ci ait été ou non précédé d'un blanchiment. Cette dégradation chimique des polyphénols explique le brunissement du poivre puisque les réactions de Maillard, malgré un « potentiel de composition » lié à la présence d'acides aminés et de sucres réducteurs, sont négligeables (pas de HMF générés). De même, la caramélisation, du fait des températures trop basses mises en œuvre, n'est pas possible. Ce brunissement non enzymatique lié à l'oxydation des polyphénols s'avère difficile à maîtriser.

Pour préserver au mieux la couleur avec nos opérations unitaires, un équilibre et des optimisations restent à trouver entre les conditions du blanchiment pour inactiver les enzymes, désorber l'oxygène des tissus, fragiliser les membranes et diminuer le temps du séchage qui suivra, et les conditions de séchage, étape durant laquelle le poivre brunit.

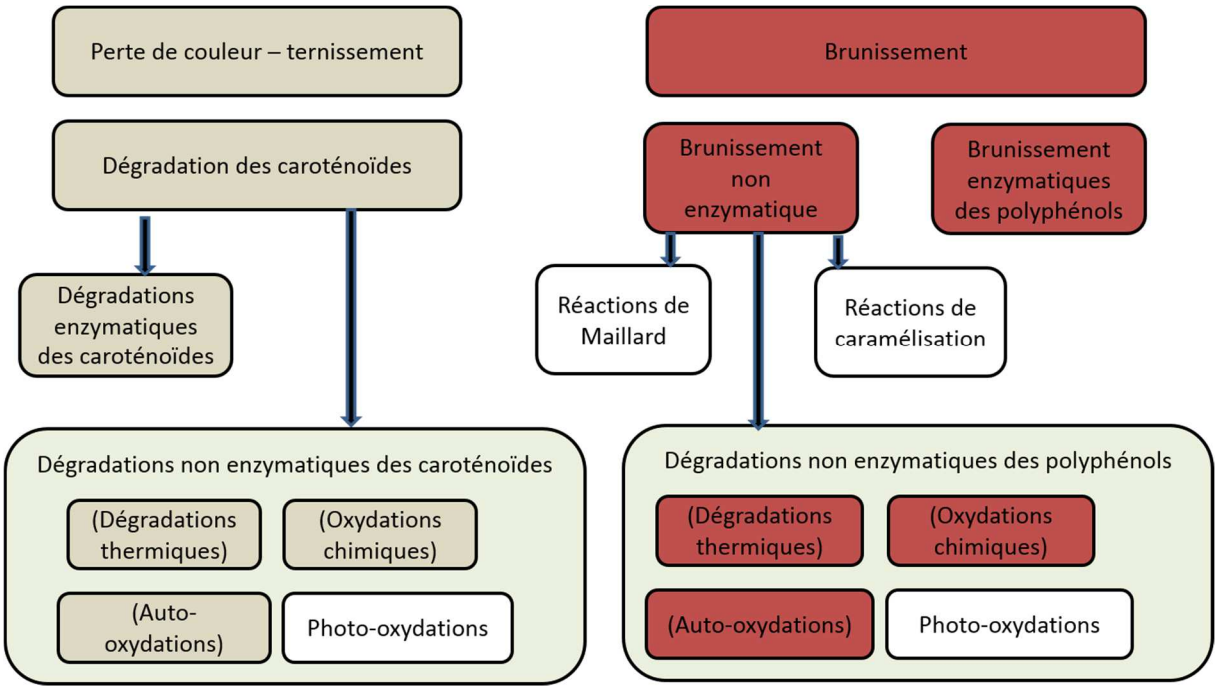

Fig 30. Mécanismes de dégradation de la couleur du *Piper borbonense*
Sur **fond rouge foncé**, les mécanismes impliqués dans le brunissement du poivre **;** **sur fond gris,** les mécanismes impiqués dans le ternissement du poivre et sur fond blanc les mécanismes non observés

On peut d'ores et déjà envisager dans le cadre d'une filière *Piper borbonense*, d'adapter le procédé de transformation selon la qualité de la matière première d'une part et par rapport au produit fini visé d'autre part, comme cela se fait pour de nombreuses transformations alimentaires. Ainsi et pour exemple, au regard de la couleur, on peut imaginer pouvoir se passer du blanchiment pour une matière première fraichement cueillie et parfaitement intègre puisque les réactions enzymatiques sont peu actives lorsque substrat et enzymes ne sont pas en contact. En revanche un blanchiment s'avèrera nécessaire pour une matière première abimée et/ou sujette à un délai important entre cueillette et traitement.

Les meilleures conditions testées ici nous ont permis de limiter mais en aucun cas d'éliminer ni même de réduire de façon satisfaisante le brunissement. On peut imaginer que des procédés différents intégrant l'acidification du milieu, l'ajout d'acide ascorbique, de sulfate de sodium (tels que pratiqués dans la transformation des fruits) ou la lyophilisation (telle qu'elle est appliquée aux plantes aromatiques) permettraient d'obtenir de bons résultats. En tant que procédé plus innovant, la friture sous vide, testée très rapidement en parallèle à ce travail, et utilisée notamment pour la

production industrielle de chips de fruits et légumes, a montré son aptitude à conserver la couleur. De tels procédés seront nécessaires ou en tous les cas à tester pour pouvoir produire un poivre *Piper borbonense* qui reste bien rouge après séchage.

4.4. Proposition d'itinéraires de transformation durables pour les poivres de Madagascar et de la Réunion

Rappelons tout d'abord, comme leur nom l'indique, que les poivres sauvages malgaches et réunionnais étudiés ne sont pas cultivés à ce jour. Rappelons ensuite que si une filière Tsiperifery (basée sur la cueillette) notamment tournée vers l'export existe à Madagascar, le *Piper borbonense* n'est pas exploité à la Réunion. Alors que la durabilité de la filière malgache est mise en péril par une exploitation anarchique de la ressource, d'où l'importance de plusieurs projets de domestication menés par ailleurs, elle est également menacée du fait d'une qualité de poivre proposée souvent hétérogène et parfois médiocre dont les consommateurs pourraient finir par se détourner. Aussi, que ce soit pour amorcer une filière réunionnaise innovante ou pour pérenniser une filière malgache existante, la qualité des poivres, centre de notre étude, est fondamentale et gage de durabilité.

Dans ce contexte, les connaissances scientifiques et techniques acquises même si incomplètes et perfectibles, peuvent d'ores et déjà servir de base à l'amélioration (concernant Madagascar) et à la mise en place (concernant la Réunion), de filières maîtrisées pour la production de poivres hauts de gamme. Le blanchiment présente de nombreux intérêts : il nettoie et décontamine, améliore l'efficacité du séchage et limite le brunissement notamment enzymatique. L'étuvage qui influence négativement la couleur et augmente très largement la charge microbienne (résultats en microbiologie obtenus mais non présentés dans le cadre de cette thèse) est à bannir. Le séchage bien qu'il impacte négativement la couleur est indispensable pour stabiliser le poivre d'un point de vue microbiologique. A l'aune de ces résultats, deux procédés de transformation peuvent être suggérés : une « voie humide » incluant un blanchiment mais débarrassée de l'étuvage et une « voie sèche » consistant en un séchage unique et intense. Le choix de se tourner vers la « voie humide » ou la « voie sèche » est à raisonner en fonction de la qualité (fraicheur, intégrité, absence de contaminations) de la matière première d'une part, et de l'accès à l'énergie, à des équipements (fut de blanchiment et passoire dédiés, chronomètre) et à de l'eau potable pour pouvoir mettre en œuvre un blanchiment d'autre part. Ainsi, si la qualité

de la matière première le permet, une « voie sèche » serait par exemple à privilégier pour les zones enclavées de Madagascar. Concernant le séchage, si une enceinte climatique (électrique) nous a permis d'obtenir de bons résultats et présente l'avantage de pouvoir être pilotée automatiquement et finement, un séchage solaire en couche mince, à l'ombre et dans une zone aérée et/ou incluant des phases de retournement, donnerait vraisemblablement de bons résultats. Cela reste à valider et présente l'inconvénient d'être tributaire du climat. Quant aux conditions opératoires à mettre en œuvre pour une « voie humide », un blanchiment à 100°C pendant 3 minutes et un séchage (en séchoir) à 60°C, 20% d'HR dans des conditions non limitantes jusqu'à obtenir 10% de matière sèche donnent, comme nous avons pu le voir dans nos travaux, de bons résultats. Ces critères peuvent vraisemblablement être optimisés.

Conclusion et perspectives

Conclusion et perspectives

L'objectif de cette thèse était d'acquérir des connaissances nouvelles sur les caractéristiques des poivres sauvages malgaches et réunionnais et d'étudier l'impact des procédés de transformation sur leur qualité, évaluée à travers le piquant, l'arôme et la couleur. L'enjeu de ce travail était, grâce aux résultats obtenus, de pouvoir proposer un ou plusieurs procédés de transformation permettant de valoriser la qualité des poivres sauvages malgaches et réunionnais. L'analyse bibliographique a démontré que le *Piper nigrum* et sa transformation sont bien décrits dans la littérature. Les travaux existants sont pour beaucoup issus d'équipes de recherche indiennes. Les éléments concernant l'impact des procédés sur le piquant et l'arôme du poivre noir restent modestes ; ceux sur la préservation de la couleur rouge inexistants. A notre connaissance, avant le démarrage de cette thèse, aucun travail n'avait jamais été publié ni sur les Tsiperifery malgaches ni sur le *Piper borbonense* de la Réunion. Souhaiter améliorer à Madagascar et amorcer à la Réunion, la mise en œuvre, de filières maîtrisées à haute valeur ajoutée nécessite un effort de recherche important. S'appuyant sur ce constat, notre travail a consisté à accroitre notre connaissance sur ces poivres sauvages (et notamment le *Piper borbonense*) et leurs transformations. Notre ambition était de prodiguer des conseils sur la conduite du procédé : choix de la matière première (espèce), état de la maturité, enchainement et rôle des opérations unitaires (blanchiment, étuvage et séchage), afin d'exprimer au mieux le potentiel exceptionnel (piquant, arôme et couleur) de ces poivres.

Pour avancer vers cet objectif, trois démarches ont été menées à bien :

- Décrire et analyser les procédés traditionnels de transformation des poivres sauvages à Madagascar
- Caractériser la composition complète du *Piper borbonense* ainsi que la teneur en composés d'intérêts pipérine et huile essentielle des poivres sauvages malgaches
- Définir un stade de maturité optimale et surtout comprendre l'impact des opérations unitaires sur l'expression ou la dégradation de la qualité du poivre exprimée à travers les composés d'intérêts et la couleur.

Deux procédés de transformation distincts ont été identifiés à Madagascar : une « voie

sèche » consistant en un simple séchage et une « voie humide » incluant blanchiment et étuvage avant séchage.

La composition globale ainsi que la composition aromatique du *Piper borbonense* de la Réunion ont été caractérisées, de même que la teneur moyenne en composés d'intérêts (pipérine et huile essentielle) des poivres malgaches. Les poivres sauvages de Madagascar et de la Réunion se différentient du poivre noir, morphologiquement, par la présence d'un pédicelle solidaire du grain (d'où le nom de poivres à queue) et chimiquement par leur faible piquant, et leur fort potentiel aromatique. Le *Piper borbonense* a pour constituant majoritaire l'amidon (41% bs). Ses composés d'arômes principaux sont le limonène, l'α- phellandrène et l'asaricin qui représentent à eux trois 50% du total de l'huile essentielle. Il se distingue du *Piper nigrum* par sa très faible teneur en pipérine (0.2% bs), sa forte teneur en huile essentielle (9.8% bs), la présence d'un pédicelle ainsi que par sa forme ovoïde. Il se différentie des Tsiperifery malgaches par sa teneur en pipérine deux fois plus faible et sa forme ovoïde. Enfin, le *Piper borbonense* se distingue du *Piper cubeba* par sa forme ovoïde. Le *Piper borbonense* présente une couleur rouge lorsqu'il est mature ; stade de maturité qui est préférable pour sa transformation parce que le poivre ainsi cueilli présente un meilleur potentiel de rendement et résiste mieux aux procédés que les poivres immatures.

L'impact et l'intérêt des étapes critiques ont été déterminés. Le blanchiment, l'étuvage et le séchage ont peu d'impact sur le piquant et l'arôme mais dégradent significativement la couleur du poivre. Le blanchiment présente de nombreux avantages : il nettoie et décontamine le poivre, augmente la vitesse du séchage et limite le brunissement notamment enzymatique. L'étuvage qui influence négativement la couleur et augmente très largement la charge microbienne du poivre est à bannir. Le séchage bien qu'il impacte négativement la couleur est indispensable pour stabiliser le poivre d'un point de vue microbiologique.

Les mécanismes conduisant à la dégradation de la couleur du poivre sont complexes et difficiles à maîtriser. Si le brunissement enzymatique du poivre peut être atténué par l'opération de blanchiment, les oxydations chimiques des polyphénols qui semblent prépondérantes dans le brunissement et qui se développent lors du séchage s'avèrent plus délicates à contrôler et limitent aujourd'hui la possibilité de conserver la couleur rouge du poivre. Si le maintien de cette couleur rouge pourrait être un objectif intéressant à atteindre en tant que critère distinctif original et supplémentaire pour les poivres sauvages étudiés, il ne s'agit pas d'un enjeu indispensable ; rappelons en effet

que le *Piper nigrum* (lui aussi pourtant rouge à maturité) est bien valorisé sous sa forme marron.

Plutôt qu'un procédé unique universel, une « voie sèche » (séchage direct) et une « voie humide » (intégrant blanchiment et séchage) sont proposées. Le choix d'en appliquer l'une ou l'autre étant à raisonner par rapport à la qualité de la matière première à traiter d'une part et en fonction du contexte et donc de critères économiques (matériels), environnementaux voire même sociaux d'autre part.

Des confirmations sont attendues pour caractériser la typicité du *Piper borbonense* et proposer des clefs, notamment chimiques, d'authentification. Des optimisations ainsi que des innovations concernant le blanchiment et le séchage seront nécessaires si l'on souhaite en particulier pouvoir conserver la couleur rouge du poivre. Les études amorcées mais non valorisées dans le cadre de cette thèse sur les aspects microbiologique et sensoriels, ainsi qu'un travail à mettre en œuvre sur l'impact du stockage sur la qualité, apporteront des éléments de réponses nécessaires à la mise en place de filières durables pour le *Piper borbonense* et les autres poivres typiques et pour l'heure, sauvages, du Sud-Ouest de l'Océan Indien. En tant que livrable associé à cette thèse, un guide de bonnes pratiques de transformation du poivre à destinations des acteurs réunionnais et malgaches, écrit en collaboration avec des collègues du dispositif en partenariat Forêts et Biodiversité de Madagascar est en cours d'élaboration. Il devrait être rapidement complété, afin que la filière soit complètement décrite de l'amont vers l'aval, d'une partie concernant les bonnes pratiques de culture. Enfin, une étude économique sera nécessaire pour décrire les conditions de viabilité d'une filière complète (intégrant culture et transformation) et maîtrisée de poivre sauvage.

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Liste des articles scientifiques

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Les résultats de ce travail de thèse ont été ou vont être publiés sous forme d'articles scientifiques.

Publication #1 : Traitements post récolte des poivres sauvages à Madagascar
(***parue dans Fruits***)
Weil M., Descroix F., Shum Cheong Sing A., Boulanger R., Hoarau M., Levesque A., Bohuon P. 2014. Postharvest treatments of wild pepper (*Piper* spp.) in Madagascar. *Fruits*, 69 (5): p. 371-380.
http://dx.doi.org/10.1051/fruits/2014025

Publication #2 : Description morphologique, anatomique et biochimique du *Piper borbonense*
Weil M., Boulanger R., Morel G., Servent A., Shum Cheong Sing A., Bohuon P.
(***soumise à Scientia Horticulturae***)

Publication #3 : Impact du stade de maturité sur le piquant, l'arôme et la couleur du *Piper borbonense*
Weil M., Shum Cheong Sing A., Hoarau M., Boulanger R., Bohuon P.
(***soumise à International Journal of Food Science and Technology***)

Publication #4 : Impact du blanchiment, de l'étuvage et du séchage sur le piquant, l'arôme et la couleur du *Piper borbonense* (***parue dans Food Chemistry***)
Weil M., Shum Cheong Sing A., Méot J.M., Boulanger R., Bohuon P.
2017. Impact of blanching, sweating and drying operations on pungency, aroma and color of *Piper borbonense*. *Food Chemistry*, 219: p. 274-281.
http://dx.doi.org/10.1016/j.foodchem.2016.09.144

Publication #5 : Etude des mécanismes biochimiques impliqués dans le brunissement du *Piper borbonense*
(***en préparation***)

Annexes

Annexes

Annexe 1 : Postharvest treatments of wild pepper (*Piper* spp.) in Madagascar (**Publication #1** - parue dans Fruits)

Annexe 2 : Impact of blanching, sweating and drying operations on pungency, aroma and color of *Piper borbonense*
(**Publication #4** - parue dans Food chemistry)

Technical paper

Postharvest treatments of wild pepper (*Piper* spp.) in Madagascar

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Resumen Español, *p. 380*

Postharvest treatments of wild pepper (*Piper* spp.) in Madagascar.

Abstract – Introduction. A study on postharvest treatments of wild peppers was carried out in Madagascar with the aim of describing the local practices and measuring their impacts on the quality of the products. **Materials and methods**. Four distinct pepper production systems (PPS) were observed, described and compared in two separate areas in East Madagascar. Major quality characteristics (piperine and essential oil) of the peppercorns were assessed in samples collected in the four systems. **Results and discussion**. Two main postharvest processes (dry and wet) were identified. The wet process differed from the dry one in that it involved two specific operations, blanching and sweating. The processes influenced the color of the pepper. Piperine contents were not affected by any of the pepper production systems, whereas essential oil contents were reduced by up to 27% by the wet process. After processing, piperine contents were up to eight times lower, whereas essential oil contents were up to six times higher than the specifications of the standard ISO 959-1 for black pepper ready for commercialization. **Conclusion**. Two main processes (dry and wet) for treatment of peppercorns in Madagascar were identified and described. The dry process, with two steps less, appeared to be easier to implement and more respectful to the product. Improving maturity control and processing according to the quality expected by the markets will be necessary to promote Malagasy peppers.

Madagascar / *Piper* / pepper / processing / essential oils / color

Traitements post récolte du poivre sauvage (*Piper* spp.) à Madagascar.

Résumé – Introduction. Une étude des traitements post récolte des poivres sauvages a été menée à Madagascar afin de décrire les pratiques locales et de mesurer leurs impacts sur la qualité des produits. **Matériel et méthodes**. Quatre systèmes de productions (PPS) ont été observés, décrits et comparés dans deux zones définies de l'est de Madagascar. Des caractéristiques qualitatives majeures (piperine et huile essentielle) du poivre ont été évaluées sur des échantillons collectés dans les quatre systèmes. **Résultats et discussion**. Deux principaux procédés post récolte (une voie sèche et une voie humide) ont été identifiés. La voie humide diffère de la voie sèche par deux opérations spécifiques : l'échaudage et l'étuvage. Les procédés ont montré une influence sur la couleur du poivre. Les teneurs en piperine n'ont pas été affectées par les systèmes de productions quels qu'ils soient alors que les teneurs en huile essentielle ont été réduites jusqu'à 27 % par la voie humide. En fin de procédés, les teneurs en piperine ont été jusqu'à huit fois plus basses et les teneurs en huile essentielle jusqu'à six fois plus élevées que celles spécifiées dans la norme ISO 959-1 pour le poivre noir prêt à la commercialisation. **Conclusion**. Deux procédés principaux (l'un « sec », l'autre « humide ») utilisés pour le traitement des poivres à Madagascar ont été identifiés et décrits. La voie sèche qui comprend deux opérations en moins, semble plus aisée à mettre en œuvre et mieux respecter le produit que la voie humide. Le respect de la maturité du poivre sauvage lors de la cueillette ainsi que la maîtrise des procédés en fonction des produits attendus par les marchés seront nécessaires pour mieux valoriser les poivres malgaches.

Madagascar / *Piper* / poivre / traitement / huile essentielle / couleur

Article published by EDP Sciences

1. Introduction

Since antiquity, spices and herbs have been used throughout the world to enhance flavor and preserve food, as well as for medicinal and cosmetic purposes [1]. They were highly sought after, much like gold. Today spices are no longer luxury items, but they are in high demand and their importance is still growing [2].

The genus *Piper* belongs to the family Piperaceae and comprises more than 700 species distributed throughout tropical and subtropical regions of the world [3]. Among this huge diversity, one species, *Piper nigrum*, represents the vast majority of the 435,000 t of pepper (*Piper* spp.) produced in the world in 2011 for a value of 900 M$[1]. This black pepper (*Piper nigrum*) is used extensively; it is known as the king of spices as it is the most popular spice worldwide. It has been the subject of several studies showing, for instance, that it can be transformed by dry or wet processes. Dhas and Korikanthimath described the various types of operations such as blanching (wet process), cleaning or drying [4]. The impacts of some of these operations on black pepper quality were assessed by Nisha *et al.* [5], who showed piperine stability after heat processing with only 5% loss after 20 min at 100 °C. Using the same process, essential oil was reduced by about 30%. Similarly, Suresh *et al.* observed a maximum piperine loss of 34% in black pepper cooked under pressure for 10 min [6].

However, most peppers remain non-cultivated wild species, mostly handpicked in limited quantities and consumed locally. To our knowledge, no scientific studies have been published on wild peppers. One or several wild pepper species that do not belong to *P. nigrum* (genetic determination is ongoing), locally named Tsiperifery, grow in Madagascar's primary rainforests. Part of the Tsiperifery production, estimated at (30 to 50) t of dry product per year (unpubl. results) is collected and transformed for local consumption or export. These Malagasy wild peppers, little known compared with *P. nigrum*, have started to gain fame in French gastronomy. The literature is thus very scarce but there is a need to acquire knowledge about the transformation processes.

In our study we describe the main local postharvest treatments of wild peppers (*Piper* spp.) in the East coast forest corridors of Madagascar and assess the impacts of these processes on some main quality characteristics, *i.e.*, essential oil and piperine contents, and visual aspect.

2. Materials and methods

2.1. Wild pepper production systems

In Madagascar, although September to December is the most suitable period, it is possible to find mature wild pepper almost throughout the year, *i.e.*, from April to January. Our study (sampling included) was carried out from July to November 2012 in two different zones (zones 1 and 2), located between the Madagascar highlands (≈ 100 km from Antananarivo, the capital) and the primary rainforests of the East coast (north of Moramanga), both at an altitude between (900 and 1300) m (*figure 1*). These zones were selected because chief local traders described them as being the main locations from which most Malagasy wild pepper is collected. Our study was not easy to carry out because the actors were difficult to reach most of the time and several hours' walking was needed to reach picking and collecting sites in both areas. Nevertheless, four distinct pepper production systems were selected as study cases in the two zones (*table I*). One pepper production system (PPS1) was located in zone 1 (Angavo forest corridor zone), and the other three pepper production systems (PPS2, PPS3 and PPS4) were in zone 2 (Ankaï forest corridor zone). The pepper production systems PPS1 and PPS2 were operated by Madépices Company (Anatananarivo).The pepper production systems PPS3 and PPS4 were operated by Cent. Techn. Hortic. Tamatave (CTHT) and SOPRAL Co. (Tamatave), respectively. These actors produce annually

[1] FAOSTAT,http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor, 30 Oct. 2013.

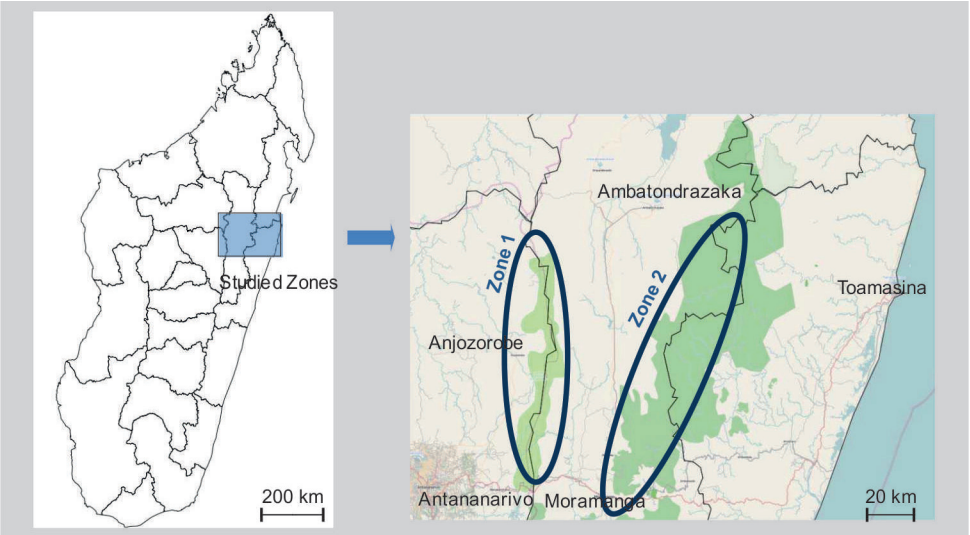

Figure 1.
Zones selected for studying postharvest treatments of wild pepper (*Piper* spp.) in Madagascar: Zone 1, Angavo forest corridor; Zone 2, Ankaï forest corridor.

Table I.
Mean values of piperine (n = 3) and essential oil (n = 2) contents at different steps (t_0 and t_1) of the postharvest processes of wild pepper with samples collected in two different zones of Madagascar (zone 1: from 40 km to 100 km northeast of Antananarivo; zone 2: from 0 km to 100 km north of Moramanga) in four distinct pepper production systems (PPS1, PPS2, PPS3 and PPS4: see *figure 2*).

Postharvest process	Zone	Reference	Process step	Content in 100 g of dry matter Piperine (g)	Essential oil (mL)
Dry process	1	PPS1	t_0	2.3	12.4
	1	PPS1	t_1	2.8	11.7
	2	PPS2	t_0	3.4	13.1
	2	PPS2	t_1	3.1	13.4
Wet process	2	PPS3	t_0	0.5	6.9
	2	PPS3	t_1	0.5	5.6
	2	PPS4	t_0	1.2	2.8
	2	PPS4	t_1	1.2	2.0

about 15 t of dry wild pepper of a total estimated to be between (30 and 50) t; this represents between 30% and 50% of the total Malagasy production.

A checklist was used for interviewing 28 actors or groups of actors (four groups of pickers, eighteen collectors and six processors-exporters) in order to characterize the pepper production systems. The checklist included the following: (i) description of activities (history, motivation, organization); (ii) pepper quality perception, evaluation and control; (iii) process description; (iv) commercialization (volumes collected or purchased, sales, prices); and (v) relations with other actors of the chain.

For the process description, we used the 5M methodology – a widely utilized tool in developing hazard analysis critical control point (HACCP) systems [7] – to describe

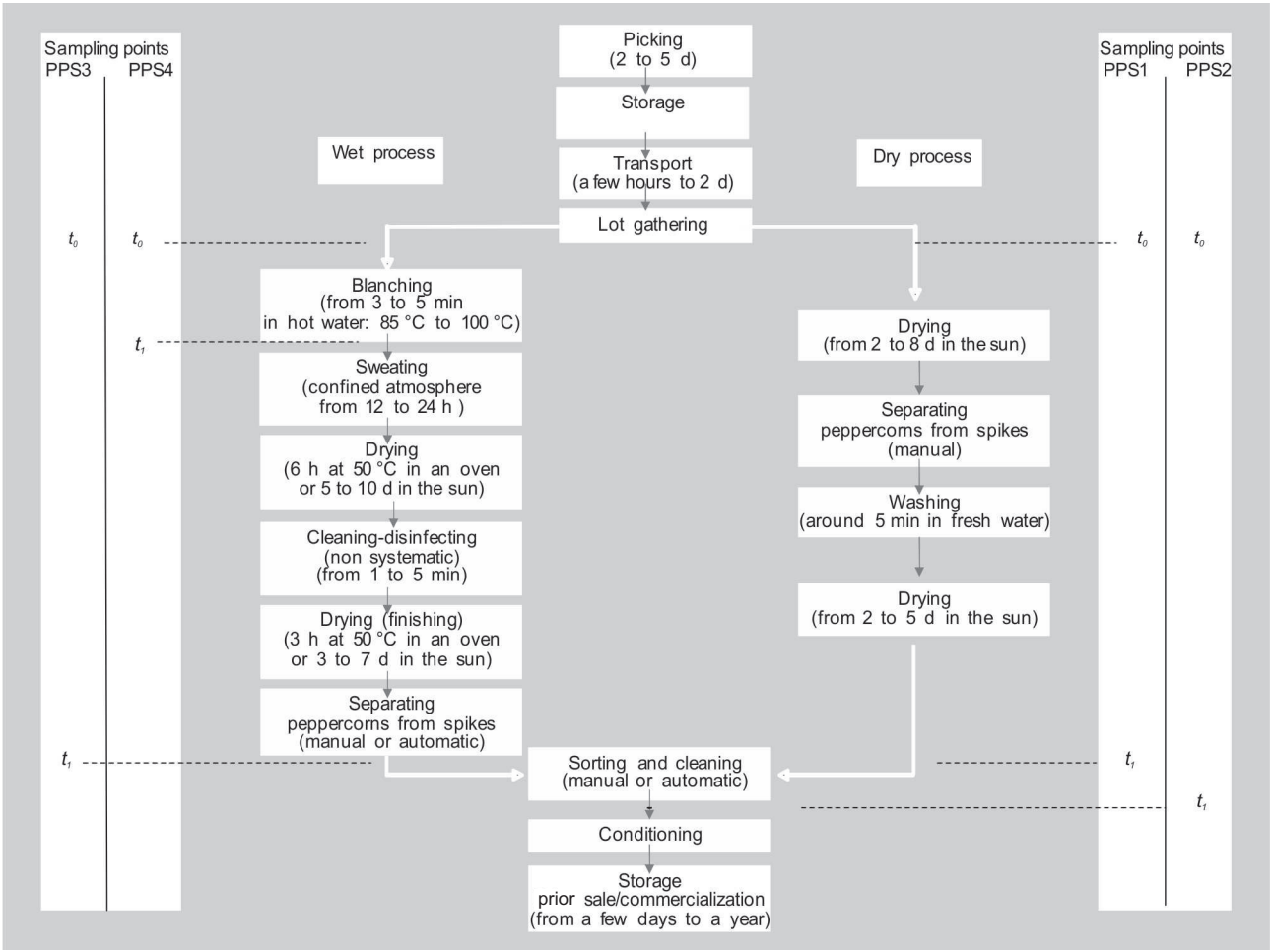

Figure 2.
Diagram of dry and wet processes used for four distinct pepper production systems (PPS1, PPS2, PPS3 and PPS4) and indication of sampling (Madagascar).

each step of the four studied pepper production systems accurately and exhaustively through five dimensions: men, materials, machines, methods and the environment ("mother nature"). At least three visits per actor (pickers, collectors, processors and exporters) were made to achieve process descriptions.

2.2. Determination of peppercorn quality

Pepper samples were collected at different steps (*figure 2*) of the four pepper production systems for quality analysis.

2.2.1. Samping procedure

Eight samples (two per pepper production system) of about 400 g each were collected at two steps (t_0 and t_1) of each of the four systems studied in zone 1 (PPS1) and zone 2 (PPS2, PPS3 and PPS4). In PPS1, PPS2 and PPS3, the two samples corresponded to the beginning (t_0) and the end (t_1) of the processes. In PPS4, as the objective was to determine the impact of blanching, t_0 and t_1 corresponded to the steps just before and just after the critical step, respectively. All samples were carried to the laboratory and stored at –80 °C before analysis. The practices observed in the field consisted of collectors gathering lots picked by different pickers in various places (in our defined zones). The samples thus collected in each zone were a mixture of peppers (various species) from different plots (with possibly different climates and soils) at various stages of maturity. We ensured, however, that peppers had not been mixed between t_0 and t_1 in the four pepper production systems.

2.2.2. Sample preparation

Each sample was thawed for 24 h at 50 °C in an oven (Memmert ULE 400, Memmert Gmbh, Germany). Peppercorns with peduncles were then manually separated from fruit stems before being ground for 10 s at 10,000 rpm with a cutting mill (Retsch - Grindomix GM 200, Retsch Gmbh, Germany).

2.2.3. Analytical methods

2.2.3.1. Dry matter content

The dry matter content was obtained by drying 5 g of ground pepper in an aluminum cup in the oven at 105 °C for 30 h (*i.e.*, until constant weight). Initial and final masses were determined with a precision balance (Scaltec SBC 22 model, Scaltec Gmbh, Germany). The maximum standard deviation of repeatability was ± 0.1% with n = 3.

2.2.3.2. Piperine content

The piperine content, expressed on a dry basis, was determined according to the spectrophotometric global method described in the standard ISO 5564 [8]. The spectrophotometer used was a Thermospectronic Helios α v4.60 (Thermo Fisher Scientific, USA). The maximum relative deviation of repeatability was ± 12% with n = 3.

2.2.3.3. Essential oil content

The essential oil content, expressed on a dry basis, was determined using a method adapted from the standard ISO 6571 [9]. One modification in the applied method was the elimination of xylene. The maximum relative deviation of repeatability was ± 11% with n = 8.

3. Results and discussion

3.1. Description of pepper postharvest treatments

Two different types of processes, one dry and one wet process, were identified in the studied zones. The dry process appeared to be easier to implement. The main difference between the dry and wet processes was that the wet process included two successive steps called blanching and sweating (*figure 2*). The 5M methodology provided information on: (i) the material, *i.e.*, maturity, size, color and state of the peppercorns (fresh, wet, dry); (ii) objects, tools or equipment, *e.g.,* bags, winnows, separators; (iii) conditions, *e.g.*, inside or outside, temperature and humidity, cleanliness; (iv) the method, *i.e.,* the way each step is handled, what method was used; and (v) the persons involved in processing pepper.

The observed processes are precisely detailed hereafter. Some process steps were common to both processes, whereas others were not.

3.1.1. Picking

Picking could last two to five days depending on the time pickers spent in the forest. The methods used consisted of (i) tree climbing up to 20 m to pick fruits directly, and, more often, (ii) uprooting vines or even (iii) cutting off live supports with machetes and axes. The last two methods are considered as having a negative impact on the pepper resource, and sometimes even on the forest after the trees have been logged . The maturity of the picked peppercorns was very heterogeneous (*figure 3*, fresh wild peppercorns at t_0) for the following reasons: vine fructification within the same area could last several months, spike maturity varied on the same vine, and fruit maturity also varied on a given spike. This heterogeneity of maturity affects the size and color of the peppercorns. After picking, the gatherers separated the spikes from the vine and leaves. Sometimes pickers kept the spikes (covered with fruits) in their hats and pockets before putting them into plastic bags. The quantity of pepper picked by one picker varied from (1 to 20) kg per day.

3.1.2. Storage

Storage was repeated between several steps of the production systems. After picking, transport or gathering, intermediary storage consisted of a period that could last of from one to five days depending on the practices, the time spent by the gatherers in the forest, and the distance between the forest and

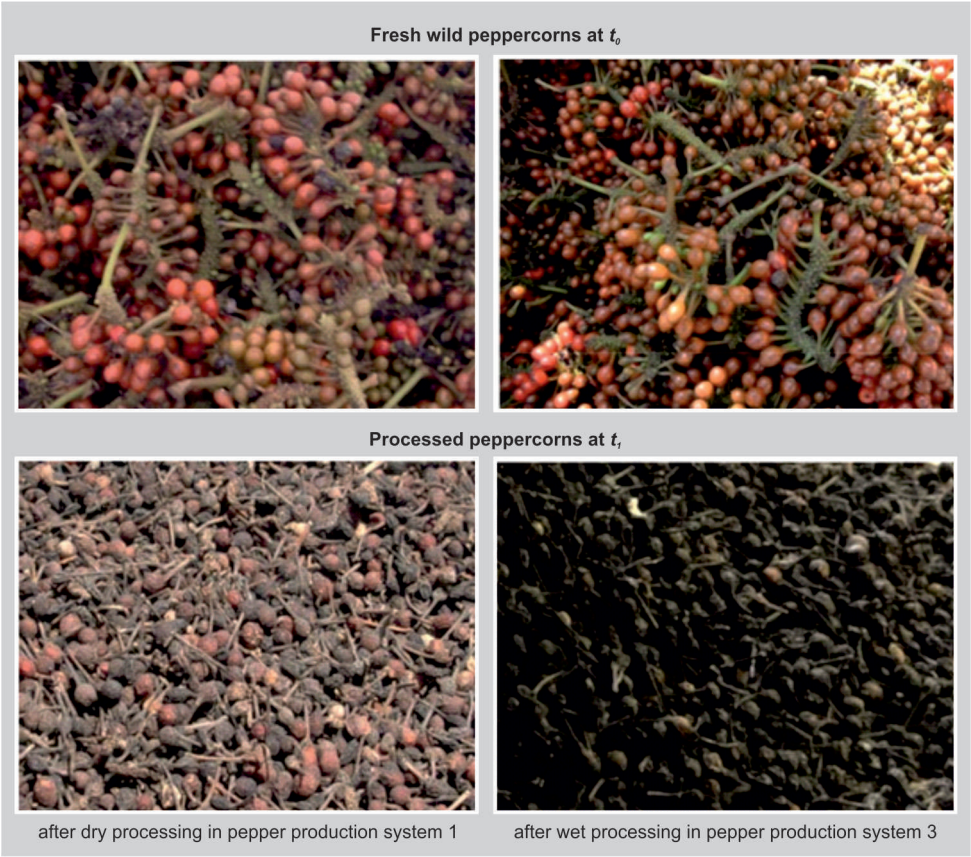

Figure 3.
Fresh wild peppercorns at t_0, and processed peppercorns at t_1 either after dry processing in pepper production system 1 or after wet processing in pepper production system 3 (Madagascar).
A color figure is available at www.fruits-journal.org.

village markets or collecting points. Pepper spikes were kept in plastic bags that were sometimes hung above ground to protect them from animals. At night, the pepper was sometimes spread on the plastic bags or on banana leaves.

In their final storage phase before conditioning or before commercialization, peppercorns could be kept for more than a year in baskets made of natural local fibers, in plastic buckets or in individual conditioning polyethylene or polypropylene bags.

3.1.3. Transport

Transport could take from a couple of hours to two days depending on the distances and means used: by foot, bicycle, motorbike, bus and, less frequently, car. It was repeated each time pepper was traded from one actor to another as gatherers, collectors and distributors were generally located in different places. Peppers, which were only partially dried, were usually kept in plastic bags during transport.

3.1.4. Gathering the lots

Gathering could be performed by primary and secondary collectors (who collected from a few to one hundred kilograms a week) as well as by distributors (up to one ton). It consisted of transferring pepper from one container to another (usually larger) without consideration of quality except at the distributors' level. The containers, usually plastic bags of different sizes, were sometimes made of natural fibers such as burlap.

3.1.5. Drying

Drying was carried out by various actors and repeated at different steps during the process: reduction in water content ensured peppercorn preservation essentially by limiting microbial growth. The dry matter content

measured in fresh peppercorns at the beginning of the drying step ranged from 26% to 30%. In PPS3, drying was conducted in thin layers on racks made of synthetic fiber in an oven at 50 °C for (6 to 12) h. In PPS1, PPS2 and PPS4 it was conducted in the sun on or above ground, and the pepper was kept inside (spread out or not) during rainy periods and at night. The support types (palm mats, drying racks in natural or synthetic fibers, or plastic sheets) and the height of the layers, that were or were not regularly turned, changed according to the actors in the various systems. Sun-drying periods, continuous or not, lasted from two to almost fifteen days depending on the pepper lots, the actors' know-how and availability, and the climate. It must be emphasized that, depending on the time of the day, the season and the zone, the temperature and humidity varied from 15 °C to 35°C and from about 40% to 90%, respectively. At the end of drying, the dry matter measured (*e.g.,* 95% in PPS1 and 93% in PPS2) was all above the 87% minimum value specified in the standard ISO 959-1 for black pepper ready for commercialization. Partial stalking and sorting (*e.g.*, removal of foreign matter, leaves and spikes) were also routinely performed during drying.

3.1.6. Separating peppercorns from spikes

This step consisted of separating berries from spikes. It was easier to perform after drying but was sometimes carried out during drying. It was usually done manually by rubbing spikes against one another, by threshing, by trampling, or by rubbing spikes on an abrasive object such as a metal colander to facilitate separation, but this last method damages peppercorns. An automatic separator (a pilot made by Sunthesis, Antananarivo, Madagascar) was used in PPS3 and PPS4.

3.1.7. Sorting and cleaning

The various actors partially sorted and cleaned the peppercorns throughout the process, activities which were usually finalized by the distributor or the exporter before conditioning and storage. Sorting served to eliminate foreign matter, dirt, and immature and lightweight aborted or broken berries. It was usually carried out manually or with a winnow and, more rarely, with a densitometric separator (such as the cyclone separator, a pilot made by Sunthesis and used in PPS3). Separate batches could then be classified into different pepper commercial categories.

3.1.8. Conditioning

The distributors conditioned peppercorns by packing them into sale units. The materials used were in direct contact with the product and were made of one or several layers of polyethylene or polypropylene. In PPS3 and PPS4 peppercorns could be packed under partial vacuum.

3.1.9. Washing (dry process only)

The washing phase, observed in PPS1 and PPS2, was carried out with fresh tap water on dry peppercorns when their dry matter content was about 93%. The peppercorns were hand-washed in a colander set inside a plastic bowl for around five minutes. Floating impurities and dust were removed with a small steel strainer. The operation was usually repeated twice before the peppercorns, whose dry matter content decreased from 93% to 61% in PPS1 and from 93% to 74% in PPS2, were set to dry again.

3.1.10. Blanching (wet process only)

Blanching (sometimes called bleaching or scalding) consisted of dipping peppercorns either directly or inside a net (mosquito-net type) or in a basket made of natural fibers or in a metal colander into simmering or boiling water (100 °C) for (3 to 5) min. The pepper was then drained. Blanching is used, according to Dhas and Korikanthimath [4], not only to remove impurities (dust and foreign matter) and decrease the microbial load but also to increase the speed of drying that follows. Blanching also allows the development of a uniform browning by promoting oxidation of phenols by phenolase enzymes [10] or by other browning mechanisms that have not yet been determined [11].

3.1.11. Sweating (wet process only)

Sweating was performed immediately after blanching and consisted of storing pepper

inside or in the shade and above ground, in a confined atmosphere, *i.e.*, burlap, fabric or plastic bag for (12 to 24) h. According to some PPS3 and PPS4 actors, the practice of combining blanching and sweating had been implemented according to the method used for traditional vanilla bean curing, which was described by Odoux *et al.* [12]. Indeed, in vanilla processing, the curing step triggers enzymatic reactions that contribute to aroma development.

3.1.12. Cleaning and disinfecting (wet process only)

Cleaning and disinfecting were not systematic. They were sometimes observed before blanching in PPS3, but were more often carried out in PPS4 after a first drying operation. In addition to washing the product, this step aimed at reducing microbiological contamination when there was presumption or proof of microorganism development (*e.g.*, presence of white mold on the surface). To do so, pepper was soaked in chlorine water in plastic bowls for (1 to 5) min. The available chlorine ranged between (6 and 50) $\mu L \cdot L^{-1}$. These concentrations are much below those proposed in the European standard EN 13697, that recommends 260 $\mu L \cdot L^{-1}$ with a contact time of 15 min for efficient disinfection [13].

3.2. Determination of some quality characteristics of the peppercorns

The visual aspect (color and size), pungency, aroma of the peppercorns and homogeneity of the batches were the quality criteria that were the most cited by the various actors. We decided to consider the visual aspect, and essential oil and piperine contents, as all three are cited in the standard ISO-959-1 [14].

There are more piperine and essential oil in the pepper used as a raw material in PPS1 and PPS2 than in PPS3 and PPS4. These differences could be due to the origins (climate and soils, for instance), maturity and species of the different lots of wild pepper.

3.2.1. Piperine content

At the beginning of the process (t_0), the piperine contents were measured in the four samples collected after gathering the lots. They ranged from 0.5% to 3.4% (dry basis) (*table I*). The rates of 0.5% to 3.1% obtained in samples after treatments (t_1) were all below (and up to eight times lower than) the 4% content recommended by the standard ISO 959-1 [14]. Our analysis also revealed that the processes, whether wet or dry, had no impact on the piperine contents of the peppercorns. This result agreed with those of Nisha *et al.* regarding the kinetic reaction rates of piperine degradation during heat treatment [5]. However, it differed from that reported by Suresh *et al.,* who obtained about 25% loss in peppercorns after heat processing [6].

3.2.2. Essential oil content

At the beginning of the process (t_0), the contents of essential oil measured in samples collected after gathering the lots (*table I*) ranged from 2.8% to 13.1% (dry basis). The 2.0% to 13.4% rates found in the samples after treatments (t_1) were all higher (and up to six times more than) the 2% rate indicated in the standard ISO 959-1 [14]. The dry process did not impact the essential oil content, whereas the wet process reduced the essential oil content of peppercorns by up to 28%. In a study on rosemary, Szumny *et al.* reported a reduction of around 40% in the essential oil content when they treated the leaves for 30 min at 60 °C [15].

3.2.3. Visual aspect

Considering the evolution of wild peppercorns at the beginning (t_0) and at the end (t_1) of PPS1 and PPS3, in both processes, the fresh peppercorns (t_0) used as raw material appeared to be heterogeneous in size and color (*figure 3*). Their lengths varied from (0.2 to 0.6) cm and their sections from (0.2 to 0.5) cm. Color ranged from green to deep purple, with red dominant. This heterogeneity reflected the many differences in maturity. After treatment (t_1), the lengths and sections of the peppercorns were all reduced to values between (0.1 and 0.4) cm. The color of peppercorns dry-processed in

PPS1 appeared lighter with a majority of gray and some light purple, whereas the peppercorns wet-processed in PPS3 appeared black and dark gray (*figure 3*). In both cases, the heterogeneity of colors was reduced by the processes, especially in the wet process.

4. Conclusion

We described the local processing practices of Malagasy wild pepper in detail through the study of four pepper production systems located in two separate areas, known as the main picking and processing zones for this product in Madagascar. Observing and describing the four pepper production systems in these areas has been quite a challenge because the systems were informal, and it was difficult to reach the locations and schedule meetings with the various actors. Despite the lack of structure for this wild pepper commodity, two main processes (dry and wet) were identified and analyzed. The dry process appeared to be more respectful to the product and easier to implement; indeed, the wet process differed from the dry one in that it included two additional operations: blanching and sweating. Piperine was not affected by the type of production system, whereas essential oil was reduced by the wet process. After processing, piperine was up to eight times lower and essential oil up to six times higher than the specifications of the standard ISO 959-1 for black pepper ready for commercialization [14]. Improving maturity control and processing according to the quality expected by the markets will be necessary to promote Malagasy peppers.

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Tratamientos postcosecha de la pimienta silvestre (*Piper* spp.) en Madagascar.

Resumen – **Introducción**. Se realizó un estudio de tratamientos postcosecha de las pimientas silvestres en Madagascar con el fin de describir las prácticas locales y de medir sus impactos en la calidad de los productos. **Material y métodos**. Se observaron, describieron y compararon cuatro sistemas de producciones (PPS) en dos zonas definidas del este de Madagascar. Se evaluaron características cualitativas mayores (piperina y aceite esencial) de la pimienta en muestras recolectadas en los cuatro sistemas. **Resultados y discusión**. Se identificaron dos procesos principales postcosecha (una vía seca y una vía húmeda). La vía húmeda difiere de la vía seca por dos operaciones específicas: el escaldado y el secado. Los procesos mostraron una influencia en el color de la pimienta. Los contenidos de piperina no fueron afectados por ningún sistema de producción, independientemente de cuál fuera, mientras que los contenidos de aceite esencial se redujeron hasta un 27 % por la vía húmeda. Al final de los procesos, los contenidos de piperina bajaron hasta ocho veces y los contenidos de aceite esencial aumentaron hasta seis veces, en comparación con aquéllos que se especifican en la norma ISO 959-1 para la pimienta negra lista para la comercialización. **Conclusión**. Se identificaron y describieron dos procesos principales (uno « seco », otro « húmedo »), empleados para el tratamiento de las pimientas en Madagascar. La vía seca, que comprende dos operaciones menos, parece más fácil de ejecutar y respetar mejor el producto que la vía húmeda. Para valorar mejor las pimientas malgaches será necesario respetar la madurez de la pimienta silvestre en el momento de la cosecha, así como controlar los procesos en función de los productos esperados por los mercados.

Madagascar / *Piper* / pimienta / procesamiento / aceites esenciales / color

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Impact of blanching, sweating and drying operations on pungency, aroma and color of *Piper borbonense*

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ABSTRACT

Low pungency, high aromatic potential and red color, give to *Piper borbonense* its originality when compared to *Piper nigrum*. Effects of blanching, sweating and drying on these characteristics were assessed. The three operations had no impact on the concentration of piperine and essential oil but affected the composition of essential oil slightly and considerably affected the color of the pepper. The "wet process", including blanching, sweating and drying, had the largest impact on the composition of aroma, increasing para-cymene content by 89% and reducing safrole content by 33% in dried pepper compared to fresh. Blanching increased the drying rate thus reducing drying time. Drying had a major impact on color, which changed from red to brown. The biggest differences observed led to reductions of 2.2, 7.9 and 8.4 units in L^*, a^* and b^* values, when chromatic values measured in fresh pepper were compared to those of dried pepper.

1. Introduction

Pepper (*Piper* spp.) is the most common spice worldwide; 472 500 tons were produced in 2013 (FAO Statistics Division., 2015). Although, also known for its medicinal properties (Ahmad et al., 2012), pepper is mainly used to enhance the taste and flavor of food. The quality of pepper as a spice is measured throughout pungency, aroma and color (Gu, Tan, Wu, Fang, & Wang, 2013). Although more than 700 species grow in tropical and subtropical regions, most of which are wild (Sumathykutty, Rao, Padmakumari, & Narayanan, 1999), one single domesticated species – *Piper nigrum* is by far the most widely consumed. A wild pepper, named *Piper borbonense* grows in Reunion Island but has not been collected until now. Some very closely related wild species of pepper, local name Tsiperifery, grow in Madagascar and are picked for both local consumption and for sale, including for export. These wild peppers differ from domesticated *Piper nigrum* in their low piperine content, high essential oil content and particular red color (Weil et al., 2014). Although they are sold at high prices in Europe, these Malagasy peppers are of heterogeneous quality which could affect their reputation and valorization. As pepper quality varies with the species, origin, agricultural system (when domesticated), climate, or maturity, it may also be influenced by postharvest treatments. Dhas and Korikanthimath (2003) described the different types of processing of pepper and the advantages of each, but few studies have focused on the impacts of processing on pepper quality. Existing studies generally tested domestic cooking, and reported contradictory results. Wild pepper is currently not processed in Reunion Island. In Madagascar, wild peppers are processed according to "dry" and "wet" processes (Weil et al., 2014). The "dry" process only consists in drying, whereas the "wet" process includes blanching and sweating prior to drying. Traditionally, sweating, i.e. keeping the hot blanched product in a blanket for 24 h is widely used in the treatment of Malagasy vanilla beans. However, it is not used elsewhere on pepper and not described either in the literature. The objective of our study was thus to assess the impact of blanching, sweating and drying in controlled conditions on the quality of wild *Piper borbonense* pepper originating from Reunion Island. The quality characteristics considered in this study were pungency (piperine content), aroma (essential oil content and composition) and color (judged by eye and through L^*, a^* and b^* chromatic values). The influence of blanching and sweating on drying kinetics was also assessed.

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2. Materials and methods

2.1. Plant material

We defined three maturity stages according to pepper color: A (immature green pepper), B (orange pepper – intermediate maturity) and C (red mature pepper). Wild mature (C) pepper spikes were picked in the south of Reunion Island. Spikes picked on different occasions were frozen at −80 °C (freezer Froilabo – Bio Memory, 690 L) before being pooled and mixed to form a single homogenous batch. Before processing, the peppercorns with their peduncles were separated from the fruit stems by hand and defrosted for two hours at room temperature. The defrosted pepper is called "fresh" pepper in the rest of this article.

2.2. Processing experiments

2.2.1. Blanching, sweating and drying

The processes consisted in three unit operations that were applied (alone or combined) to obtain different samples (Fig. 1). F: fresh pepper; B: blanched (B1: 60 °C/30 s; B2: 75 °C/180 s; B3: 100 °C/300 s); S: sweated (35 °C, 99% RH, 24 h); D: dried (60 °C, 20% RH, 39 h). Blanching consisted in soaking the peppercorns in a hot water bath (Memmert Gmbh type WB 22 Schwabach, Germany) at a ratio of 1:36 peppercorns to water in three different conditions: at 60 °C for 30 s; 75 °C/180 s; and 100 °C/300 s. Sweating consisted of storing the peppercorns in a climatic chamber (BIA Climatic – Type CL 125, Conflans Sainte Honorine, France) at 35 °C and 99% RH for 24 h. Drying was performed by placing aluminum trays (300 cm^2) containing 250 g of peppercorns arranged in a compact 1 cm thick layer for 39 h at 60 °C ± 1 °C, RH 20% ± 2% in the same climatic chamber. Hot air (60 ± 1 °C, RH 20 ± 2%) was circulated over the surface of the layer.

2.2.2. Drying kinetics

Drying of peppercorn samples used a cross flow pilot dryer, developed in our laboratory. In the treatment chamber (0.25 m long × 0.25 m wide × 0.92 m high), 150 g of peppercorns were placed on a sieve (0.25 × 0.25 m^2) in a single thin non-compact layer. Hot air (60 ± 1 °C, RH 20 ± 2%) was circulated downwards at 2.7 ± 0.1 ms^{-1} through the layer of peppercorns by a high-capacity fan. The air velocity was just high enough to have no significant effect on temperature when passing through the layer of peppercorns to ensure a proper treatment, and to enable statistical analyses. When the heat treatment was complete, the peppercorns were cooled by ventilation with air at ambient temperature. The water content, which was measured on a dry basis (noted X) as a function of time, was estimated in line, using the mass reading of the sieve. Water content kinetics $X^{(t)}$ were fitted with a cubic smoothing spline (Matlab® Version 5.2, The Mathworks Inc., USA). The drying rate (dX/dt) was calculated as the direct analytical derivative of the cubic smoothing spline function on $X^{(t)}$.

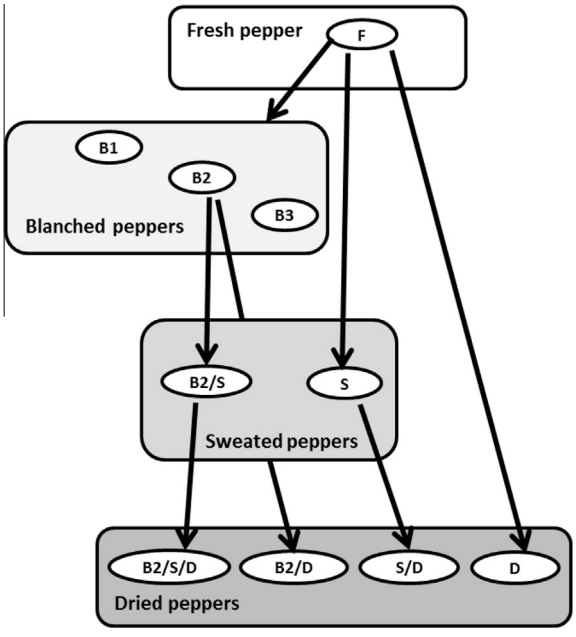

Fig. 1. Processes applied to pepper F: fresh pepper; B: blanched (B1:60 °C/30 s; B2: 75 °C/180 s; B3: 100 °C/300 s); S: sweated (35 °C, 99% RH, 24 h); D: dried (60 °C, 20% RH, 39 h).

2.3. Sample preparation

The samples resulting from the different processing operations were frozen at −80 °C for further preparation and analysis. The pepper samples were ground for 10 s at 10,000 rpm in mill (Retsch – Grindomix GM200, Retsch Gmbh, Germany) for all analyses except color which was measured on whole peppercorns.

2.4. Analytical methods

2.4.1. Dry matter content

The dry matter content (mean "essential oil free dry matter") was obtained by drying 5 g of ground pepper in an aluminum cup in the oven (ULE 400, Memmert Gmbh, Germany) at 105 °C for 30 h (i.e., until constant weight). Initial and final mass was determined with a precision balance (Scaltec SBC 22 model, Scaltec Gmbh, Germany). The mean standard deviation of repeatability was ±0.6% (n = 3). Water content expressed on a dry basis was deduced from essential oil and dry matter content.

2.4.2. Piperine content

The piperine content, expressed on a dry basis, was determined according to 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 deviation of repeatability was ±7.3% (n = 3).

2.4.3. Essential oil content

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

2.4.4. Color measurements

Color measurements (CIE L^*, a^* and b^* values, representing lightness, redness and yellowness, respectively) were made on whole peppercorns using a Minolta CR 400 and utility software. Ten measurements were made on each sample of peppercorns spread in a 1-cm layer in an uncovered Petri dish. The mean relative deviation of repeatability was 1.2%, 2.3% and 3.6% respectively for L^*, a^*, b^* (n = 10).

2.4.5. Identification and quantification of essential oil compounds

2.4.5.1. Separation on a polar column. Volatile compounds were analyzed 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 in Section 2.4.3. above) were injected into the GC–MS in split mode (1:30). The injector's temperature was 250 °C.

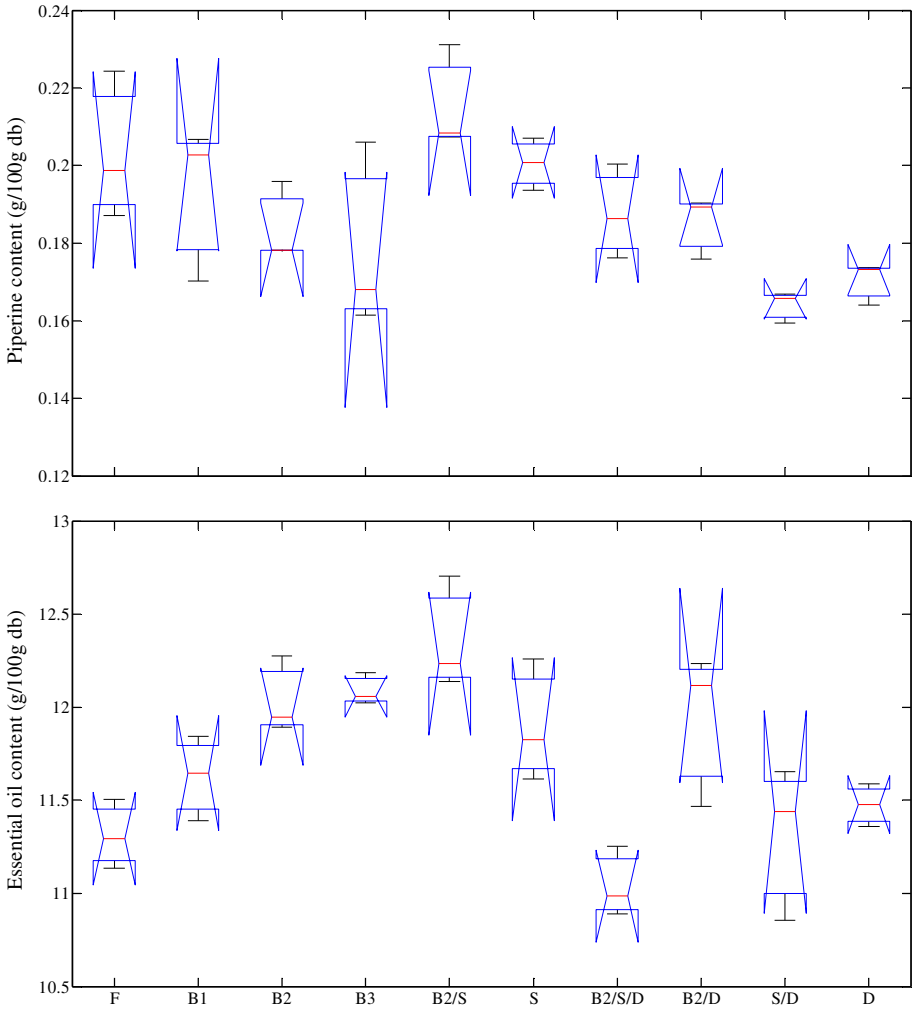

Fig. 2. Impact of processing on piperine and essential oil contents F: fresh pepper; B: blanched (B1: 60 °C/30 s; B2: 75 °C/180 s; B3: 100 °C/300 s); S: sweated (35 °C, 99% RH, 24 h); D: dried (60 °C, 20% RH, 39 h). Boxplots provide a statistic test of group medians: the line in the middle of each box is the sample median (n = 3); the tops and bottoms of each box are the 25th and 75th percentiles of the samples.

The temperature of the transfer line was 250 °C and the flow rate of the gas carrier (Helium) was 0.8 mL/min. The temperature program was as follows: initial temperature 60 °C, heating rate of 4 °C/min 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) which functions in electron impact (70 eV) mode. The mass range was between 25 and 350 m/z.

2.4.5.2. Separation on a non-polar column. Volatile compounds were analyzed with a GC (HP 6890), equipped with a 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 in Section 2.4.3 above) were injected into the GC–MS in split mode (1:50). The injector's temperature was 250 °C. The temperature of the transfer line was 250 °C and the flow rate of the gas carrier (Helium) was 0.7 mL/min. The temperature program was as follows: initial temperature 60 °C, heating rate of 4 °C/min until final temperature of 250 °C was reached then maintained constant for 50 min. The molecules were identified using a GC/MS (HP 6890) which functions in electron impact (70 eV) mode. The mass range was between 20 and 400 m/z.

2.4.5.3. Identification. The aromatic compounds separated on the two columns, were identified by comparing their mass spectrum to those available in commercial libraries (NIST02, WILEY) or constituted under our care and by comparison of their retention indexes calculated relative to those available in the literature (Adams, 1995; Jennings & Shibamoto, 1980; Kondjoyan & Berdagué, 1996) and Internet databases. (2014).

2.4.5.4. Quantification on non-polar column. The aromatic compounds were quantified by a GC (HP 5890), equipped with a 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 in Section 2.4.3.above) and internal standard terpinolene (20:2; v/v) were injected into the GC–FID in split mode (1:33). The injector's temperature was 250 °C. The flow rate of the gas carrier (Helium) was 0.7 mL/min. The oven temperature program was as follows: initial temperature 60 °C, rate of 4 °C/min until a final temperature of 250 °C was reached then maintained constant for 20 min. The mean relative deviation of repeatability was ±2.5% (n = 3).

2.5. Statistical analysis

Differences in the mean values of piperine content, essential oil content, essential oil composition and L*, a* and b* values were tested by analysis of variance (ANOVA); the significance of

differences between samples was determined using Fisher's test. The level of significance was $P < 0.05$.

3. Results

Two levels of observation were considered: unit operations and full processes. In this paper, we considered four full processes: three "wet processes" including blanching and/or sweating and drying and a "dry process" consisting in one drying single operation.

3.1. Impacts of the unit processing operations

Here we describe the impacts of blanching, sweating and drying operations (Fig. 1) on piperine and essential oil contents (Fig. 2), color (Fig. 3) and on drying kinetics (Fig. 4).

3.1.1. Impact of blanching

Blanching had no impact on piperine content (Fig. 2). There was no significant difference in the results obtained (ranging from 0.18% to 0.20%, dry basis) in samples F, B1, B2, and B3. Blanching had no impact on essential oil content (Fig. 2) as evidenced by the absence of a significant difference in the results obtained for essential oil (ranging from 11.31% to 12.09%, dry basis) in the same samples.

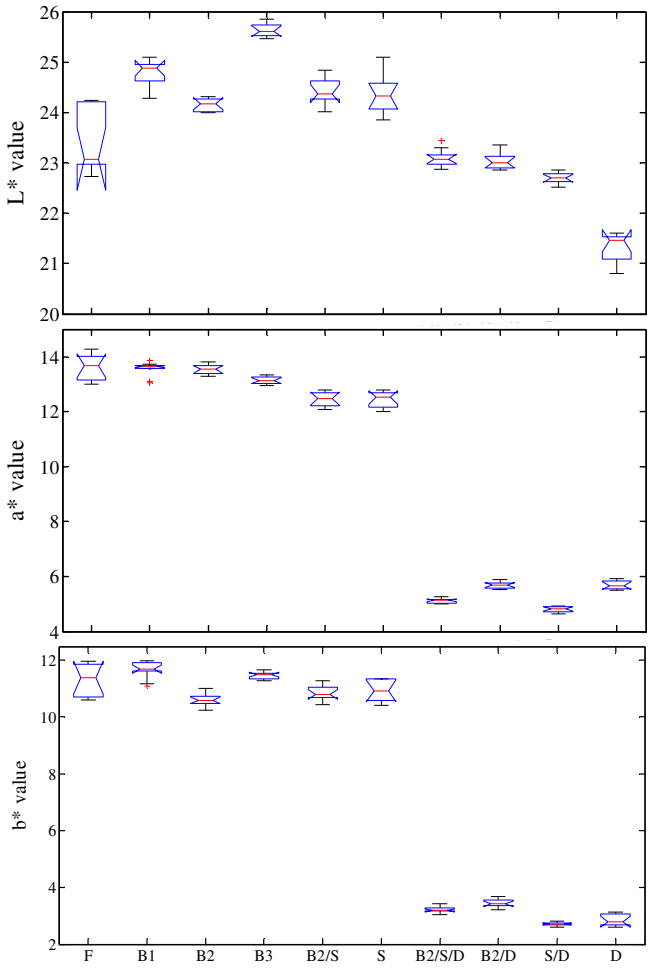

Fig. 3. Impact of processes on L*, a* and b* chromatic values F: fresh pepper; B: blanched (B1: 60 °C/30 s; B2: 75 °C/180 s; B3: 100 °C/300 s); S: sweated (35 °C, 99% RH, 24 h); D: dried (60 °C, 20% RH, 39 h). Boxplots provide a statistic test of group medians: the line in the middle of each box is the sample median ($n = 10$); tops and bottoms of each box are the 25th and 75th percentiles of the samples.

Blanching did have a slight impact on color (Fig. 3) as some slight yet significant differences were observed. The L* value of sample B3, which was subjected to the most drastic treatment, differed from all the other samples: +2.1 units compared to sample F. The biggest differences in a* and b* values were respectively lower than 0.5 and 1 in samples F, B1, B2, and B3. Fig. 4 shows the impact of blanching on the drying curves. Two hours were required to obtain a 50% reduction in the initial water content of fresh pepper and 1 h20 min for blanched pepper. The comparison of the drying curves showed a much higher initial drying rate (1.44 ± 0.10 kg.kg^{-1}.h^{-1}) in blanched pepper than in fresh pepper (0.84 ± 0.02 kg.kg^{-1}.h^{-1}). The differences between the drying rates were no longer significant when the water content was below 0.5 kg.kg^{-1}.h^{-1}. This water content was obtained after 4 h in fresh pepper and 3 h15 min in blanched pepper. Blanching greatly increased the drying rate and consequently reduced total drying time.

3.1.2. Impact of sweating

Sweating had no impact on piperine content (Fig. 2). There was no significant difference between sample B2 and sample B2/S on the one hand, and between sample F and sample S on the other hand. Sweating had no impact on essential oil content (Fig. 2) as there was no significant difference between the same samples. However, sweating did have an impact on color (Fig. 3) as sample B2 differed (+1 unit) from sample B2/S in a* value, and sample F differed from sample S in L* (−0.9 units) and a* (+1.2 units) values. Fig. 4 shows the impact of sweating after blanching on the drying curves. One hour twenty minutes was required to obtain a 50% reduction in the initial pepper water content of blanched pepper and 1 h15 min for "blanched plus sweated" pepper. Comparison of the drying curves revealed very similar drying rates in blanched plus sweated pepper (1.47 ± 0.16 kg.kg^{-1}.h^{-1}) and in blanched pepper (1.44 ± 0.10 kg.kg^{-1}.h^{-1}). Sweating after blanching did not increase the drying rate; consequently the combined operation did not significantly reduce drying time compared to blanching alone.

3.1.3. Impact of drying

Drying had no impact on piperine content (Fig. 2), as there was no significant difference between samples B2/S and B2/S/D, B2 and B2/D, S and S/D, F and D considered in pairs. Drying had no impact on essential oil content (Fig. 2) as no significant visible difference was observed between the different samples except a slight difference between sample B2/S and sample B2/S/D (relative loss of 11%). Drying did have a marked impact on color (Fig. 3) as there were significant differences in all values (L*, a*, b*) in all dried samples. The greatest differences were observed between samples F and D (Fig. 5) with reductions of 2.2, 7.9 and 8.4 units for L*, a* and b* values respectively (Fig. 3).

3.2. Impacts of "full" processes

In this study a 'full' process referred either to drying alone (dry process) or a succession of processing operations (wet processes) including blanching and/or sweating before drying (Fig. 1). Here we describe the impacts of these 'full' processes on piperine and essential oil contents (Fig. 2), essential oil composition (Table 1 and Fig. 6), and pepper color (Figs. 3 and 5).

3.2.1. Impact of the "full" processes on piperine and essential oil contents, and on color

None of the "full" processes had an impact on piperine content or on essential oil content (Fig. 2). There was no significant difference between samples F, B2/S/D, B2/D, S/D and D. However, "full" processes did have a marked impact on color, confirming the major impact of the drying operation on all chromatic values (Fig. 3). The

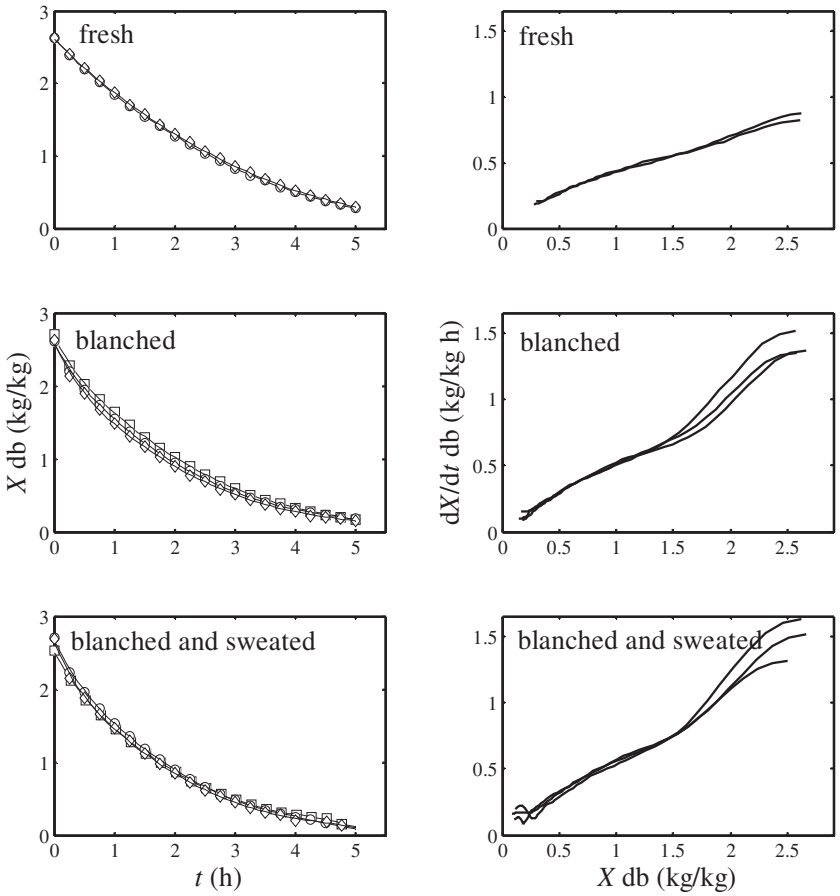

Fig. 4. Drying curves of fresh, blanched and blanched/sweated pepper. Water content (X) on a dry basis as a function of time (t) and drying rate (dX/dt) as a function of X. Drying curves of a "single thin non-compact layer" recorded by an air dryer (60 °C, RH 20%, air velocity 2.7 ± 0.1 ms^{-1}). Blanching (75 °C/180 s); Sweating (35 °C, 99% RH, 24 h). The different symbols on curves correspond to different trials.

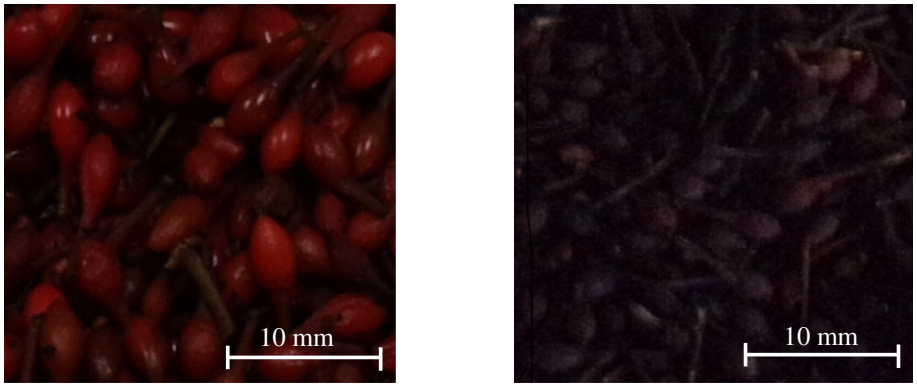

Fig. 5. Impact of drying on the color of the pepper Sample F (fresh pepper); Sample D (dried pepper). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

chromatic dimensions L*, a* and b* were impacted irrespective of the operation considered concerned, as clearly shown by comparing the values obtained for fresh pepper (sample F) to values obtained for processed peppers (samples B2/S/D, B2/D, S/D and D) or by comparing the values obtained for processed-peppers among themselves. When drying was preceded by blanching and or/sweating, the L* value decreased less than during drying alone ($\leqslant$0.8 compared to 2.2 units). The greatest differences (detailed in Section 3.1.3.), mostly due to drying, were observed between sample F and sample D (Fig. 5) and between F and S/D while small but significant differences (all <1 unit) were observed for a* and b* values between samples B2/S/D, B/2/D, S/D and D.

3.2.2. Impact of the "full" processes on aromatic composition

Taking sample F (fresh pepper) as a reference, we were able to identify 25 aromatic compounds representing 97% (m/m) of the total essential oil. Among these, 15 major compounds, all of which were present at a rate of more than 1%, represented 93% (Table 1) of the total essential oil. The monoterpenoïd family represented 55% of the total aromatic compounds. Limonene (27% of the total),

Table 1
Major volatile compounds in *Piper borbonense* fresh pepper (F) essential oil.

Aromatic compounds	Concentrations (g/100 g)
Limonene + Eucalyptol*	29.54 ± 0.05
Alpha-phellandrene	14.38 ± 0.09
Asaricin	13.94 ± 0.33
Beta-pinene	6.46 ± 0.17
Alpha-pinene	6.00 ± 0.36
Dillapiole	4.32 ± 0.13
Safrole	3.89 ± 0.09
Delta-3-Carene	3.39 ± 0.03
Elemicin	1.95 ± 0.05
Myristicin	1.49 ± 0.24
para-cymene	1.70 ± 0.01
Myrcene	1.70 ± 0.01
Delta-elemene	1.35 ± 0.04
Sabinene	1.41 ± 0.03
Camphene	1.45 ± 0.07
Total	92.98 ± 0.12

Mean values ($n = 3$).
* Eucalyptol represents around 2.5 g/100 g of essential oil.

was the most abundant compound followed by alpha phellandrene and asaricin (14% each). As the amounts of the compounds in sample F (fresh pepper) were very close to the amounts of compounds in samples B2/S/D, B2/D, S/D or D (processed peppers), we can conclude that globally, the composition of the flavor of the pepper was little affected by any of the processes (Fig. 6). Nevertheless, two monoterpens (para-cymene and camphene) were found at higher concentrations in processed peppers (samples B2/S/D, B2/D, S/D and D) than in fresh pepper (sample F). The most remarkable increase (89%) was obtained for para-cymene between samples F and B2/S/D. Conversely, the concentration of safrole, a non-monoterpenic compound, was lower in processed peppers (samples B2/S/D, B2/D, S/D and D) than in fresh pepper (sample F). The most remarkable decrease (33%) for safrole was observed in sample F versus sample B2/S/D. The least affected compounds were delta 3-carene, myrcene and sabinene; no differences of more than 9% in these compounds were found between fresh (sample F) and processed peppers (samples B2/S/D, B2/D, S/D and D). The impacts of single processing operations could be deduced from "full" processes comparison. For example, the concentrations of some monoterpens (alpha phellandrene, beta pinene and delta-elemene) were reduced by sweating, as shown by the values obtained for samples B2/D and B2/S/D (this sample including sweating) on the one hand and for samples D and S/D (this sample including sweating) on the other hand. The most remarkable significant difference (a drop of 13%) was observed for delta-elemene between samples B2/D and B2/S/D. The aromatic profiles of sample B2/S/D, which included blanching, sweating and drying (full wet process), and sample D (dry process), which only underwent single drying, were very similar. No significant differences were found in 13 out of 15 compounds, while significant

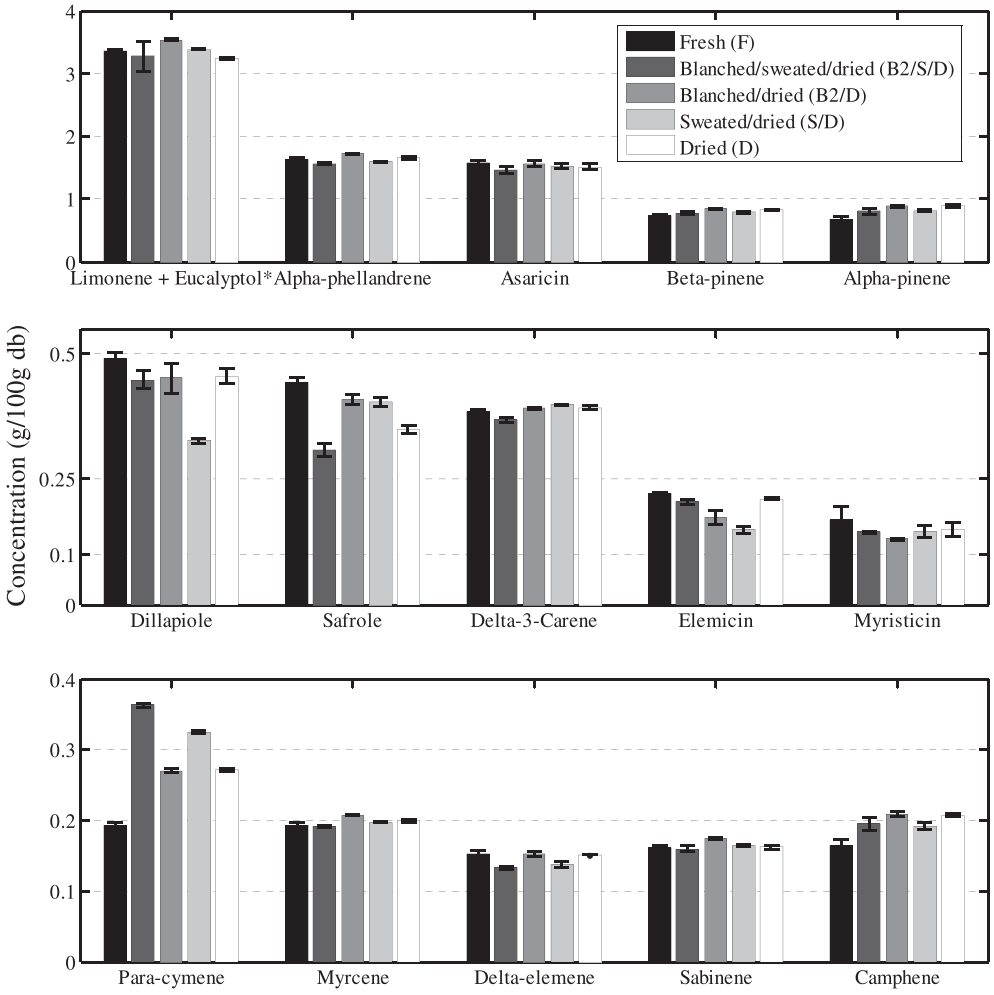

Fig. 6. Composition of essential oil of fresh and processed pepper corns. The process conditions were: blanching (75 °C/3 min), sweating (35 °C, 90% RH, 24 h) and drying (60 °C, 20% RH, 39 h). The error bars represent the standard error ($n = 3$). *Eucalyptol represents 0.2 to 0.3 g/100 g (db) of pepper.

differences were found for two compounds, alpha phellandrene and para-cymene, which was 25% higher in sample B2/S/D than in sample D.

4. Discussion

4.1. Originality of Piper borbonense composition

The 11.3% (db) rate of essential oil in fresh *Piper borbonense* is more than 5 times higher than the 2% content recommended by standard ISO 959-1 (International Standard Organization., 1998) for black pepper. It is also very close to the highest concentration of 13.1% (db) found in the richest Malagasy wild pepper species, local name Tsiperifery (Weil et al., 2014). The rate of 0.20% (db) piperine obtained in fresh *Piper borbonense* is 20 times lower than the value of 4% indicated by the same standard. It is also 2.5 times lower than the lowest concentration of 0.5% found in Malagasy wild pepper species. These low pungency and high aroma values give *Piper borbonense* its originality compared to cultivated black pepper (*Piper nigrum*) and Malagasy wild peppers.

4.2. Impact of the processes on piperine and essential oil contents, essential oil composition and color

The different unit operations and 'full' processes tested had no impact on piperine, nor on essential oil contents of the pepper. The results we obtained for piperine are in agreement with those of Nisha, Singhal, and Pandit (2009) but not with those reported by Suresh, Manjunatha, and Srinivasan (2007). Nisha et al. (2009) reported piperine to be stable to heat processing, with only 2.5% loss after 20 min at 100 °C, whereas Suresh et al. (2007) reported 28% losses of piperine in black pepper in the same conditions. Our results concerning essential oil differ from those of Nisha et al. (2009) who reported a 38% reduction in the essential oil content after 20 min at 100 °C, and from those of Schweiggert, Mix, Schieber, and Carle (2005) who reported a 75% loss after 10 min at 90 °C. These differences could be explained by the fact that both Nisha and Suresh used ground pepper and applied blanching conditions that were more drastic than ours. The fact that we observed no drop in piperine and essential oil contents in our study, even after 5 min at 100 °C, could be because the pericarp of the peppercorn acts as a barrier against mass transfer.

In our study, Limonene, alpha phellandrene and asaricin were shown to be the most abundant compounds in *Piper borbonense*. These compounds are also present in cultivated black pepper and play a role in the appreciation of its quality. According to Schulz, Baranska, Quilitzsch, Schutze, and Losing (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 analyzed in our study contained 55% of monoterpenoïds excluding pinenes, which represented only 13% of the total, we can conclude that the aroma of wild pepper *Piper borbonense* is of good quality and is preserved even after heat treatment. According to Jirovetz, Buchbauer, Ngassoum, and Geissler (2002), limonene, β-pinene, α-phellandrene, δ-carène, asaricin and elemicin give black pepper its characteristic aroma. In our study, each of these compounds represented more than 1% of the total essential oil; together they represented over 65% of the essential oil of wild pepper *Piper borbonense*. According to Jagella and Grosch (1999), α-pinene, α-phellandrene, myrcene, and limonene are key odorants in *Piper nigrum*. These four compounds represented 48% of essential oil in *Piper borbonense* in our study. The composition of essential oil as expressed by the relative amounts of the 15 major compounds in the different samples was almost not affected by any of the processing operations tested. The biggest decrease (33%) we observed for safrole after the 'full' wet process is in agreement with the results of Farag and Abo-Zeid (1997) who reported a loss of more than 90% after boiling the seeds for 30 min or drying the seeds for 30 min at 70 °C. Safrol, which is known to be carcinogenic, could have been degraded during blanching by hydroxylation of the dioxolane ring. Our results which unexpectedly showed that, the volatile compounds remained stable during processing, differ from those of Asekun, Grierson, and Afolayan (2007) who analyzed essential oil extracted from *Mentha longifolia* leaves. These authors observed significant differences in the chemical composition of the essential oils obtained using different drying methods. Four monoterpenoïd compounds (alpha pinene, beta pinene, limonene, 1,8-cineole), stable in our study, were all widely affected (increased or reduced) by the three drying methods tested by these authors. In their case, the fact that the essential oil is known to be stored on or near the surface of the leaf may explain this sensitivity. Argyropoulos and Muller (2014), who studied lemon balm (*Melissa Officinalis L.*) reported pronounced changes in the relative composition of essential oil when the leaves were dried at 60 °C. Most of these changes occurred during the initial period of drying. Apart from the sensitivity of the essential oil constituents to temperature, these authors also attributed these losses to the structure of the leaves. In our case the structure of the peppercorn, and in particular the pericarp, may protect the volatile compounds from transfer during processing.

The three different unit operations tested, especially drying, impacted pepper color, reducing, in particular, a^* and b^* chromatic values leading to browning that was visible to the naked eye. Even blanching, which should reduce enzyme activity and hence limit browning, had a slight but significant impact on color. As suggested by Gu et al. (2013), aside from enzymatic browning described by several authors (Dhas & Korikanthimath, 2003; Mangalakumari, Sreedharan, & Mathew, 1983; Variyar, Pendharkar, Banerjee, & Bandyopadhyay, 1988), other browning mechanisms may be involved in the change in the color of the pepper.

4.3. Role and interest of each unit processing operation

Blanching significantly increased the drying rate of the pepper. By itself, this result, which can be explained by the partial destruction of the pepper cell walls, thus facilitating water transfer (Kaymak-Ertekin, 2002), justifies this step. Indeed, a blanching step, which reduces drying time, could save energy or limit climate-dependence in the case of sun drying. Blanching is also useful as it removes any dust from the pepper. Dhas and Korikanthimath (2003), suggested that moderate blanching could contribute to uniform browning by promoting the oxidation of phenols by phenolase enzymes. Depending on the length and temperature applied, as a thermal treatment, blanching could be a critical sanitary step as it reduces the microbial load as well as the safrole content of the pepper. Sweating affected the color of the pepper, systematically reducing the a^* value, which corresponds to red, validating the hypothesis that the conditions (24 h at 35 °C in a water saturated atmosphere) used for sweating favor enzymatic browning, as described by Mangalakumari et al. (1983). The favorable humidity and temperature conditions could also stimulate the growth of microorganisms. Considering these results, we question the interest of this operation, which is used in Madagascar (Weil et al., 2014) for wild pepper species that are close to our *Piper borbonense,* as well as for *Piper nigrum*. Drying is crucial; it not only stabilizes the product but is also critical for the sanitary and sensorial quality of the pepper. Drying had a major influence on color as all values (L^*, a^*, b^*) were affected, and the color turned

from red to brown. Referring to Agudelo-Laverde, Schebor, and del Pilar Buera (2013) who demonstrated that browning increased with an increase in water content on strawberry slices, we hypothesize that accelerating drying, by rapidly reducing water activity, could help preserve the red color.

5. Conclusion

The originality of dry *Piper borbonense* is based on its high aromatic potential, low pungency and red color. Separate or combined, the blanching, sweating and drying operations had no impact on piperine and essential oil content and only a slight impact on essential oil composition. The three unit operations influenced color, drying having the most impact. To preserve the color, sweating should be avoided, while blanching and drying could be optimized. Indeed, as demonstrated in this study, right blanching parameters could reduce drying time as well as limit enzymatic browning. Enhancing and innovating drying conditions would also help reduce both enzymatic and non-enzymatic oxidative reactions.

Conflict of interest

The authors of this article certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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Résumé

L'objectif de cette thèse était d'acquérir des connaissances nouvelles sur les caractéristiques des poivres sauvages malgaches (Tsiperifery) et réunionnais (*Piper borbonense*) et d'étudier l'impact des procédés de transformation sur leur qualité, évaluée à travers le piquant, l'arôme et la couleur. L'enjeu étant, grâce aux résultats obtenus, de pouvoir proposer un ou plusieurs procédés de transformation revisités permettant de valoriser la qualité de ces poivres. Ce travail de thèse a consisté dans un premier temps à étudier les procédés traditionnels de transformation du poivre sauvage mis en œuvre à Madagascar. Deux procédés de transformation distincts ont été identifiés : une « voie sèche » consistant en un simple séchage et une « voie humide » incluant blanchiment et étuvage avant séchage. Ensuite, des expérimentations ont été menées en conditions maîtrisées à la Réunion sur du poivre *Piper borbonense*. Ainsi, la morphologie, l'anatomie et la composition biochimique du poivre sauvage réunionnais ont été caractérisées. Enfin, dans la mesure où c'est la couleur, rouge, du poivre sauvage, qui est la plus affectée, les mécanismes impliqués dans l'altération de la couleur ont été analysés. Le *Piper borbonense* de la Réunion se distingue du *Piper nigrum* par sa très faible teneur en pipérine (0,2 % bs), sa forte teneur en huile essentielle (9,8 % bs), la présence d'un pédicelle solidaire du grain ainsi que par sa forme ovoïde. Il se différentie aussi des poivres sauvages malgaches, notamment par sa teneur en pipérine deux fois plus faible. Les composés d'arômes principaux mesurés sont le limonène, l'α-phellandrène et l'asaricin qui représentent à eux trois 50 % du total de l'huile essentielle. C'est à pleine maturité, lorsque le *Piper borbonense* est de couleur rouge vif, qu'il est préférable de le récolter pour maximiser le rendement massique. Le blanchiment, l'étuvage et le séchage ont peu d'impact sur le piquant et l'arôme mais dégradent significativement la couleur du poivre. Les oxydations chimiques des polyphénols qui semblent prépondérantes dans le brunissement du poivre s'avèrent délicates à contrôler. Le blanchiment présente de nombreux avantages : il nettoie et décontamine le poivre, augmente la vitesse du séchage et limite le brunissement enzymatique. L'étuvage est à bannir car il dégrade la couleur et augmente les risques microbiens. Le séchage par entrainement bien qu'il impacte négativement la couleur reste indispensable pour stabiliser le poivre. Plutôt qu'un procédé universel, une « voie sèche » (séchage direct) et une « voie humide » (intégrant blanchiment et séchage) sont proposées. Le choix d'en appliquer l'une ou l'autre est à raisonner par rapport à la qualité de la matière première d'une part et en fonction du contexte, c'est-à-dire selon des critères économiques, environnementaux voire même sociaux d'autre part.

Mots clefs : poivre, procédés, qualité, huile essentielle, piperine, caroténoïdes

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Abstract

The objective of this thesis work was to acquire new knowledge on the characteristics of Malagasy (Tsiperifey) and Réunion (*Piper borbonense*) wild peppers. The impact of transformation processes on their quality was evaluated by measuring pungency, aroma and color. The challenge was to propose, according to the data obtained, one or more revisited transformation processes to valorize the quality of the peppers. This PhD work initially consisted of studying the traditional transformation processes of the wild peppers implemented in Madagascar. Two distinct transformation processes have been identified: a "dry process" consisting of simple drying step and a "wet process" including blanching and steaming steps before drying. Then, experiments were carried out under controlled conditions in Reunion Island on *Piper borbonense*. Thus, the morphology, anatomy and biochemical composition of Reunion wild pepper were characterized. Finally, since the red color of wild pepper appear to be the most affected by the process, the mechanisms involved in color alteration were investigated. The *Piper borbonense* of Réunion Island can be distinguished from *Piper nigrum* according to its very low content of piperine (0.2% bs), its high content of essential oil (9.8% bs), the presence of a pedicel and its ovoid shape. *Piper borbonense* can also be differentiated from Malagasy wild peppers, notably by its lower piperine content. The main volatile compounds found are limonene, α-phellandrene and asaricin, which together account for 50% of the total essential oil composition. *Piper borbonense* should be harvested at full maturity stage, bright red-colored, as this allows optimum mass yield. Blanching, sweating and drying steps have little impact on pungency and aroma but significantly degrade the color of the pepper. The chemical oxidation of polyphenols, which seems to be preponderant in the browning of the pepper, is difficult to control. Blanching has many advantages: it helps cleaning and decontaminating pepper, reduces drying time and limits enzymatic browning. The sweating step has to be banned because it degrades the color and increases the microbial risk. Drying, although negatively impacting the color, is essential to stabilize the pepper. As opposed to a universal process, a "dry way" (direct drying) and a "wet way" (integrating blanching and drying) are proposed. The choice to apply one or the other could be made according to the quality of the raw material on the one hand and to the context of production (economic, environmental and even social criteria) on the other hand.

Key words: pepper, processes, quality, essential oil, piperine, carotenoids