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**CRISTINE VANZ BORGES**

PERFIL BIOQUÍMICO NA PÓS-COLHEITA DE FRUTOS DE *Musa* spp., ÊNFASE EM COMPOSTOS BIOATIVOS

**Botucatu**
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Tese apresentada à Faculdade de Ciências Agronômicas da Unesp Campus de Botucatu, para obtenção do título de Doutora em Agronomia (Horticultura).

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  Igor Minatel
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**Botucatu**

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## CERTIFICADO DE APROVAÇÃO

TÍTULO DA TESE: PERFIL BIOQUÍMICO NA PÓS-COLHEITA DE FRUTOS DE *Musa* spp. ÊNFASE EM COMPOSTOS BIOATIVOS

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Aprovada como parte das exigências para obtenção do Título de Doutora em AGRONOMIA (HORTICULTURA), pela Comissão Examinadora:
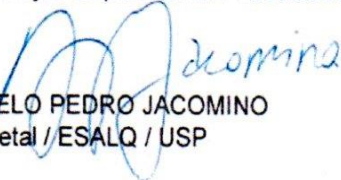
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*Aos meus pais, por compartilharem os seus princípios, essenciais para a formação do meu caráter.*

*Ao meu marido, pelo companheirismo, amor, paciência e ajuda nas horas mais difíceis.*

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**DEDICO**

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# RESUMO

O objetivo desse trabalho foi avaliar o perfil físico-químico e bioquímico, de genótipos de bananeiras (*Musa* spp.), com ênfase em amido resistente, carotenoides, aminas bioativas, compostos fenólicos e minerais. Todos os genótipos (bananas e plátanos) fazem parte do programa de seleção de germoplasmas da Embrapa que visa obter cultivares biofortificados e com melhor qualidade na pós-colheita. Os frutos obtidos de cada genótipo foram avaliados ao longo do amadurecimento em 3 estádios (verde: estádio 2; maduro: estádio 5 e super maduro: estádio 7) e 4 métodos de cocção (fervura em água – com e sem casca, micro-ondas – com e sem casca e fritura). No primeiro capítulo, constam as análises físico-químicas e bioquímicas de 19 genótipos de bananas e plátanos. Foi verificada ampla variação no comprimento, diâmetro dos frutos, massa fresca dos frutos, relação polpa casca e atributos físico-químicos, diferenciando os subgrupos e/ou diferentes tipos de bananas. A banana de sobremesa 'Ney Poovan' contém alto teor de sólidos solúveis totais e relação polpa-casca, um resultado interessante para a promoção do consumo *in natura* desse fruto. Os resultados encontrados mostram que bananas e plátanos são fontes importantes de compostos fenólicos. Os genótipos 'Ney Poovan', 'Ouro da Mata', 'Pelipita' e 'Tiparot' foram os que se destacaram nestes compostos antioxidantes. Níveis elevados de carotenoides foram encontrados em plátanos e/ou bananas de cocção e elevados teores de vitamina C foram verificados em plátanos (AAB) e na banana de sobremesa 'Prata' (AAB), principalmente nos frutos maduros. Os genótipos 'Pelipita' e 'Samurá B' são promissores para o uso industrial, principalmente para o processamento de *chips* de banana, tanto em frutos verdes, quanto maduros. No segundo capítulo, polpas e cascas de 22 genótipos foram analisados para a obtenção do perfil de carotenoides e potencial provitamina A. O conteúdo de pró-vitamínicos A foi variável entre os genótipos, e os plátanos foram os que apresentaram os maiores teores (e.g. 'Samurá B.'). O teor de carotenoides é afetado pelo estádio de amadurecimento e teores elevados são encontrados nos frutos maduros (estádio 5). Entre os métodos de cocção avaliados é possível destacar a ebulição dos frutos com casca, que aumentam a bioacessibilidade dos compostos bioativos, independente da cultivar utilizada. No capítulo 3, é apresentado o perfil de aminas bioativas em 20 genótipos ao longo do processo de amadurecimento e após o processamento térmico. As aminas tiramina, histamina, dopamina, serotonina, espermidina e espermina diminuíram ao longo do

amadurecimento e a amina putrescina aumentou. No entanto, em plátanos a serotonina e a dopamina não diminuíram no estádio 7, demonstrando uma possível diferenciação do perfil destas aminas por grupo de consumo. A casca é um subproduto importante na indústria de alimentos como fonte potencial de aminas bioativas, principalmente dopamina e serotonina. Além disso, o processamento térmico altera o conteúdo de aminas nos frutos, dependendo do composto e do genótipo analisado, principalmente a ebulição dos frutos com a casca, que deve ser o método preferido em preparações domésticas, principalmente para o consumo de maiores quantidades de catecolaminas e indolaminas. No quarto e último capítulo, foram avaliados os teores de amido, amido resistente, minerais e compostos fenólicos dentre os 22 genótipos. Os plátanos e as bananas de cocção se destacaram no teor de amido e amido resistente (até 49,9%). As polpas dos frutos das bananas de sobremesa 'Khai' e 'Ouro da Mata', e os frutos da banana de cocção 'Pacha Nadam', destacaram-se na maioria dos minerais analisados (P, K e Fe; Zn e Fe; Ca, Mg e Zn, respectivamente). O conteúdo de compostos fenólicos totais foi elevado nas bananas de sobremesa (e.g., 'Ney Poovan') e nas bananas de cocção (e.g., 'Tiparot'), principalmente nos frutos maduros (estádio 5), conferindo a estes frutos uma maior capacidade antioxidante. Além disso, o processamento térmico aumentou o valor funcional e nutricional dos frutos, principalmente quando fervidos (ebulição) com casca, o qual deve ser o preferido em preparações domésticas.

Palavras-chave: Processamento térmico. Amadurecimento. Biofortificação. Compostos bioativos. Antioxidantes.

# ABSTRACT

The objective of this work was to evaluate the physico-chemical and biochemical profile of 22 accessions of banana trees (*Musa* spp), with emphasis on resistant starch, carotenoids, bioactive amines, phenolic compounds and minerals. All accesses (bananas or plantains) are part of the Embrapa germplasm selection program, which aims to obtain cultivars biofortified and with improved postharvest quality. Fruits obtained from each access were evaluated at 3 ripening stages (green: stage 2; ripe: stage 5; and super-ripe, stage 7) and 3 cooking methods (boiling, microwaving and stir-frying). In the first chapter, the physical-chemical and biochemical analyzes of 19 accessions were included. It was verified a wide variation in length, fruit diameter, fresh mass, pulp ratio and physical-chemical attributes, differentiating the subgroups and/or bananas types.  The banana for dessert 'Ney Poovan' contain high total soluble solid content and pulp-to-peel ratio, an interesting result to promotion the *in natura* consumption of this fruit. Results show that bananas and plantains are important sources of phenolic compounds. The genotypes 'Ney Poovan', 'Ouro da Mata', 'Pelipita' and 'Tiparot' were the ones with remarkable antioxidant compounds. Increased levels of carotenoid were found in cooking bananas and/or plantains. High levels of vitamin C were observed in plantains (AAB) and dessert banana 'Prata' (AAB), especially in ripe fruits. The genotypes 'Pelipita' and 'Samurá B' are promising for industrial use, mainly for the production of banana chips, in both green and ripe fruits. In the second chapter, pulp and peel of 22 accessions were analyzed to obtain the profile of carotenoids and pro-vitamin A potential. The provitamin A carotenoids (pVACs) content varied according to the genotypes and high quantities were identified in plantains (e.g. 'Samurá B.'). Carotenoid content is affected by ripening stage and highest pVACs quantity was verified in the ripe fruit (stg 5). Among the evaluated cooking methods, it´s possible to emphasize the boiling of fruits with peel, which increased the bioaccessibility of the bioactive compounds, regardless of the cultivar used. In chapter 3, the profile of bioactive amines in 20 accessions is presented along the ripening and after the thermal processing. The amines tyramine, histamine, dopamine, serotonin, spermidine and spermine decreasing during the fruit ripening and the amine putrescine increased. However, in plantains serotonin and dopamine did not decrease in stage 7, showing a possible differentiation of the profile of these amines

by consumption group. Peel is an important by-product in the food industry as a potential source of bioactive amines, mainly dopamine and serotonin. In addition, the thermal processing changes the amine content in the fruits, depending on the compound and the genotype analyzed, mainly when fruits with peel are boiled, which should be the favorite in domestic preparations, mainly for the consumption of larger amounts of catecholamines and indolamines, regardless of the cultivar used. In the fourth and last chapter, the contents of starch, resistant starch, minerals and phenolic compounds among the 22 accessions were evaluated. Plantain and cooking bananas highlighted in terms of starch and resistant starch (up to 49.9%). The pulps of dessert bananas 'Khai' and 'Ouro da Mata', and cooking banana 'Pacha Nadam', stand out in most of the analyzed minerals (P, K and Fe, Zn and Fe, Ca, Mg and Zn, respectively). The content of total phenolic compounds was elevated in dessert bananas (e.g., 'Ney Poovan') and cooking bananas (e.g., 'Tiparot'), mainly in ripe fruits (stg 5), giving to these fruits a higher antioxidant capacity. In addition, the thermal processing increases the functional and nutritional values of the fruits, mainly by boiling with peel, which should be the favorite in domestic preparations.

# SUMÁRIO

# INTRODUÇÃO GERAL

A bananicultura destaca-se como uma atividade de grande importância econômica e social, sendo cultivada em uma extensa região tropical em todo mundo, geralmente por pequenos agricultores. Com uma produção mundial de cerca de 107 milhões de toneladas, as bananas e os plátanos são o quarto alimento mais produzido no mundo, juntamente com o arroz, o trigo e o milho. Ocupam a primeira posição no ranking mundial de frutas, sendo produzidas em larga escala e amplamente cultivadas na Índia, China, Filipinas, Equador e Brasil, os quais são os maiores produtores (FAO, 2017).

O Brasil é o quinto produtor mundial de banana, tendo produzido aproximadamente 7,0 milhões de toneladas em 2012, em uma área aproximada de 500 mil hectares (FAO, 2017). Novos híbridos com melhor resistência a doenças são desenvolvidos constantemente pelo programa de melhoramento genético de bananas e plátanos da Embrapa os quais, consequentemente, aumentam a diversidade de bananas. Esse programa foi iniciado em 1985, a partir de coletas nacionais e internacionais, criando a coleção de germoplasma com aproximadamente 400 acessos de *Musa* spp. A Embrapa desenvolve um programa de melhoramento genético via cruzamentos de indivíduos diploides ricos em compostos funcionais (carotenoides e compostos fenólicos), visando o desenvolvimento de diploides superiores para uso como genitores masculinos em esquemas de hibridização com triploides e tetraploides para a obtenção de novas cultivares e/ou uso direto, nos casos de desempenho agronômico positivo. Os primeiros estudos mostraram que existe grande diversidade no conteúdo de compostos funcionais ou bioativos dentro do germoplasma de *Musa* spp*.,* sendo que há acessos com quantidades apreciáveis destes compostos, principalmente quando comparados com as cultivares mais comercializadas atualmente (AMORIM et al., 2011; BORGES et al., 2014). Esta diversidade poderia ser explorada para identificar genótipos potencialmente adequados para serem utilizados em programas de melhoramento genético com foco na biofortificação da cultura e/ou para serem promovidos e incorporados dentro de sistemas agrícolas existentes para diversidade da cultura e, consequentemente, da dieta brasileira.

Contudo, apesar destes trabalhos iniciais, o conjunto de informações acerca da composição química e do valor nutricional daquele germoplasma e de genótipos

adaptados ao cultivo em diferentes regiões é considerado incipiente, sendo necessária a caracterização de mais acessos, bem como a análise de mais compostos bioativos (e.g., poliaminas). Bananas e plátanos destacam-se por serem consumidos em todos os estádios de maturação e após diferentes processamentos térmicos (por exemplo, fritos e assados). A compreensão das transformações bioquímicas que ocorrem com o amadurecimento dos frutos, irá permitir o conhecimento de como essas mudanças metabólicas afetam a qualidade e vida pós-colheita dos frutos de novos híbridos promissores desenvolvidos pela empresa, além de estabelecer o melhor ponto de consumo. Devido à escassez de informações na literatura científica, suscita-se uma demanda pela investigação dos reais benefícios à saúde advinda do consumo de biomassas biofortificadas de bananas, tornando necessária a averiguação de sua autentica ação nutracêutica. O acesso às peculiaridades químicas destes genótipos ao longo do processo de maturação e após o processamento térmico aprofunda o conhecimento sobre suas singularidades metabólicas, contribuindo para melhor caracterizar o germoplasma disponível com foco na qualidade pós-colheita de frutos, além de ampliar o conhecimento das alterações destes compostos antioxidantes na forma de consumo, permitindo uma melhor avaliação da qualidade nutricional e ou funcional de frutos de *Musa* spp.

Outro fator relevante para diminuir as perdas pós-colheita, assim como os resíduos gerados, é que a polpa e a casca da banana poderiam ser exploradas pelas indústrias alimentícia e farmacêutica, agregando valor ao produto e aumentando a renda do produtor rural. A casca corresponde a 40% do peso total dos frutos e causa um grande problema nas indústrias de processamento de alimentos hoje; dessa forma, é essencial encontrar aplicação para o uso desse subproduto (EMAGA et al., 2007; PEREIRA; MARASCHIN, 2015).

Na alimentação humana, a banana adquire importância não só por ser consumida em todas as faixas etárias, mas também pelo seu valor energético (i.e., rica em amido, banana – 89 Kcal/100g e plátanos - 122 Kcal/100g) e pelo conteúdo de vitaminas e sais minerais (e.g., banana – 358 mg de K/100g e plátanos – 459 mg de K/100g). Destaca-se das demais fruteiras de clima tropical, devido a seu elevado consumo e isso se dá tanto pela sua versatilidade em termos de modalidades de uso (consumo *in natura*, processamento, frita, cozida, entre outras), quantos pelos seus caracteres de sabor e aroma. Em algumas regiões de maior carência alimentar (i.e., África) o consumo pode chegar a 162 Kg/indivíduo/ano (FAO, 2017), sendo que essa

dependência de milhões de pessoas sobre as bananas e seus produtos derivados, a coloca como um alimento muito importante como fonte de nutrientes (principalmente carboidrato – amido) e com grande potencial de uso como alimento funcional.

O constituinte majoritário da polpa da banana é o amido e é o principal polissacarídeo componente da dieta humana. O acúmulo de amido em bananas ocorre sob a forma de grânulos parcialmente cristalinos, cuja morfologia, composição química e estrutura supramolecular são peculiares a dada espécie/variedade (BORGES et al., 2014; WANG et al., 2014). Além de possuir elevado teor de amido na sua composição, a banana verde destaca-se como alimento funcional, devido aos elevados teores de amido resistente (AR), um componente de baixa digestibilidade no intestino delgado, i.e., fibra insolúvel, com efeito, prebiótico e preventivo de doenças inflamatórias intestinais. Adicionalmente, a lenta digestão do AR também pode melhorar a resposta glicêmica e insulinêmica com efeito importante no controle da síndrome metabólica, responsável por alguns dos maiores problemas de saúde atualmente (obesidade, doenças cardiovasculares e diabetes – GRAHAM et al., 2012).

O arranjo estrutural e os conteúdos relativos dos constituintes amídicos nos grânulos, i.e., amilose e amilopectina, definem a funcionalidade do amido, bem como afetam atributos qualitativos e o tempo de meia-vida do fruto em pós-colheita. Durante o armazenamento pós-colheita, inúmeras enzimas transformam o amido em diferentes açúcares e, consequentemente, o teor de amido resistente (AR) em bananas nos diferentes estádios de maturação é diferente. Além disso, há relatos que os processos de degradação da amilopectina e amilose diferem em função da cultivar analisada (WANG et al., 2014). Entretanto, poucos estudos centraram-se sobre as mudanças do AR durante o armazenamento de *Musa* sp. nos diferentes cultivares, sendo que estas informações são valiosas para as potenciais aplicações do AR de banana.

Os frutos e as cascas de bananas e plátanos, bem como outras partes das plantas de *Musa* spp., que inclui raízes, pseudocaule, cascas e flores têm sido utilizadas há muito tempo na medicina popular na África, Índia, Ásia e América (TSAMO et al., 2015). Estudos destacam a ação antioxidante, antibactericida, anti-ulcerogenica, anti-hipertensiva, antidiabética e anticâncer dos frutos de *Musa* spp. (IMAN; AKTER, 2011). Além disso, há relatos na literatura de genótipos de bananas

ricos em pró-vitamínicos A (DAVEY et al., 2009; BORGES et al., 2014), destacando o potencial do uso da cultura em programas de biofortificação como alimento funcional.

A biofortificação dos alimentos, por meio da introdução de variedades melhoradas, além de complementar as intervenções em nutrição existente, proporciona maior sustentabilidade e baixo custo para produtores e consumidores. Realmente, pesquisas indicam que a deficiência de micronutrientes, principalmente a deficiência de zinco (Zn), ferro (Fe) e vitamina A afeta milhões de pessoas, sendo um dos principais problemas nutricionais em países em desenvolvimento (WHO, 2017). Como medidas de controle de curto prazo, suplementos encapsulados são utilizados, além da fortificação dos alimentos. Porém, esses processos dependem de infraestrutura de mercado e sistemas de saúde altamente funcionais que permitam o acesso das populações aos produtos gerados.

A investigação destinada à biofortificação tem sido realizada principalmente com mandioca (*Manihot esculenta*), arroz (*Oryza sativa*), milho (*Zea mays*), batata-doce (*Ipomoea batatas*) e feijão comum (*Phaseolus vulgaris*). Estas culturas foram incluídas na primeira fase do projeto coordenado pelo CGIAR HarvestPlus, com a participação de mais de 70 cientistas de 46 instituições de pesquisa em todo o mundo, incluindo a Embrapa. A biofortificação da banana (*Musa* spp.) está incluída na segunda fase, juntamente com culturas como a cevada (*Hordeum vulgare*), feijão caupi (*Vigna unguiculata*), lentilha (*Lens culinaris*), milheto (*Panicum miliaceum*), ervilha (*Cajanus cajan*), batata (*Solanum tuberosum*), sorgo (*Sorghum bicolor*) e inhame (*Dioscarea* spp.) (Pfeiffer & Bonnie, 2007).

A pesquisa no melhoramento genético de bananeira atualmente está focada, entre outros objetivos, na identificação e desenvolvimento de genótipos com quantidades significativas de amido resistente e fitoquímicos para produzir e acumular nutrientes importantes como vitaminas (e.g., vitamina A) e minerais, por estratégias de biofortificação (GHAG; GANAPATHI, 2018). Os frutos de *Musa* spp. são considerados boas fontes de minerais (i.e., potássio e fósforo) (EMAGA et al., 2007; SULAIMAN et al., 2014), e micronutrientes como o Zn e o Fe estão sendo amplamente pesquisados nestes programas (e.g. Harvest Plus), pelos grandes problemas de desnutrição relacionados a esses minerais, principalmente em países em desenvolvimento (GENC et al., 2005). A falta de um padrão de procedimento para medir a deficiência de Zn impede estimativas do número de pessoas com deficiências em Zn, mas cerca de 20% da população mundial está em risco (WUEHLER et al.,

2005). A deficiência de Fe é a mais comum, atingindo 1/3 da população mundial e as estimativas do WHO (World Health Organization) é que a maioria das crianças em idade pré-escolares e mulheres grávidas de países em desenvolvimento apresentam deficiência deste mineral (GENC et al., 2005).

Dentro do germoplasma *Musa* spp. foram identificados acessos com quantidades significativas de compostos bioativos, tais como o amido resistente, carotenoides, ácidos fenólicos e os flavonoides, que agem como antioxidantes no organismo, o que torna um alimento funcional perfeito para a melhoria da saúde humana (BORGES et al., 2014; GHAG; GANAPATHI, 2018; PEREIRA; MARASCHIN, 2015). A maioria dos estudos de identificação destes compostos bioativos em frutos de bananeira são focados em cultivares comerciais (e.g. subgrupo Cavendish) e algumas cultivares locais (ANYASI et al., 2018; GONZALEZ-MONTELONGO et al., 2010), o que torna interessante mais estudos dentro do extenso germoplasma de *Musa* spp. nos diferentes locais de pesquisa. Essa diversidade poderia ser explorada, a fim de identificar genótipos potencialmente adequados para serem utilizados em programas de melhoramento genético com foco na biofortificação da cultura e/ou para ser promovidos e incorporados nos sistemas agrícolas existentes para a diversificação da cultura e, consequentemente, do consumo popular.

A Embrapa Mandioca e Fruticultura (Cruz das Almas/BA) caracterizou parte da sua coleção de germoplasma quanto aos seus compostos nutricionais e funcionais dentro do programa de melhoramento genético, incluindo o aumento de vitamina A, i.e., biofortificação, introduzindo um importante objetivo no programa de melhoramento genético da cultura (AMORIM et al., 2011; BORGES et al., 2014). Os carotenoides são considerados compostos bioativos por desempenharem papéis fundamentais na saúde humana e são reconhecidos como as principais fontes de vitamina A. Essa vitamina pode ser consumida na forma biologicamente ativa, i.e., obtida de alimentos de origem animal ou na forma de provitamina, a partir de alimentos de origem vegetal. Cabe ressaltar, entretanto, que mais de 80% do consumo de vitamina A em países em desenvolvimento é derivado de fontes vegetais, sob a forma de carotenoides pro-vitamínicos A (pVACs), os quais são convertidos em retinol (vitamina A) no organismo humano. Dentre os precursores de vitamina A o β-caroteno é o que apresenta maior atividade. Além disso, possuem potencial de eliminar ou manter em equilíbrio as espécies reativas de oxigênio e são relacionadas com a diminuição da incidência de cânceres, doenças oculares e cardiovasculares, atuando

principalmente na interação com as membranas biológicas, afetando a sua estabilidade e a sua fluidez de diferentes maneiras para a eliminação e proteção contra os radicais livres (JOMOVA; VALKO, 2013). As cultivares comerciais de *Musa* spp., especialmente as do subgrupo Cavendish, não contêm quantidades significativas destes compostos. Entretanto, foram identificados acessos, dentro de bancos ativos de germoplasma, com alta quantidade de carotenoides (AMORIM et al., 2011; ARORA et al., 2008; BORGES et al., 2014; DAVEY et al., 2009; ENGLBERGER et al., 2010).

Estudos iniciais realizados com 61 acessos da coleção de germoplasma de banana da Embrapa encontraram uma ampla variabilidade genética no conteúdo de carotenoides totais entre os acessos analisados, principalmente em diploides (1,41 µg/g a 13,04 µg/g), indicando que o melhoramento para esse caractere pode ser obtido com êxito (AMORIM et al., 2011). BORGES et al. (2014) trabalhando com 12 acessos do mesmo banco ativo, verificaram grande quantidade de carotenoides pró-vitamina A (84,57%). A média de pVACs (provitamínicos A) foi de 231,15 µg/g (97,88 µg/g de *trans* β-caroteno), sendo que o valor mínimo encontrado foi para a cultivar 'Williams' (subgrupo Cavendish) com quantidades traços destes compostos (t-AC – *trans* α-caroteno e t-BC – *trans* β-caroteno). O diploide 'Jari Buaya', por sua vez, apresentou o maior conteúdo daqueles metabólitos e também luteína e zeaxantina, os quais, apesar de não terem função de pró-vitamina A, possuem ação antioxidante, protetora do sistema cardiovascular, antitumoral e contra a degeneração macular (RODRIGUEZ-AMAYA, 2001). Concomitantemente, verificou-se que a coloração da polpa é uma característica fenotípica que pode indicar a quantidade de carotenoides pVACs na banana. De fato, cultivares com coloração mais clara tendem a ter menores quantidades de carotenoides pró-vitamina A (AMORIM et al., 2011; BORGES et al., 2014; DAVEY et al., 2009; ENGLBERGER et al., 2010).

Pelos resultados de pesquisa é possível afirmar que a maioria dos carotenoides presentes nos frutos de banana são α-caroteno e β-caroteno (90%), os quais são precursores de vitamina A (DAVEY et al., 2009; BORGES et al., 2014). Essa proporção é bem diferente do que é evidenciado em outras culturas consideradas ricas em carotenoides, como o milho, onde os carotenoides precursores de vitamina A representam somente 10-20% e a maioria é luteína e zeaxantina (ORTIZ-MONASTERIO et al., 2007). Esta característica, somada ao alto consumo da banana em regiões onde problemas de hipovitaminose A são significativos (e.g., África - 162

kg/pessoa/ano – FAO, 2017), faz da banana uma fruta com grande potencial de uso em programas de biofortificação como alimento funcional.

Estudos de retenção realizados com diferentes genótipos de bananas e plátanos consumidas na África mostraram alta retenção de carotenoides (provitamínicos A) em diferentes formas de preparo utilizadas nas regiões estudadas (EKESA et al., 2012). As retenções de carotenoides após o processo de ebulição podem variar de 40 a 95%, dependendo da cultivar analisada. Aumento de duas vezes o total de pVACs foi descrito no genótipo 'Musilongo' após o tratamento em ebulição quando comparado ao valor *in natura* (EKESA et al., 2012). Os carotenoides podem sofrer modificações químicas e alterações em seus conteúdos em função do processo térmico nos diferentes métodos de preparo (EKESA et al., 2012). Estes estudos de retenção realizados até o momento foram feitos com cultivares locais e processamentos térmicos e modo de preparo específicos utilizados em algumas regiões na África, sendo necessários mais estudos para verificação do teor real nutricional e funcional de diferentes genótipos de *Musa* spp. e com diferentes processamentos térmicos, utilizados nos vários locais de consumo do fruto.

De forma similar aos carotenoides, os compostos fenólicos (e.g., flavonoides e ácidos fenólicos) constituem uma importante classe de metabólitos secundários notadamente em função de sua reconhecida atividade antioxidante, a qual confere qualidade ao alimento e potencial benefício à saúde humana. Estes antioxidantes neutralizam os radicais livres, inibindo a cadeia de iniciação ou interrompendo a cadeia de propagação das reações oxidativas, convertendo os radicais livres em moléculas menos prejudiciais e reparando os danos oxidativos nas células humanas (DU et al., 2009).

A polpa de banana e de plátanos, bem como as suas cascas, tem potencial para serem exploradas nas indústrias alimentares e farmacêuticas, principalmente pelo seu conteúdo de catequinas e de rutina (BORGES et al., 2014; TSAMO et al., 2015). Estes estudos mostram que bananas e plátanos são fontes tão importantes de compostos fenólicos, como outros vegetais considerados ricos nestes compostos, e.g. batata (90,85 mg EAG/100 g f.w.) (NAYAK et al., 2011) e beterraba (257 mg EAG/100g f.w.) (LIN; TANG, 2006), e a variação destes compostos em diferentes estádios de amadurecimento é genótipo-dependente. Além disso, há genótipos com quantidades superiores de compostos fenólicos, quando comparados com as cultivares mais comercializadas atualmente no Brasil (e.g., Grande Naine e Prata-Anã), sendo

interessante a promoção e incorporação de novos genótipos obtidos de bancos ativos de germoplasma com teores superiores destes bioativos, ou mesmo o uso dos mesmos em programas de melhoramento genético da cultura (BORGES et al., 2014).

Estudos *in vitro* e *in vivo* sugerem que os compostos fenólicos desempenham um papel importante contra uma ampla gama de distúrbios fisiológicos, e.g. câncer, diabetes, deficiências neurodegenerativas, desordens cardiovasculares, lesões gastrointestinais e danos ósseos (RODRÍGUEZ-MORATÓ et al., 2015). Os benefícios destes compostos à saúde humana são resultado de várias atividades biológicas como antioxidante, anti-inflamatória, modulação da expressão enzimática e quimioprevenção (MANOSROI et al.,2013). Em bananas, há estudos investigando suas habilidades para exercer essas atividades de promoção da saúde em modelos *in vitro*. No entanto, poucos estudos consideraram que muitas bananas passam por processos de cocção antes do consumo. Mudanças nos compostos fenólicos são de grande complexidade porque variam de acordo com sua estrutura, material alimentar original e o método de cozimento utilizado. Estudos inicias indicam que os tratamentos térmicos enfraquecem a parede celular e facilitam a liberação de compostos fenólicos (TSAMO et al., 2015). Em estudos com plátanos na África verificou-se que o método de ebulição em água com e sem casca aumenta a quantidade de fenóis em polpas, principalmente a ebulição dos frutos com a casca. No entanto, mais estudos sobre a biodisponibilidade destes compostos fenólicos e suas formas conjugadas devem ser realizados para confirmar se ferver com casca é realmente mais vantajoso do que ferver sem casca, já que são raros os estudos com bananas e processamentos térmicos. Além disso, vale a pena estudar outros processos de cozimento como vapor, assar e fritar para determinar qual destes processos de cozimento preserva melhor os compostos fenólicos em frutos de bananeira.

Além destes agentes quimiopreventivos, as poliaminas também têm sido objeto de muitos estudos, devido ao grande interesse com relação ao paradoxo nutricional e a sua possível ação como antioxidante. Estes compostos desempenham importantes funções metabólicas e fisiológicas em animais, vegetais e microrganismos. Em plantas, aminas como espermidina e espermina estão associadas a processos de crescimento e divisão celular, e por apresentarem o mesmo precursor do etileno, estão relacionadas com amadurecimento e senescência (KALAC, 2014). Algumas aminas estão envolvidas no controle e na regulação às respostas ao estresse biótico e abiótico que limitam a qualidade e vida útil pós-colheita de frutos (AGUDELO-

ROMERO et al., 2013; LIU et al., 2006). Outras aminas como histamina e serotonina, podem agir como substâncias protetoras contra insetos e fungos, enquanto que dopamina e noradrenalina estão relacionadas ao escurecimento enzimático em certos frutos, como bananas (MARRIOTT, 1980). Além disso, pesquisas indicam que as poliaminas e as aminas biogências (ABs) também podem contribuir para a atividade antioxidante em frutos e hortaliças (Adão & Glória, 2005; Lima et al., 2008). Estudos têm mostrado e.g., que a dopamina apresenta maior capacidade antioxidante *in vitro* (pelo ensaio DPPH), comparativamente a outros antioxidantes naturais, como ácido ascórbico, glutationa reduzida e vários compostos fenólicos, tal como a galocatequina (GONZÁLEZ-MONTELONGO et al., 2010).

Aminas bioativas são encontradas nos alimentos e, dependendo de suas concentrações, pode ser relevante não somente para o *shelf life* e qualidade final do produto, mas também para a saúde humana (KALAC, 2014). A serotonina tem sido detectada em quantidades elevadas em frutos de *Musa* spp., principalmente quando comparados com outras frutas e hortaliças (ISLAM et al., 2016). Segundo Xiao et al. (1998), com a ingestão de banana, considerada relativamente rica em serotonina, ocorre um aumento do nível de serotonina no sangue. Considerando os efeitos antiobesidade da serotonina, é importante na ciência hortícola, a investigação do conteúdo deste composto em diferentes genótipos de frutos e hortaliças.

A banana tem sido apontada também como uma fonte promissora de pesquisa de dopamina (L-DOPA) para o futuro desenvolvimento de formulações farmacêuticas para o tratamento de doenças, como Mal de Parkinson (PEREIRA; MARASCHIN, 2015). Além da importância da determinação do conteúdo destes compostos nos alimentos, em prol dos benefícios acarretados para a saúde humana, a determinação de aminas bioativas em alimentos também assume grande importância pelo efeito tóxico de algumas aminas, como histamina e tiramina, dependendo da quantidade. Quando consumidas em excesso, podem causar distintos efeitos farmacológicos, fisiológicos e tóxicos. Os níveis das aminas bioativas são influenciados pela composição do alimento, flora microbiana, armazenamento (e.g., temperatura, amadurecimento e armazenamento) e tipo de processamento (ADÃO; GLÓRIA, 2005; BOMTEMPO et al., 2016; PLONKA; MICHALSKI, 2017) e é de suma importância estudos do plantio até o consumo final.

Estudos indicam que os níveis das aminas variam conforme os estádios de desenvolvimentos dos frutos, sendo que essa variação depende do genótipo

analisado, sendo necessários estudos para verificar a ocorrências dessas aminas e seu papel na fisiologia dos frutos e nas propriedades promotoras da saúde de acessos de *Musa* spp. Estudos de retenção das aminas bioativas em diferentes processamentos térmicos indicam que a exposição prolongada aos processamentos térmicos resulta em perdas substanciais desses compostos bioativos (PLONKA; MICHALSKI, 2017). Raros são os estudos encontrados em frutos após o processamento térmico, incluindo em banana, o que torna essencial pesquisas com diferentes cultivares e os diferentes modos de preparo domésticos realizados nas diferentes regiões de consumo, e com isso, conhecer o real papel destes compostos amínicos na dieta humana.

Sendo assim, a presente proposta enfoca a determinação do perfil bioquímico, com ênfase na composição de amido resistente, carotenoides, compostos fenólicos e aminas bioativas de genótipos e/ou novos híbridos melhorados de bananeira pertencentes a coleção de germoplasma da Embrapa (Latitude 12º40'12''S; Longitude 39º06'07''W) ao longo do processo de maturação dos frutos e após o processamento térmico, em conexão com a seleção de genótipos superiores ao programa de melhoramento genético daquela empresa, visando à geração de cultivares biofortificadas e/ou com melhor qualidade na pós-colheita de *Musa* spp.

## OBJETIVOS

**Geral:**

Caracterizar o perfil bioquímico de genótipos de bananeira do banco ativo de germoplasma da Embrapa, ao longo do processo de amadurecimento dos frutos, bem como nos diferentes processamentos térmicos domésticos, com ênfase em amido, amido resistente, minerais, carotenoides, compostos fenólicos e aminas bioativas.

**Específicos:**

- Caracterizar os diferentes genótipos quanto ao teor de amido e amido resistente;
- Determinar os minerais K, Na, Zn e Fe por espectrômetro de absorção atômica durante o processo de amadurecimento dos frutos;

- Caracterizar o perfil carotenoídico por cromatografia líquida de ultra eficiência em fase reversa (HPLC) e a concentração de carotenoides totais por meio de espectrofotometria UV-visível durante o processo de amadurecimento dos frutos;

- Determinar o perfil de compostos fenólicos por UHPLC e a absorção espectral e a concentração de fenóis totais, flavonoides totais por meio de espectrofotometria UV-visível durante o processo de amadurecimento dos frutos;

- Determinar o conteúdo de aminas livres via UHPLC, durante a maturação dos frutos, ao longo do processo de armazenamento;

- Determinar a atividade antioxidante *in vitro* (DPPH, FRAP e ABTS) do extrato metanólico obtido da polpa dos frutos dos genótipos em estudo e correlacionar com os compostos bioativos analisados;

- Avaliar os frutos na sua forma de consumo e/ou em diferentes formas de processamento térmico, para investigar a retenção dos compostos analisados;

- A partir do conjunto total de dados bioquímicos de frutos de *Musa* spp. e de técnicas de bioinformática e quimiometria (ACP), desenvolver uma ferramenta de discriminação da variabilidade química dos acessos, auxiliando a identificação e seleção de genótipos de interesse, i.e., melhoramento genético assistido bioquimicamente.

## Capítulo 1

**Post-harvest physicochemical profile and bioactive compounds of 19 bananas and plantains genotypes**

(artigo aceito para publicação no periódico científico Bragantia)

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**ABSTRACT:** Nineteen genotypes of bananas and plantains were analyzed in order to differentiate the subgroups and/or groups of consumption or industrial use. Genotypes of banana and plantain from different genomic groups and in three ripening stages (2, 5 and 7) were studied in relation to physical and physicochemical characteristics, including bioactive compounds. Furthermore, with the obtained data analysed by multivariate statistical analyses (Principal Component Analysis) it was possible to relate all analyzed characteristic profile of samples with the different genotype. The three ripening stages were differentiate by total soluble solids, titratable acidity, chrome (C*) and the carotenoids contents. 'Ney Poovan' contain high total soluble solid content and pulp-to-peel ratio, an interesting result for the promotion of this genotype for *in natura* consumption. 'Ney Poovan', 'Ouro da Mata', 'Pelipita' and 'Tiparot' are sources of antioxidant compounds. The genotypes 'Pelipita' and 'Samurá B' are promising for the industrial use, mainly for the processing of banana chips, for both green and ripe fruit.

**Key words:** *Musa* spp., carotenoids, vitamin C, cooking banana

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## 1.1 Introduction

Banana and plantains cultivation is an activity of great economic and social importance. Their worldwide production represents around 107 million tons and are the fourth most produced food in the world (FAO 2017). In comparison to other tropical fruits, the consumption of bananas and plantains is high, mainly due to its versatility in use (*in natura* consumption, processing, fried, cooked, among others), and flavor and aroma characteristics. The edible bananas (*Musa* spp.) are generally classified according to the consumption mode (dessert or cooking bananas) and the constitution of their genome (AA, AB, BB, AAA, AAB, ABB, AAAA, AAAB, and ABBB) (Jesus et al. 2013). The botanic classification is based in morphologic characteristics that help in the differentiation of dessert bananas (AA, AAA and AAB), cooking bananas (AAA, AAB and ABB) and plantains (AAB). During the ripening, there are modifications in the physicochemical characteristics associated to the organoleptic and nutritional alterations. Dessert bananas are consumed *in natura* in advanced ripening stages (5, 6 and 7), depending on the consumer preference. However, cooking bananas are consumed in many ripening stages, going through a cooking process and are not generally appreciated in its *in natura* form (e.g., absence of sweetness and unpleasant firmness) (Gibert et al. 2009).

Physicochemical and biochemical characteristics are influenced by many factors, such as the genotype and ripening stage, which contribute to the differentiation and variation of these characteristics. Furthermore, these parameters help to identify the best application for each genotype (e.g., banana for *in natura* consumption, banana candy, banana chips, banana pulp, among others). Studies have indicated that banana and plantain fruit contain appreciable quantities of antioxidant compounds, such as carotenoids (Yan et al. 2016) and phenolic compounds (e.g. flavonoids). Analysis of physicochemical and biochemical parameters (fruit quality) such as peel color, pulp firmness, soluble solids (SS), pH, titratable acidity (TA), and bioactive compounds, will be useful for the characterization and selection of genotypes with superior characteristics for genetic improvement, as well as for the introduction of new varieties in existent agricultural systems. Thus, the aim of this study was to analyze physicochemical and biochemical characteristics in different banana fruit genotyped of dessert, nonplantain cooking and plantain cooking in three different stages of ripening, in order to differentiate the subgroups and/or groups of consumption.

## 1.2 Materials and Methods

The plant material consisted of 19 banana genotypes from different genomic groups maintained in the Active Germplasm Bank of Embrapa Cassava & Fruits (Latitude 12º40'12'' S; Longitude 39º06'07'' W; Altitude 225 m) (Table 1). This working collection was organized in six genotypes groups based on their consumption mode, genomic constitution and morphological characters. When the fruits reached the ripening stage (stg) 1, central bunches of each genotype were harvested (2 bunches = 40 fruit) and they were stored at room temperature (20 ± 2 °C) and relative humidity (80 ± 2 %), without ethylene treatment, until complete the desired ripening stage. The three ripening stages assessed, 2, 5 and 7, corresponded to the scale described by Soltani et al. (2011) and Yan et al. (2016) and were: stage 2 - all green; stage 5 - yellow with green ends; and stage 7 - yellow with brown areas.

**Table 1.** Accessions of banana belonging to the Active Germplasm Bank of Embrapa Cassava & Fruits.

| Accesses | Ploidy | Subgroup/Subspecies | Use form |
|---|---|---|---|
| Yangambi Km5 (Ykm5) | AAA | Ibota | In natura |
| Grande Naine (GN) | AAA | Cavendish | In natura |
| Khai (Kh) | AAA | Ibota | In natura |
| Prata-Anã (PA) | AAB | Prata | In natura |
| Pisang Kepok Bung (PKB) | AAB | Peyan | In natura |
| Ney Poovan (NP) | AB | Ney Poovan | In natura |
| Ouro da Mata (OM) | AAAB | - | In natura |
| Monthan 172 (M172) | ABB | Monthan | Cooked |
| Simili Radjah (SR) | ABB | Peyan | Cooked |
| Pelipita (PPT) | ABB | Bluggoe | Cooked |
| Pacha Nadan (PN) | ABB | Saba | Cooked |
| Namwa Khom (NK) | ABB | Pisang Awak | Cooked |
| Muisa Tia (MT) | ABB | Pisang Awak | Cooked |
| FC06-02 (F02) | AABB | Figo | Cooked |
| Tiparot (TPT) | ABBB | Klue Teparod | Cooked |
| D'Angola (DA) | AAB | Plantain | Cooked |
| Terra Sem Nome (TSN) | AAB | Plantain | Cooked |
| Terra Anã Branca (TAB | AAB | Plantain | Cooked |
| Samurá B (SB) | AAB | Plantain | Cooked |

The fruits were washed and separated into pulp and peel. The pulps were ground to a fine powder (IKA, A.11, Germany) in liquid nitrogen, lyophilized and stored at - 80 °C. The pulp was cut in the length and across the width, creating four quarters. With the quarters, two groups were created, one for biochemical analysis, and other for physicochemical analysis. Three banana/plantain fruits constituted each analysis (n = 3) and all analyses were performed in triplicate.

Fruit firmness (N) was determined using a TA-XT2i texture analyzer (Stable Micro System Ltd., Gidalming, UK), with an 8 mm diameter probe at a speed of 2 mm s$^{-1}$ and penetration of 10 mm (two measures in the central part of unpeeled fruit). The SS content was obtained using a manual refractometer (Atago, model N-1E, Atago Co. Ltd., Japan) and the results were expressed in ° Brix (AOAC 2005). The pH was determined in aqueous solution, using approximately 10 g of banana pulp in 100 mL of distilled water (IAL 2008) and same aqueous extract it was measured the titratable acidity, with standardized solution (0.0996 N NaOH) (IAL, 2008). Dry weight (%) was determined by oven drying for 24 h at 105 °C (AOAC 2005). CIE colour values of luminosity (*L\**), chromaticity (*C\**) and angle Hue (H\*) for each fruit on both peel and pulp were determined using the spectrocolorimeter (CR 410 Chroma Meter, Konica Minolta, Osaka, Japan). Peel thickness was measured with a digital caliper (Jomarca®, São Paulo, Brazil) in a central portion of the peel. The pulp-to-peel ratio was determined by pulp fresh weight and peel fresh weight.

Total carotenoid contents were determined according to Lichtenthaler (1987), with minor modifications. Dry samples (200 mg) were extracted twice with 80 % acetone by sonication for 30 min. The extracts were combined and centrifuged at 3,800 rpm (10 min) and the absorbance (A663, A646 and A455) of the acetone extracts was measured at 663, 646, and 455 nm, respectively, using a UV-Vis spectrophotometer Ultrospec 3000 (Pharmacia Biotech, Uppsala, Sweden) and expressed in μg g$^{-1}$ DW (dry weight).

Ascorbic acid (AA) and dehydroascorbic acid (DHAA) were measured according to the method of (Pertuzatti et al. 2015), with modifications. In fifty mg of banana pulp were added 5 mL of cold extraction solution, consisted of 10 g of metaphosphoric acid (4.5% w/v) and 40 mL of glacial acetic acid. Afterwards, the tubes were homogenized in vortex (1 min) and incubated for 30 min in ultrasonic bath at 5 °C. The samples were centrifuged at 3,800 rpm for 15 min. The residue was twice subjected to similar procedures of extraction, and the supernatants were combined to reach a final volume of 15 mL. The sample was transferred to a 1.5 mL vial, and 20 μL were injected into a UPLC system (Ultimate 3000, Dionex-Thermo Fisher Scientic Inc., San Jose, USA) equipped with a diode array detector and Ace 5 C18 (Advanced Chromatography Technologies, ACT, UK) column (5μm, 250 x 4.6 mm). The mobile phase was 2% acetic acid in an isocratic flow of 0.5 mL min$^{-1}$. The column temperature was set to 25 °C, and the detection wavelength was 248 nm for ascorbic acid and 240 nm for the

dehydroascorbic acid. The results were expressed in mg AA or Vitamin C 100 g$^{-1}$ (DW). The total flavonoids was performed according to Popova et al. (2005) with adjustments. Fresh pulp or peel powdered in liquid nitrogen was homogenized with 10% acidified methanol. After 30 min in ultrasonic bath, 5 % AlCl$_3$ (w/v) was added and the samples were centrifuged for 20 min at 3,800 rpm (Mikro220R, Hettich Zentrifugen, Tuttlingen, Germany). Finally, the samples were filtered, and the absorbance was measured at 425 nm.  The results were expressed as mg of quercetin equivalents (QE) per 100 g dry weight (DW).

All analysis were conducted in entirely randomized design, with factorial scheme 19 x 3 (genotypes x ripening stages), with three repetitions (three fruit by parcel). The data were collected, summarized and submitted to the variance analysis (ANOVA), followed by Scott Knott averages comparison test among the genotypes, and Tukey test among the ripening stages, using the SISVAR program. Pearson's correlation and principal component analysis (PCA) were performed for the physicochemical and biochemical data using the statistical analysis software XLSTAT Version 2014.2.03 (STATCON, Witzenhausen, Germany).

## 1.3 Results and Discussion

Significant differences were observed in the indexes of color L$^*$ and C$^*$ of the peel and pulp, firmness (with peel and unpeeled), soluble solids (SS), pH and dry weight pulp, total flavonoids and carotenoids and vitamin C content (ascorbic and dehydroascorbic acid), during the ripening process and in different genotypes. Aiming to establish a descriptive model of grouping the ripening stage in function of physicochemical characteristics, we compared the results by principal components analysis (PCA). The dispersion of the genotypes, according to the PC1 and PC2 axis (Figure 1a, 1b), show the existence of three groups, correspondent to each ripening stage analyzed. PC1 and PC2 explained 63.96 % of the data variance. The three groups are separated by the first principal component (PC1) in ascending order from left to right. The PC1 axis represents 43.11 % of the total variance, separating the green fruit from the ripe ones. SS, TA, C$^*$ pulp, carotenoids and vitamin C content are positively correlated with the ripening stage, suggesting that these parameters

increase during the ripening process. L\* and C\* peel also have a positive correlation with the ripening stage, but in less extension.
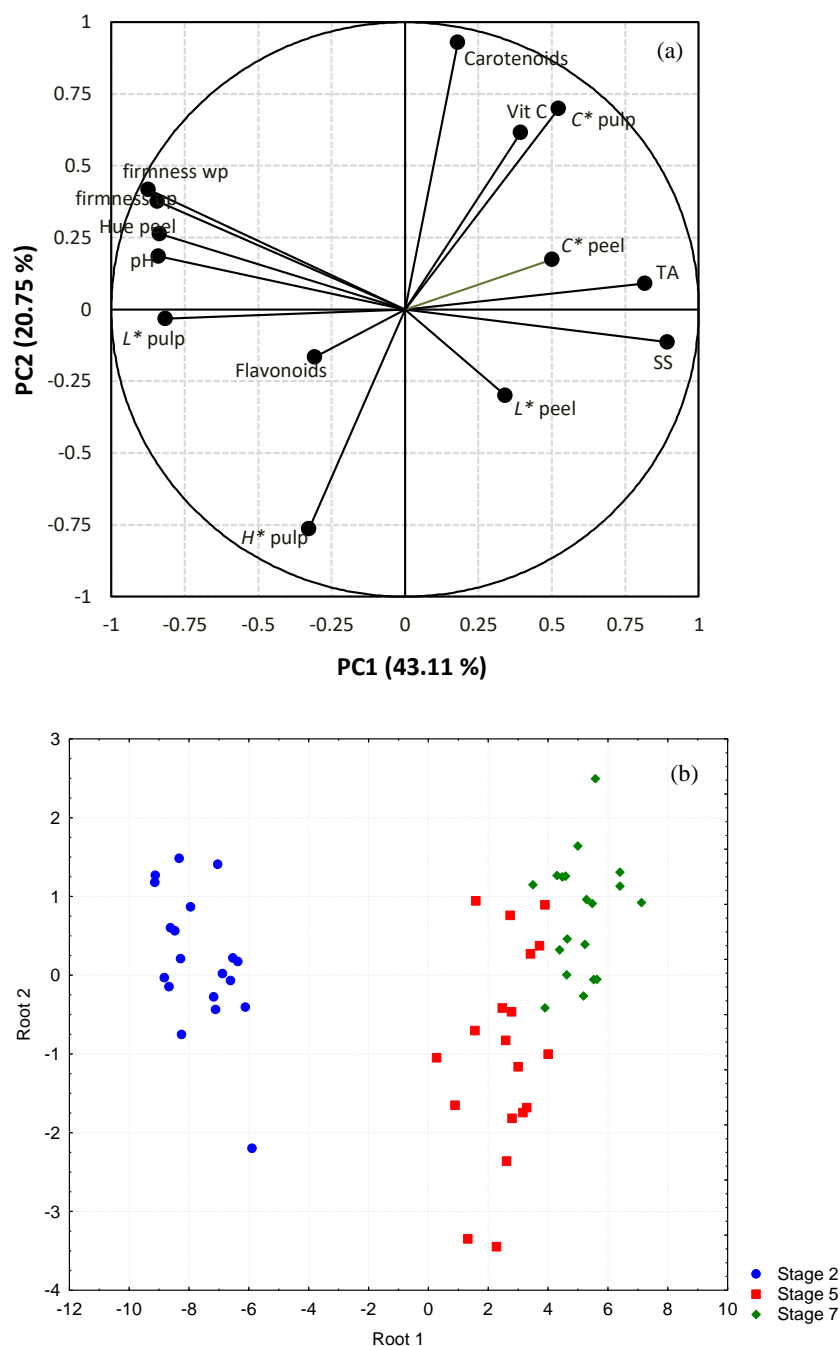


**Figure 1.** Two-dimensional projection (a) and scores (b) of physicochemical and biochemical attributes in the two first main components among the 19 genotypes of banana fruit evaluated in three ripening stages. Stage 2 – all green; stage 5 – yellow with green ends; and stage 7 – yellow with brown.

There was an increase in L\* peel values during the banana fruit ripening process (until stg 5) due to the change of color from the unripe (green) to ripe (coloration completely yellow) (Table 2).

**Table 2.** Coordinate luminosity ($L^*$), Chrome ($C^*$) and angle Hue ($H^*$) at ripening stages 2, 5 and 7 in the post-harvest (20 ± 2 °C and RH 85 ± 2 %) of the peel of *Musa* spp. genotypes.

| Genotypes | $L^*$ | | | $C^*$ | | | $H^*$ | | |
|---|---|---|---|---|---|---|---|---|---|
| | Stg 2 | Stg 5 | Stg 7 | Stg 2 | Stg 5 | Stg 7 | Stg 2 | Stg 5 | Stg 7 |
| Dessert bananas | | | | | | | | | |
| Yagambi Km5 | 57.4 ± 1.2$^{bC\#}$ | 65.4 ± 2.4$^{aA}$ | 62.9 ± 3.5$^{aA}$ | 22.3 ± 2.3$^{aB}$ | 32.0 ± 1.7$^{bA}$ | 32.9 ± 3.0$^{aA}$ | 116.6 ± 0.5$^{bA}$ | 80.4 ± 1.8$^{bB}$ | 79.7 ± 3.5$^{aC}$ |
| Grande Naine | 49.8 ± 1.2$^{dB}$ | 56.6 ± 0.3$^{cA}$ | 54.1 ± 3.9$^{cA}$ | 28.24 ± 4.3$^{aA}$ | 28.0 ± 0.6$^{bA}$ | 27.9 ± 2.5$^{bA}$ | 122.9 ± 1.7$^{aA}$ | 82.8 ± 3.6$^{aB}$ | 75.0 ± 2.7$^{bC}$ |
| Khai | 63.8 ± 0.6$^{aB}$ | 68.6 ± 1.7$^{aA}$ | 58.1 ± 1.2$^{bC}$ | 27.1 ± 2.7$^{aB}$ | 36.0 ± 2.3$^{aA}$ | 27.5 ± 2.3$^{bB}$ | 111.2 ± 1.5$^{cA}$ | 84.6 ± 1.4$^{aB}$ | 79.9 ± 0.8$^{aB}$ |
| Prata-Anã | 49.4 ± 0.2$^{dC}$ | 62.5 ± 1.4$^{bA}$ | 55.4 ± 2.5$^{bB}$ | 18.8 ± 1.1$^{bC}$ | 39.2 ± 2.9$^{aA}$ | 30.4 ± 3.3$^{aB}$ | 126.0 ± 1.4$^{aA}$ | 86.2 ± 1.2$^{aB}$ | 77.4 ± 2.5$^{aC}$ |
| Pisang K. Bung | 49.8 ± 1.3$^{dB}$ | 55.4 ± 3.9$^{cA}$ | 51.5 ± 1.2$^{cB}$ | 11.7 ± 4.3$^{cB}$ | 25.0 ± 5.3$^{cA}$ | 16.9 ± 1.8$^{cB}$ | 121.1 ± 1.0$^{bA}$ | 80.3 ± 3.4$^{bB}$ | 70.3 ± 2.9$^{cC}$ |
| Ney Poovan | 54.4 ± 2.7$^{bA}$ | 56.6 ± 1.8$^{cA}$ | 48.6 ± 1.8$^{dB}$ | 32.2 ± 6.8$^{aA}$ | 24.4 ± 2.6$^{cB}$ | 14.7 ± 1.3$^{cC}$ | 110.9 ± 3.3$^{cA}$ | 77.8 ± 1.2$^{bB}$ | 52.9 ± 0.6$^{eC}$ |
| Ouro da Mata | 56.6 ± 2.0$^{bC}$ | 67.2 ± 0.2$^{aA}$ | 61.2 ± 0.9$^{aB}$ | 24.1 ± 3.3$^{aB}$ | 37.2 ± 1.2$^{aA}$ | 31.5 ± 0.2$^{aA}$ | 120.2 ± 1.3$^{bA}$ | 88.3 ± 1.4$^{aB}$ | 78.9 ± 0.5$^{aC}$ |
| Mean | 54.5 | 61.7 | 56 | 23.4 | 27.7 | 26.0 | 85.5 | 82.9 | 73.5 |
| Nonplantain cooking | | | | | | | | | |
| Monthan 172 | 53.8 ± 1.0$^{bB}$ | 61.7 ± 2.2$^{bA}$ | 52.4 ± 0.8$^{cB}$ | 15.1 ± 3.8$^{bB}$ | 27.8 ± 2.5$^{bA}$ | 19.1 ± 1.8$^{cB}$ | 117.4 ± 0.6$^{bA}$ | 80.1 ± 0.8$^{bB}$ | 72.9 ± 2.5$^{bC}$ |
| Simili Radjah | 49.3 ± 0.3$^{dA}$ | 50.1 ± 3.4$^{cA}$ | 47.58 ± 3.4$^{dA}$ | 10.0 ± 2.7$^{cB}$ | 19.3 ± 3.1$^{dA}$ | 18.5 ± 1.7$^{cA}$ | 102.4 ± 4.1$^{dA}$ | 64.7 ± 4.5$^{dB}$ | 53.4 ± 3.5$^{eC}$ |
| Pelipita | 51.3 ± 1.2$^{cB}$ | 56.2 ± 1.1$^{cA}$ | 52.8 ± 1.2$^{cB}$ | 12.2 ± 1.2$^{cB}$ | 31.7 ± 3.7$^{bA}$ | 28.6 ± 3.2$^{bA}$ | 125.9 ± 0.1$^{aA}$ | 67.1 ± 4.3$^{dB}$ | 65.4 ± 2.0$^{dB}$ |
| Pacha Nadan | 56.4 ± 1.5$^{bB}$ | 65.8 ± 2.4$^{aA}$ | 57.6 ± 5.0$^{bB}$ | 26.0 ± 2.4$^{aA}$ | 32.5 ± 3.8$^{bA}$ | 26.6 ± 3.8$^{bA}$ | 114.7 ± 0.3$^{cA}$ | 83.8 ± 1.9$^{aB}$ | 77.5 ± 3.7$^{aC}$ |
| Namwa Khom | 52.5 ± 2.5$^{cB}$ | 54.7 ± 3.2$^{cA}$ | 51.6 ± 4.1$^{cB}$ | 16.4 ± 3.0$^{bA}$ | 18.6 ± 5.2$^{dA}$ | 17.66 ± 3.2$^{cA}$ | 111.3 ± 2.0$^{cA}$ | 80.2 ± 5.4$^{bB}$ | 74.2 ± 2.1$^{bC}$ |
| Muísa Tia | 51.6 ± 1.9$^{cC}$ | 59.3 ± 0.8$^{cA}$ | 55.6 ± 2.0$^{bB}$ | 20.0 ± 7.3$^{bA}$ | 26.0 ± 3.8$^{cA}$ | 19.38 ± 3.8$^{cA}$ | 117.7 ± 3.2$^{bA}$ | 85.6 ± 1.1$^{aB}$ | 68.8 ± 3.1$^{cC}$ |
| FC06-02 | 54.5 ± 0.9$^{bA}$ | 53.2 ± 0.4$^{cA}$ | 46.6 ± 1.8$^{dB}$ | 17.7 ± 3.3$^{bB}$ | 24.6 ± 2.1$^{cA}$ | 15.26 ± 2.6$^{cB}$ | 104.8 ± 4.4$^{dA}$ | 68.2 ± 1.9$^{dB}$ | 53.6 ± 3.4$^{eC}$ |
| Tiparot | 51.2 ± 1.7$^{cB}$ | 56.4 ± 1.2$^{cAB}$ | 54.1 ± 1.9$^{bA}$ | 15.1 ± 2.7$^{bB}$ | 24.0 ± 2.3$^{cA}$ | 17.34 ± 1.7$^{cB}$ | 127.4 ± 3.0$^{aA}$ | 77.3 ± 4.3$^{bB}$ | 66.0 ± 2.9$^{dC}$ |
| Mean | 52.6 | 57.2 | 52.3 | 16.6 | 25.6 | 20.3 | 85.2 | 75.9 | 66.5 |
| Plantain cooking | | | | | | | | | |
| D'Angola | 47.7 ± 1.8$^{dB}$ | 54.6 ± 2.3$^{cA}$ | 51.1 ± 1.0$^{cB}$ | 22.26 ± 3.3$^{aA}$ | 29.0 ± 3.6$^{bA}$ | 23.3 ± 1.7$^{cA}$ | 118.6 ± 2.2$^{bA}$ | 81.3 ± 1.1$^{bB}$ | 72.3 ± 1.8$^{bC}$ |
| Terra S. N. | 52.6 ± 3.6$^{cA}$ | 56.5 ± 2.6$^{cA}$ | 53.5 ± 0.7$^{cA}$ | 27.44 ± 2.1$^{aA}$ | 28.2 ± 3.1$^{bA}$ | 25.3 ± 1.7$^{bA}$ | 111.9 ± 1.7$^{cA}$ | 81.2 ± 2.3$^{bB}$ | 75.5 ± 3.1$^{bC}$ |
| Terra A. B. | 48.5 ± 1.8$^{dB}$ | 56.5 ± 1.3$^{cA}$ | 54.16 ±1.7$^{cA}$ | 27.15 ± 4.0$^{aA}$ | 28.2 ± 5.0$^{bA}$ | 24.6 ± 2.3$^{bA}$ | 119.6 ± 1.0$^{bA}$ | 73.5 ± 3.8$^{cB}$ | 76.5 ± 0.3$^{aB}$ |
| Samurá B | 48.3 ± 1.8$^{dB}$ | 57.7 ± 0.4$^{cA}$ | 52.23 ± 0.7$^{cB}$ | 25.83 ± 4.2$^{aB}$ | 31.1 ± 2.9$^{bA}$ | 33.9 ± 1.6$^{aA}$ | 119.6 ± 1.8$^{bA}$ | 80.3 ± 0.4$^{bB}$ | 75.5 ± 1.4$^{bB}$ |
| Mean | 49.3 | 56.3 | 52.7 | 25.6 | 29.1 | 26.7 | 87.5 | 79.0 | 75.0 |

$\#$Values in the same column followed by different lower case (genotypes) and in the same row followed by different upper case letters (ripening stages), for each parameter, differ by the Scott Knott test ($p<0.01$) (genotypes) and by Tukey test ($p<0.01$) (ripening stages). Stage 2 – all green; stage 5 – yellow with green ends; and stage 7 – yellow with brown.

In stg 7, there was a decrease in L* peel values, due to the increase of dark pigmentation, characteristic of ripening stage. Changes in the peel and in the pulp color (Tables 2 and 3) associated with the ripening can be described by the evolution of L* and C*. Luminosity (L*) decrease and chrome (C*) increase reflect the decrease of whiteness and the raise of the color intensity. An increase of L* and C* of the peel to a maximum level at stg 5 expresses the change from green to yellow, due to the degradation of chlorophyll and the accumulation of carotenoids. The genotypes Simili Radjah and Namwa Khom showed lower values of C* and higher Hue* angle, which demonstrates to be the genotypes with the lightest pulp color (Table 3). With the ripening there is an increase of color intensity (*C), while the Hue* angle decreases, indicates that the pulp gets more yellow/orange with the ripening (Aquino et al. 2017). The genotype Namwa Khom showed the highest intensity only when the fruit reached the stage 7. The highest *C indicates that the pulp has a higher intensity of the yellow/orange color (Table 3). The genotypes Khai, Simili Radjah and FC06-02 presented the highest color intensity by the Hue* angle in the stages 5 and 7. In addition, SS, pH and TA acidity strongly influenced the fruit separation in the stages 5 and 7 (Figure 1). Fruit softening, SS, pH and TA acidity content are factors that indicate the ripening and quality of fruit. The increase of SS and decrease of pH was observed during the ripening process and the content depends on genotype and on ripening stage. During the ripening, the lower pH was found in fruit in stages 5 and 7, similar with the results found in other genotypes of *Musa* spp. In this process, pH decrease and acidity increase, inducing a raise in the acid flavor on the fruit (Youryon and Supapvanich 2017) (Figure 2). Pulp pH decrease is associated with the accumulation of some acids, mainly malic acids in bananas, promoting acidity alteration and inducing acid flavor (Newilah et al. 2009).

According to previous studies, fruit with high SS levels are the ones that present the highest possibility of acceptance (Gibert et al. 2009). There were SS variations, as 4.51 to 23.38 ° Brix in dessert bananas (Figure 2a), 7.18 to 25.37 in cooking bananas (Figure 2b) and 7.19 to 26.87 in plantains (Figure 2c).

The fruit green (e.g. plantain) also present higher percentage dry weight (Figure 2i) and firmness (Figure 3). A clear differentiation was verified among the subgroup and/or genotype of bananas in relation to SS, dry weight and firmness, based on its consumption.

**Table 3.** Coordinate luminosity (*L\**), Chrome (*C\**) and angle Hue (*H\**) at ripening stages 2, 5 and 7 in the post-harvest (20 ± 2 °C and RH 85 ± 2 %) of the pulp of *Musa* spp. genotypes.

| Genotypes | *L\** | | | *C\** | | | *H\** | | |
|---|---|---|---|---|---|---|---|---|---|
| | Stg 2 | Stg 5 | Stg 7 | Stg 2 | Stg 5 | Stg 7 | Stg 2 | Stg 5 | Stg 7 |
| Dessert bananas | | | | | | | | | |
| Yagambi Km5 | 81.2 ± 0.4[aA#] | 74.1 ± 0.4[cB] | 73.0 ± 0.3[cB] | 24.7 ± 0.3[eB] | 30.9 ± 1.7[bA] | 30.9 ± 0.7[bA] | 93 ± 0.3[aA] | 89.4 ± 0.6[bB] | 88.7 ± 0.1[aB] |
| Grande Naine | 80.8 ± 1.4[aA] | 73.0 ± 0.6[cB] | 71.8 ± 0.7[dC] | 29.6 ± 1.1[dC] | 34.01 ± 4.5[bB] | 36.2 ± 7.0[aA] | 90.6 ± 0.5[bA] | 84.0 ± 1.0[dB] | 83.5 ± 0.6[dB] |
| Khai | 78.8 ± 0.4[bA] | 74.1 ± 0.4[cB] | 71.9 ± 4.5[dB] | 25.9 ± 0.9[eB] | 29.02 ± 0.5[cA] | 30.7 ± 0.7[bA] | 92.7 ± 0.2[aA] | 90.8 ± 0.4[aB] | 90.3 ± 1.7[aB] |
| Prata-Anã | 81.7 ± 1.5[aA] | 76.8 ± 0.4[bB] | 75.3 ± 0.6[bB] | 26.9 ± 3.9[dB] | 32.12 ± 0.1[bA] | 32.8 ± 2.9[bA] | 88.6 ± 0.8[cA] | 86.4 ± 0.3[cB] | 86.4 ± 1.0[bB] |
| Pisang K. B. | 81.9 ± 0.4[aA] | 76.4 ± 2.1[bB] | 72.8 ± 1.7[cC] | 23.4 ± 2.2[eB] | 26.5 ± 0.2[cA] | 27.7 ± 0.6[cA] | 85.2 ± 1.6[dA] | 85.2 ± 1.2[dA] | 82.2 ± 2.1[dB] |
| Ney Poovan | 77.2 ± 1.3[bA] | 76.5 ± 1.4[bA] | 75.8 ± 1.6[bA] | 28.5 ± 1.2[dA] | 29.1 ± 2.2[cA] | 27.3 ± 1.1[cA] | 87.6 ± 1.4[cA] | 87.3 ± 2.1[cA] | 87.0 ± 1.3[bA] |
| Ouro da Mata | 82.7 ± 1.3[aA] | 71.5 ± 1.4[dB] | 71.9 ± 1.3[dB] | 21.0 ± 0.5[fC] | 24.9 ± 0.6[dB] | 31.5 ± 0.3[bA] | 89.2 ± 0.8[cA] | 86.5 ± 0.5[0B] | 85.7 ± 0.5[cB] |
| Mean | 80.6 | 74.7 | 73.2 | 25.7 | 29.5 | 31.0 | 89.6 | 87.1 | 86.3 |
| Nonplantain cooking | | | | | | | | | |
| Monthan 172 | 80.8 ± 0.3[aA] | 76.5 ± 0.3[bB] | 76.3 ± 0.3[bB] | 27.1 ± 0.2[dA] | 26.8 ± 0.7[cA] | 29.9 ± 0.8[bA] | 85.1 ± 0.4[dA] | 84.7 ± 0.5[dA] | 85.0 ± 0.3[cA] |
| Simili Radjah | 84.5 ± 1.2[aA] | 78.2 ± 1.6[aB] | 75.1 ± 0.7[bC] | 16.5 ± 0.5[gB] | 24.4 ± 0.9[dA] | 24.7 ± 0.6[dA] | 91.9 ± 0.9[aA] | 91.1 ± 1.2[aA] | 89.5 ± 0.0[aB] |
| Pelipita | 82.4 ± 1.1[aA] | 76.0 ± 0.2[bB] | 73.7 ± 1.4[cB] | 28.7 ± 0.2[dB] | 36.2 ± 1.2[aA] | 36.1 ± 1.8[aA] | 81.5 ± 0.9[eA] | 77.5 ± 0.2[fB] | 78.3 ± 0.2[eB] |
| Pacha Nadan | 81.1 ± 1.0[aA] | 74.6 ± 0.1[cB] | 74.2 ± 0.7[cB] | 24.6 ± 1.6[eB] | 29.1 ± 1.6[cA] | 32.1 ± 0.2[bA] | 89.0 ± 0.6[cA] | 85.4 ± 0.5[dB] | 86.9 ± 1.3[bB] |
| Namwa Khom | 79.5 ± 0.3[bA] | 76.6 ± 1.2[bB] | 77.3 ± 1.4[aAB] | 22.2 ± 0.6[eA] | 22.5 ± 1.9[eA] | 22.9 ± 1.6[dA] | 88.3 ± 0.2[cA] | 89.2 ± 0.3[bA] | 89.2 ± 0.5[aA] |
| Muísa Tia | 81.7 ± 0.8[aA] | 78.3 ± 1.2[aB] | 77.8 ± 1.4[aB] | 16.4 ± 0.4[gB] | 19.8 ± 0.6[fA] | 21.3 ± 2.8[dA] | 87.7 ± 0.9[cA] | 87.6 ± 1.4[cA] | 88.8 ± 0.3[aA] |
| FC06-02 | 79.7 ± 1.3[bA] | 78.8 ± 0.8[aA] | 75.6 ± 0.7[bB] | 25.5 ± 1.3[eA] | 25.4 ± 1.0[dA] | 27.3 ± 1.2[cA] | 90.6 ± 0.6[bA] | 91.1 ± 0.8[aA] | 89.7 ± 0.6[aA] |
| Tiparot | 82.3 ± 0.4[aA] | 71.18 ± 1.1[dB] | 73.4 ± 0.8[cB] | 16.4 ± 0.5[gB] | 22.9 ± 0.6[eA] | 23.3 ± 0.2[dA] | 80.8 ± 0.6[eA] | 76.7 ± 0.3[gB] | 76.6 ± 0.5[fB] |
| Mean | 81.5 | 76.3 | 75.4 | 22.2 | 25.91 | 27.2 | 86.9 | 85.4 | 85.5 |
| Plantain cooking | | | | | | | | | |
| D'Angola | 80.3 ± 1.2[aA] | 78.6 ± 2.4[aA] | 78.2 ± 0.5[aA] | 38.4 ± 2.2[bA] | 37.0 ± 0.8[aA] | 35.7 ± 1.0[aA] | 82.6 ± 2.06[eA] | 80.7 ± 0.4[eB] | 79.4 ± 0.1[eB] |
| Terra S. N. | 74.6 ± 1.9[cA] | 74 ± 0.3[cA] | 72.6 ± 0.5[cA] | 40.9 ± 1.1[aA] | 38.2 ± 0.9[aA] | 38.2 ± 0.9[aA] | 77.5 ± 1.9[fA] | 76.7 ± 1.6[gA] | 76.6 ± 0.9[fA] |
| Terra A. B. | 77.6 ± 0.4[bA] | 74.6 ± 1.9[cB] | 74.8 ± 1.6[bB] | 37.4 ± 1.5[bA] | 38.4 ± 0.2[aA] | 37.1 ± 2.2[aA] | 78.2 ± 0.7[fA] | 78.0 ± 0.3[fA] | 76.3 ± 0.1[fB] |
| Samurá B | 78.6 ± 2.6[bA] | 74.5 ± 0.3[cB] | 71.5 ± 0.4[dC] | 32.8 ± 5.9[cB] | 33.8 ± 0.5[bB] | 36.2 ± 0.3[aA] | 78.3 ± 1.2[fA] | 75.6 ± 0.7[gB] | 72.2 ± 0.2[gC] |
| Mean | 77.8 | 75.4 | 74.3 | 37.4 | 36.9 | 36.8 | 79.2 | 77.7 | 76.1 |

[#]Values in the same column followed by different lower case (genotypes) and in the same row followed by different upper case letters (ripening stages), for each parameter, differ by the Scott Knott test (p<0.01) (genotypes) and by Tukey test (p<0.01) (ripening stages). Stage 2 – all green; stage 5 – yellow with green ends; and stage 7 – yellow with brown.
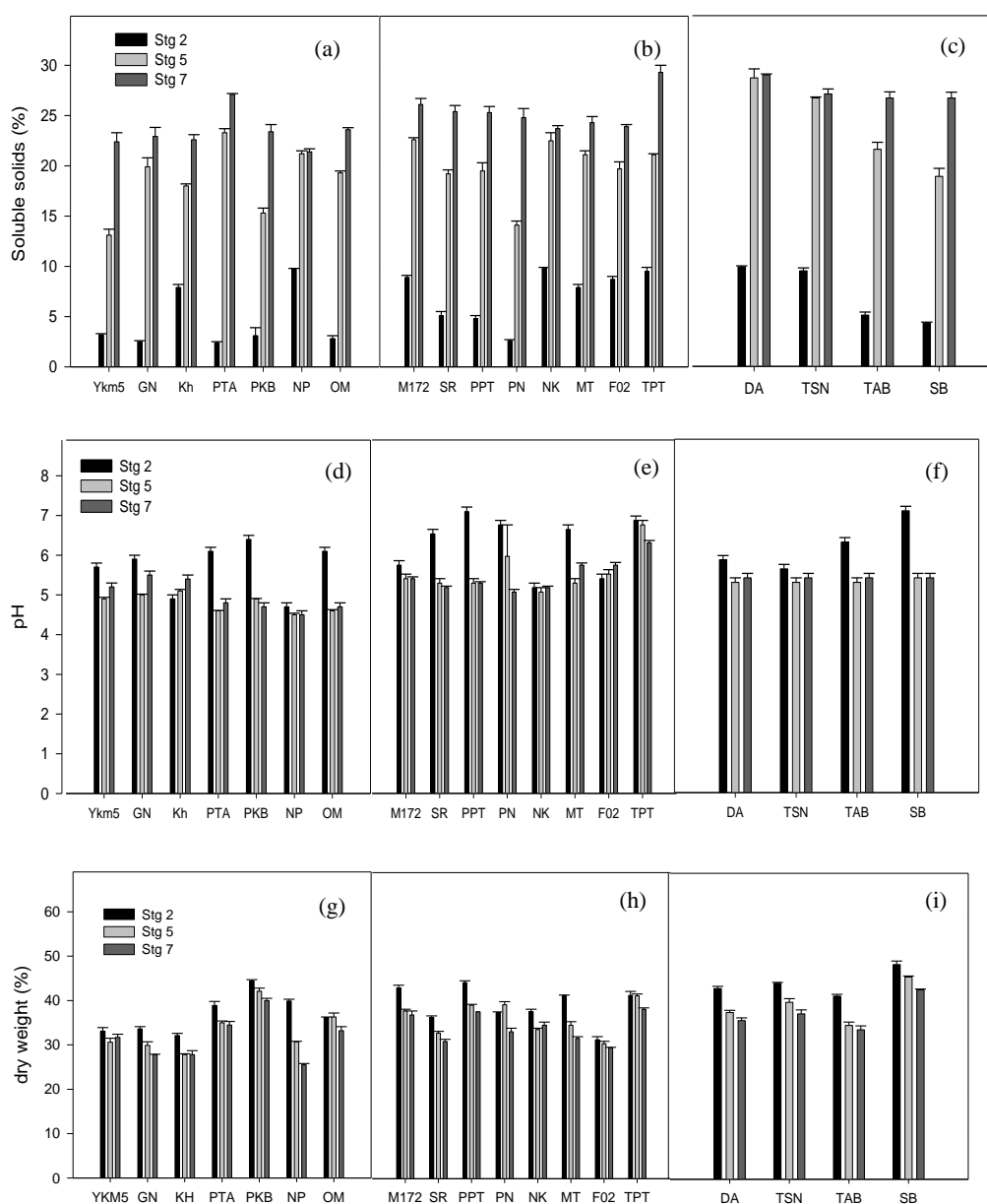
* Ykm5: Yangambi Km5; GN: Grande Naine; Kh: Khai; PA: Prata-Anã; PKB: Pisang Kepok Bung; NP: Ney Poovan; OM: Ouro da Mata; M172: Monthan 172; SR: Simili Radjah; PPT: Pelipita; PN: Pacha Nadan; NK: Namwa Khom; MT: Muisa Tia; F02: FC06-02; TPT: Tiparot; DA: D'Angola; TSN: Terra Sem Nome; TAB: Terra Anã Branca; SB: Samurá B.

**Figure 2.** Soluble solids (°Brix), pH and dry weight (%) in banana fruit at ripening stages 5, 6 and 7 of *Musa* spp. genotypes, separated by subgroup and/or consumption mode (a, d and g) dessert bananas, (b, e and h) nonplantain cooking and (c, f and i) plantain. Stage 2 – all green; stage 5 – yellow with green ends; and stage 7 – yellow with brown.

Plantains, generally present the highest SS contents (Figure 2c), dry weight (Figure 2i) and firmness (Figure 3e, 3f) with values even superior when the fruit where green. Firmness change is one of the most perceptible attribute, resulting from the ripening process.
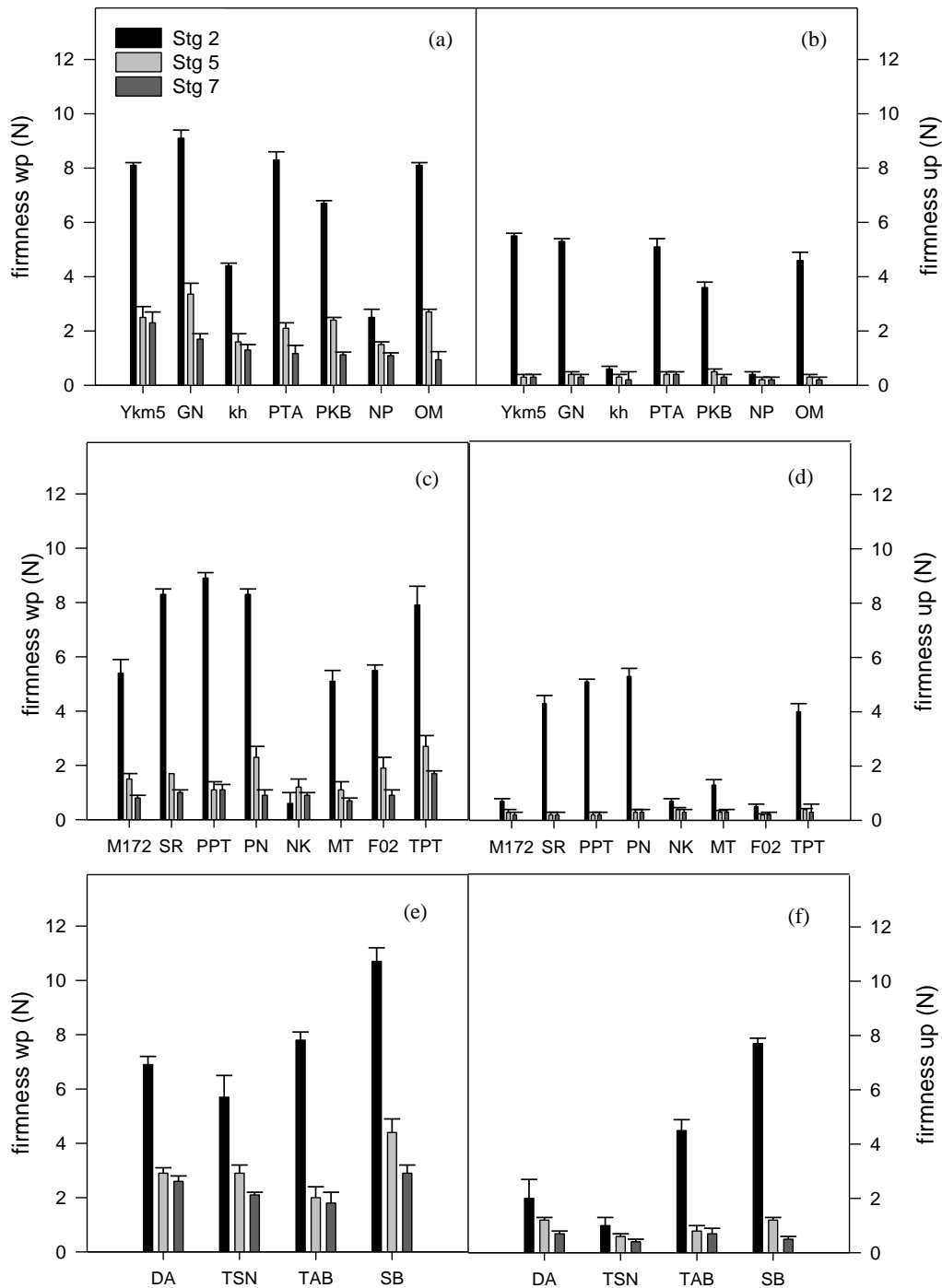
* Ykm5: Yangambi Km5; GN: Grande Naine; Kh: Khai; PA: Prata-Anã; PKB: Pisang Kepok Bung; NP: Ney Poovan; OM: Ouro da Mata; M172: Monthan 172; SR: Simili Radjah; PPT: Pelipita; PN: Pacha Nadan; NK: Namwa Khom; MT: Muisa Tia; F02: FC06-02; TPT: Tiparot; DA: D'Angola; TSN: Terra Sem Nome; TAB: Terra Anã Branca; SB: Samurá B.

**Figure 3.** Firmness (N) of fruit with peel (Firmness WP) and unpeeled banana fruit (Firmness UP) at ripening stages 5, 6 and 7 of *Musa* spp. genotypes, separated by subgroup and/or consumption mode (a and b) dessert bananas, (c and d) nonplantain cooking and (e and f) plantain. Stage 2 – all green; stage 5 – yellow with green ends; and stage 7 – yellow with brown.

There was a difference in the fruit among the genotypes and in the different ripening stages (Figure 3). Genotypes with firmer pulps (e.g. plantain 'Samurá B') are for industrial use, mainly for the preparation of fried products e.g. banana chips. In addition, fruit with higher firmness are more resistant to transport and durable after the harvest (Pereira et al. 2004). During the ripening process, the pulp percentage dry weight decreases (Figure 2g, 2h, 2i). This characteristic is important to the selection of genotypes for industry (or even for the domestic consumption for the preparation of cooked and/or fried dishes), mainly in cooking bananas, which are preferable in many countries.

The highest carotenoids levels occur in plantains, except for the nonplantain cooking banana 'Pelipita', mainly in the green fruit (stg 2). The carotenoids content showed variations among the genotypes (2.90 µg/g in 'Muisa Tia' at stg 5 to 53.82 µg/g in 'Samurá' B at stg 5), influenced by ripening and we verified that it is a genotype-depending characteristic (Figure 4a, 4b, 4c).

In bananas, studies describe a wide variability among the genotypes inside active germplasm banks of *Musa* spp. (Borges et al. 2014). Previous studies indicate that carotenoids content in banana fruit is mainly constituted of pro-vitamin A compounds and the pulp coloration is a phenotypic characteristic that can indicate the quantity of pro-vitamin A carotenoids (pVACs) (Borges et al. 2014). Using the correlation analysis, we observed that $C^*$ presented a positive linear correlation with the total carotenoids content (r = 0.78, p < 0.05), showing a strong negative correlation with the hue angle ($H^*$). These results show that bananas with high $C^*$ and pulp yellow/orange, present higher contents of pro-vitamin A compounds. Genotypes with lighter coloration tend to have lower quantities of carotenoids, mainly the pVACs. However, these genotypes generally present higher proportions of antioxidant compounds such as the lutein and zeaxanthin (Englberger et al. 2010).
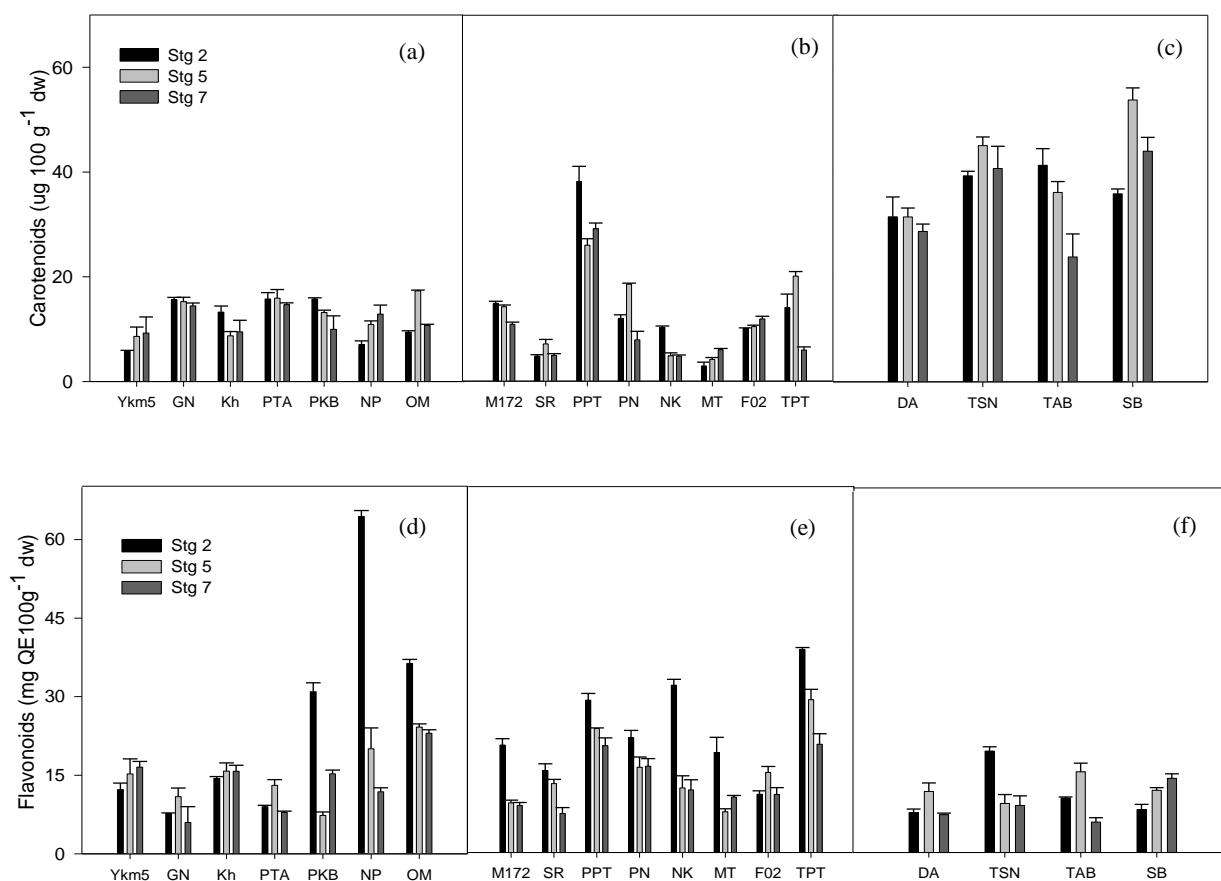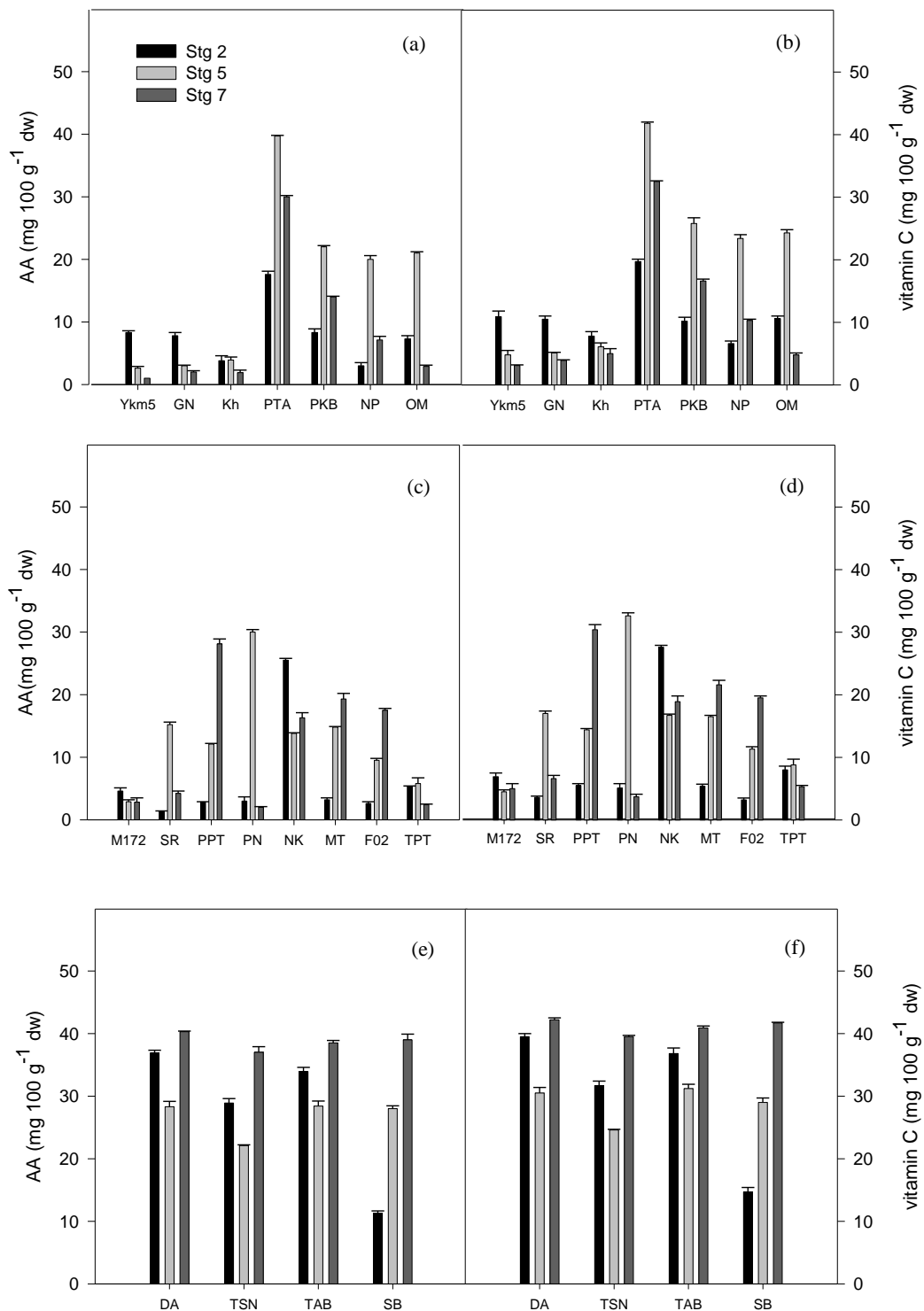
\* Ykm5: Yangambi Km5; GN: Grande Naine; Kh: Khai; PA: Prata-Anã; PKB: Pisang Kepok Bung; NP: Ney Poovan; OM: Ouro da Mata; M172: Monthan 172; SR: Simili Radjah; PPT: Pelipita; PN: Pacha Nadan; NK: Namwa Khom; MT: Muisa Tia; F02: FC06-02; TPT: Tiparot; DA: D'Angola; TSN: Terra Sem Nome; TAB: Terra Anã Branca; SB: Samurá B.

**Figure 4.** Carotenoids (µg 100 g$^{-1}$) and flavonoids (mg 100 g$^{-1}$) content in pulp of *Musa* spp. genotypes, at ripening stages 5, 6 and 7, separated by subgroup and/or consumption mode (a and d) dessert bananas, (b and e) nonplantain cooking and (e and f) plantain. Stage 2 – all green; stage 5 – yellow with green ends; and stage 7 – yellow with brown.

The vitamin C and ascorbic acid (AA) data showed a variation among the genotypes and the influence by the fruit ripening stage (Figure 5), i.e., a genotype-dependent characteristic. Plantain generally also present higher carotenoids contents (i.e., pVACs). There are no variations in plantains during the ripening and in most of the genotypes (Figure 5e, 5f).  In the dessert (Figure 5a, 5b) and cooking bananas (Figure 5c, 5d), the variation of vitamin C and AA are higher than other ones. Dessert bananas presented a decrease of vitamin C content during the ripening (Figure 5b). The dessert banana 'Prata-Anã' (Figure 5b) presented the highest vitamin C contents in the ripe fruit (stg 5), higher than Cavendish banana ('Grand Naine'). Most of the edible bananas are genetic triploids, results of the genomic combination of the wild species *Musa acuminata* (A) and *M. balbisiana* (B), or a combination of both.

* Ykm5: Yangambi Km5; GN: Grande Naine; Kh: Khai; PA: Prata-Anã; PKB: Pisang Kepok Bung; NP: Ney Poovan; OM: Ouro da Mata; M172: Monthan 172; SR: Simili Radjah; PPT: Pelipita; PN: Pacha Nadan; NK: Namwa Khom; MT: Muisa Tia; F02: FC06-02; TPT: Tiparot; DA: D'Angola; TSN: Terra Sem Nome; TAB: Terra Anã Branca; SB: Samurá B.
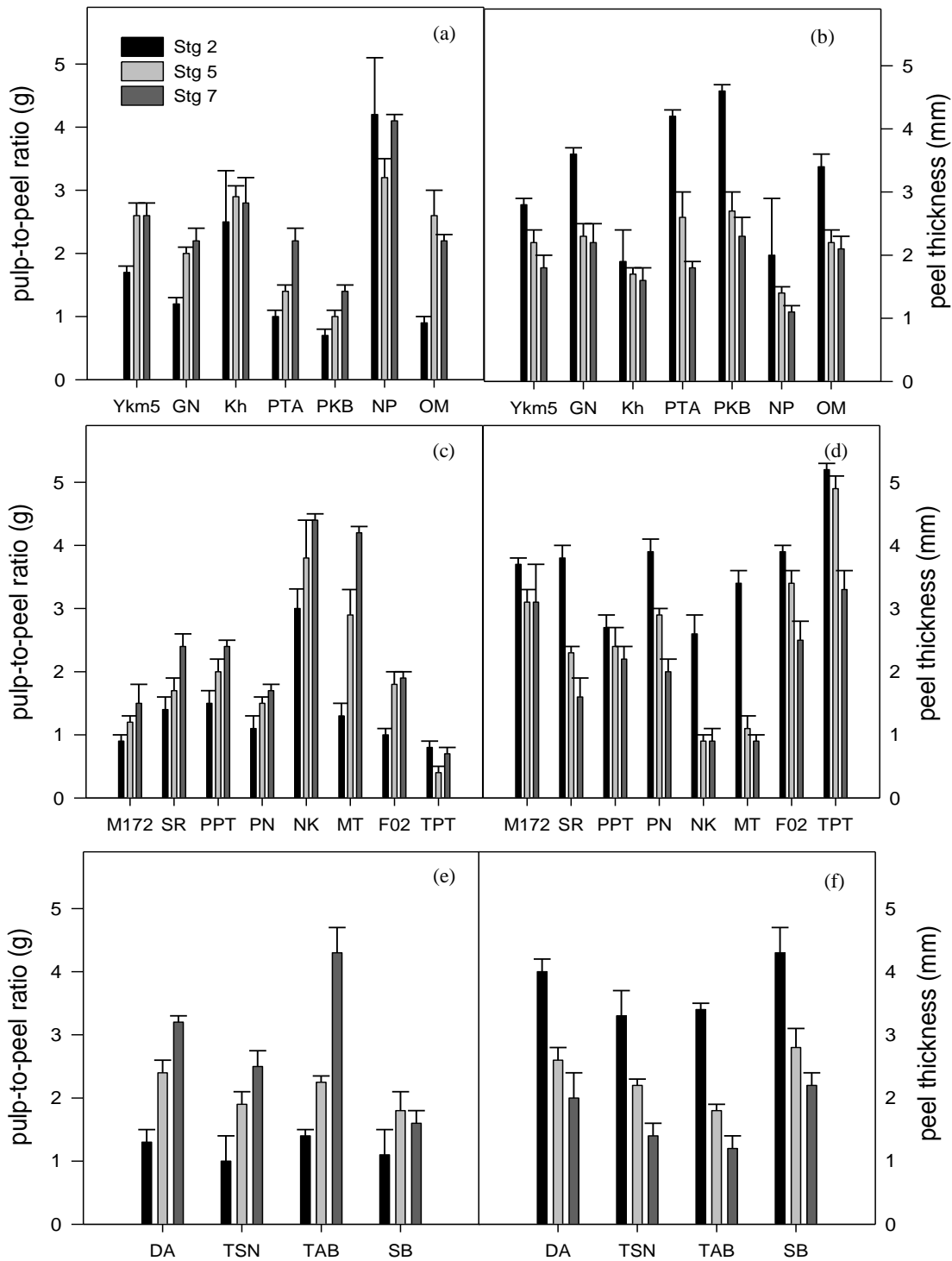
**Figure 5.** Ascorbic acid (AA) and vitamin C in pulp of *Musa* spp. genotypes, at ripening stages 5, 6 and 7, separated by subgroup and/or consumption mode (a and b) dessert bananas, (c and d) nonplantain cooking and (e and f) plantain. Stage 2 – all green; stage 5 – yellow with green ends; and stage 7 – yellow with brown.

The 'Prata-Anã' have a genomic constitution AAB (same of the plantains), while the ´Grand Naine´ is AAA, demonstrating a possible importance of the B genome in the content of these compounds. (Wall 2006) also verified that ripe fruit (yellow coloration) of bananas with genomic constitution AAB ('Dwarf Brazilian') had superior values (up to 3 times) of vitamin C than genotypes of the Cavendish subgroup AAA. Thus, it is evident the importance of the genomic group in the ascorbic acid content in *Musa* ssp., proving that genotypes with genomic constitution AAB have higher contents (until 13 times more than the most commercialized ones) of these antioxidant compound. Among the cooking bananas (nonplantain cooking), we can observe that the highest AA and vitamin C values occur at stages 5 and 7 (Figure 5c, 5d). However, in 'Namwa Khom', the profile was different with high contents of vitamin C in the three stages. 'Pacha Nadan' and the 'Pelipita' showed the highest contents in the stages 5 and 7, respectively.

Higher total flavonoid content, was found in dessert bananas and some nonplantain cooking in their initial ripening stages (Figure 4d, 4e), presenting a positive correlation with the Hue angle (H*) of the peel (r = 0.354, p < 0.05) and a negative correlation with the SS content (r = -0.326, p < 0.05), differently from the other antioxidants analyzed (data not shown). This effect is attributed to the ripening process, with apparent gradual decrease of the contents of these compounds in fruit, which can be associated to the oxidative process (Parr and Bolwell 2000). Tsamo et al. (2014) verified increase in the total phenolic compounds in plantains until the stg 5 of ripening and a decrease at stg 7, similar to obtained in this study with plantains ('D'Angola' and 'Terra Anã Branca') and in some dessert bananas ('Grand Naine' and 'Prata-Anã').

However, in some genotypes (e.g., 'Ney Poovan' and 'D'Angola') there was an increase in the total flavonoids content at stages 5 and 7 (Figure 4d, 4f). The total flavonoids contents varied widely among the analyzed genotypes (Figure 4d, 4e, 4f). In all the genotypes of bananas, there was a wide variation of the flavonoids content. Plantains presented the lowest contents of these compounds (Figure 4f) and high values of total flavonoids were found in dessert bananas (e.g., 'Ney Poovan') (Figure 4d) and non-plantains bananas ('Pelipita' and 'Tiperot') (Figure 4e). Among the plantains, 'Terra Sem Nome' showed the highest total flavonoids values in stg 2 (green fruit) (Figure 4f). In addition, there are genotypes with superior quantities of flavonoids, when compared to the most commercialized genotypes (e.g., dessert bananas 'Grand

Naine' and 'Prata-Anã' and the plantain 'D'Angola'). This result is interesting for the promotion and incorporation of genotypes (e.g., 'Ney Poovan', 'Pelipita' and 'Tiparot') with superior contents of these bioactives or even, for the use in programs of genetic improvement of the culture. In addition, the dessert genotype 'Ney Poovan' also showed the highest pulp-to-peel ratio, interesting result for the promotion of this genotype for *in natura* consumption (Figure 6a). The pulp-to-peel ratio varied among the genotypes and among the ripening stages (Figure 6a, 6c, 6e). During the ripening, this relation showed variations, which resulted in a higher pulp yield (Aquino et al. 2017). In general, there was a decrease of the peel thickness during the fruit ripening (Figure 6b, 6d, 6f). In addition, the increase of the pulp-to-peel ratio can be attributed to the migration of water from peel to the pulp because of the osmotic gradient, due to the increase of the sugar contents in the pulp, in relation to the peel (Aquino et al. 2017). In dessert banana genotype, the lower values of the pulp-to-peel ratio (highest peel thickness values) were verified in 'Pisang Kepok Bung', 'Khai', 'Grand Naine' and 'Yangambi Km5' (Figure 6a, 6b). The genotype Ney Poovan showed the highest pulp-to-peel ratio and, consequently, highest pulp yield. In nonplantain cooking bananas, 'Namwa Khom' and 'Muisa Tia' presented the highest values (Figure 6c, 6d). The pulp yield is an important quality parameter for the industry and *in natura* consumption.

* Ykm5: Yangambi Km5; GN: Grande Naine; Kh: Khai; PA: Prata-Anã; PKB: Pisang Kepok Bung; NP: Ney Poovan; OM: Ouro da Mata; M172: Monthan 172; SR: Simili Radjah; PPT: Pelipita; PN: Pacha Nadan; NK: Namwa Khom; MT: Muisa Tia; F02: FC06-02; TPT: Tiparot; DA: D'Angola; TSN: Terra Sem Nome; TAB: Terra Anã Branca; SB: Samurá B.

**Figure 6.** Pulp-to-peel ratio (g) and peel thickness (mm) at ripening stages 2, 5 and 7 in the post-harvest (20 ± 2°C and RH 85 ± 2%) of the *Musa* spp. genotypes, separated by subgroup and or consumption mode (a and b) dessert bananas, (c and d) nonplantain cooking and (e and f) plantain. Stage 2 – all green; stage 5 – yellow with green ends; and stage 7 – yellow with brown.

## 1.4 Conclusion

The physicochemical and biochemical (bioactive compounds) characteristics evaluated in this study vary according the genotype and ripening stage. There are genotypes with superior quantities of SS, dry weight, firmness, flavonoids, carotenoids and vitamin C, especially compared with the main genotype that are currently marketed. Higher content the SS, dry weight, firmness, carotenoids and vitamin C were found in plantain subgroup. Among the all genotypes, 'Samurá B' (plantain) and 'Pelipita' (nonplantain cooking) showed the highest firmness and both genotypes, green or ripe, are promising for the industrial use, mainly for the processing of banana chips. Plantains and/or non-plantain cooking bananas contain high carotenoids values, while dessert genotype (the most consumed worldwide) contain lowest amounts of these bioactives and present a strong correlation with the pulp color intensity ($^*C$).

High vitamin C contents are verified in plantains (AAB) and dessert banana 'Prata-Anã' (AAB), mainly in the ripe fruit. It is evident the importance of the genomic group in the vitamin C content in the *Musa* ssp. germplasm, considering that genotypes of genomic constitution AAB have high levels (up to 13 times higher than the most commercialized genotype) of vitamin C. In addition, the dessert banana 'Ney Poovan' contain high SS content, pulp-to-peel ratio and flavonoid content, an interesting result for the promotion of this genotype for *in natura* consumption. Our result leads us to suggest the promotion and incorporation of these genotypes in programs of genetic improvement of the culture and/or incorporation inside the existent agricultural systems.

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## Capítulo 2

**Ripening and cooking processes influence the carotenoid content in bananas and plantain (*Musa* spp.)**

(artigo publicado no periódico científico Food Research International)

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**ABSTRACT:** In bananas, the major carotenoids are α- and β-carotene, which give this fruit great potential in biofortification programs. The carotenoid content in pulp and peel of 22 banana and plantain genotypes was analyzed in order to evaluate the impact of ripening on the carotenoid content as well as its retention after different thermal processes. Fruits were ripened at stage 2 (green), 5 (yellow) and 7 (yellow with dark spots). The provitamin A content (pVACs) varied from 20.8 ('Muisa Tia' stg 7) to 6341.5 µg/100 g f.w. ('Samurá B' stg 5). High quantities were identified in plantains, which have yellow pulp, a phenotypic characteristic that can indicate the quantity of pVACs in *Musa* spp fruit. The 'Samurá B' plantain showed the highest pVACs (6341.5 µg/100 g f.w.) and *trans*-β-carotene (5220.0 µg/100 g f.w.) content in pulp, especially when compared to the 'D'Angola' plantain (pVACs: 3214.0 µg/100 g f.w.), the most common in Brazil, and with the dessert cultivar from the Cavendish subgroup ('Grand Naine' – pVACs: 230.6 µg/100 g f.w.), the most consumed worldwide. The highest pVACs quantity was verified in the ripe fruit (stg 5), decreasing during the fruit ripening (stg 7). In the peels, lutein was the majority compost, with contents higher than the pulp. The highest lutein content was identified in the green fruit peels from the 'Terra Anã Branca' cultivar (1602.1 µg/100 g d.w.), almost ten times higher than the content found in the pulp of the same cultivar. However, the pulp of all the cultivars showed superior values of pVACs, *trans*-β-carotene and *trans*-α-carotene, mainly in the ripe stage 5. The plantain 'Samurá B' has nutritional and/or functional properties, and its promotion/incorporation in existing agricultural systems is of interest for use in

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biofortification programs. In addition, thermal processing can increase the functional and nutritional value of the *Musa* spp. fruit, mainly by boiling, which should be the favorite in domestic preparations, regardless of the cultivar used.

*Keywords*: provitamin A, β-carotene, boiling, lutein.


## 2.1 Introduction

Carotenoids are bioactive compounds with fundamental roles in human health. They have the potential to eliminate or maintain in balance, the levels of reactive oxygen species (ROS) and are related to decreased incidence of cancer, cardiovascular and eye diseases (Schieber & Weber, 2016). These benefits are associated with the ability of carotenoids to interact with biological membranes, preserving their stability and fluidity in various ways by eliminating free radicals (Jomova & Valko, 2013). In addition, the carotenoids are also recognized as the main precursor source of vitamin A. Despite it being found in foods of animal origin, it is worthwhile to point out that more than 80% of vitamin A consumption in developing countries is derived from vegetable sources in the form of provitamin A carotenoids (pVACs), which are converted into retinol (vitamin A) in humans. Among the vitamin A precursors, β-carotene is the one that presents the highest activity (Rodriguez-Amaya, Delia B., Rodriguez, Evelyn B, Amaya-Farfan, 2006).

Vitamin A deficiency is a serious public health problem worldwide and is most pronounced in developing countries, affecting mainly children and women who are at reproductive age or pregnant. One of the sustainable ways to mitigate the vitamin A deficiency problem is to encourage the consumption of foods naturally rich in carotenoids, such as fruits and dark green leafy vegetables. For example, the consumption of some banana genotypes can provide high amounts of vitamin A precursors, approximately 90% of α- and β-carotene (Borges et al., 2014; Saini, Nile, & Park, 2015). Initial studies showed that there is great diversity in the content of bioactive compounds inside the *Musa* spp. and that there are cultivars with appreciable quantities of these compounds, mainly when compared to those most commercialized presently (Amorim et al., 2011; Borges et al., 2014). This diversity can be explored in

order to identify potentially adequate genotypes to be used in genetic improvement programs focusing on culture biofortification and/or to be promoted and incorporated in existing agricultural systems for culture diversification and consequently, popular consumption.

Bananas and plantains stand out for being consumed in all ripening stages and after many different thermal treatment processes (e.g., fried and baked). For different genotypes, biochemical peculiarities at ripeness generate additional possibilities for the use of *Musa* spp. fruit, once their consumption does not necessarily occur *in natura* but also after processing or through use of processed byproducts at different ripening stages. In addition, comprehension of the biochemical transformations that occur during ripening allows us to understand how these metabolic changes affect fruit quality and post-harvest life and establish what the best consumption point is. Another relevant factor for decreasing post-harvest losses, as well as the generated residues, is that the banana pulp and peel could be explored by the food and pharmaceutical industries, adding value to the product and increasing rural producer income. The peel corresponds to 40% of the total fruit weight and causes a great problem in food processing industries today; thus, it is essential to find applications for this byproduct (Emaga et al., 2007; Pereira & Maraschin, 2015).

The levels of carotenoids in bananas and plantains are variable, and due to the lack of information in the literature about some genotypes cultivated in Brazil, it is important to investigate their content before recommending their introduction in the human diet. In general, for plantains and bananas, there is a demand for investigation of the real benefits to health provided by the consumption of biofortified biomasses of bananas, making the confirmation of their authentic nutraceutical action necessary. Cooking bananas and plantains are consumed after cooking processes, which makes studies of the retention of compounds essential after different thermal processes used in the consumption regions, since many factors influence the retention, absorption and bioconversion of antioxidant and provitamin compounds (carotenoids). Thus, the aims of this study were to evaluate (1) the impact of ripening on the carotenoids content of pulps and peels and (2) the retention of the analyzed compounds after different thermal processes.

## 2.1 Materials and Methods

### 2.1.1 Harvest location of the analyzed genotypes

Bunches of 22 genotypes of banana trees were harvested between March and June 2016 from different genomic groups belonging to the Active Germplasm Bank (banana AGB) (Embrapa Cassava & Fruits, Cruz das Almas, Bahia, Brazil). All studied genotypes were grown at the same location (Latitude 12º40'12'' S; Longitude 39º06'07'' W; Altitude 225 m), to exclude experimental errors due to environmental factors regarding differences in composition (Table 1). These genotypes (20 plants per genotype) were planted over 3 replications. Field planting was done from April 2015. These cultivars are undergoing agronomic evaluation. When branches reached ripening stage 1, we harvested the 2nd and 3rd hand (each containing between 10 fingers) of each genotype (± 2 bunches = 40 fruit) and stored them at room temperature (20 ± 2°C) at a relative humidity of 80 ± 2% until they reached the complete ripening stage (stage 7).

**Table 1.** Banana tree accesses belonging to the Active Germplasm Bank (banana AGB) from Embrapa Mandioca e Fruticultura, Cruz das Almas, Bahia, Brazil.

| Accesses | Ploidy | Subgroup/Subspecies | Form of use |
|---|---|---|---|
| Yangambi Km5 | AAA | Ibota | *In natura* |
| Grand Naine | AAA | Cavendish | *In natura* |
| Khai | AAA | Ibota | *In natura* |
| Prata-Anã | AAB | Prata | *In natura* |
| Pisang Kepok Bung | AAB | Peyan | *In natura* |
| Ney Poovan | AB | Ney Poovan | *In natura* |
| Ouro da Mata | AAAB | * | *In natura* |
| Monthan 301 | ABB | Monthan | Cooked |
| Monthan 172 | ABB | Monthan | Cooked |
| Simili Radjah | ABB | Peyan | Cooked |
| Pelipita | ABB | Bluggoe | Cooked |
| Pacha Nadan | ABB | Saba | Cooked |
| Namwa Khom | ABB | Pisang Awak | Cooked |
| Muisa Tia | ABB | Pisang Awak | Cooked |
| FC06-02 | AABB | Figo | Cooked |
| Tiparot | ABBB | Klue Teparod | Cooked |
| D'Angola | AAB | Plantain | Cooked |
| Curare Enano | AAB | Plantain | Cooked |

| Terra Sem Nome | AAB | Plantain | Cooked |
| Tipo Velhaca | AAB | Plantain | Cooked |
| Terra Anã Branca | AAB | Plantain | Cooked |
| Samurá B | AAB | Plantain | Cooked |

*2.1.2 Ripening stage determination*

The fruit were divided into seven ripening stages during storage, according to the banana color scale described by Soltani, Alimardani, & Omid (2011). These indices are based on the peel color: 1 = green; 2 = green with yellow traces; 3 = more green than yellow; 4 = more yellow than green; 5 = yellow with green; 6 = completely yellow; 7 = yellow with coffee color areas. The stages 2, 5 and 7 were chosen for all analyses, which are those most used for processing and/or *in natura* consumption (Ekesa et al., 2012).

The pulps and peels of the 22 genotypes were sliced, powdered in liquid nitrogen, lyophilized and stored in an ultra-low temperature freezer (-80°C). The pulps of all genotypes were analyzed and we chose the peels of the genotypes with significant carotenoid quantities; that is, all the plantains and one cooking banana ('Pelipita'), which had a yellow/orange pulp color.

*2.1.3. Retention analysis*

To study the retention after thermal processing, we chose ripe fruit (stg 5) from the D'Angola plantain, which is already commercially used, and fruit from the cooking banana 'Pelipita', which showed the best results for pVACs in relation to the other cooking bananas (Table 2). The analyses were performed with and without peel. The preparation methods tested included boiling in water, microwaving and stir-frying. Bananas with or without peel (stage 5) were boiled for 10 min in a stainless-steel pan covered with a lid. For the boiling method, we used three fingers (with and without peel), in approximately 300 mL of water. The remaining water was drained and the fruit were cooled. For the microwaving process, the whole bananas were microwaved (with and without peel) for 2 min, using a commercial microwave oven set at the highest level. In these fruits, we made a longitudinal cut in the peels to maintain the internal steam pressure. Stir-frying was performed in fruit longitudinally cut, using a stainless-

steel frying pan greased with soybean oil (± 2 mL), for 5 min. The pulps were ground to a fine powder (IKA, A.11, Germany) in liquid nitrogen, lyophilized and stored at - 80 °C until analysis.

### 2.1.4. Carotenoids extraction

Banana and plantain samples (500 mg) that had been lyophilized and ground were homogenized in 2.5 mL MeOH (HPLC grade) using a vortex for 1 min and ultrasonicated for 30 min at 25 °C. After centrifugation (3800 x g, Hettich Zentrifugen, Mikro220R) for 10 min, the supernatant was transferred to amber tubes. The residual pellet was submitted to one more process of extraction with 2.5 mL MeOH and two more similar extractions with tetrahydrofuran (THF) (2.5 mL in each extraction). The supernatants (10 mL) were completely evaporated under $N_2$. The dry organic solvent extract was resolubilized in 1 mL ethanol and centrifuged (5 min, 800 x g at 4°C) (Monaco et al., 2016), and 20 $\square$L of supernatant was injected into the HPLC/DAD system.

### 2.1.5. Carotenoids analysis by reversed phase high performance liquid chromatography (HPLC)

For the carotenoids analysis, we used the method described by Yeum et al. (1996). Briefly, the system consisted of a reversed phase HPLC Thermo Scientific Ultimate 3000 (Thermo Scientific, USA), equipped with a diode array detector and C30 column for carotenoids (5 µm, 150 x 4.6 mm, YMC, Wilmington, NC). HPLC mobile phase conditions were the same as previously described by Li et al. (2009), which consisted of methanol: methyl tert-butyl ether (MBTE): water (85:12:3, v/v/v) with 1.5% ammonium acetate in water for solvent A and methanol:MTBE:water (8:90:2 v/v/v) with 1.0% ammonium acetate in water for solvent B. The solvent gradient was as follows: 90% A and 10% B (2 min), 85% A and 15% B (5 min), 75% A and 25% B (9 min), 60% A and 40% B (12 min), 17%A and 83%B (16 min), 5% A and 95% B (23 min), 5% A and 95% B (25 min), 60% A and 40% B (28 min) at a flow rate of 0.4 mL min$^{-1}$ (10 °C). The carotenoids were quantified at 450 nm by determining peak areas under the curve in the HPLC calibrated against known amounts of standards. We adjusted the results using a standard curve for α, β-carotene, and lutein (Sigma-Aldrich Co., St. Louis, MO)

of 99.98% purity. The 13-*cis*-α-carotene (*c*-AC) and 13-*cis*-β-carotene (*c*-BC) were identified by the spectrum and calculated by the all *trans*-α-carotene (*t*-AC) and all *trans*-β-carotene (*t*-BC) curve. The recovery standard average was 97%. The results were expressed in µg per 100 g and correspond to the average of three consecutive injections per sample (n = 3). All analyses were performed in triplicate.

### 2.1.6. Retinol activity equivalent (RAE) and vitamin A

The components of the provitamin A carotenoids have different vitamin. An activities, due to the differences in chemical structure. The concept of retinol activity equivalent (RAE) considers that one retinol activity equivalent corresponds to 1 µg of retinol or 12 µg of *t*-BC or 24 µg of other provitamin A carotenoids (pVACs) (K.-J. Yeum & Russell, 2002). As there is still no certainty about the conversion in RAE of other isomers of *t*-BC (e.g., *cis*-beta-carotene), we considered the conversion value 24:1 per gram of ingestion of carotenoids (Davey, Van den Bergh, Markham, Swennen, & Keulemans, 2009). Based on the conversions, we determined the retinol activity equivalent (RAE) and consequently, the nutritional value of vitamin A for each analyzed genotype.

### 2.1.7 Statistical analysis

We used the entirely randomized design, with factorial scheme 22 x 3 (genotypes – pulp x ripening stages) and 7 x 3 (genotypes – peel x ripening stage), with three repetitions (three fruit per parcel), for the analyzed genotypes in the three ripening stages and after thermal processing ('Pelipita' and 'D'Angola'). The carotenoids data were collected, summarized and submitted to variance analysis (ANOVA), followed by the Scott-Knott averages comparison test among the genotypes, and Tukey and Scott-Knott tests among the ripening stages for pulps and peels, respectively. For the analyses after thermal processing, the data were also submitted to variance analysis (ANOVA), followed by the Tukey test using average comparison. The analysis of the variance in the data was performed using the statistics software SISVAR (Ferreira, 2011). Principal component analysis (PCA, software XLSTAT - version 2017 (Addinsoft, France) was used to visualize the possible correlation between ripening, physicochemical data (texture unpeeled, texture with

peel, soluble solids, titratable acidity, luminosity, chromaticity and hue angle) and biochemical analysis (total carotenoids, lutein, all trans-α-carotene, all trans-β-carotene and pro-vitamin A carotenoids). PCA was also used to visualize the effects of thermal processing on carotenoid content (lutein, all trans-α-carotene, all trans-β-carotene and pro-vitamin A carotenoids) in the different genotypes.

## 2.2 Results and Discussion

### 2.2.1 Impact of the ripening stage on the carotenoids content of the pulps of the Musa spp. fruit

In all genotypes assessed there was a wide variation in the carotenoids content, as also described in other studies with *Musa* spp. (Amorim et al., 2011; Borges et al., 2014). The average of provitamin A carotenoids (pVACs) varied from 61.0 µg/100 g d.w. (20.91 µg/100 g f.w. in 'Muisa Tia' stg 7) to 13598.5 µg/100 g d.w. (6341.5 µg/100 g f.w. in 'Samurá B' stg 5), which represents up to 98.9% of carotenoids identified, mainly in plantains (Table 2). These levels are within the range of values reported for bananas from Micronesia that have the highest carotenoids levels in the world (up to 6360 µg/100 g f.w.) (Englberger et al., 2010).

In a tentative attempt to establish a descriptive model for grouping the ripening stages as a function of the total carotenoids and allow a better visualization of these compounds' profiles during the ripening process, we opted for comparing the obtained results through PCA. The dispersion of varieties according to PC1 and PC2 are shown in Figure 1.

PC1 and PC2 explained 70.22% of the data variance. The PC1 axis represents 39.53% of the total data variance, separating the green fruit (stg 2 – PC1) from the ripe ones (stg 5 and 7 – PC1 +). The total carotenoids content, lutein, *trans*-α-carotene (*t*-AC) and *trans*-β-carotene (*t*-BC) grouped in the PC1 positive quadrants (PC1+), suggesting that these compounds increase during the fruit ripening process. The samples with higher carotenoids contents were grouped in PC1 and PC2 (+), together with the C* value of the pulp, confirming that the yellow/orange color of the pulp is positively related to the carotenoids content (e.g., pVACs – r = 0.7, p <0.05).

**Table 2.** Lutein and pVACs (*t*-BC, *t*-AC and *c*-BC) content, percentage of pVACs of the total carotenoids and RAE in pulp of *Musa* spp.

| Accesses | Ripening stage | pVACs (µg/100g d.w.) | pVACs (µg/100g d.w.) | | | Lutein (µg/g d.w.) | ∑ Carotenoids (µg/100g d.w.) | pVACs (%) of total carotenoids | *t*-BC (%) of pVACs total | RAE (µg/100g d.w.) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | *t*-AC | *t*-BC | *c*-BC | | | | | |
| *Dessert bananas* | | | | | | | | | | |
| Yagambi | 2 | 107.4 | 43.2[iA*] | 63.3[nA] | 0.9[gB] | 104.0[fC] | 211.4 | 50.8 | 58.9 | 7.11 |
| | 5 | 217.8 | 89.7[kA] | 106.7[nA] | 21.4[iA] | 160.6[eA] | 378.4 | 57.5 | 49.0 | 13.5 |
| | 7 | 102.5 | 36.9[hA] | 58.9[mA] | 6.7[iB] | 130.1[eB] | 232.6 | 44.1 | 57.4 | 6.7 |
| Grande Nine | 2 | 642.4 | 372.7[gA] | 247.2[lA] | 22.5[fB] | 195.8[bC] | 838.2 | 76.6 | 38.5 | 37.1 |
| | 5 | 768.5 | 458.0[iA] | 272.6[lA] | 37.9[hAB] | 136.4[gA] | 904.9 | 84.9 | 35.5 | 43.4 |
| | 7 | 470.6 | 208.2[fB] | 233.7[kA] | 28.7[gA] | 128.8[eB] | 599.4 | 78.5 | 49.7 | 29.3 |
| Khai | 2 | 122.2 | 62.4[iA] | 44.6[nA] | 15.2[fA] | 317.5[aA] | 439.7 | 27.8 | 36.5 | 6.9 |
| | 5 | 138.2 | 51.1[kA] | 65.5[nA] | 21.6[iA] | 169.8[eC] | 308.0 | 44.9 | 47.6 | 8.5 |
| | 7 | 118.1 | 45.7[hA] | 56.2[mA] | 16.2[hA] | 254.6[bB] | 372.7 | 31.7 | 47.6 | 7.3 |
| Prata-Anã | 2 | 2362.3 | 809.4[eB] | 1492.1[hA] | 60.8[dB] | 165.2[dB] | 2527.5 | 93.5 | 63.2 | 160.6 |
| | 5 | 2742.7 | 1034.9[cA] | 1572.4[hA] | 135.4[dA] | 284.9[aA] | 3027.6 | 90.6 | 57.3 | 179.8 |
| | 7 | 1201.7 | 396.0[fC] | 763.9[hB] | 41.8[fC] | 281.0[aA] | 1482.7 | 81.0 | 63.6 | 81.9 |
| Pisang K. B. | 2 | 1556.2 | 170.5[hAB] | 1365.3[iB] | 20.4[fB] | 152.1[dA] | 1708.3 | 91.1 | 87.7 | 121.7 |
| | 5 | 1799.5 | 268.1[jA] | 1487.1[iA] | 44.3[gA] | 145.9[fA] | 1945.4 | 92.5 | 82.6 | 136.9 |
| | 7 | 404.9 | 70.8[hB] | 328.2[iC] | 5.9[iC] | 107.1[fB] | 512.0 | 79.1 | 81.0 | 30.5 |
| Ney Poovan | 2 | 110.0 | 32.1[iA] | 75.3[nB] | 2.6[gA] | 118.0[eC] | 228.0 | 48.2 | 68.4 | 7.7 |
| | 5 | 479.1 | 178.6[jA] | 296.2[lA] | 4.3[jA] | 193.5[dA] | 672.6 | 71.2 | 61.8 | 32.3 |
| | 7 | 197.1 | 46.9[hA] | 146.4[lA] | 3.8[iA] | 150.1[dB] | 342.2 | 57.6 | 74.3 | 14.3 |
| Ouro da Mata | 2 | 273.0 | 100.1[iB] | 150.6[mB] | 22.3[fB] | 108.8[fB] | 381.8 | 71.5 | 55.2 | 17.6 |
| | 5 | 738.5 | 257.3[jA] | 429.9[kA] | 51.3[gA] | 262.1[bA] | 1000.6 | 73.8 | 58.2 | 48.7 |
| | 7 | 286.6 | 96.2[hB] | 178.8[kB] | 11.6[hC] | 112.5[fB] | 399.1 | 71.8 | 62.4 | 19.4 |
| *Cooking bananas* | | | | | | | | | | |
| Monthan 301 | 2 | 98.6 | 19.5[iA] | 78.1[nA] | 1.0[gA] | 98.7[fC] | 197.3 | 49.9 | 79.2 | 7.4 |
| | 5 | 152.9 | 25.1[kA] | 122.3[nA] | 5.5[jA] | 151.7[fB] | 304.6 | 50.2 | 79.9 | 11.5 |
| | 7 | 125.7 | 22.5[hA] | 100.0[lA] | 3.2[iA] | 186.7[cA] | 312.4 | 40.2 | 79.5 | 9.4 |
| Monthan 172 | 2 | 667.8 | 142.2[hA] | 504.9[jA] | 20.7[fB] | 123.4[eAB] | 791.2 | 84.4 | 75.6 | 48.9 |
| | 5 | 730.3 | 180.7[jA] | 516.2[jA] | 33.4[hA] | 124.5[gA] | 854.8 | 85.4 | 70.7 | 51.9 |
| | 7 | 401.2 | 87.2[hA] | 293.7[iB] | 20.3[hA] | 101.2[fB] | 502.4 | 79.8 | 73.2 | 28.9 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Simili Radjah | 2 | 75.7 | 15.9$^{iA}$ | 58.9$^{nA}$ | 0.9$^{gA}$ | 98.2$^{fB}$ | 173.9 | 43.5 | 77.8 | 5.6 |
| | 5 | 89.1 | 20.9$^{kA}$ | 63.6$^{nA}$ | 4.6$^{jA}$ | 133.3$^{gA}$ | 222.4 | 40.1 | 71.4 | 6.4 |
| | 7 | 68.8 | 18.2$^{hA}$ | 48.3$^{mA}$ | 2.3$^{iA}$ | 124.7$^{eA}$ | 193.5 | 35.6 | 70.2 | 4.9 |
| Pelipita | 2 | 5634.8 | 584.8$^{fB}$ | 4992.8$^{bB}$ | 57.2$^{dA}$ | 133.5$^{eA}$ | 5768.3 | 97.7 | 88.6 | 442.8 |
| | 5 | 8247.2 | 820.1$^{hA}$ | 7369.1$^{dA}$ | 58.0$^{fA}$ | 141.2$^{fA}$ | 8388.4 | 98.3 | 89.3 | 650.7 |
| | 7 | 4890.9 | 335.4$^{fC}$ | 4517.8$^{cC}$ | 37.7$^{fB}$ | 127.7$^{eA}$ | 5018.6 | 97.5 | 92.4 | 392.0 |
| Pacha Nadan | 2 | 658.1 | 211.5$^{hA}$ | 423.8$^{kA}$ | 22.8$^{fB}$ | 144.2$^{dB}$ | 802.3 | 82.0 | 64.4 | 45.1 |
| | 5 | 876.6 | 280.1$^{iA}$ | 548.5$^{jA}$ | 48.0$^{gA}$ | 216.0$^{cA}$ | 1092.6 | 80.2 | 62.6 | 59.4 |
| | 7 | 510.7 | 51.9$^{hB}$ | 454.9$^{jA}$ | 3.9$^{iC}$ | 110.7$^{fC}$ | 621.4 | 82.2 | 89.1 | 40.2 |
| Namwa | 2 | 140.9 | 32.7$^{iA}$ | 95.2$^{nB}$ | 13.0$^{fA}$ | 175.3$^{cA}$ | 316.2 | 44.6 | 67.6 | 9.8 |
| | 5 | 357.9 | 97.8$^{kA}$ | 246.9$^{lA}$ | 13.2$^{iA}$ | 146.5$^{fB}$ | 504.4 | 70.9 | 69.0 | 25.2 |
| | 7 | 73.0 | 19.6$^{hA}$ | 52.5$^{mB}$ | 0.9$^{iB}$ | 125.1$^{eB}$ | 198.1 | 36.8 | 72.0 | 5.3 |
| Muisa Tia | 2 | 61.2 | 16.5$^{iA}$ | 42.5$^{nA}$ | 2.2$^{gA}$ | 95.8$^{gB}$ | 155.8 | 38.5 | 70.7 | 4.3 |
| | 5 | 81.6 | 27.7$^{kA}$ | 50.3$^{nA}$ | 3.6$^{jA}$ | 123.5$^{gA}$ | 205.1 | 39.8 | 61.6 | 5.5 |
| | 7 | 61.0 | 16.0$^{hA}$ | 43.0$^{mA}$ | 2.1$^{iA}$ | 137.5$^{eA}$ | 199.7 | 31.1 | 70.2 | 4.4 |
| FC06-02 | 2 | 229.5 | 60.1$^{iA}$ | 152.7$^{mB}$ | 16.7$^{fB}$ | 151.6$^{dAB}$ | 381.1 | 60.2 | 66.5 | 15.9 |
| | 5 | 543.0 | 107.3$^{kA}$ | 417.5$^{kA}$ | 18.2$^{hA}$ | 137.1$^{gB}$ | 680.1 | 79.8 | 76.9 | 40.0 |
| | 7 | 260.1 | 51.9$^{hA}$ | 196.7$^{kB}$ | 11.5$^{hB}$ | 165.9$^{dA}$ | 426.0 | 61.0 | 75.6 | 19.0 |
| Tiparot | 2 | 187.9 | 17.7$^{iA}$ | 170.2$^{mA}$ | nd | 102.1$^{fA}$ | 290.0 | 64.7 | 90.6 | 14.9 |
| | 5 | 193.0 | 18.1$^{kA}$ | 174.9$^{mA}$ | nd | 88.8$^{hA}$ | 281.8 | 68.5 | 90.6 | 15.3 |
| | 7 | 143.4 | 16.8$^{hA}$ | 126.6$^{lA}$ | nd | 110.8$^{fA}$ | 254.2 | 56.4 | 88.3 | 11.2 |
| *Plantains* | | | | | | | | | | |
| D'Angola | 2 | 3048.6 | 669.1$^{fB}$ | 2308.9$^{gC}$ | 70.6$^{cB}$ | 190.2$^{bA}$ | 3238.8 | 94.2 | 75.7 | 223.2 |
| | 5 | 8457.9 | 1579.4$^{eA}$ | 6771.3$^{gA}$ | 107.2$^{eA}$ | 191.1$^{dA}$ | 8649.0 | 97.8 | 80.0 | 634.5 |
| | 7 | 4885.1 | 637.8$^{eB}$ | 4198.9$^{eB}$ | 48.4$^{eC}$ | 184.9$^{cA}$ | 5070.0 | 96.3 | 86.0 | 378.5 |
| Curare Enano | 2 | 4095.0 | 824.5$^{eB}$ | 3227.8$^{fC}$ | 42.7$^{eC}$ | 118.5$^{eB}$ | 4213.5 | 97.2 | 78.8 | 305.1 |
| | 5 | 8412.0 | 1279.8$^{fA}$ | 7018.0$^{fA}$ | 114.2$^{eA}$ | 168.8$^{eA}$ | 8580.8 | 98.0 | 83.4 | 642.9 |
| | 7 | 4675.5 | 815.5$^{dB}$ | 3798.2$^{fB}$ | 61.8$^{dB}$ | 164.5$^{dA}$ | 4840.0 | 96.6 | 81.2 | 353.1 |
| Terra S. N. | 2 | 7153.3 | 2300.1$^{b}$ | 4684.7$^{dC}$ | 168.5$^{bC}$ | 167.5$^{dC}$ | 7320.8 | 97.7 | 65.5 | 493.2 |
| | 5 | 11061.3 | 3247.1$^{aA}$ | 7493.8$^{cA}$ | 320.4$^{aA}$ | 256.4$^{bA}$ | 11317.7 | 97.7 | 67.7 | 773.1 |
| | 7 | 8099.7 | 2752.1$^{aB}$ | 5107.7$^{aB}$ | 239.9$^{aB}$ | 193.4$^{cB}$ | 8293.1 | 97.7 | 63.1 | 550.3 |
| Tipo velhaca | 2 | 5161.9 | 1218.1$^{dCB}$ | 3870.0$^{eB}$ | 73.8$^{cB}$ | 118.1$^{eB}$ | 5280.0 | 97.8 | 75.0 | 376.3 |
| | 5 | 9185.2 | 1827.3$^{dA}$ | 7215.4$^{eA}$ | 142.5$^{jA}$ | 161.6$^{eA}$ | 9346.8 | 98.3 | 78.5 | 683.4 |
| | 7 | 4056.3 | 803.2$^{dC}$ | 3169.7$^{gC}$ | 83.4$^{cB}$ | 161.5$^{dA}$ | 4217.8 | 96.2 | 78.1 | 301.1 |
| Terra A. B. | 2 | 8647.2 | 2901.1$^{dB}$ | 5486.5$^{aB}$ | 259.6$^{aA}$ | 172.6$^{cAB}$ | 8819.8 | 98.0 | 63.4 | 588.9 |
| | 5 | 11281.3 | 3041.6$^{bA}$ | 8073.5$^{bA}$ | 166.2$^{cB}$ | 194.5$^{dA}$ | 11475.8 | 98.3 | 71.6 | 806.4 |
| | 7 | 6014.9 | 933.9$^{cC}$ | 4972.5$^{bC}$ | 108.5$^{bC}$ | 171.5$^{dB}$ | 6186.4 | 97.2 | 82.7 | 457.8 |
| Samurá B | 2 | 6765.0 | 1987.9$^{aB}$ | 4733.4$^{cB}$ | 43.7$^{eC}$ | 107.4$^{fB}$ | 6872.4 | 98.4 | 70.0 | 479.1 |
| | 5 | 13598.5 | 2268.1$^{cA}$ | 11106.5$^{aA}$ | 223.9$^{bA}$ | 152.1$^{fA}$ | 13750.6 | 98.9 | 81.7 | 1029.4 |
| | 7 | 5744.2 | 1245.3$^{bC}$ | 4396.4$^{dC}$ | 102.5$^{bB}$ | 146.8$^{dA}$ | 5891.0 | 97.5 | 76.5 | 422.5 |

*The same lower case letters (genotypes) and uppercase letters (ripening stages) do not differ by Scott and Knott test (5%) and Tukey test (5%), respectively

Thus, we can suggest that genotypes with high pulp C* value (e.g*.,* with pulp of higher yellow/orange intensity (Chacón-Ordóñez et al., 2017), present higher content of these provitamin compounds. High levels of provitamin compounds were found in cooking bananas and mainly in the plantains, which present a pulp with visually more yellowish coloration (some orange) compared to the others. Cultivars with lighter coloration tend to have lower carotenoids quantities, mainly the pVACs. The ripe fruit (stg 5 and 7) of the plantains and the cooking banana 'Pelipita' grouped in the PC1 and PC2 positive quadrants, together with the C* pulp variables, *t*-AC, *t*-BC, pVACs and total carotenoids, presenting the highest values of these compounds (Fig. 1).
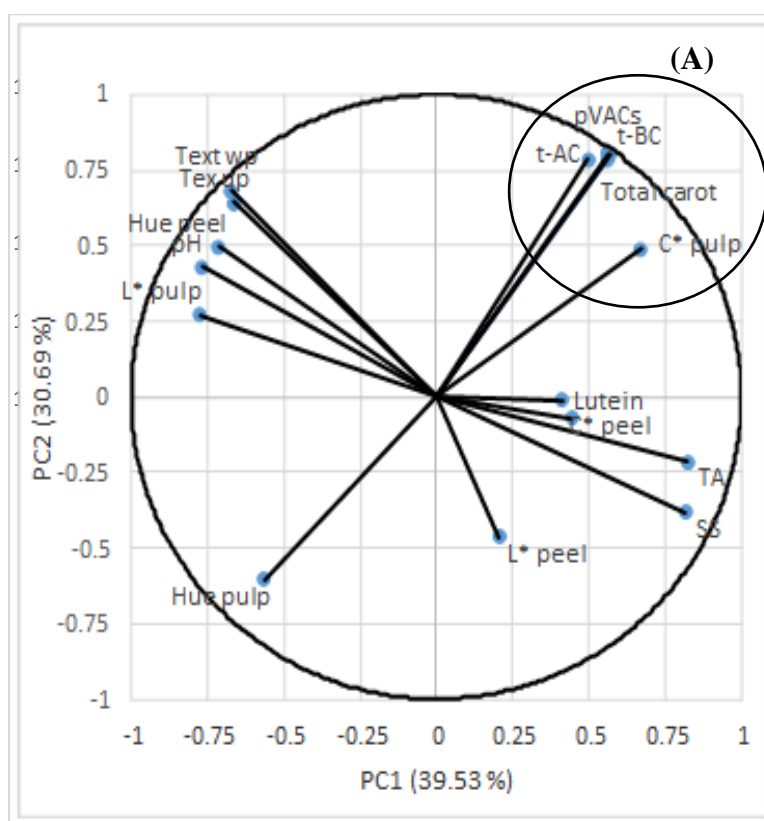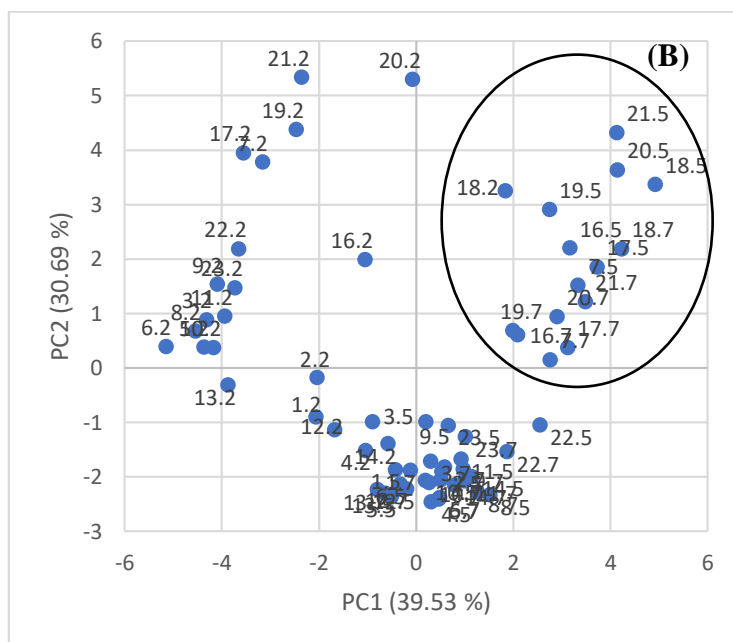
*1: FC06-02; 2: Mont. 172; 3: Tiparot; 4: Khai; 5: Mont. 301; 6: Simili; 7: Pelipita; 8: Ouro; 9: Pisang; 10: Yagambi; 11: Pacha; 12: Namwa; 13: Muisa T.; 14: Ney Poovan; 16: D'Angola; 17: Curare E.; 18: Terra S.N.; 19: Terra A. B.; 20: Tipo V.; 21: Samurá B.; 22: Prata A.; 23: Grand Naine; Stage 2: 2; Stage 5: 5; Stage 7: 7.

**Fig. 1**. Two-dimensional projection (A) and scores (B) from physicochemical (texture unpeeled: Text up, texture with peel: Text wp, soluble solids: SS, titratable acidity: TA, luminosity: L*, chromaticity: C*, angle Hue: H*) and biochemical attributes (lutein, total carotenoids: total carotenoids, pro-vitamin A carotenoids: pVACs, trans-β-carotene: t-BC, trans-α-carotene: t-AC) in the two first principal components among 22 bananas and plantains genotypes (pulp) evaluated during the fruit ripening stage (stg 2, 5 and 7).

The 'Samurá B' presented the highest pVACs contents (13598.5 µg/100 g d.w. and 6341.5 µg/100 g f.w.) and *t*-BC (11106.5 µg/100 g d.w. and 5220.04 µg/100 g f.w.) compared to the others, mainly the 'D'Angola' plantain (pVACs: 8457.9 µg/100 g d.w. and 3214 µg/100 g f.w.), which is more used in Brazil, and the dessert cultivar from the Cavendish subgroup ('Grand Naine' – pVACs: 768.5 µg/100 g d.w. and 239.30 µg/100 g f.w.), which is the most consumed in the world (Table 2). Generally, the highest quantity of pVACs was verified in the ripe fruit (stg 5), and in many cultivars in stg 7, there was a decrease in these compounds, probably due to degradation processes, oxidation and/or isomerization of these bioactive forms (Schieber & Carle, 2005). Afterwards, during ripening, carotenoid degradation occurs by enzymatic cleavage and produces other compounds, such as volatiles that contribute to the flavor (Enzell, 1985). Only the green fruit from the 'Samurá B' plantain also presented superior values of these compounds in relation to the other genotypes and were grouped into the ripe fruit category (PC1 + and PC2 +) (Fig. 1). In this study, all plantains and the cooking
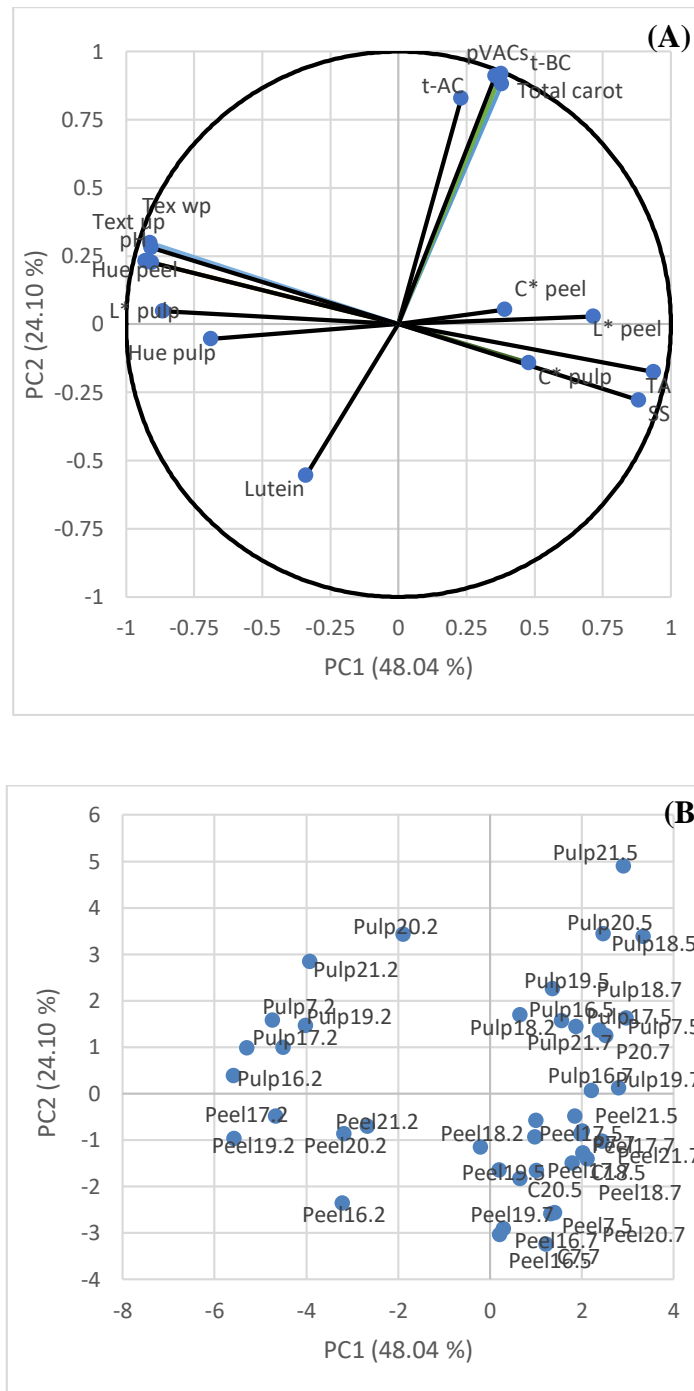
banana 'Pelipita' presented the highest RAE values (223.2 to 1029.4 μg /100 g f.w.) in relation to other studied genotypes (Table 2), with larger vitamin A quantities.

In dessert bananas, the pVACs levels varied from 98.6 μg/100 g d.w. (78.1 μg/100 g f.w. of $t$-BC) for the 'Monthan 301' (stg 2) to 2742.7 μg/100 g d.w. (1572 μg/100 g f.w.) for the 'Prata-Anã' (stg 5). The 'Prata-Anã' (AAB), the most consumed cultivar in Brazil, and the 'Pisang K. Bung' (AAB), presented 3 and 2 times more pVACs and almost 6 and 5 times more $t$-BC, respectively, in the ripe fruit (stg 5), when compared to the variety of the Cavendish subgroup (Grand Naine AAA, most consumed in the world) (Table 2).

*2.2.2 Carotenoids in pulps and peels in different ripening stages with significant pro-vitamin A (pVACs) quantities*

A principal components analysis was conducted in order to show the possible differences in the carotenoids distribution in peel and pulp. Due to the diversity in the carotenoid patterns, depending on the fruit localization, distinct groups were formed (Fig. 2). Generally, pigments such as the carotenoids are found in larger quantities in the peels compared to the pulps (Rodriguez-Amaya, 2001). The fruit and vegetable peels are more exposed to sunlight than the pulp, which induces carotenoids synthesis (Prasad et al., 2011). However, in the present study, we detected variations in the carotenoids content in the different fractions (pulp and peel), depending on the type of carotenoid found. PC1 and PC2 explained 72.14% of the data variance (Fig. 2). The CP2 axis represents 24.10% of the total data variance, separating the pulps (PC2 +) from the peels (PC2 -).

Lutein was the major compound found in the peels, with content superior to the pulp and was the compound responsible for the graphical separation between peel and pulp. The highest lutein content was identified in the green fruit peel from the Terra Anã Branca cultivar (1602.1 μg/100 g d.w. – Table 3), almost ten times greater than the content found in the pulp of the same cultivar. However, the pulps of all the cultivars presented superior values of pVACs, $t$-BC and $t$-AC, mainly in the ripe (stg 5) and overripe (stg 7) (PC1 + and PC2 +) fruit. The pVACs content in the peel (8122.10 μg/100 g d.w.) of plantain 'Samurá B' was lower than that in the pulp (13598.5 μg/100 g). PC1 was the principal component responsible for the separation of the green fruit from the ripe ones (pulp and peel), with 48.04% of the data variance.

Fig. 2. Two-dimensional projection (A) and scores (B) from physicochemical (texture unpeeled: Text up, texture with peel: Text wp, soluble solids: SS, titratable acidity: TA, luminosity: L*, chromaticity: C*, angle Hue: H*) and biochemical attributes (lutein, total carotenoids: total carot, pro-vitamin A carotenoids: pVACs, trans-β-carotene: t-BC, trans-α-carotene: t-AC) in the first two principal components among the peels and pulps from 7 accesses (plantains and cooking banana 'Pelipita').

*16: D'Angola; 17: Curare E.; 18: Terra S.N.; 19: Terra A. B.; 20: Tipo V.; 21: Samurá B.; Stage 2: 2; Stage 5: 5; Stage 7: 7.

The highest lutein content was verified in the peels of the fruit in stg 2 (green fruit) (PC1- and PC2 -) (Fig. 2). It is interesting that in the peel, during the ripening

process (stg 5 and 7), there is the presence of an α-carotene isomer (*cis*-α-carotene – c-AC), different from what was observed in the pulp. Similar to the pulp, the pVACs content increases in the peels, with little decrease when they reach stage 7 (Table 3). In addition to the stimulant effects of the solar exposure on carotenogenesis, photoisomerization can lead to a larger proportion of isomers (e.g., α-carotene) in the peel than in the pulp (Etzbach, Pfeiffer, Weber, & Schieber, 2018).

It is also worthwhile to point out that the peel is an important byproduct in the food industry and can be a potential source of antioxidant compounds. Some phytochemical and pharmacological studies describe the use of *Musa* spp. peels for presenting a pharmacological potential attributed to its chemical compounds, e.g., carotenoids. However, the use of this byproduct for industrial purposes depends on its chemical composition, a characteristic that can be strongly affected by genetic factors and the ripening stage, as demonstrated in this study.

**Table 3.** Lutein and pVACs (*t*-BC, *t*-AC and *c*-BC) content, percentage of pVACs of the total carotenoids and RAE in peels of different accesses of *Musa* spp. (plantains).

| Accesses | Ripening stage | pVACs (µg/100g d.w.) | pVACs (µg/100g d.w.) | | | | Lutein (µg/100g d.w.) | ∑ Carotenoids (µg/100g d.w.) | pVACs (%) of total carotenoids | *t*-BC (%) of pVACs total | RAE (µg/100g d.w.) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | *t*-AC | *c*-AC | *t*-BC | *c*-BC | | | | | |
| Pelipita | 2 | 1891.4 | 403.7$^{eA}$* | nd** | 1456.1$^{eA}$ | 31.6$^{eB}$ | 1285.1$^{cA}$ | 3176.5 | 59.5 | 77.0 | 139.5 |
| | 5 | 2191.5 | 417.9$^{eA}$ | 153.2$^{bA}$ | 1476.4$^{eA}$ | 50.9$^{eA}$ | 1188.1$^{aB}$ | 3379.6 | 64.8 | 67.4 | 152.8 |
| | 7 | 1113.0 | 153.4$^{fB}$ | 57.4$^{eB}$ | 873.0$^{Eb}$ | 29.2$^{eB}$ | 971.3$^{aC}$ | 2084.3 | 53.4 | 78.4 | 82.8 |
| D'Angola | 2 | 849.8 | 278.9$^{fB}$ | nd | 550.8$^{fA}$ | 20.1$^{fA}$ | 1021.2$^{eA}$ | 1871.0 | 45.4 | 64.8 | 58.4 |
| | 5 | 1044.7 | 323.2$^{fAB}$ | 88.7$^{eA}$ | 608.6$^{fA}$ | 24.2$^{fA}$ | 861.7$^{bB}$ | 1906.4 | 54.8 | 58.2 | 68.9 |
| | 7 | 1001.8 | 350.2$^{dA}$ | 87.7$^{dB}$ | 543.9$^{fA}$ | 20.0$^{fA}$ | 609.0$^{dC}$ | 1610.8 | 62.2 | 54.3 | 64.4 |
| Curare Enano | 2 | 4040.3 | 903.4$^{cB}$ | nd | 3088.4$^{aB}$ | 38.6$^{dC}$ | 1397.9$^{bA}$ | 5438.2 | 74.3 | 76.4 | 297.0 |
| | 5 | 5220.5 | 993.5$^{cA}$ | 93.7$^{eA}$ | 4015.6$^{bA}$ | 98.5$^{dA}$ | 311.5$^{cB}$ | 5532.0 | 94.4 | 76.9 | 384.8 |
| | 7 | 3758.5 | 575.0$^{dC}$ | 81.8$^{dB}$ | 3040.3$^{aB}$ | 48.4$^{dB}$ | 285.6$^{eB}$ | 4044.1 | 92.9 | 81.0 | 283.3 |
| Terra S. N | 2 | 3402.5 | 1008.9$^{bB}$ | nd | 2335.7$^{cB}$ | 57.9$^{cB}$ | 1164.9$^{dA}$ | 4567.4 | 74.5 | 68.6 | 239.1 |
| | 5 | 4087.3 | 1152.0$^{bA}$ | 129.6$^{dB}$ | 2650.4$^{cA}$ | 155.3$^{aA}$ | 918.6$^{bB}$ | 5005.9 | 81.6 | 64.8 | 280.7 |
| | 7 | 3749.9 | 1201.7$^{aA}$ | 126.6$^{cB}$ | 2264.4$^{bB}$ | 157.2$^{aA}$ | 665.5$^{cC}$ | 4415.4 | 84.9 | 60.4 | 250.6 |
| Tipo Velhaca | 2 | 2712.3 | 816.1$^{dA}$ | nd | 1809.5$^{dA}$ | 68.8$^{bB}$ | 1262.6$^{cA}$ | 3974.9 | 68.2 | 66.7 | 188.4 |
| | 5 | 3079.2 | 835.8$^{dA}$ | 130.0$^{dA}$ | 1946.1$^{dA}$ | 101.6$^{dA}$ | 908.6$^{bB}$ | 3987.8 | 77.2 | 63.2 | 209.4 |
| | 7 | 2992.8 | 820.6$^{cA}$ | 123.2$^{cA}$ | 1968.4$^{cA}$ | 70.1$^{cB}$ | 855.7$^{bB}$ | 3848.5 | 77.8 | 65.8 | 206.7 |
| Terra A. B | 2 | 2928.0 | 975.7$^{bB}$ | nd | 1773.9$^{dA}$ | 178.4$^{aA}$ | 1602.1$^{aA}$ | 4530.1 | 64.6 | 60.6 | 195.9 |
| | 5 | 3385.7 | 1190.8$^{bA}$ | 138.8$^{cA}$ | 1802.4$^{dA}$ | 149.8$^{bB}$ | 850.1$^{bC}$ | 4235.8 | 80.0 | 53.2 | 216.2 |
| | 7 | 2264.5 | 154.1$^{fC}$ | 133.0$^{bA}$ | 1741.2$^{dA}$ | 98.0$^{bC}$ | 930.0$^{aB}$ | 3194.5 | 71.0 | 76.9 | 166.9 |
| Samurá B. | 2 | 4350.7 | 1429.1$^{aB}$ | nd | 2698.4$^{bB}$ | 37.2$^{dC}$ | 879.9$^{fA}$ | 5230.6 | 83.2 | 62.0 | 293.7 |
| | 5 | 8122.1 | 1558.6$^{aA}$ | 208.7$^{aA}$ | 6245.4$^{aA}$ | 109.4$^{cA}$ | 865.2$^{bA}$ | 8987.3 | 90.5 | 75.4 | 605.4 |
| | 7 | 4185.4 | 998.7$^{bC}$ | 157.5$^{aB}$ | 2899.2$^{aB}$ | 94.2$^{bB}$ | 590.6$^{dB}$ | 4776.0 | 87.6 | 69.3 | 295.2 |

*The same lower case letters (genotypes) and uppercase letters (ripening stages) do not differ by Scott and Knott test (5%) and Tukey test (5%), respectively.

** nd – not detected

*2.2.3 Impact of thermal processing on plantains and/or cooking bananas*

Retention studies of the pVACs in different thermal processes indicate that the prolonged exposure to the heat of frying and cooking processes results in substantial losses of these bioactive compounds. However, the reported data are conflicting and many times difficult to interpret. Generally, the processing conditions are partially described and, in addition, the time and temperature conditions are used in a different form in the same processing method, making the comparisons among studies more difficult (Ekesa et al., 2012).

With the principal components analysis, we could verify the profile of these compounds in the different thermal processes in the genotypes studied. The retention study of carotenoids after thermal processing has been one of the main concerns, both in the industrial process and home preparations, which can generate great losses of these nutrients depending on the preparation method used (Rodriguez-Amaya, Delia B., Rodriguez, Evelyn B, Amaya-Farfan, 2006). The dispersion of the varieties according to the PC1 and PC2 axis are shown in Figure 3. PC1 and PC2 explained 93.2% of the data variance. The PC2 axis represents 11.09% of the total data variance and was responsible for the genotypes separation, in the function of different chemical compounds observed (i.e., lutein, total carotenoids, provitamin A carotenoids, *t*-BC and *t*-AC).

The 'Pelipita' grouped with the total carotenoids, pVACs, *t*-BC and lutein (PC1+ and PC2-), showing the highest content of those compounds (Table 3). However, the 'D'Angola' presented the highest *t*-AC contents.

PC1 represents 82.07% of the total data variance, grouping the thermal treatments used (Fig. 3). Few studies have focused on plantains and/or cooking bananas after the different thermal processes, that is, how the fruit are consumed. The *in natura* samples microwaved without peel, and boiled bananas with peels, presented the best results regardless of the genotype used (Fig. 3). The 'Pelipita' boiled with peel or cooked in microwave without peel presented 1.12 and 0.93 times more pVACs than the *in natura* fruit, respectively (Table 4). Boiling, baking and frying are the principal cooking methods used in bananas (e.g., plantains and cooking bananas) with a preference for boiling. These fruit can be boiled with or without peel, according to the preparations (Ekesa et al., 2012).
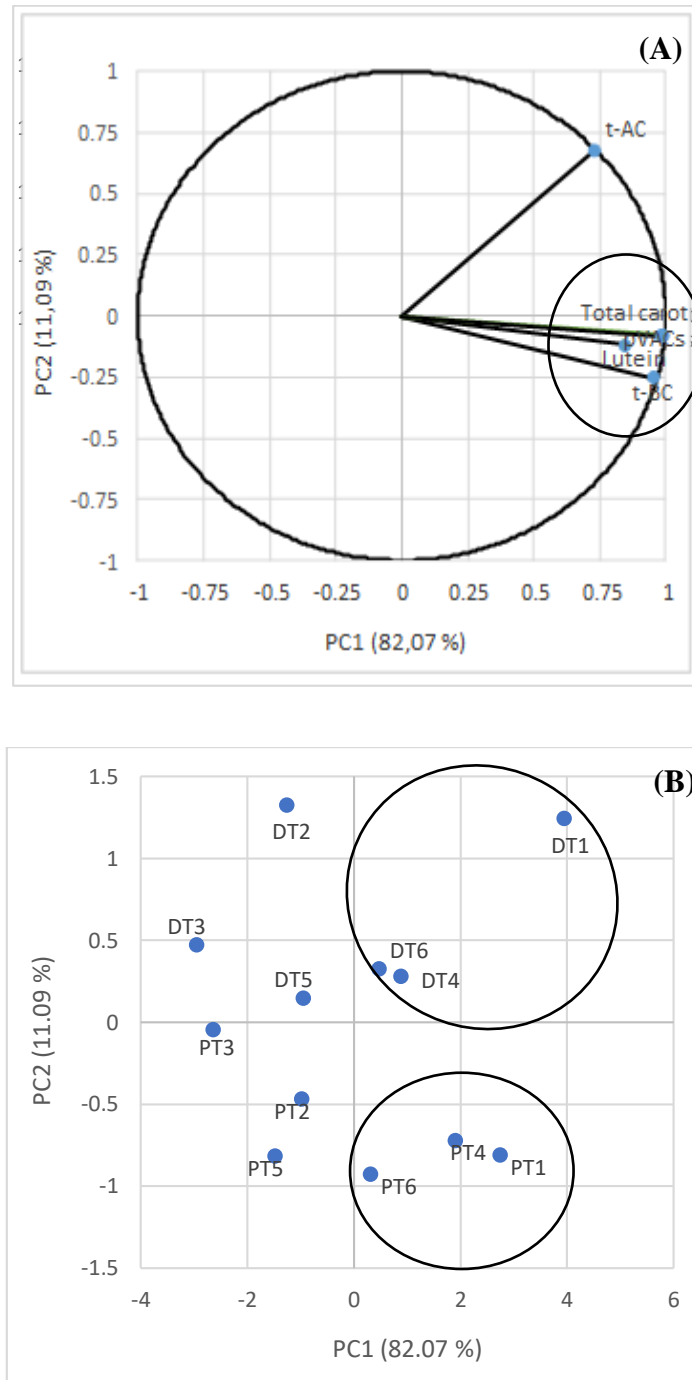
*T1: boiling with peel; T2: boiling without peel; T3: microwaving with peel; T4: microwaving without peel; T5: styr-frying; T6: *in natura.*

**Fig. 3**. Two-dimensional projection (A) and scores (B) of biochemical attributes (lutein, total carotenoids: total carot, pro-vitamin A carotenoid: pVACs, trans-β-carotene: t-BC, trans-α-carotene: t-AC) in the two first principal components among 'D'Angola' (D) plantain and 'Pelipita' (P) cooking banana submitted to thermal processes.

For the 'D'Angola', cooking with the peel also resulted in increase of the provitamin compounds when compared to the *in natura* fruit, increasing the nutritional and functional values. However, differently from 'Pelipita', 'D'Angola' without peel presented losses of pVACs in the microwaving, besides the verified retention (90.6%).

These differences found in the carotenoids content under the same thermal treatment, between cooking banana 'Pelipita' and the 'D'Angola' plantain, may be a result of differences inherent between genotypes. 'Pelipita' (cooking banana) presented less firmness (1.06 N with peel and 0.25 N unpeeled) compared to 'D'Angola' (2.80 N with peel and 0.80 N without peel) at the time of thermal processing, which may have been a contributing factor for the extraction of carotenoids. Similar results were described by (Ekesa et al., 2012) in boiled bananas and plantains. The authors attributed the results to the level the cellulose is broken down in the genotypes, consequently improving the carotenoid extraction.

Retention studies performed with different genotypes of cooking bananas and plantains consumed in Africa showed high carotenoids retention (provitamin) in different forms of preparation used in the regions studied (Ekesa et al., 2012). The carotenoids retentions after the boiling process can vary from 40 – 95%, depending on the analyzed cultivar. In addition, an increase of two times the total of pVACs was described in the 'Musilongo' genotype after the boiling treatment when compared to the *in natura* value (Ekesa et al., 2012), similar to what was found in this study. The carotenoids can suffer chemical modifications and alterations in their contents as a function of the thermal process in the different methods of preparation (Ekesa et al., 2012). The thermal treatment affects the cell wall of the food matrix, which can lead to better extraction of the antioxidant compounds from the cell matrix (Blessington et al., 2010; Murador, Braga, Da Cunha, & De Rosso, 2018), similar to the results found in some treatments (boiling with peel and microwaved without peel) performed in this study.

**Table 4.** Lutein and pVACs (*t*-BC, *t*-AC and *c*-BC) content, percentage of t-BC and pVACs of total carotenoids and RAE after cooking processes of the 'D'Angola' plantain in the 'Pelipita' cooking banana.

| Cooking processes | pVACs (µg/100g d.w.) | pVACs (µg/100g d.w.) | | | Lutein (µg/100g d.w.) | ∑ Carotenoids (µg/100g d.w.) | pVACs (%) of total carotenoids | *t*-BC (%) of total carotenoids | REA (µg/100g f.w.) |
|---|---|---|---|---|---|---|---|---|---|
| | | *t*-AC | *t*-BC | *c*-BC | | | | | |
| *D'Angola* | | | | | | | | | |
| Boiling with peel | 10128.1 | 1913.1 (828.5**)[a*] | 7981.0 (3456.4)[a] | 234.0 (101.3)[a] | 181.1 (78.4)[a] | 10309.2 | 98.2 | 78.8 | 326.8 |
| Boiling without peel | 6705.9 | 1601.9 (546.9)[b] | 4929.2 (1682.7)[d] | 174.8 (59.7)[b] | 143.3 (48.9)[b] | 6879.2 | 97.5 | 73.5 | 165.5 |
| Microwave with peel | 2651.3 | 815.8 (326.3)[f] | 1744.8 (880.0)[f] | 90.7 (36.3)[d] | 150.2 (60.1)[b] | 2801.5 | 97.1 | 81.9 | 88.4 |
| Microwave without peel | 6939.9 | 1209.8 (515.4)[d] | 5538.8 (2365.9)[c] | 191.3 (81.5)[b] | 176.7 (75.3)[a] | 7116.6 | 97.5 | 79.8 | 222.0 |
| Stir-frying | 5186.2 | 898.6 (393.1)[e] | 4146.2 (1813.5)[e] | 141.4 (88.4)[c] | 144.9 (63.4)[b] | 5331.1 | 97.3 | 79.9 | 171.2 |
| *In natura* | 7656.0 | 1318.3 (500.9)[c] | 6206.0 (2394.0)[b] | 131.7 (49.5)[c] | 174.1 (66.2) [a] | 7830.0 | 97.8 | 81.1 | 222.4 |
| *Pelipita* | | | | | | | | | |
| Boiling with peel | 8496.6 | 1010.6 (466.8)[a] | 7410.9 (3423.2)[a] | 75.1 (34.7)[a] | 178.1(82.3)[a] | 8674.7 | 97.8 | 87.2 | 306.2 |
| Boiling without peel | 7020.7 | 819.4 (307.1)[b] | 6146.5 (2303.7)[c] | 54.8 (20.0)[c] | 136.4 (51.1)[c] | 7157.1 | 98.1 | 87.5 | 205.6 |
| Microwave with peel | 4038.1 | 654.1 (274.7)[c] | 3360.1 (1411.1)[d] | 23.9 (10.0)[e] | 131.3 (55.1)[c] | 4169.4 | 95.7 | 77.0 | 129.4 |
| Microwave without peel | 8366.6 | 980.7 (432.9)[a] | 7235.3 (3194.1)[a] | 150.6 (28.8)[b] | 168.3 (74.3)[ab] | 8534.9 | 98.0 | 86.5 | 285.4 |
| Stir-frying | 5149.1 | 529.9 (227.1)[d] | 4568.1 (1958.1)[d] | 51.1 (21.9)[cd] | 139.9 (60)[c] | 5289.0 | 97.4 | 88.7 | 186.2 |
| *In natura* | 7794.8 | 783.9 (313.6)[b] | 6963.1 (2785.2)[b] | 47.8 (19.1)[d] | 155.7 (62.3)[b] | 7950.5 | 98.0 | 89.3 | 245.9 |

*The same lower case letters do not differ by Tukey test (5%)

** fresh weight (f.w.)

## 2.3 Conclusion

This study contributes carotenoids characterization in different *Musa* spp. genotypes. The presence of β-carotene, α-carotene and lutein in green, ripe and over-ripe fruit (peel and pulp) was confirmed. The pulps of all the cultivars presented superior values of provitamin A, mainly in the ripe and over-ripe fruit. The pVACs contents vary according to the genotype and high levels of carotenoids are found in plantains. The plantain 'Samurá B' presents nutritional properties superior to the other analyzed genotypes, which makes its promotion or incorporation in agricultural systems or biofortification programs interesting. Lutein was the major compound found in the peels and was identified mainly in the green fruit peel. Peel is an important byproduct in the food industry and can be a potential source of bioactive compounds (e.g., lutein). The use of genotypes whose pulps and peels present high levels of antioxidants could be explored by the food and pharmaceutical industries, adding value to the product and at the same time increasing the income of the rural producer. However, it is worthwhile to point out that carotenoids content is affected by ripening stage and revealed decreased nutritional values (reduced pVACs) of fruit at ripening stage 7. These results indicate that choices for yellow fruits (stg 5) become interesting when consumers are looking for higher quantities of bioactive compounds, mainly provitamin A. In addition, thermal processing can increase the functional and nutritional values of the *Musa* spp. fruit. The carotenoids can suffer chemical modifications and alterations in their contents as a function of the thermal process used in the different methods of fruit preparation, mainly boiling with water, which should be preferred in domestic preparations, regardless of the cultivar used.

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**Capítulo 3**

**Effect of ripening and cooking processes on the bioactive amine content in bananas and plantain (*Musa* spp.)**

(artigo submetido ao periódico científico Food Research International)

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**ABSTRACT:** Bioactive amines are found in food and, depending on its concentrations, can be relevant not only for the product shelf life of the product and final quality, but also for the human health. The bioactive amines content in pulp and peel of 20 banana and plantain genotypes was analyzed in order to evaluate the impact of ripening on the carotenoid content as well as its retention after different thermal processes. Fruits were ripened at stage 2 (green), 5 (yellow) and 7 (yellow with dark spots). The bioactive amines profile evaluated in this study variated according to the genotype, ripening stage and the analyzed plant tissue. In most of the analyzed genotypes, tyramine, histamine, dopamine, serotonin, spermidine and spermine decreased during the ripening process in pulps. In opposition, during the ripening, there was accumulation of putrescine. In many genotypes of bananas, the serotonin and dopamine content in the pulp was increased until the stage 5 (ripe fruit) and decreased in the stage 7 (super ripe fruit/yellow with black spots). However, in the pulp of most of the plantains genotypes there was an accumulation of serotonin and dopamine in the stage 7, showing a possible differentiation from the profile of these amines by consume group. Dopamine and serotonin occur in superior quantities in the peels when compared to the pulp of the *Musa* spp. fruit. In addition, thermal processing affects the content of amines present in the fruit depending on the compounds and analyzed genotype. The 'D'Angola' plantain contain superior quantities of catecholamines (tyramine and dopamine), in *in natura* fruit as well as in the ones submitted to thermal processing (i.e. boiling with peel). Boiling without peel, microwave with peel and stir-frying) induced losses of dopamine, differently form the observed with the tyramine, which did not show variation after the processing, regardless of the analyzed genotype. The cooking

banana 'Pelipita' presented high contents of serotonin and spermine, mainly when the fruit were submitted to cooking processes (microwave and boiling). The boiling and microwaving with peel increased the serotonin contents in this genotype. The boiling with peel should be preferred in domestic preparations, mainly when the aim is to consume higher quantities of catecholamines and indolamines, regardless of the cultivar used.

Keywords: dopamine, serotonin, antioxidant, cooking processes

## 3.1 Introduction

The bioactive or biologically active amines perform important metabolic and physiological functions in animals, vegetables and microorganisms. In plants, amines as spermidine and spermine are associated to growth processes and cell division, and for presenting the same precursor as the ethylene, are related to ripening and senescence (Kalač, 2014). Some amines are involved in the control and in the regulation of the responses to the biotic and abiotic stress that limit the quality and post-harvest useful life of the fruit (Agudelo-Romero, Bortolloti, Pais, Tiburcio, & Fortes, 2013; Liu et al., 2006). Other amines such as the histamine and serotonin can act as protective substances, while dopamine and noradrenaline are related to the enzymatic darkening in some fruit, as the bananas for example (Marriott, 1980). In addition, recent researches indicate that the bioactive amines (BAs) can also contribute to the antioxidant activity in fruit and vegetables (Adão & Glória, 2005; Lima, Da Rocha, Takaki, Ramos, & Ono, 2008). Studies have shown e.g., dopamine presents higher antioxidant capacity *in vitro* (by DPPH test), in comparison to the other natural antioxidants, such as ascorbic acid, reduced glutathione and many phenolic compounds, as the gallocathecol (González-Montelongo, Gloria Lobo, & González, 2010).

Bioactive amines are found in food and, depending on its concentrations, can be relevant not only for the product shelf life of the product and final quality, but also for the human health (Kalač, 2014). The serotonin has been detected in high quantities in *Musa* spp. fruit, mainly when compared with other fruit and vegetables (Islam, Shirakawa, Nguyen, Aso, & Komai, 2016). According to Xiao et al. (1998), with the

ingestion of banana, considered relatively rich in serotonin, there is a raise in the level of serotonin in the blood. Considering the antiobesity effects of the serotonin, the investigation of the content of this compound in different genotypes of fruits and vegetables is important in the horticulture. In addition, the banana has also been appointed as a promising source of dopamine (L-DOPA) research for the future development of pharmaceutical formulations for diseases treatment, as the Parkinson disease (Pereira & Maraschin, 2015).Besides the importance of determining the content of these compounds in foods, on behalf of the benefits for human health, the determination of biogenic amines in foods also assume great importance due to the toxic effect of some amines, as histamine and tyramine, depending on the quantity. When consumed in excess, can cause different pharmacological, physiological and toxic effects. The levels of the bioactive amines are influenced by the food composition, microbial flora, storage (i.e., ripening and packaging) and processing type (Adão & Glória, 2005; Bomtempo, Costa, Lima, Engeseth, & Gloria, 2016; Plonka & Michalski, 2017). It is also of great importance the study from the planting to the final consumer.

Bananas and plantains stand out for being consumed in all stages of ripening and after different thermal processes (i.e., fried and baked). The additional biochemical peculiarities during the ripening of different genotypes generate additional possibilities for the use of *Musa* spp. fruit, because its consumption does not occur necessarily in the *in natura* form, but also after the processing and/or use of byproducts processed in different ripening stages. In addition, the comprehension of biochemical transformations that happen during the ripening allow us to understand how these alterations affect the quality of fruit and the post-harvest life, establishing the best consumption point. Another relevant factor to decrease the post-harvest losses, as well as the generated residues, is that the banana pulp and peel can be explored by the food and pharmaceutical industries, adding value to the product and increasing the rural producer income. The peel correspond to 40% of the total weight of the fruit and causes a great problem in the food processing industries today; thus, it is essential to find an application for the use of this byproduct  (Emaga et al., 2007; Pereira & Maraschin, 2015). Therefore, the aims of this study were to evaluate (1) the impact of ripening in the bioactive amine sin pulps and peels and (2) the retention of analyzed compounds after different thermal processes in banana tree fruit of different genotypes.

## 3.2 Materials and Methods

### 3.2.1 Harvest location of the analyzed genotypes

Bunches of 20 genotypes of banana trees were harvested between March and June 2016 from different genomic groups belonging to the Active Germplasm Bank (banana AGB) (Embrapa Cassava & Fruits, Cruz das Almas, Bahia, Brazil). All studied genotypes were grown at the same location (Latitude 12º40'12'' S; Longitude 39º06'07'' W; Altitude 225 m), to exclude experimental errors due to environmental factors regarding differences in composition (Table 1). These genotypes (20 plants per genotype) were planted over 3 replications. Field planting was done from April 2015. The genotypes are undergoing agronomic evaluation. When branches reached ripening stage 1, we harvested the 2nd and 3rd hand (each containing between 10 fingers) of each genotype (± 2 bunches = 40 fruit) and stored them at room temperature (20 ± 2°C) at a relative humidity of 80 ± 2% until they reached the complete ripening stage (stage 7).

**Table 1**. Banana tree genotypes belonging to the Active Germplasm Bank (banana AGB) from Embrapa Mandioca e Fruticultura, Cruz das Almas, Bahia, Brazil.

| Genotypes | Ploidy | Subgroup/Subpecies |
|---|---|---|
| *Dessert bananas* | | |
| Yangambi Km5 | AAA | Ibota |
| Khai | AAA | Ibota |
| Pisang Kepok Bung | AAB | Peyan |
| Ney Poovan | AB | Ney Poovan |
| Ouro da Mata | AAAB | * |
| *Cooking bananas* | | |
| Monthan 301 | ABB | Monthan |
| Monthan 172 | ABB | Monthan |
| Simili Radjah | ABB | Peyan |
| Pelipita | ABB | Bluggoe |
| Pacha Nadan | ABB | Saba |
| Namwa Khom | ABB | Pisang Awak |
| Muisa Tia | ABB | Pisang Awak |
| FC06-02 | AABB | Figo |
| Tiparot | ABBB | Klue Teparod |
| *Plantains* | | |
| D'Angola | AAB | Plantain |

| | | |
|---|---|---|
| Curare Enano | AAB | Plantain |
| Terra Sem Nome | AAB | Plantain |
| Tipo Velhaca | AAB | Plantain |
| Terra Anã Branca | AAB | Plantain |
| Samurá B | AAB | Plantain |

### 3.2.2. Ripening stage determination

The fruit were divided into seven ripening stages during storage, according to Soltani, Alimardani, & Omid (2011) (banana color scale). The indices are based on the peel color: 1 = green; 2 = green with yellow traces; 3 = more green than yellow; 4 = more yellow than green; 5 = yellow with green; 6 = completely yellow; 7 = yellow with coffee color areas.  We choose the stages 2, 5 and 7 for this study, because they are the most used ones for the consumption *in nature*, for the processing. The pulps and peels were powdered in liquid nitrogen, lyophilized and stored in freezer (-80°C).  The pulps of all bananas and plantains were analyzed. Only banana peels with higher levels of bioactive amines were analyzed, that is, all the plantains and one cooking banana ('Pelipita').

### 3.2.3. Bioactive amines content analysis by reverse phase high performance liquid chromatography (HPLC)

The polyamines extraction (Lima et al., 2008) was performed from lyophilized samples. Samples were homogenized for one minute in cold perchloric acid 5% (v/v) and centrifuged in 12.000 g for 20 minutes, at 4°C. To the supernatant (200μL) we added 400μL dansyl chloride and 200μL saturated sodium carbonate and, after 1 hour at 60°C, we added 200μL proline and the mixture were maintained in the dark for 30 minutes, at room temperature. After the addition of 1000 μL toluene and after homogenization, the supernatant was separated and submitted to drying in gaseous nitrogen. The samples were resuspended in 1.5 mL acetonitrile, stirred in vortex and, after 1 minute in ultrasonic bath, were filtrated (0.25 Mm) before the injection in HPLC, according to Dadáková et al. (2009). We injected 20 μL of sample in one system of DionexUltiMate 3000 Thermo Scientic (Thermo Fisher Scientific Inf.; MA, EUA), attached to a quaternary pump, with automatic final sampler 3000RS and diode array detector (DAD-3000RS). The polyamines were separated in column C18 (4.6 x 250

mm; 5 um) at 25ºC. The analysis was monitored at 280 nm and the integration peak and calibrations were realized between 210 and 350 nm, using the Chromeleon Dionex software. For determining the peaks of each compounds, we evaluated the areas below the curve, obtained through the calibration with the standards for each compounds. We accepted the maximum, inter and intra-test coefficient variation of 5%. We evaluated the contents of putrescine, spermidine, spermine, histamine, tyramine, dopamine and serotonin.

### 3.2.4. Retention analysis

D'Angola plantain in the stage 5 (ripe fruit) and the banana 'Pelipita' with and without peel were analyzed regarding the retention of bioactive amines after the thermal processing (boiling in water, microwaving and stir-frying). Three fingers (with and without peel) were boiled for 10 min in 300 mL of water in a stainless-steel pan, or microwaved for 2 min (oven set at the highest level). Fruit longitudinally cut were stir-frying in a pan grassed with soybean oil (± 2 mL), for 5 min. All pulps were powdered (IKA, A.11, Germany), lyophilized and stored at - 80 °C.

### 3.2.5. Statistical analysis

For the polyamines analyses in non-processed fruit, we used the entirely randomized design, differently for the pulp and peel. For the pulps we used a factorial scheme 20 x 3 (pulp x ripening stages), in the three ripening stages. Only two genotypes were used for the thermal processing ('Pelipita' and 'D'Angola'), which were analyzed in triplicate. The bioactive amines data were collected, summarized and submitted to variance analysis (ANOVA), followed by the Scott-Knott averages comparison test among the genotypes. Both Tukey and Scott-Knott tests were used among the ripening stages for pulps and peels, respectively. The data of the fruit submitted to the thermal processing were also submitted to variance analysis (ANOVA), followed by Tukey test using average comparison. The analysis of the variance in the data was performed using the statistics software *SISVAR (Ferreira, 2011)*. Principal component analysis (PCA, software XLSTAT - version 2017 (Addinsoft, France) was applied in order to visualize the possible correlation between ripening and bioactive amines analysis (pulp and peel). PCA was also used to visualize

the effects of thermal processing on bioactive amines content in the different genotypes.

## 3.3 Results and Discussion

### 3.3.1. Impact the ripening stage on the amines contents of the pulps of the Musa spp. fruit

We found 7 bioactive amines inside the germplasm of *Musa* spp., i.e., tyramine, histamine, dopamine, serotonin, spermine, spermidine and putrescine. The content of each amine varied during the ripening process in all the genotypes. Generally, the content of tyramine, histamine, dopamine, serotonin, spermidine and spermine decrease during the ripening process. The levels of putrescine increased, mainly in plantains, in more advanced ripening stages (Table 2).

In an attempt of establishing a descriptive model of grouping the ripening stages in functions of the total of analyzed amines for a better visualization of the profiles of these compounds during the ripening process, we opted for comparing the results obtained through the PCA. The dispersion of varieties according to PC1 and PC2 are shown in Figure 1 and reveals the existence of two groupings, correspondents to the green and ripe/super ripe fruit (stg 5 and 7). PC1 and PC2 explained 62.70% of the data set variance. The PC1 axis represents 41.20% of the total variance, separating the green fruit from the ripe ones. There is an accumulation of putrescine during the ripening process and a strong correlation with the soluble solids and titratable acidity (TA) (PC1- and PC2+). Spermine, spermidine, histamine, tyramine, dopamine and serotonin were positively related to the green fruit, decreasing along with the ripening process (Fig. 1). However, the cv. Ouro da Mata also presented a high dopamine content in stg. 5 (ripe fruit), standing out from the other genotypes in this stage.

As it was previously highlighted, the polyamines spermidine and spermine decrease with the ripening, while there is an accumulation of putrescine (Fig. 1 and Table 2). It is widely documented that for the spd and pm biosynthesis, the utilization of S-adenosilmetionina (SAM) is necessary, which is a common precursor of the ethylene (Pandey & Penna, 2017).

**Table 2**. Bioactive amines content (mg/100 g d.w.) in pulp of different genotypes of *Musa* spp.

| Genotypes | Ripening stage | Putrescine | Spermidine | Spermine | Histamine | Tyramine | Dopamine | Serotonin |
|---|---|---|---|---|---|---|---|---|
| *Dessert bananas* | | | | | | | | |
| Yangambi Km5 | 2 | 15.3[dB] | 17.4[bA] | 14.8[aA] | 7.3[aA] | 10.0[bA] | 38.1[aA] | 14.8[bA] |
| | 5 | 26.0[cA] | 16.1[cB] | 13.9[bB] | 6.9[eB] | 9.3[aB] | 31.3[bB] | 8.6[dB] |
| | 7 | 25.9[fA] | 15.3[dC] | 13.6[bB] | 6.8[cB] | 9.1[dC] | 26.5[eC] | 9.0[cB] |
| Khai | 2 | 15.3[dB] | 14.3[dB] | 14.5[bA] | 6.9[cB] | 8.9[fB] | 29.4[eB] | 10.0[eA] |
| | 5 | 24.7[cA] | 17.2[aA] | 14.0[bB] | 7.5[bA] | 9.4[aA] | 30.7[bA] | 10.6[cA] |
| | 7 | 24.9[fA] | 16.6[cA] | 13.8[aB] | 7.4[aA] | 9.1[dB] | 27.6[dC] | 9.1[cB] |
| Pisang K. Bung | 2 | 14.6[dC] | 16.8[cA] | 14.7[aA] | 7.1[bA] | 9.6[dA] | 30.1[eA] | 10.4[eA] |
| | 5 | 20.1[dB] | 17.2[aA] | 13.8[cB] | 6.9[eB] | 9.3[aB] | 29.6[cA] | 7.7[dB] |
| | 7 | 26.1[fA] | 17.8[aA] | 13.8[aB] | 6.8[cB] | 9.2[dB] | 27.0[eB] | 8.5[dB] |
| Ney Poovan | 2 | 20.6[bB] | 18.1[aA] | 13.7[dA] | 6.9[cA] | 9.5[dA] | 37.0[aA] | 8.5[fA] |
| | 5 | 20.8[dB] | 16.9[aB] | 13.6[cA] | 7.0[dA] | 9.4[aA] | 30.1[cB] | 9.4[dA] |
| | 7 | 24.6[fA] | 16.5[cB] | 13.5[bA] | 6.7[cB] | 9.3[cB] | 28.4[dC] | 9.4[cB] |
| Ouro da Mata | 2 | 14.3[dB] | 16.3[cB] | 14.8[aA] | 7.4[aB] | 10.3[aA] | 35.4[aA] | 20.3[aA] |
| | 5 | 27.1[bA] | 17.5[aA] | 14.0[bB] | 8.6[aA] | 9.5[aB] | 34.4[aA] | 13.7[bB] |
| | 7 | 25.7[fA] | 17.1[bA] | 13.9[aB] | 7.1[bC] | 9.2[dC] | 27.7[dB] | 9.0[cC] |
| *Cooking bananas* | | | | | | | | |
| Monthan 301 | 2 | 14.9[dB] | 17.5[bB] | 14.2[cA] | 7.3[aA] | 9.5[dA] | 27.9[fA] | 7.9[fA] |
| | 5 | 26.1[cA] | 17.3[aA] | 13.7[cB] | 7.2[cAB] | 9.4[aA] | 28.3[dA] | 7.7[dA] |
| | 7 | 27.5[eA] | 16.3[cB] | 13.7[aB] | 7.1[bB] | 9.3[cB] | 28.0[dA] | 8.0[cA] |
| Monthan 172 | 2 | 16.0[dB] | 17.3[bA] | 13.8[dA] | 7.0[cA] | 9.1[eB] | 26.8[fA] | 8.8[fA] |
| | 5 | 19.4[dA] | 15.9[cB] | 13.8[cA] | 6.9[eA] | 9.3[aA] | 27.2[eA] | 8.5[dA] |
| | 7 | 18.9[hAB] | 15.6[dB] | 13.6[bA] | 6.7[cB] | 9.0[dB] | 27.2[eA] | 7.9[dA] |
| Simili Radjah | 2 | 15.8[dC] | 17.0[cA] | 14.0[cA] | 7.2[aA] | 9.3[eA] | 27.8[fA] | 11.0[dA] |
| | 5 | 25.4[cB] | 16.0[cB] | 13.7[cB] | 6.9[eA] | 9.2[bA] | 28.0[dA] | 8.0[dB] |
| | 7 | 29.3[eA] | 16.8[cA] | 13.5[bB] | 6.7[cB] | 9.1[dA] | 26.3[eB] | 8.0[dB] |
| Pelipita | 2 | 14.8[dB] | 17.5[bA] | 14.3[cA] | 7.4[aA] | 9.2[eA] | 27.4[fA] | 13.1[cB] |
| | 5 | 19.4[dA] | 15.8[cB] | 14.2[aA] | 7.1[cB] | 9.3[aA] | 27.1[eA] | 16.7[aA] |
| | 7 | 22.2[gA] | 15.6[dB] | 13.8[aB] | 7.1[bB] | 9.2[dA] | 27.1[eA] | 8.2[dC] |
| Pacha Nadam | 2 | 17.2[dB] | 18.2[aA] | 14.4[bA] | 7.4[aA] | 9.8[cA] | 30.6[eA] | 20.2[aA] |
| | 5 | 21.0[dA] | 16.4[bB] | 13.8[cB] | 7.3[cA] | 9.4[aB] | 30.8[bA] | 12.9[bB] |
| | 7 | 23.1[gA] | 16.2[cB] | 13.7[aB] | 7.2[bB] | 9.2[dC] | 26.7[eB] | 9.9[cC] |
| Namwa | 2 | 21.4[bA] | 17.9[bA] | 13.9[cA] | 7.0[cA] | 10.3[aA] | 34.6[bA] | 8.9[fA] |
| | 5 | 21.2[dA] | 16.4[bB] | 13.5[cB] | 6.8[eB] | 9.5[aB] | 27.9[dB] | 7.8[dB] |
| | 7 | 17.5[hB] | 15.8[dB] | 13.6[bB] | 6.8[cB] | 9.3[cC] | 27.4[eB] | 9.6[cA] |
| Muísa Tia | 2 | 20.1[bA] | 18.5[aA] | 14.1[cA] | 6.9[cA] | 9.6[dA] | 27.3[fB] | 8.0[fA] |
| | 5 | 20.3[dA] | 16.4[bB] | 13.7[cB] | 6.9[eA] | 9.6[aA] | 28.2[dA] | 8.7[dA] |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | 7 | 19.3[hA] | 16.7[cB] | 13.8[aB] | 6.9[cA] | 9.5[bB] | 28.3[dA] | 8.8[cA] |
| FC06-02 | 2 | 17.19[dA] | 19.0[aA] | 13.8[dA] | 7.2[aA] | 9.2[eA] | 27.3[fA] | 9.7[eA] |
| | 5 | 17.48[dA] | 16.7[bB] | 13.7[cA] | 6.8[eB] | 9.1[bB] | 26.7[eA] | 7.5[dB] |
| | 7 | 17.44[hA] | 16.5[cB] | 13.5[bB] | 6.6[cB] | 8.9[dB] | 26.5[eA] | 7.6[dB] |
| Tiparot | 2 | 13.95[dC] | 16.0[cB] | 13.9[cA] | 7.4[aA] | 9.6[dA] | 31.5[dA] | 8.2[fA] |
| | 5 | 20.62[dB] | 16.3[bB] | 13.8[cA] | 7.1[dB] | 9.5[aA] | 29.4[cB] | 8.2[dA] |
| | 7 | 31.48[dA] | 17.1[bA] | 13.7[aA] | 6.8[cC] | 9.1[dB] | 26.5[eC] | 8.2[dA] |

*Plantains*

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| D'Angola | 2 | 24.9[aB] | 18.6[aA] | 14.1[cA] | 6.9[cB] | 9.5[dA] | 30.5[eA] | 11.6[dA] |
| | 5 | 24.7[cB] | 17.2[aB] | 13.9[bA] | 7.2[cA] | 9.4[aA] | 28.6[dB] | 9.8[cB] |
| | 7 | 28.2[eA] | 16.8[cB] | 13.7[aB] | 6.8[cB] | 9.2[dB] | 28.9[dB] | 11.0[bA] |
| Curare Enano | 2 | 14.7[dC] | 16.6[cB] | 14.4[bA] | 6.9[cA] | 9.5[dB] | 31.4[dA] | 13.9[cA] |
| | 5 | 40.4[aB] | 16.7[bB] | 13.8[cB] | 6.8[eA] | 9.2[bC] | 29.8[cB] | 10.9[cB] |
| | 7 | 63.6[aA] | 17.8[aA] | 13.7[aB] | 6.9[cA] | 10.0[aA] | 31.2[bA] | 13.2[aA] |
| Terra S. N. | 2 | 21.1[bB] | 17.5[bA] | 13.8[dA] | 6.9[cB] | 9.2[eA] | 33.7[cA] | 15.5[bA] |
| | 5 | 23.4[cB] | 17.5[aA] | 13.9[bA] | 7.2[cA] | 9.3[aA] | 30.3[bB] | 11.1[cB] |
| | 7 | 38.7[cA] | 17.9[aA] | 13.7[aA] | 6.8[cB] | 9.3[cA] | 33.5[aA] | 14.5[aA] |
| Tipo Velhaca | 2 | 18.4[cC] | 17.9[bA] | 14.0[cA] | 6.9[cB] | 9.3[eA] | 29.8[eB] | 13.3[cA] |
| | 5 | 25.9[cB] | 17.8[aA] | 13.9[bA] | 7.2[cA] | 9.3[aA] | 28.8[dB] | 11.5[cB] |
| | 7 | 34.3[dA] | 17.9[aA] | 13.8[aA] | 6.9[cB] | 9.4[bA] | 33.1[aA] | 13.0[aA] |
| Terra A. B | 2 | 18.3[cB] | 18.3[aA] | 14.0[cA] | 7.1[bA] | 9.4[dA] | 32.1[dA] | 14.2[bA] |
| | 5 | 19.9[dB] | 15.7[cB] | 13.8[cA] | 7.0[dA] | 9.3[aA] | 27.3[eC] | 8.9[dB] |
| | 7 | 33.6[cA] | 17.9[aA] | 13.7[aA] | 6.9[cA] | 9.3[cA] | 29.9[cB] | 12.5[aA] |
| Samurá B. | 2 | 14.8[dC] | 16.7[cB] | 14.2[cA] | 6.8[dA] | 9.2[eA] | 31.2[dA] | 14.5[bA] |
| | 5 | 29.7[bB] | 17.4[aA] | 13.7[cB] | 6.9[eA] | 9.2[bA] | 29.3[cB] | 10.4[cB] |
| | 7 | 45.1[bA] | 17.9[aA] | 13.5[bB] | 6.8[cA] | 9.2[dA] | 32.3[aA] | 13.1[aA] |

*the same lower case letters (genotypes) and uppercase letters (ripening stage) do not differ by Scott and Knott test (1%) and Tukey test (1%), respectively

With the simultaneous decrease of spd and spm and increase of the ethylene synthesis and of the ripening, there is an accumulation of putrescine, as observed in this study (Fig. 1). This raise occurs due to the decrease of the substrate needed for the formation of both spd and spm by the action of the enzymes spermidine synthase and spermine synthase (Kalač, 2014).
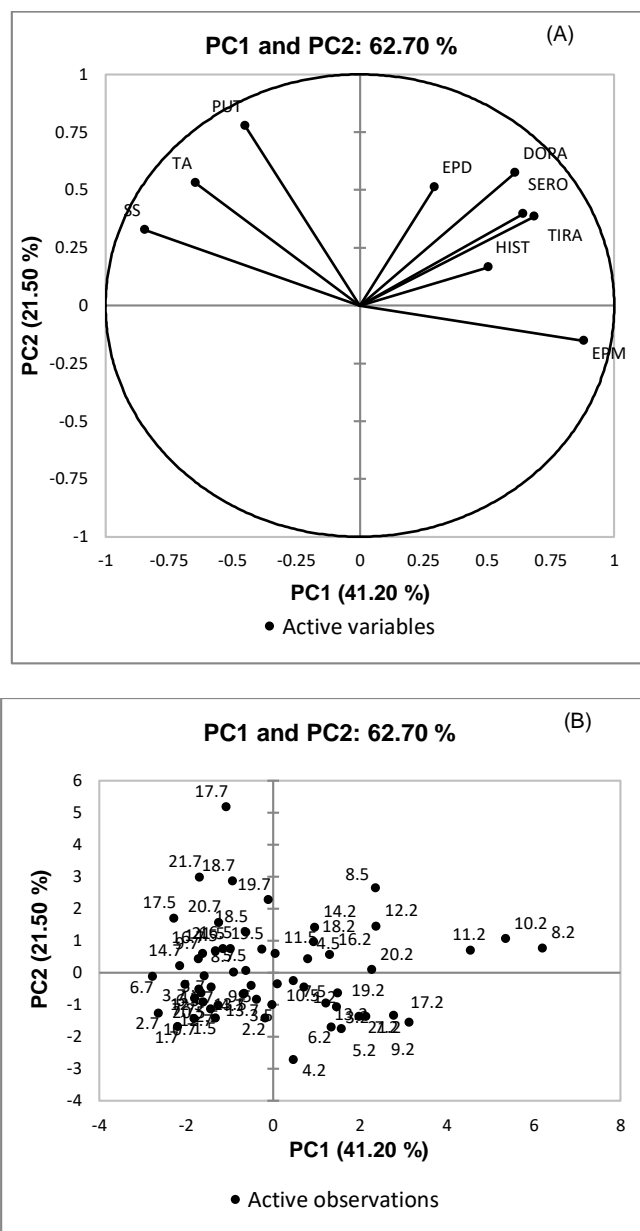


*1: FC06-02; 2: Mont. 172; 3: Tiparot; 4: Khai; 5: Mont. 301; 6: Simili; 7: Pelipita; 8: Ouro; 9: Pisang; 10: Yangambi; 11: Pacha; 12: Namwa; 13: Muisa T.; 14: Ney Poovan; 16: D'Angola; 17: Curare E.; 18: Terra S.N.; 19: Terra A. B.; 20: Tipo V.; 21: Samurá B.; 2: Stage 2, 5: Stage 5, 7: Stage 7.

**Fig. 1.** Two-dimensional projection (A) and scores (B) from bioactive amines in the two first principal components among 20 bananas and plantains genotypes (pulp) evaluated during the fruit ripening stage (stg 2, 5 and 7).

Higher putrescine contents were found in overripe plantain 'Curare E.' (63.6 mg/100g) (Table 2). High levels of spermidine were found in green fruit of dessert bananas 'Ney Poovan' (18.1 mg/100 g) and in cooking bananas 'FC06-02' (19.0 mg/100 g), 'Muísa Tia' (18.5 mg/100 g) and 'Pacha Nadam' (18.2 mg/100 g). For the plantains the higher contents of this polyamine were obtained in green fruit of 'D'Angola' (18.6 mg/100 g) and 'Terra Anã Branca' (18.3 mg/100 g). Adão and Glória (2005), working with commercial banana 'Prata' found levels up to 1.0 mg/100g putrescine e 1.50 mg/100g f.w spermidine, below of the ones obtained in this study. Comparing to the other genotypes, green fruit of the dessert bananas Ouro da Mata' (14.8 mg/100 g d.w.), 'Yangambi km5' (14.8 mg/100 g) and 'Pisang K. B.' (14.7 mg/100 g) presented the highest spermidine levels.

Depending on the genotype and of the analyzed ripening stage, there was a variation in the serotonin content (8.0 mg/100 g to 20.2 mg/100 g d.w.) (Table 2). In the green fruit of 'Ouro da Mata' (dessert banana), we found the highest content for this bioactive amine. We can highlight the serotonin content in ripe fruit (stg. 5) of the cooking banana 'Pelipita' (16.7 mg/100g d.w.), when compared to other genotypes and with other studies performed with plantains and bananas (Adão & Glória, 2005; Foy & Parratt, 1960). In this genotype, we observed an increase of serotonin until the stage 5 and a significant decrease when the fruit was overripe (0.7 mg/100 g) (Fig. 1 and Table 2). The profile of this compound in the post-harvest is similar to what was found in another study performed with plantains, where the contents between 4.99 and 5.67 mg/100g f.w occurs during the ripening and there was a steep decrease when the fruit was overripe (1.20 mg/100 g) (Foy & Parratt, 1960; National et al., 2014). However, the literature results are divergent, depending on the genotype in study, as verified in this study.

Several studies demonstrate that the serotonin content in banana 'Prata' (most consumed cultivar in Brazil) vary between 0.50 – 1 mg/100 g f.w. and there are no variations until the stage 6, with a little decrease after reaching the end of the ripening (Adão & Glória, 2005). Serotonin has been described as antioxidant and presents anti senescent action in vegetal tissues, besides having beneficial effects (neurotransmitter) related to the human health (Islam et al., 2016). The decrease in the serotonin levels in many banana genotypes can be related to the increase of oxidation in the tissues, which occurs during the senescence (cytoprotective) (Mukherjee, David, Yadav, Baluška, & Bhatla, 2014), besides having demonstrated an anti-darkening

effect due to the inhibition of the enzyme polyphenoloxidase (PPO) (Bajwa, Shukla, Sherif, Murch, & Saxena, 2015). Thus, this bioactive amine can be considered a biochemical marker of senescence in bananas. However, it is worth to stress out that this profile was not verified in some genotypes. i.e., plantains, there was a raise of this amine in stage 7, consequently showing the necessity of more studies (Table 2).

The dopamine content varied form 26.5 mg/100g d.w. ('Tiparot' stg. 7) to 38.1 mg/100g d.w. ('Yagambi stg. 2') (Table 2). The genotypes 'Yagambi' and 'Ouro da Mata' presented the highest contents, both in the green fruit (38.1 and 35.4 mg/100g, respectively) as in the ripe fruit (stg. 5 – 31.3 and 34.4 mg/100g, respectively). There as a decrease of this amines content during the ripening, also demonstrating to be genotype dependent (Table 2). In plantains there is a little raise of this compounds in the stage 7, as also verified for the serotonin, occurring a possible separation of the amine profile by consumption group.

Both dopamine as serotonin have been described for presenting antioxidant potential (Mukherjee et al., 2014), similar to the ascorbic acid, besides presenting anti-inflammatory effect (Bajwa et al., 2015). In addition, the dopamine has been related to the decrease of Parkinson disease symptoms in human beings (Patil, Apine, Surwase, & Jadhav, 2013). Studies performed with the commercial banana 'Prata' show very low levels of these compounds (Adão & Glória, 2005). In our study, we found genotypes with superior levels of serotonin and dopamine, compared to the commercial cultivars cited in the literature, both in green fruit and in the ripe ones (ripe and overripe), which could be selected for being used in genetic improvement programs with focus in the culture biofortification and/or for being promoted and incorporated in existent agricultural systems for culture diversification and, consequently, popular consumption (Borges et al., 2014).

Histamine and tyramine are related to allergenic and intoxication processes and the determination of these compounds is of great importance for the quality analysis in the food (Larqué, Sabater-Molina, & Zamora, 2007). Only a few studies describe the presence of histamine and tyramine in fruit, including bananas. In some studies, significant quantities of tyramine are described in fruit of *Musa* spp. and attribute some flavor and aroma characteristics in ripe bananas to the presence of tyramine and histamine (Plonka & Michalski, 2017). These studies prove that tyramine and histamine increase during the ripening process. However, it is possible to realize that the profile of these amines is genotype dependent. In most of the analyzed genotypes in the

present study, these amines decreased during the ripening process (Fig. 1 and Table 2). The tyramine contents varied from 9.0 ('Monthan 172' - stg. 7) to 10.3 mg/100g d.w. ('Ouro da Mata' stg. 2) and the histamine contents varied from 6.6 mg/100g d.w. ('FC06-02' – stg. 7) to 8.6 mg/100g d.w. ('Ouro' - stg. 5) (Table 2). It is worth to stress out that the quantity of amines found in the fruit of all the genotypes are below of what is considered toxic for the human health (histamine and tyramine: 10 to 100 mg/100g f.w.) (Larqué et al., 2007).

### 3.3.2. Differences in the amines distribution in pulps and peels in ripening stages

The principal component analysis was conducted in order to show the possible differences in the distribution of amines in peel and pulp. Generally, some bioactive compounds as the bioactive amines (i.e. dopamine and serotonin), are found in larger quantities in the peels in comparison to the pulps (Pereira & Maraschin, 2015).

PC1 and PC2 explained 61.03% of the data variance (Fig. 2). The CP1 axis represents 34.94% of the total data variance, separating the peels (PC1 +) from the pulps (PC1 -). The peels presented the highest contents of most of the bioactive amines, presenting high correlation with these variables (Fig. 2 and Table 3). In fact, the fruit and vegetable peels are more exposed to sunlight than the pulp and may protect itself from the oxidative stress caused by strong sunshine and high temperature by producing large amouts of antioxidants. The putrescine presented no significant variations, depending on the analyzed vegetal tissue. However, the levels of this amines we a little superior in the pulp, comparing to the other bioactive amines. It is possible to observe that most of the green peels show a high correlation with dopamine, tyramine, spermidine and spermine (PC1 + and PC2-) and presented the highest contents of these amines. Serotonin and histamine presented high correlation (r = 0.8 p < 0,05) and high contents were found in peels (Table 3), mainly in the ripe and super ripe stages of the plantains (i.e. 'Tipo Velhaca' and 'Samurá B.'), differently from the profile verified in pulps (Fig. 1). We can highlight the plantains 'Samurá B.', 'Tipo Velhaca' and 'Terra S. N.' in relation to the serotonin and dopamine levels, which presented high correlation with these amines (ripe and green fruit, respectively).
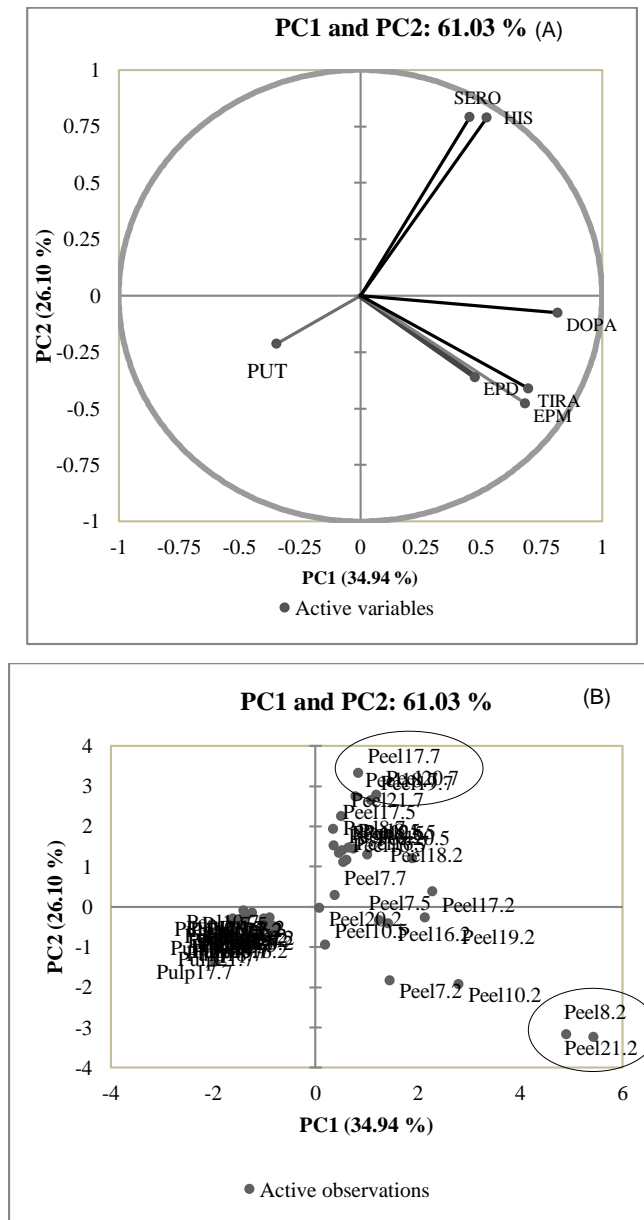
* 7: Pelipita; 8: Ouro; 9: Pisang; 10: Yangambi; 16: D'Angola; 17: Curare E.; 18: Terra S.N.; 19: Terra A. B.; 20: Tipo V.; 21: Samurá B.; 2: Stage 2, 5: Stage 5, 7: Stage 7.

**Fig. 2.** Two-dimensional projection (A) and scores (B) from bioactive amines in the first two principal components among the peels and pulps from 9 accesses.

## 3.3. Impact of thermal processing on cooking bananas (plantain and non-plantain)

Few studies concentrate in plantains and/or cooking bananas after the different thermal processes, that is, the form the fruit are consumed. Retention studies of bioactive amines in different thermal processes indicate that the prolonged exposition to high temperature results in substantial losses of these bioactive compounds (Plonka & Michalski, 2017).

With the principal components analysis, we could verify the profile of these compounds in the different thermal processes in the genotypes studied. PC1 represents 42.93% of the total data variance and was responsible for separating the different analyzed genotypes (Fig. 3).
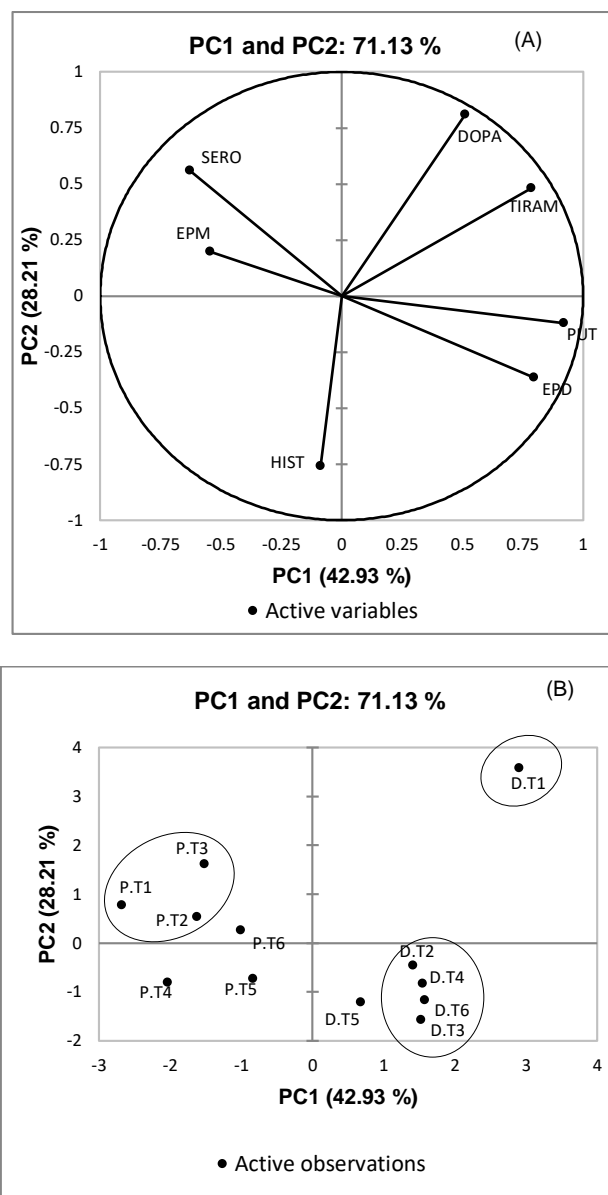


*T1: boiling with peel; T2: boiling without peel; T3: microwaving with peel; T4: microwaving without peel; T5: stir-frying; T6: *in natura.*

**Fig. 3.** Two-dimensional projection (A) and scores (B) of biochemical attributes (bioactive amines) in the two first principal components among 'D'Angola' (D) plantain and 'Pelipita' (P) cooking banana submitted to thermal processes.

Dopamine and tyramine presented high positive correlation (r = 0,8, p < 0,05), grouping in PC1+ and PC2+. The plantain 'D'Angola' contains superior quantities of

these amines (mainly dopamine) both in the *in natura fruit*, as in the ones submitted to thermal processes (i.e. boiling with peel). It is worth to point out that some thermal processes (boiling without peel, microwave with peel and stir-frying) induced dopamine losses, differently for the result found for tyramine, that is, there was no variation after the processing, regardless of the analyzed genotype (Table 4 and Fig.3).

Serotonin, representative of the indolamines, presented positive correlation with spermine ($r = 0,3$ $p < 0,05$), grouping in the PC1 – and PC2 +. The cooking banana 'Pelipita' contains high levels of serotonin and spermine, mainly when the fruit were submitted to cooking processes (microwaving and boiling) (Table 4). High serotonin contents in 'Pelipita' were found in the cooking treatments with peel (boiling and microwaving with peel). It is worth to stress out that the stir-frying process induced losses in the serotonin content. The sharp drop of serotonin was also observed in other processes used in sample bananas (frozen, sorbet and nectar after pasteurization process) (Plonka & Michalski, 2017). The cooking without peel (boiling without peel) resulted in fruit with superior quantities of spermine (PC1 - and PC2 +, Fig. 3). The microwaving of 'Pelipita' without the peel resulted in an increase of histamine.

Putrescine and spermidine presented high positive correlation ($r = 0.85$, $p < 0.05$) and the plantains D'Angola presented superior values of these amines (Fig. 3). Fruit boiled and peeled contain high putrescine contents, while in the other cooking processes occurred a reduction in this amine content. Spermidine decrease happened when the fruit were submitted to some cooking processes (boiling with peel and stir-frying) and superior quantities of this polyamine were found in the D'Angola fruit (*in natura*, microwave with and without peel, and boiling without peel) (PC1 + and PC2 -, Fig. 3). Retention studies of the bioactive amines reported that most of these amines are no thermo stable. However, as we can verify in the present study, this will depend on the analyzed amine, food matrix (i.e., genotype) and used processing.

**Table 4.** Bioactive amines content (mg/100 g d.w.) after cooking processes of the 'D'Angola' plantain in the 'Pelipita' cooking banana.

| Cooking processes | Putrescine | Spermidine | Spermine | Histamine | Tyramine | Dopamine | Serotonin |
|---|---|---|---|---|---|---|---|
| *D'Angola* | | | | | | | |
| Boiling with peel | 60.3[b] | 21.6[c] | 13.9[a] | 7.4[b] | 9.6[a] | 59.5[a] | 12.3[a] |
| Boiling without peel | 67.1[a] | 22.9[a] | 13.9[bc] | 7.5[ab] | 9.3[b] | 32.6[c] | 12.4[a] |
| Microwave with peel | 58.1[b] | 22.9[a] | 14.1[a] | 7.8[a] | 9.5[b] | 29.8[d] | 10.3[d] |
| Microwave without peel | 54.1[c] | 23.1[a] | 13.8[c] | 7.7[a] | 9.4[b] | 33.6[b] | 11.4[c] |
| Stir-frying | 44.1[d] | 22.6[b] | 14.0[ab] | 7.6[a] | 9.3[b] | 30.2[d] | 10.3[d] |
| *In natura* | 58.1[b] | 23.9[a] | 13.9[bc] | 7.8[a] | 9.3[b] | 34.2[b] | 11.8[b] |
| *Pelipita* | | | | | | | |
| Boiling with peel | 21.2[c] | 18.0[c] | 14.1[bc] | 7.7[bc] | 9.2[a] | 39.0[a] | 13.7[b] |
| Boiling without peel | 37.7[a] | 21.0[ab] | 14.5[a] | 7.5[bc] | 9.3[a] | 29.1[b] | 12.9[c] |
| Microwave with peel | 37.2[a] | 21.2[a] | 14.2[b] | 7.5[bc] | 9.2[a] | 40.0[a] | 15.1[a] |
| Microwave without peel | 32.4[b] | 18.6[a] | 14.1[bcd] | 7.8[a] | 9.2[a] | 28.3[b] | 13.3[c] |
| Stir-frying | 33.7[b] | 20.7[b] | 13.9[d] | 7.6[bc] | 9.2[a] | 28.0[b] | 11.7[d] |
| *In natura* | 36.6[a] | 20.7[b] | 14.0[cd] | 7.5[c] | 9.2[a] | 28.1[b] | 13.2[c] |

*the same lower case letters do not differ by Tukey test (5%)

The thermal treatments affects affects the cell wall of the food matrix, which can lead to better extraction of the antioxidant compounds from the cell matrix (C. V. Borges et al., 2018), similar to the results found in some treatments performed in this study. Banana fruit can be boiled with or without peel, according to the preparation and the boiling with peel resulted in increased in the content of serotonin (up to 2.3%) and dopamine (up to 73%), regardless of the analyzed genotype (Table 4). This effect can be attributed to the high quantity of these compounds found in the peels, that is, a migration of serotonin and dopamine from the peel to the pulp might have occurred, as already verified with other compounds (i.e. phenolic compounds) analyzed in *Musa spp.* fruit (Borges et al., 2018; Tsamo et al., 2015).

## 3.4 Conclusion

The bioactive amines profiles evaluated in this study varies according to the genotype and ripening stage. Amines such as tyramine, histamine, dopamine, serotonin, spermidine and spermine decrease during the ripening process in most of the analyzed genotypes. In opposition, during the fruit ripening process, there is an accumulation of putrescine, caused by the increase of the ethylene biosynthesis, mainly in plantains. Serotonin and dopamine have been described by presenting antioxidant properties and the increase of these amines in stage and posterior decrease in stage 7 can be related to the oxidation of tissues. Thus, they can be used as biochemical markers of *Musa* spp. senescence. However, there is an accumulation of serotonin and dopamine in most of the plantains in stage 7, showing a possible differentiation of these amines profile by consumption group. Peel is an important byproduct in the food industry and can be a potential source of bioactive amines, mainly dopamine and serotonin, which occurred in superior quantities when compared to the pulp of the *Musa* spp. fruit. The use of genotypes whose pulps and peels present high levels of amines could be explored by the food and pharmaceutical industries, adding value to the product and at the same time increasing the income of the rural producer. The consumption and/or use of the banana, both in green stage and ripe stage can be a good source of polyamines, dopamine and serotonin, which are important molecules for the human health. In addition, some genotypes present high values, mainly when compared to the most consumed varieties nowadays. In addition,

thermal processing affects the content of amines present in the fruit, depending on the compounds and on the analyzed genotype. The bioactive amines can suffer chemical modifications and alterations in their contents as a function of the thermal process used in the different methods of fruit preparation. For *Musa* spp. fruit, boiling with peel might be preferred in domestic preparations, mainly when the objective is to consume higher quantities of catecholamines and indolamines, regardless of the cultivar used.

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**Capítulo 4**

**Nutritional value and antioxidant compounds during the ripening and after domestic cooking of bananas and plantains**

(artigo submetido ao periódico científico Food Research International)

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## ABSTRACT

Nowadays, the research with banana fruit is focused in identifying and exploring genotypes with significant quantities of nutritional compounds and antioxidants to produce and accumulate important nutrients, such as resistant starch, phenolic compounds and minerals by biofortification strategies. The aim of this study was to identify banana and plantain accesses with significant content of resistant starch, phenolic compounds and minerals and to evaluate the impact of the ripening stage and domestic thermal processing in the phenolic compounds content. All the subgroups and/or group of consumption (dessert bananas, cooking bananas and plantains) demonstrated a wide content variation of phenolic compounds, resistant starch and minerals in the fruit. The peel has the highest nutritional and functional value (i.e., phenolic compounds and minerals). The total starch content in the pulp varied from 42.3% ('FC06-02') to 80.6% ('Pelipita'). Generally, the plantains and cooking bananas contain the highest contents of starch (up to 68.2%) and resistant starch (up to 49.9%). The dessert bananas (e.g. Ney Poovan: 448 mg/100 g) and cooking bananas (e.g. Tiparot: 509.7 mg/100 g) were the ones that presented the highest total phenolic compounds levels, mainly in the ripe fruit (stg.5). There was a not a clear separation from the consumption group in function of the minerals content. The pulps of the dessert genotype 'Khai' and 'Ouro da Mata' and of the cooking genotype 'Pacha Nadam' stood out due to their high contents in most of the analyzed minerals (P, K, Fe and Ni; Zn and Fe; Ca, Mg and Zn, respectively). The phenolic compounds were the ones that contributed the most to antioxidant activity in the analyzed *Musa* spp.

germplasm. However, the content of these compounds was affected by the ripening stage and superior values were found in the ripe fruit (stg. 5). In addition, the thermal processing can increase the functional and nutritional values of the *Musa* spp. fruit, mainly the cooking of fruit with peel by ebullition, which should be preferred in domestic preparations.

## 4.1 Introduction

The consumers are worried in ingesting food that, besides nourishing, bring benefits for the human health. Bananas and plantains are the fourth most produced food in the world, with annual production of 107 millions of tons and the greatest producers are India, China, Philippines, Ecuador and Brazil (FAO, 2017). They stand out from the other tropical climate fruit, mainly due to the high consumption and their versatility of use (*in natura*, processed, fried, cooked, among others), besides being considered a low cost food and with high energetic value (rich in amide). These peculiarities make the banana a very important food as a source of nutrients and with great potential of use as a functional food.

Recently, the research in genetic improvement of banana tree is focused, among other objectives, in identifying and exploring genotypes with significant quantities of resistant starch and phytochemicals in order to produce and accumulate important nutrients by biofortification strategies (Ghag & Ganapathi, 2018). The *Musa* spp. fruit are truly considered good source of antioxidants, such as the phenols and the minerals (i.e., potassium and phosphate) (Emaga et al., 2007; Sulaiman et al., 2011). In addition, micronutrients as Zn and Fe are being widely researched in biofortification programs (e.g. Harvest Plus), due to the great malnutrition problems related to these minerals (Genc, Humphries, Lyons, & Graham, 2005).

Inside the *Musa* spp. germplasm, we identified accesses with significant quantities of bioactive compounds, such as phenolic acids and flavonoids that act as antioxidants in the organism, which makes a functional food perfect for improving the human health (Borges et al., 2014; Ghag & Ganapathi, 2018; Pereira & Maraschin, 2015). Many papers pointed out the antioxidant, antiulcerogenic, antihypertensive and anticarcinogenic actions of consuming the *Musa* spp. fruit due to the presence of compounds as vitamins A, B, C and E, β-carotene, phenolic compounds (catechins,

lignins, tannins and anthocyanins) and minerals (C. V. Borges et al., 2018; Pereira & Maraschin, 2015; Sulaiman et al., 2011). In addition, the green banana is an important source of resistant starch (RS), a low digestibility compound in the small intestine, with prebiotic effect and prevents inflammatory intestinal diseases. The low digestion of RS can also increase the glycemic and insulinemic response with important action in controlling the metabolic syndrome, responsible for some of the worst health problems nowadays (Graham, Zhang, & Dixon, 2012).

Most of the studies for identifying bioactive compounds in banana tree fruit are focused in commercial cultivars (e.g. Cavendish subgroup) and some local cultivars (Anyasi, Jideani, & Mchau, 2018; González-Montelongo, Gloria Lobo, & González, 2010), which makes the studies more interesting inside the vast *Musa* spp. germplasm in the different research places. This diversity could be explored aiming to identify potentially adequate genotypes to be used in genetic improvement programs focused in the biofortification of the culture and/or to be promoted and incorporated in the agricultural systems existent for the diversification of the culture and, consequently, of the popular consumption. It is worth to stress out that the content of these phytochemicals, mainly the phenolic compounds, vary significantly during the fruit ripening and after the different types of domestic processing, causing losses or gains. Thus, it is of fundamental importance the determination of these antioxidant compounds in the different ripening stages and after thermal processing (e.g. cooking bananas and plantains), when the aim is to ingest or even extract significant quantities of these beneficial compounds for human health. Therefore, the aims of this study were to (1) characterize the different genotypes regarding the contents of resistant starch, minerals and phenolic compounds in pulps and peels, (2) evaluate the ripening impact on the phenolic compounds content and (3) evaluate the retention of these antioxidant compounds after the different types of thermal processing in cooking bananas and plantains.

## 4.2 Material and Methods

### 4.2.1 Harvest of the analyzed genotypes

Bunches of 22 genotype of banana trees were harvested between March and June of 2016 from different genomic groups belonging to the Active Germplasm Bank (banana AGB) from Embrapa Cassava & Fruits (Cruz das almas, Bahia, Brazil). All the studied genotypes were cultivated in the same place (Latitude 12º40'12 '' S; Longitude 39º06'07 '' W; Altitude 225 m) in order to exclude experimental errors, due to the environmental factors related to differences in the composition (Table 1). These genotypes (20 plants per genotype) were planted over 3 replications. Field planting was done from April of 2015. When the branches reached ripening stage 1, we harvested the second and third hand (each containing about 10 fingers) of each genotype (± 2 bunches = 40 fruit) and stored in room temperature (20 ± 2 ° C) and relative humidity of 80 ± 2%, until reaching ripening stage 7 (Borges et al., 2018).

**Table 1.** Genotypes of the Active Germplasm Bank (banana AGB) from Embrapa Cassava & Fruits, Cruz das Almas, Bahia, Brazil.

| Genotypes | Ploidy | Subgroup/Subspecies | Form of use |
|---|---|---|---|
| Yangambi Km5 | AAA | Ibota | *In natura* |
| Khai | AAA | Ibota | *In natura* |
| Pisang Kepok Bung | AAB | Peyan | *In natura* |
| Ney Poovan | AB | Ney Poovan | *In natura* |
| Ouro da Mata | AAAB | * | *In natura* |
| Prata Anã | AAB | Prata | *In natura* |
| Grande Nine | AAA | Cavendish | *In natura* |
| Monthan 301 | ABB | Monthan | Cooked |
| Monthan 172 | ABB | Monthan | Cooked |
| Simili Radjah | ABB | Peyan | Cooked |
| Pelipita | ABB | Bluggoe | Cooked |
| Pacha Nadan | ABB | Saba | Cooked |
| Namwa Khom | ABB | Pisang Awak | Cooked |
| Muisa Tia | ABB | Pisang Awak | Cooked |
| FC06-02 | AABB | Figo | Cooked |
| Tiparot | ABBB | Klue Teparod | Cooked |
| D'Angola | AAB | Plantain | Cooked |
| Curare Enano | AAB | Plantain | Cooked |
| Terra Sem Nome | AAB | Plantain | Cooked |
| Tipo Velhaca | AAB | Plantain | Cooked |
| Terra Anã Branca | AAB | Plantain | Cooked |
| Samurá B | AAB | Plantain | Cooked |

*4.2.2 Ripening stage determination*

The fruit were divided in seven ripening stages during the ripening, according to the banana color scale described by Soltani, Alimardani, & Omid (2011). These indices are based on the peel color: 1 = green; 2 = green with yellow traces; 3 = more green than yellow; 4 = more yellow than green; 5 = yellow with green; 6 = completely yellow; 7 = yellow with coffee color areas. For the characterization analyses of the analyzed compounds we analyzed the fruit in stage 2. The pulps and peels of the 20 genotypes were sliced, powdered in liquid nitrogen, lyophilized and stored in an ultra-low temperature freezer (-80 ° C). For studying the ripening stage impact on the phenolic compounds content, we chose the stages 2, 5 and 7, which are the most used for processing and/or *in nature* consumption (Borges et al., 2018). The genotypes that stood out regarding the total phenolic compounds content were also analyzed via HPLC.

*4.2.3 Analyzes of starch and resistant starch*

Total starch (TS) content was determined according to Goñi, Garcia-Alonso, Saura-Calixto (1997). The samples (50 mg) were suspended in 2M KOH to disperse starch and shaken at room temperature for 30 min. The samples were then incubated (60 °C, 45 min, pH 4.75) with amyloglucosidase (1 mL (300 U/mL), Sigma A-7255) to hydrolyze starch. The free glucose was determined using glucose oxidase, peroxidase, and ABTS assay (Bergmeyer & Bernet, 1974). The total starch was calculated as glucose × 0.9. The wheat starch (Sigma S-1514) was used as reference standard.

The resistant starch determination (Goñi, García-Diz, Mañas, & Saura-Calixto, 1996) was performed using 100 mg of samples passed through sieve number 100-ABNT and placed in a 50 mL erlenmeyer flask. It was added 10 mL of KCl-HCl buffer (pH 1.5, 0.2M) and 0.2 mL of pepsin solution (0.1 g of pepsin Sigma P-7012 in 10 mL KCl-HCl buffer solution, pH 1.5). The samples were kept in a water bath (40°C) with shaking and after 60 minutes, they were cooled to room temperature. 9 ml of 0.1M tris-maleate buffer (pH 6.9, 0.1 M) and 1 ml α-amylase (4 g α-amylase Sigma A-3176 in 100 ml tris-maleate buffer) were added and the sample was then kept in a water bath at 37 ° C for 16 hours under stirring. Then, the samples were filtered on 110mm filter paper. The filtrate was discarded and the material trapped in the filter was transferred

to 50 mL erlenmeyers and 3 mL of distilled water and 3 mL of 2.0 mol L-1 KOH were added. The sample was held for 30 minutes, with occasional shaking. 4.5 mL of 1M HCl, 3 mL of sodium acetate buffer (pH 4.75) and 80 µL of amyloglucosity (0.144 g of Sigma A-7255 amyloglucosidase in 10 mL of water) were added. The sample was kept in a water bath at 60 ° C for 45 minutes under stirring. After further filtration (11.0 / 12.5 filter paper), 20 µL of each sample was placed in a test tube and used for reaction with 2 mL of glucose-oxidase solution. The tubes were capped and held in a water bath at 37 ° C for 10 minutes. The samples were cooled to room temperature and the absorbances were read in a spectrophotometer at 505 nm. The quantification of the RS (%) was calculated by multiplying the value found (glucose released) by 0.9.

### 4.2.4 Phenolic compounds analyses

### 4.2.4.1 Preparation of the extract for the analyses of total phenols, antioxidants and specific phenols (HPLC)

The lyophilized and ground samples (500 mg) of bananas and plantains were homogenized in 2.5 mL MeOH: formic acid: water (80, 0.1, 19.9, v/v/v) in vortex during 1 min and sonicated during 30 min at 25 °C. After centrifugation (3800 x g, Hettich Zentrifugen, Mikro220R) during 10 min, the supernatant was transferred to amber tubes. The residual sediment was submitted to one more extraction process with 2.5 mL MeOH: formic acid: water: (80, 0.1, 19.9, v/v/v). The supernatants were mixed and used for analysis.

### 4.2.4.2 Total phenolic compounds analyses by spectrophotometry

The analyses were performed using the Folin-Ciocalteau reagent (Singleton & Rossi, 1965). The values were calculated with a standard curve of gallic acid and were expressed in mg equivalent of gallic acid (EAG) by 100 g of dry weight (d.w.).

### 4.2.5 Minerals analyses

The determination of the minerals K, Na, Zn and Fe was performed by an atomic absorption spectrophotometer (Shimadzu, model AA 7000) using the more intense

resonance lines for each mineral. The minerals K and Na were determined by atomic emission and the microelements Cu, Zn and Fe were determined by atomic absorption using its respective hollow cathode lamps. For the calibration curves, standard mineral solutions of 1000 mg $L^{-1}$ (Specsol, Brazil) were used after stepwise dilution to the desired concentration.

### 4.2.6 In vitro analyses of the antioxidant activity (DPPH, FRAP and ABTS)

### 4.2.6.1 DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay

The capacity of bananas and plantains extracts to reduce the radical DPPH was assessed using the method of Brand-Williams et al. (1995), with modifications. The evaluation system of the antioxidant activity consisted in adding 290 µL DPPH solution and 10 µL of 80% methanol acidified with 0.1% formic acid for the control (differences remaining between 0.5 – 0.6). The same volume for DPPH solution with 10 µL of the extract sample was used. The samples readings were performed in spectrophotometer UV/VIS Ultrospec 3000 (Pharmacia Biotech, Uppsala, Sweden) at 515 nm after 60 min of incubation. The percentage of inhibition was calculated using the following equation: % inhibition = $(1-Af/A0) \times 100$, where: Af is the absorbance after 60 min and A0 is the absorbance of DPPH at time 0. The antioxidant activity of the bananas and plantains was expressed as Trolox equivalent, using a calibration curve with Trolox.

### 4.2.6.2 FRAP (Ferric Reducing Antioxidant Power) assay

The determination of the antioxidant activity by the reduction of iron was evaluated according to Benzie & Strain (1996). The evaluation system of the antioxidant activity by the FRAP test consisted in adding 900 µL of the fresh FRAP reagent [25 mL acetate buffer solution (300 nM, pH 3.6), 2,5 mL TPTZ solution (2,46-tris (2-pyridyl)-5-triazine/ 0,8ml-HCL) (10 nM in 40 mM HCl) and 2.5 mL $FeCl_3$ solution] in 30 µL of the sample. The absorbance was performed using spectrophotometer UV/VIS Ultrospec 3000 (Pharmacia Biotech, Uppsala, Sweden) at 595 nm. The absorbance values were compared to a calibration curve with ferrous sulphate ($FeSO_4$) and the values were expressed in µmol of Fe equivalent by gram of the dry weight (d.w.).

### 4.2.6.3 ABTS (Radical Scavenging Activity) assay

The antioxidant activity determination by the sequestration of the radical 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was performed according to Re et al., (1999). 30 µL of sample were mixed with 3 mL ABTS solution (n = 3). After stirring for 30 seconds, the samples were kept in the dark for 6 minutes. The absorbance was performed using spectrophotometer UV/VIS Ultrospec 3000 (Pharmacia Biotech, Uppsala, Sweden) at 734 nm. The results were expressed in mmol of Trolox equivalente (TE) by 100g of dry weight (d.w.).

### 4.2.7 Study of the thermal processing impact on the specific phenolic compounds content (HPLC)

In order to study the impact of the thermal processing on the phenolic compounds content, we used ripe fruit (stage 5) of the D'Angola plantain, which is already used commercially and ripe fruit of the 'Pelipita' (cooking banana). The analyses were performed with peel and without peel. The preparation methods included the boiling in water, microwaving and stir-frying (with 2 mL soybean oil). Peeled and unpeeled bananas and plantains (stage 5) were boiled for 10 min in a stainless-steel pan covered with a lid, in approximately 300 mL of water. The remaining water was drained and the fruit was cooled. For the microwaving process, the whole bananas were microwaved (with and without peel) for 2 min, using a commercial microwave oven (highest power). In these fruit, we sliced the peels longitudinally in order to maintain the internal steam pressure. Stir-frying was performed with the pulps sliced longitudinally, using a stainless steel frying pan greased with soybean oil (± 2 mL), for 5 min. The pulps and peels were ground to a fine powder (IKA, A.11, Germany) in liquid nitrogen, lyophilized and stored at - 80 C° until the analysis (Borges et al., 2018).

### 4.2.7.1 Phenolic compounds content analysis by High Performance Liquid Chromatography in reverse phase (HPLC)

The dry extract (item 2.4.1) was resolubilized in 500 µL of the mobile phase [½ solvent A (acidified water: trifluoracetic acid, 99.9:0.1, v/v/v) and ½ solvent B

(Acetonitrile 100%)] and centrifuged (5 min, 5000 rpm). The supernatant was separated for analysis. For quantifying and qualification of the phenolic compounds, we injected 10 μL of sample into HPLC system (Thermo Scientific Ultimate 3000, Bremen, Germany) coupled to photo-diodo array detector and C18 column (150 x 4.6 mm, Kinetex 2.6 μ F5 100 A, Phenomenex). The mobile phases were consisted in acidified water: trifluoracetic acid (99.9:0.1, v/v) (solvent A) and acetonitrile (100%) (solvent B). The running conditions were as follows: 90% A and 10% B (5 min), 60% A and 40% B (5 min.), 30% A and 60% B (3 min), 90% A and 10% B (2 min) at a flux rate of 0,75 mL min$^{-1}$ (22 ° C). The phenolic compounds were determined at 270 nm by determining peak areas under the curve in the HPLC calibrated against known amounts of standards. We adjusted the results using a standard curve for gallic acid, hydroxybenzoic acid, epigallocatechin gallate, catechin and quercetin (Sigma-Aldrich Co., St. Louis, MO; 99.98%). The results were expressed in μg/g of dry weight (d.w.) and corresponded to the average of 3 consecutive injections by sample (n = 3).

### 4.2.8 Statistical analyses

We used the completely randomized design, with factorial scheme 22 x 3 (genotypes – pulp/peel x ripeness) and three repetitions (three fruit per experimental unity). The fruit characterization data (starch, resistant starch, total phenolic compounds, DPPH, FRAP and ABTS) were obtained and submitted to the variance analysis (ANOVA), followed by Scott Knott ($p < 0.01$) averages comparison test among the genotypes and Tukey test for separating the averages among the ripening stages (e.g., phenolic compounds, pulps and peels). For the analyses after the thermal processing, the data were also submitted to the variance analysis (ANOVA), followed by the Tukey test for separating the averages. The data variance analysis was performed through the SISVAR software (Ferreira, 2011). Principal component analysis (PCA, software XLSTAT - version 2017 (Addinsoft, France) was used to visualize the possible correlation among the data in the characterization analyses and between ripening (phenolic compounds). In addition, we used the PCA in order to visualize the correlation among the different thermals processes with the specific phenolic compounds content (phenolic acids and flavonoids) in the different genotypes.

## 4.3 Results and Discussion

*4.3.1 Nutritional composition of the Musa spp. genotypes*

There was a wide variation in the content of all analyzed compounds inside the *Musa* spp. germplasm (Table 2). The total starch content varied from 42.3% ('FC06-02') to 80.6% (Pelipita). In general, the plantains showed the higher contents of total starch (TS) and resistant starch (RS) and the ones that stood out were 'Terra Anã Branca' (TS: 68.2% and RS: 49.9%) and 'Samurá B.' (TS: 63.3% and RS: 45.6%). These genotypes presented superior RS values than the D'Angola cultivar (41.2%), which is one of the most used plantains commercially in Brazil. The cooking bananas 'Pelipita' (48.1%), 'Monthan 301' (59.9%), 'Monthan 172' (47.6%) and 'Simili R.' (48.2%) also presented high RS percentages. Among the dessert bananas, the 'Prata Anã' (47.5%) was the one that stood out regarding the RS content, followed by the Pisang K. B. (39.7%). These values are higher when compared to the Grande Nine (27.5%), which is the most used commercially in the world.

Studies demonstrate that the RS content present in the green bananas varies according to the variety and that commercial cultivars (e.g., Grande Naine and Nanicão) used for the production of banana flour, generally show contents below 20% (Ramos, Portes & Leonel, 2009). It is worth to point out that, in the present study, we verified that there are genotypes with superior RS quantities inside the *Musa* spp. germplasm, mainly when compared to the most used cultivars nowadays, which could be explored for increasing the contents of these compounds by conventional improvement or by introducing them directly in productive systems.

The highest averages of total phenolic compounds were observed in the dessert bananas and in the cooking bananas (non plantains) (Fig. 3).

**Table 2**. Total starch (TS), resistant starch (RS), total phenolic (TP) and antioxidant activity in *Musa* spp.

| Genotypes | TS (%) | RS (%) | TP pulp (mg/100 g d.w.) | TP peel (mg/100 g d.w.) | DPPH pulp (mg TE/g d.w.) | FRAP pulp (µmol Fe/g d.w.) | ABTS pulp (mmol TE/100g d.w.) |
|---|---|---|---|---|---|---|---|
| *Dessert bananas* | | | | | | | |
| Yangambi Km5 | 67.6[a] | 27.5[e] | 409.6[e] | 544.1[e] | 3.4[e] | 190.1[f] | 503.5[j] |
| Khai | 56.6[b] | 26.7[e] | 408.8[e] | 482.8[g] | 3.9[d] | 159.1[g] | 587.5[i] |
| Pisang K. Bung | 66.6[a] | 39.7[b] | 332.5[h] | 486.5[g] | 9.6[b] | 59.2[j] | 174.8[m] |
| Ney Poovan | 44.7[c] | 27.2[e] | 448.0[b] | 654.4[c] | 7.1[c] | 397.2[b] | 3650.9[a] |
| Ouro da Mata | 63.7[a] | 32.5[d] | 436.3[c] | 532.5[f] | 4.9[d] | 219.5[e] | 1008.4[f] |
| Prata Anã | 72.0[a] | 47.5[a] | 333.3[h] | 374.2[k] | 3.3[e] | 157.9[g] | 1256.8[d] |
| Grande Nine | 54.7[b] | 27.5[e] | 224.3[m] | 409.1[j] | 2.5[e] | 185.6[f] | 529.7[j] |
| *Cooking bananas* | | | | | | | |
| Monthan 301 | 67.1[a] | 45.5[a] | 439.5[c] | 462.8[h] | 5.7[c] | 315.4[c] | 1480.8[c] |
| Monthan 172 | 59.9[b] | 47.6[a] | 165.9[o] | 463.0[h] | 2.7[e] | 16.5[k] | 224.9[l] |
| Simili Radjah | 73.3[a] | 48.2[a] | 270.5[l] | 467.5[h] | 9.3[b] | 85.5[j] | 350.2[k] |
| Pelipita | 80.6[a] | 48.1[a] | 506.1[a] | 519.1[f] | 4.5[d] | 253.1[d] | 886.5[g] |
| Pacha Nadam | 66.4[a] | 38.2[c] | 398.7[f] | 534.0[f] | 4.2[d] | 204.1[e] | 800.1[h] |
| Namwa | 59.1[b] | 22.9[f] | 404.6[e] | 557.9[e] | 1.9[f] | 143.4[g] | 449.9[j] |
| Muísa Tia | 73.2[a] | 32.6[d] | 418.3[d] | 647.4[c] | 6.1[c] | 66.8[j] | 450.9[j] |
| FC06-02 | 42.3[c] | 32.4[d] | 130.2[p] | 439.9[i] | 1.4[f] | 5.7[k] | 104.5[m] |
| Tiparot | 53.3[b] | 40.9[b] | 509.7[a] | 464.4[h] | 74.4[a] | 749.3[a] | 3494.6[b] |
| *Plantains* | | | | | | | |
| D'Angola | 63.9[a] | 41.2[b] | 322.3[i] | 615.9[d] | 2.9[e] | 78.9[j] | 451.5[j] |
| Curare Enano | 63.0[a] | 39.7[b] | 390.0[g] | 655.4[c] | 9.7[b] | 139.4[g] | 1181.3[e] |
| Terra S. N. | 63.3[a] | 45.3[a] | 298.9[k] | 703.0[a] | 6.5[c] | 95.4[i] | 765.1[h] |
| Tipo Velhaca | 79.0[a] | 42.4[b] | 304.1[k] | 687.7[b] | 6.1[c] | 119.2[h] | 499.6[j] |
| Terra A. B | 68.2[a] | 49.9[a] | 188.3[n] | 676.8[b] | 5.3[d] | 72.6[j] | 250.5[l] |
| Samurá B. | 63.3[a] | 45.6[a] | 315.1[j] | 615.0[d] | 7.5[c] | 209.7[e] | 651.4[i] |

*The same lower case letters (genotypes) and uppercase letters (ripening stages) do not differ by Scott and Knott test (5%) and Tukey test (5%), respectively. TS: total starch; RS: resistant starch; TP: total phenolic.

Superior values of phenolic compounds in dessert bananas (247 mg/100g f.w.) were also reported in other studies performed with *Musa* spp. (Tsamo et al., 2014).

Curiously, this study showed that the phenolic compounds content is one of the most important attributes to differentiate dessert bananas (genomic constitution A) from the cooking bananas (non plantains – genomic constitution B). However, in the present study, besides the high content found in the dessert bananas, high content of phenolic compounds were also found in non plantains cooking cultivars, e.g. 'Pelipita' and 'Tiparot', as previously cited, with no clear division in the content of these compounds regarding subgroup and/or mode of consumption.

Besides the pulps, we also analyzed the content of these antioxidants in the peels of all the genotypes. Studies indicate that the peels contain various antioxidants, many times above the quantities found in the banana pulps (Pereira & Maraschin, 2015). The peels presented superior values of phenolic compounds compared to the pulps in all of the analyzed genotypes. The phenolic compounds content in the peels varied from 374 ('Prata Anã') to 703.04 mg EAG/100 g ('Terra S.N.') (Table 2). In the pulps, the levels varied from ('Monthan 172') to 509.74 mg EAG/100 g ('Tiparot'). The cooking genotypes Tiparot' (509.74 mg/100 g EAG) and Pelipita (506.12 mg/100 g EAG) were the ones that stood out inside the analyzed *Musa* spp. germplasm (Table 2).

Other nutritive components of bananas and plantains are the mineral elements, which are involved in several vital functions of the human body. The concentration of minerals in pulps and peels are presented in the Tables 3 and 4. We can notice that, in general, the pulps have more content of minerals than the pulps, as also described by Sulaiman et al., (2011) in different genotypes of banana from Malaysia. In addition, there was a wide variation in the analyzed genotypes, regardless of the analyzed plant tissue.

The edible part of the banana fruit is considered a good source of K in the human diet (Wall, 2006). The high level was reported by Anyasi et al. (2018) banana flours of different commercial (cv. Williams) and non-commercial genotypes (South Africa) from 9117.32 to 14,746.73 mg/Kg, values that are a little superior than the one found in the present study (Table 3). According to the dietary reference intake (DRI), the adequate diary intake of K for an adult is 4700 mg (IOM, 2000; Wall, 2006). Thus, 100 g of pulp and peel of the analyzed genotypes in this study would provide from 12 to 27% ('Khai') and 33 to 89% ('Terra sem nome') of K, respectively. These values are higher than the

ones reported by the studies with bananas from Malaysia, where there were detected genotypes with values between 10% (pulp) to 29% (peel) in the maximum of the diary K need (Sulaiman et al., 2011). K is an essential nutrient in the diet and constitutes around 70% of the positive ions in the cells and is fundamental for the regulation of the acid-base and hydric balance of the cells, demonstrating the importance of analyzing the *Musa* spp. germplasm, in order to detect genotypes with superior values of this mineral (Sulaiman et al., 2011).

The Phosphorus (P) was the second most abundant mineral found in the analyzed pulps and peels. The Khai genotype (251.9 mg/100 g) in the pulps and the 'Tiparot' genotype (350.4 mg/100 g) in the peels were the ones that presented the highest contents of this mineral, standing out from the others (Table 3 and 4). Considered that the DRI for P is 700 mg, the consumption of 100 f of pulp (d.w.) from the Khai genotype would provide 36% of the DRI for normal adults, which is a quite superior value compared to what was verified by Sulaiman et al. (2011). However, in indigenous bananas, values higher than the ones found in the present study were detected (Anyasi et al., 2018). It is worth to point out that, besides the genotype, other factors influence the minerals content, such as the microclimate (e.g. soil, climate) where the plants were cultivated as well as agricultural practices used in plantings (Forster, Rodríguez Rodríguez, Darias Martín, & Díaz Romero, 2002).

The magnesium (Mg) also presented high values (pulps and peels) (Table 3 and 4), when compared to other minerals. Pulps of 'Pacha' (166.56 mg/100 g) and peels of the Tiparot genotype (334.9 mg/100 g) presented the highest contents. These values are superior from the ones detected in studies performed with other *Musa* spp. genotypes (Sulaiman et al., 2011) and are similar to the values found in genotypes originating from South African communities (Anyasi et al., 2018). According to the results, 100 g of peels of the 'Tiparot' genotype, for example, would provide the total DRI for women (320 mg) and 84% of the DRI for adult men (400 mg) (Sulaiman et al., 2011), emphasizing the banana fruit as food rich in this mineral. The peels of all of the genotypes also presented superior values of Ca. Pulps of the Pisang genotype (70.9 mg/100 g) stood out among the others and the peels of 'Prata Anã' presented values of 277.4 mg/ 100 g, higher than the values obtained in the other analyzed genotypes.

Among the evaluated microelements, Zn and Fe are being widely researched in biofortification programs (e.g. Harvest Plus) due to the great problems of malnutrition related to these minerals, mainly in developing countries.

**Table 3**. Minerals content (mg/100 g d.w.) in pulp of *Musa* spp.

| Genotypes | P | K | Na | Ca | Mg | Fe | Cu | Zn |
|---|---|---|---|---|---|---|---|---|
| *Dessert bananas* | | | | | | | | |
| Yangambi Km5 | 195.4[d] | 1006.7[b] | 43.7[g] | 52.9[d] | 118.0[d] | 18.1[b] | 0.34[a] | 1.03[e] |
| Khai | 251.9[a] | 1244.9[a] | 61.6[b] | 44.4[e] | 120.6[d] | 23.9[a] | 0.25[c] | 1.24[c] |
| Pisang K. Bung | 178.7[e] | 758.8[h] | 71.3[c] | 70.9[b] | 120.6[d] | 20.0[b] | 0.26[c] | 1.18[c] |
| Ney Poovan | 225.2[c] | 807.3[f] | 53.6[e] | 39.9[f] | 127.3[c] | 18.8[b] | 0.22[c] | 1.17[c] |
| Ouro da Mata | 190.8[d] | 996.3[c] | 53.2[e] | 47.1[e] | 133.5[b] | 23.5[a] | 0.20[d] | 1.53[a] |
| Prata Anã | 167.3[f] | 764.9[g] | 45.8[g] | 38.9[f] | 129.2[c] | 19.8[b] | 0.27[c] | 1.01[e] |
| Grand Nine | 135.3[i] | 961.3[d] | 48.2[f] | 38.9[f] | 131.3[c] | 19.4[b] | 0.37[a] | 1.37[b] |
| *Cooking bananas* | | | | | | | | |
| Monthan 301 | 236.0[b] | 829.1[e] | 43.9[g] | 43.5[e] | 121.7[d] | 18.9[b] | 0.29[b] | 1.36[b] |
| Monthan 172 | 132.8[i] | 648.9[l] | 60.9[b] | 46.4[e] | 127.2[c] | 17.3[b] | 0.18[d] | 1.03e |
| Simili Radjah | 153.3[g] | 752.1[h] | 37.4[h] | 36.9[f] | 118.8[d] | 15.9[c] | 0.12[f] | 0.70[g] |
| Pelipita | 134.0[i] | 684.7[j] | 37.1[h] | 25.5[g] | 93.4[e] | 15.9[c] | 0.12[f] | 0.83[f] |
| Pacha Nadam | 234.6[b] | 647.8[l] | 50.7[f] | 76.0[a] | 166.6[a] | 23.7[a] | 0.17[d] | 1.54[a] |
| Namwa | 127.0[i] | 591.2[m] | 82.7[a] | 39.3[f] | 95.5[e] | 20.3[b] | 0.30[b] | 0.90[f] |
| Muísa Tia | 130.4[i] | 652.2[l] | 59.9[d] | 43.9[e] | 85.4[g] | 20.5[b] | 0.10[f] | 1.43[b] |
| FC06-02 | 199.6[d] | 765.3[g] | 56.3[e] | 57.5[c] | 136.5[b] | 20.1[b] | 0.15[e] | 1.33[b] |
| Tiparot | 141.7[h] | 655.6[k] | 56.7[e] | 36.8[f] | 121.4[d] | 18.9[b] | 0.24[c] | 1.11[d] |
| *Plantains* | | | | | | | | |
| D'Angola | 119.7[j] | 702.5[i] | 93.3[a] | 22.2[g] | 96.9[e] | 19.2[b] | 0.14[e] | 1.02[e] |
| Curare Enano | 115.5[k] | 647.6[l] | 48.0[f] | 23.9[g] | 98.2[e] | 19.1[b] | 0.10[f] | 1.09[d] |
| Terra S. N. | 115.6[k] | 661.8[k] | 62.7[b] | 24.8[g] | 96.3[e] | 17.9[b] | 0.04[g] | 0.98[e] |
| Tipo Velhaca | 110.7[k] | 690.2[j] | 47.1[f] | 24.3[g] | 91.4[f] | 21.3[b] | 0.11[f] | 0.88[f] |
| Terra A. B | 112.1[k] | 686.5[j] | 55.8[e] | 23.4[g] | 95.5[e] | 19.8[b] | 0.25[c] | 0.75[g] |
| Samurá B. | 121.1[j] | 646.2[l] | 45.2[g] | 22.3[g] | 98.3[e] | 19.3[b] | 0.22[c] | 0.99[e] |

*The same lower case letters do not differ by Scott and Knott test (5%).

**Table 4**. Minerals content (mg/100 g d.w.) in peel of *Musa* spp.

| Genotypes | P | K | Na | Ca | Mg | Fe | Cu | Zn |
|---|---|---|---|---|---|---|---|---|
| *Dessert bananas* | | | | | | | | |
| Yangambi Km5 | 248.5[d] | 2224.0[n] | 62.1[f] | 180.5[h] | 152.4[j] | 26.9[e] | 0.9[b] | 2.9[g] |
| Khai | 220.4[e] | 3065.6[e] | 90.2[c] | 299.6[a] | 145.7[k] | 35.8[a] | 1.1[a] | 4.8[c] |
| Pisang K. Bung | 256.3[c] | 2670.6[k] | 157.7[a] | 190.5[g] | 290.7[b] | 30.0[c] | 0.5[d] | 6.1[a] |
| Ney Poovan | 247.8[d] | 2862.7[h] | 60.5[f] | 239.5[e] | 216.9[e] | 23.4[h] | 0.5[d] | 5.1[b] |
| Ouro da Mata | 204.6[f] | 2216.07 | 77.3[d] | 159.7[b] | 184.4[h] | 27.6[e] | 0.7[c] | 2.5[g] |
| Prata Anã | 243.2[d] | 2839.9[i] | 71.2[e] | 277.4[b] | 194.7[g] | 25.9[f] | 0.3[e] | 6.2[a] |
| Grande Nine | 311.8[b] | 3243.6[c] | 104.5[b] | 253.5[d] | 172.8[i] | 33.8[b] | 0.7[c] | 4.7[d] |
| *Cooking bananas* | | | | | | | | |
| Monthan 301 | 134.2[j] | 2077.8[o] | 57.7[f] | 211.6[f] | 186.3[h] | 24.9[g] | 0.8[c] | 3.8[f] |
| Monthan 172 | 218.2[e] | 2046.1[p] | 55.8[f] | 200.8[g] | 190.3[g] | 26.2[f] | 0.7[c] | 2.8[g] |
| Simili Radjah | 213.4[e] | 2648.9[l] | 75.57[d] | 250.7[d] | 191.9[g] | 27.6[e] | 0.5[d] | 4.9[b] |
| Pelipita | 263.4[c] | 3075.1[e] | 64.9[e] | 107.8[k] | 200.6[f] | 25.9[f] | 0.7[c] | 2.2[i] |
| Pacha Nadam | 174.3[g] | 1575.0[s] | 60.4[f] | 261.4[c] | 232.8[d] | 24.0[h] | 0.7[c] | 2.9[g] |
| Namwa | 244.4[d] | 3418.7[b] | 68.8[e] | 111.7[k] | 241.7[c] | 25.8[f] | 0.4[d] | 4.8[c] |
| Muísa Tia | 151.4[i] | 3161.4[d] | 80.46[d] | 122.1[j] | 204.3[f] | 30.9[c] | 0.5[d] | 4.0[e] |
| FC06-02 | 167.7[h] | 1870.9[q] | 78.13[d] | 205.9[f] | 209.7[f] | 28.9[d] | 0.7[c] | 2.9[g] |
| Tiparot | 350.4[a] | 2590.12[m] | 69.9[e] | 249.1[d] | 334.9[a] | 25.3[g] | 0.6[c] | 4.7[d] |
| *Plantains* | | | | | | | | |
| D'Angola | 163.3[h] | 2986.4[f] | 78.98[d] | 91.1[l] | 121.8[m] | 24.9[g] | 0.3[e] | 4.6[d] |
| Curare Enano | 130.8[j] | 2787.6[j] | 55.44[f] | 71.0[m] | 120.8[m] | 20.9[i] | 0.6[c] | 2.2[i] |
| Terra S. N. | 180.3[g] | 4192.7[a] | 61.63[f] | 122.4[j] | 142.3[k] | 29.1[d] | 0.5[d] | 4.6[d] |
| Tipo Velhaca | 149.3[i] | 2921.3[g] | 107.7[b] | 126.1[j] | 131.6[l] | 24.5[g] | 0.7[c] | 2.8[g] |
| Terra A. B | 139.6[j] | 2999.3[f] | 86.67[c] | 66.9[m] | 119.4[m] | 25.9[f] | 0.7[c] | 2.7[g] |
| Samurá B. | 137.3[j] | 1685.4[r] | 63.8[f] | 56.6[n] | 98.8[n] | 24.9[g] | 0.5[d] | 1.9[j] |

*The same lower case letters do not differ by Scott and Knott test (5%).

The lack of a pattern of procedure to measure the deficiency of Zn hinder estimates about the number of people with deficiency of Zn, but about 20% of the world population is in risk (Wuehler, Peerson, & Brown, 2005). The higher Zn contents were detected in peels of all of the analyzed genotypes, mainly in the dessert banana 'Prata-Anã', which stood out from the others (6.20 mg/100 g d.w.). In the pulps, the values varied from 0.70 ('Simili R.') to 1.54 mg/100 g ('Pacha N.'). The cooking banana 'Pacha Nadam' (1.54 mg/100g) and the dessert banana 'Ouro da Mata' (1.53 mg/100g) were the ones that presented the highest contents of this microelement (Table 3). Fe was the microelement found in the highest content the analyzed germplasm, mainly in the fruit peels. Peels of dessert bananas 'Khai' (35.8 mg/100 g) presented the highest levels of this mineral. The Fe deficiency is the most common, affecting 1/3 of the world population and WHO (World Health Organization) estimates that most of the children in pre-school age and pregnant women in developing countries present Fe deficiency (Genc et al., 2005). The pulps of the dessert bananas 'Ouro da Mata' (23.5 mg/100 g) and of the cooking banana 'Pacha Nadam' (23.7 mg/100 g) excelled regarding the Fe content as well as regarding the Zn content. Besides these genotypes, the dessert banana Khai (23.9 mg/100 g) also showed high Fe levels in the fruit composition, standing out in the analyzed germplasm.

It is possible to observe clearly that the pulps of the genotypes Khai, Pacha Nadam and Ouro da Mata showed the higher content in relation to the mineral and thus, it is interesting to incorporate these genotypes in the diet (Table 3). The dessert banana Khai showed pulps with high levels of P, K and F and moderate levels of Na, Ca, Mg, Zn and Cu and also presented peels with high contents of Ca, Fe, Cu and Zn (Table 3 and 4). High values of Zn and Fe were found, mainly, in the pulps of the dessert banana 'Ouro da Mata'. The cooking banana 'Pacha N.' contains high contents of Ca, Mg and Zn (Table 3).

*4.3.2 Correlation among the phenolic compounds, minerals and antioxidant activity in Musa spp. fruit.*

The phenolic compounds content and the antioxidant activity were correlated to the mineral content of the pulps of the analyzed genotypes. Studies have indicated that a correlation exists among minerals with the antioxidant capacity of foods (Escuredo, Fernández-gonzález, & Seijo, 2013; Seraglio et al., 2017). The results showed that the

phenols content presented a negative correlation with Mg and Na and a low correlation with the others. PC1 and PC2 explained 60.13% of the data variance and the second principal component (PC2) was responsible for separating the minerals (PC2-) from the total phenols content (PT) and from the antioxidant methods (FRAP, DPPH and ABTS) (PC2+) (Fig. 1). P, Mg and Ca were the minerals that showed the highest correlation with the PC1 (0.9, 0.8 and 0.7, respectively). Zn also showed high positive correlation with this principal component (0.7). The genotypes Pacha Nadam, Khai and Ouro da Mata were the ones with the highest correlation with this principal component. The genotype 'Pacha N.' was the one that presented the highest content of these minerals (Fig. 1 e Table 3).

Polyphenols have been suggested as anti-nutritional factors (ANFs) and as strong inhibitors of minerals, such as Fe and Zn (Mascitelli & Goldstein, 2011; Raes, Struijs, & Camp, 2014). Due to that, the polyphenols have been described as the main ANFs responsible for the low bioavailability of minerals in plant based food (Raes et al., 2014). In this study, a weak and/or negative correlation was observed for all the minerals, phenolic compounds and antioxidant activity in pulps in the analyzed *Musa* spp. germplasm. When phenolic compounds are liberated from the food during the digestion, they have the property of combining with minerals such as Zn and Fe in the intestinal lumen, which then become unavailable for absorption (Raes et al., 2014).

Despite the abundance of bibliographical information about the contents of chemical compounds in fruits and vegetables, the studies on the correlation between the mineral content and the extracts' antioxidant activity are rare. When the minerals are complexed with the phenolic compounds, they can show considerable synergism in antioxidant capacity, because many minerals can act as electrons donors and have their charges quickly stabilized by the polyphenolic structures (Sant'Ana et al., 2012). Sulaiman et al. (2011) studied the minerals content and antioxidant activity in banana cultivars from Malaysia and verified no correlation of the minerals with antioxidant activity in the analyzed extracts, corroborating to the present study. Some authors suggest an inverse relation between the minerals content (e.g., Mg) and the antioxidant activity. In addition, an imbalance of minerals can change the flavonoids content, a potent antioxidant presents in *Musa* spp. fruit. Tewari, Kumar, & Sharma, (2006) verified an increase in the activities of enzymes, such as superoxide dismutase (SOD) in mulberry plants deficient in Mg. In addition, the deficiency of P can lead to an increase in the flavonoids level (Lillo, Lea, & Ruoff, 2008), which can also help to

explain, partially, the negative correlation between the phenolic compounds and the minerals found in the present study. Generally, cultivars with lower mineral contents tend to have higher phenols content and higher antioxidant activity.
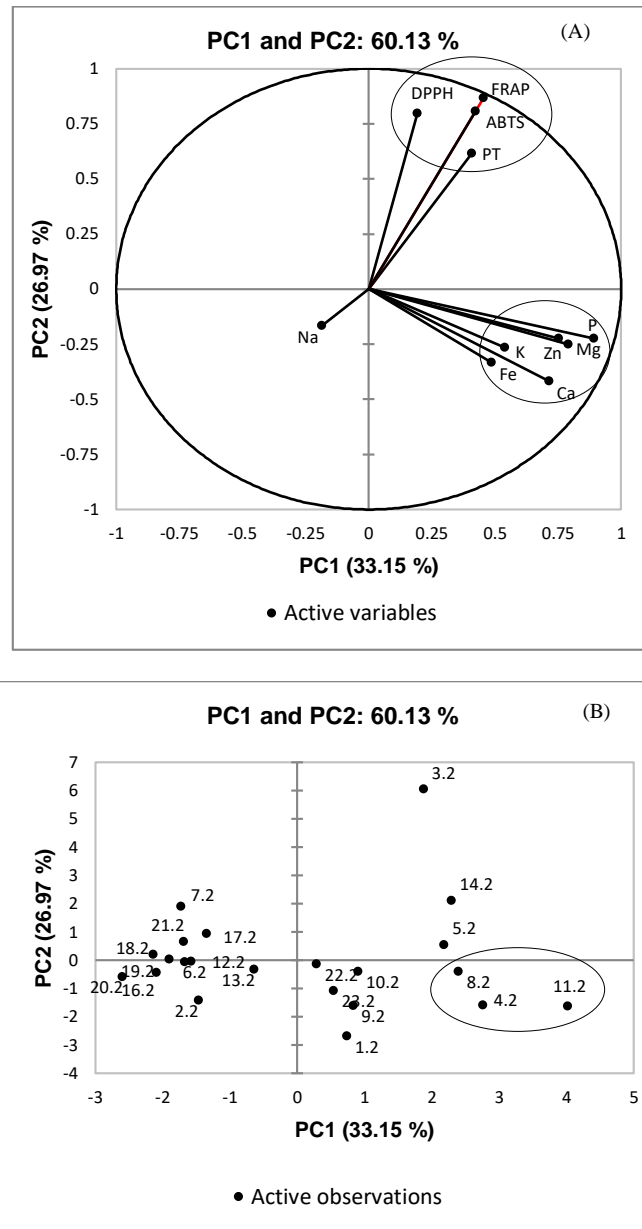


*1: FC06-02; 2: Mont. 172; 3: Tiparot; 4: Khai; 5: Mont. 301; 6: Simili; 7: Pelipita; 8: Ouro; 9: Pisang; 10: Yagambi; 11: Pacha; 12: Namwa; 13: Muisa T.; 14: Ney Poovan; 16: D'Angola; 17: Curare E.; 18: Terra S.N.; 19: Terra A. B.; 20: Tipo V.; 21: Samurá B.; 22: Prata A.; 23: Grand Naine.

**Fig. 1.** Two-dimensional projection (A) and scores (B) of biochemical attributes (phenolic compounds, antioxidant activity and minerals) in the two first principal components among the pulps (stage 2) of the *Musa* spp. genotypes.

*4.3.3 Impact of the ripening stage on the phenolic compounds content in pulps of Musa spp. fruit.*

The total phenolic compounds content varied widely among the analyzed genotypes (74.64 to 506.12 mg GAE/100g, Fig. 2). The ripening stage, which is a genotype-dependent characteristic, influenced on the content of these compounds among the cultivars. In general, lower contents of phenolic compounds were found in bananas during the stage 7, mainly in dessert bananas. This probably occurred due to the ripening metabolic process. With the ripening, there are an apparent decrease in the total phenolic content, which might be associated to the activity of oxidative enzymes, such as the polyphenol oxidase (Parr & Bolwell, 2000). In addition, in more advanced ripening stages, a decrease on the quantity of phenolic compounds may occur caused by the increase of water in the cells and decrease of the enzymatic activity involved in the biosynthesis of phenolic compounds (i.e. PAL – phenylalanine ammonia lyase EC 4.3.1.5) (Starrett & Laties, 1991). Tsamo et al. (2015) verified in plantains that the total phenolic compounds content in pulps of *Musa* spp. fruit increased until de ripening stage 5, decreasing in the stage 7, corroborating with the profile of many genotypes analyzed in this study (plantains - 'Terra sem nome' and 'Samurá B'; non plantain cooking bananas: 'Simili Radjah', 'Pacha Nadan' and 'Tiparot'; and dessert bananas: Grande Naine', 'Prata-Anã', and 'Ouro da Mata') (Fig. 2). However, in some cultivars (e.g. Ney Poovan and D'Angola), there was an increase in these compounds in stage 7 and more studies are necessary in order to verify this variation during the ripening process in specific cultivars. Ummarat et al. (2011) also reported increases in phenolic compounds and free flavonoids in 'Kluai Hom Thong' bananas during the ripening process. The differences of profiles might be associated to specific phenolic compounds. For example, fruit rich in anthocyanins show an increase in the total phenolic with the ripening, while many other phenolic compounds decrease (Rogez, Pompeu, Akwie, & Larondelle, 2011). These results demonstrate the importance of the specific phenolic compounds analyses in order to differentiate the genotypes, as well as for comprehending the changes that occur in the profile of these compounds during the ripening process in bananas.

The cooking genotypes 'Tiparot' and 'Pelipita' and the dessert bananas 'Poovan' were the ones that stood out among all of the analyzed genotypes. The 'Pelipita' presented high content of total phenolic compounds, mainly in the green fruit (506.12

mg EAG/100 g) (Fig. 2). In contrast, the 'Tiparot' contain larger contents of phenolic compounds, both in green and in ripe fruit (stage 5 – 658.80 mg EAG/100g). It is worth to point out the genotype Ney Poovan showed an increase in these compounds during the ripening (460.19 mg GAE /100g d.w.). In studies with 'Kluai Leb Mue Nang' bananas (AA group), increases in the total phenolic compounds were verified in more advanced ripening stages (stage 5 and 7, with no difference among them) (Youryon & Supapvanich, 2017).

Among the plantains, 'Samurai B' presented the highest total phenolic content (432.06 mg GAE / 100 g d.w.) in mature fruits (stage 5). In green and overripe fruits (yellow with black spots), 'D'Angola' showed the highest amounts of these compounds (Fig. 2). Similar levels of phenolic compounds verified in our study were described by Tsamo et al., (2014) in Niangafelo (198.4 mg GAE / 100 g fw) and Moto Ebanga (182.10 mg GAE / 100 g fw) bananas and Pelipita (319.5 mg GAE / 100 g fw). However, in 'Pelipita' we found 506.12 mg EAG / 100g d.w. (223.76 mg GAE / 100 g f.w.) at stage 2; 405.09 mg GAE / 100g d.w. (158.47 mg GAE / 100 g f.w.) at stage 5 and 432.28 mg / 100g d.w. (162.62 mg GAE / 100g f.w.) at stage 7, slightly below the reported levels in the literature (Tsamo et al., 2014). Differences in values may be due to different extraction methods and analyzes, as well as the cultive conditions (e.g., climate, soil, altitude, etc.).

Our results show that bananas and plantains are important sources of phenolic compounds, as other fruits and vegetables considered rich in these compounds, e.g. potato (90.85 mg EAG/100 g f.w.) (Nayak, Berrios, Powers, Tang, & Ji, 2011) and beetroot (257 mg EAG/100g f.w.) (Lin & Tang, 2006) and the variations of these compounds in different ripening stages are genotype-dependent (Figure 2). In addition, there are genotypes with superior quantities of phenolic compounds, when compared to the most consumed cultivars nowadays (e.g., Grande Naine and Prata-Anã). Thus, the promotion and incorporation of genotypes with superior quantities of these bioactives (e.g., 'Poovan', 'Pelipita' and 'Tiparot') would be interesting, or at least their use in programs of genetic improvement of the culture. Using the correlation analysis, we can verify that the phenolic compounds are the ones that most contribute to the antioxidant activity in the *Musa* spp. germplasm (DPPH: r = 0.55, p <0.01 e FRAP: r=0.71, p<0.01).
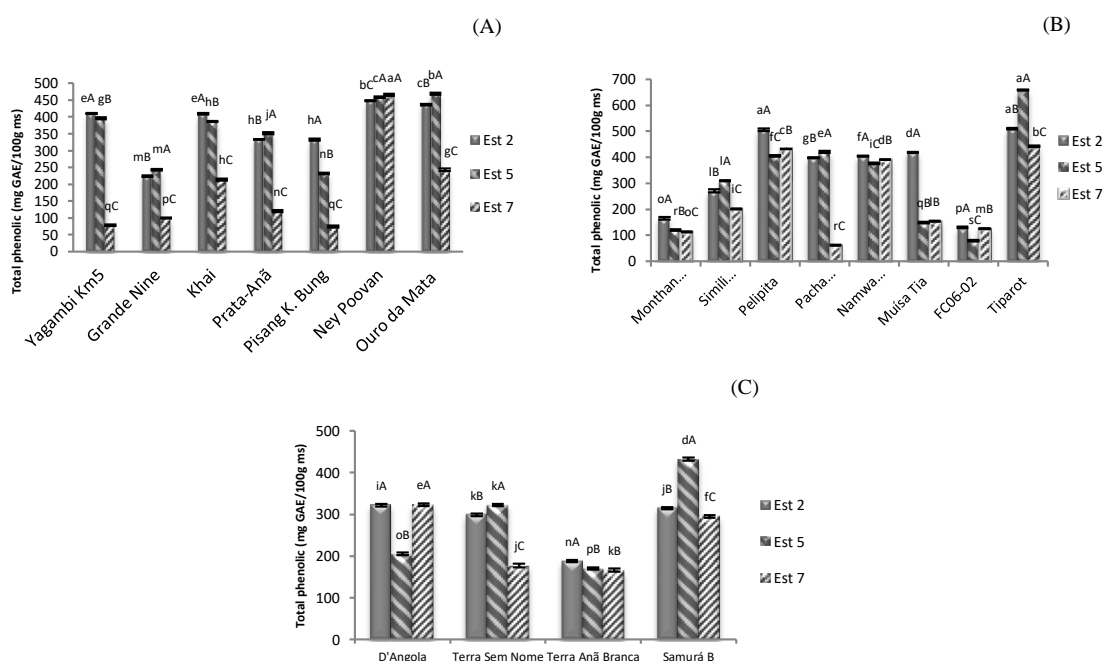
(A)

(B)

(C)

*The same lower case letters (genotypes) and uppercase letters (ripening stages) do not differ by Scott and Knott test (5%) and Tukey test (5%), respectively.

**Fig. 2.** Mean and standard deviation of total phenolic compounds of pulp (stage 2, 5 and 7). Genotypes separated by dessert bananas (A), cooking bananas (B) and plantain (C).

### 4.3.4 Impact of the ripening stage on the specific phenolic compounds content in pulps of Musa spp. fruit

We chose 8 genotypes that stood out regarding the total phenolic compounds content for analysis if profile by HPLC. We detected gallic and hydroxybenzoic acid; and the flavonoids catechin, epigallocatechin and quercetin (Table 5). The dessert banana 'Ney Poovan' and the cooking genotype 'Tiparot' were the ones that presented the highest flavonoids and phenolic acids contents, regardless of the ripening stage. Among the plantains, the genotype Samurá B presented the highest levels.

**Table 5.** Phenolic content via HPLC (µg/g d.w.) in pulp of *Musa* spp.

| Genotypes | Ripening stage | Phenolic acids | | | Flavonoids | | | |
|---|---|---|---|---|---|---|---|---|
| | | Gallic acid | Hydroxybenzoic acid | Mean | Epigallocatechin | Catechin | Quercetin | Mean |
| *Dessert banana* | | | | | | | | |
| Ney Poovan | 2 | 241.7[bB] | 39.9[eC] | 140.8 | 53.6[dC] | 262.9[bC] | 75.1[bB] | 130.5 |
| | 5 | 254.4[aA] | 85.9[aA] | 170.2 | 60.8[dB] | 288,1[bA] | 98.1[bA] | 149.0 |
| | 7 | 184.9[bC] | 68.9[aB] | 126.9 | 78.5[dA] | 269,4[aB] | 79.9[aB] | 142.6 |
| Prata | 2 | 183.5[cA] | 67.6[bA] | 125.5 | 19.2[eB] | 88.0[eB] | 68.4[cA] | 58.5 |
| | 5 | 146.5[bB] | 58.2[bB] | 102.4 | 28.2[eA] | 106.5[eA] | 44.6[dB] | 59.8 |
| | 7 | 145.1[cB] | 50.8[bC] | 97.9 | 15.2[gC] | 51,1[fC] | 18.9[cC] | 28.4 |
| Grande Nine | 2 | 101.2[eA] | 53.5[dA] | 77.4 | 19.2[eA] | 88.5[eA] | 13.1[fA] | 40.3 |
| | 5 | 86.6[dB] | 44.1[dB] | 65.4 | 19.8[fA] | 78.4[fB] | 10.0[gA] | 36.1 |
| | 7 | 86.7[eB] | 34.3[dC] | 60.5 | 11.2[hB] | 23.3[gC] | 6.9[dB] | 13.8 |
| *Cooking banana* | | | | | | | | |
| Tiparot | 2 | 352.0[aA] | 28.5[fB] | 190.3 | 121.0[bB] | 459.1[aB] | 142.0[aB] | 240.7 |
| | 5 | 249.2[aB] | 58.2[bA] | 153,7 | 149.8[bA] | 641,6[aA] | 153.0[aA] | 314.8 |
| | 7 | 193.9[aC] | 28.2[eB] | 111.1 | 119.5[bB] | 117.2[cC] | 32.9[bC] | 89.9 |
| Pelipita | 2 | 70.5[f C] | 14.1[gC] | 42.3 | 14.8[fB] | 118.3[dB] | 35.3[dA] | 56.1 |
| | 5 | 80.7[dA] | 43.3[dA] | 62.0 | 28.9[eA] | 122.1[dB] | 28.2[eB] | 60.0 |
| | 7 | 67.8[fC] | 29.1[eB] | 48.5 | 31.4[fA] | 117.1[cA] | 17.6[cC] | 55.4 |
| *Plantain* | | | | | | | | |
| D'Angola | 2 | 151.6[dA] | 72.7[aA] | 112.2 | 77.5[cA] | 82.8[fA] | 20.1[eA] | 60.1 |
| | 5 | 95.6[cB] | 56.9[bB] | 76,3 | 57.7[dB] | 70.6[gB] | 16.1[gB] | 48.1 |
| | 7 | 79.7[eC] | 38.6[cC] | 59.2 | 59.6[eB] | 52.9[fC] | 23.2[cA] | 45.2 |
| Terra S. N | 2 | 159.9[dB] | 12.7[gB] | 86.3 | 51.2[dC] | 24.0[gC] | 66.4[cA] | 47.2 |
| | 5 | 248.5[aA] | 16.7[eA] | 132.6 | 134.5[cB] | 42.6[hB] | 26.4[eB] | 67.8 |
| | 7 | 146.5[cC] | 10.7[fB] | 78.6 | 170.3[aA] | 79.9[eA] | 26.2[bB] | 92.1 |
| Samurá B. | 2 | 154.9[dA] | 72.3[aA] | 113.6 | 147.9[aB] | 164.2[cA] | 65.1[cB] | 125.7 |
| | 5 | 142.0[bB] | 54.7[cB] | 98.4 | 158.5[aA] | 160.5[cA] | 82.1[cA] | 133.7 |
| | 7 | 123.6[dC] | 37.5[cC] | 80.5 | 105.7[cC] | 107.2[dB] | 31.6[bC] | 81.5 |

*The same lower case letters (genotypes) and uppercase letters (ripening stages) do not differ by Scott and Knott test (5%) and Tukey test (5%), respectively.

In a tentative attempt to stablish a descriptive model of grouping the ripening stages as a function of the phenolic found, we opted for comparing the obtaining results using a PCA. The dispersion of varieties according to PC1 and PC2 are shown in Figure 3.
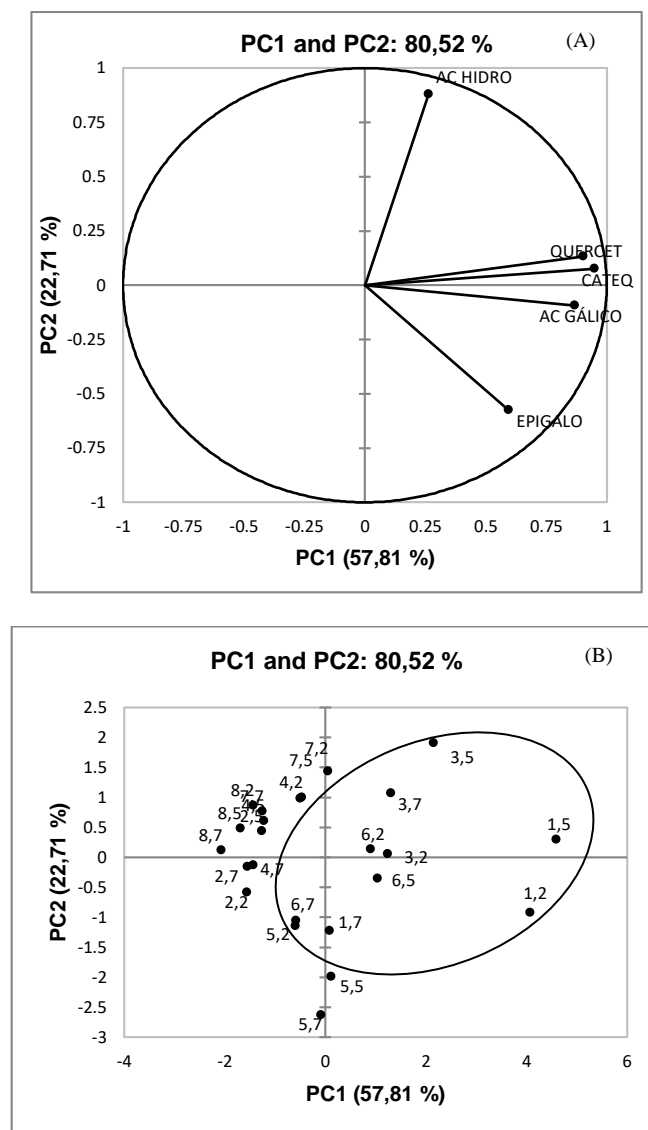


*1: Tiparot; 2: Pelipita; 3: Ney Poovan; 4: D'Angola; 5: Terra S. N.; 6: Samurá B.; 7: Prata Anã; 8: Grand Nine; Stage 2: 2; Stage 5: 5; Stage 7: 7.

**Fig. 3.** Two-dimensional projection (A) and scores (B) of phenolic compounds (gallic acid, hydroxybenzoic acid, epigallocatechin, catechin and quercetin) in the first two principal components among eight genotypes of bananas and plantains evaluated during the ripening (stages 2, 5 and 7).

PC1 and PC2 explained 80.52% of the data variance. The PC1 explained 57.81% of the data variance and was responsible for separating the genotypes with

higher phenolic compounds content (PC1+) from the genotypes with lower contents (PC1-). 'Ney Poovan', 'Tiparot' and 'Samurá B. grouped with the phenolic acids and flavonoids, presenting the highest content of these compounds, regardless of the analyzed ripening stage (Fig. 2). PC2 explained 22.7% of the data variance and was responsible for separating the genotypes in function of the phenolic acids and flavonoids. The dessert banana 'Ney Poovan' (stg. 5 and 7), the plantain Samurá B. (stg. 2) and the cooking banana Tiparot (stg. 5) were the genotypes that present the highest content of hydroxybenzoic acid, grouping in PC2+. The genotype Tiparot (stg. 2 and 5) also presented the highest content of catechin, quercetin and gallic acid. Superior levels of epigallocatechin were found in more advanced stages of the plantain 'Samurá B'. (stg. 5) and in the plantain 'Terra S. N.' (stg.7), which were grouped in PC2- (Fig. 3).

It was not possible to verify a clear grouping of the different ripening stages in function of the phenolic compounds content found in the different analyzed genotypes by the PCA. However, in average, the phenolic acids and flavonoids content increased until the stage 5 (ripe fruit), decreasing in more advanced ripening stages (stage 7) (Table 5). Some authors report that there is an increase of these compounds with the ripening and a decrease in overripe stages (Campuzano, Rosell, & Cornejo, 2018; Youryon & Supapvanich, 2017), similar to the results found in the present study. In addition, other antioxidant compounds verified in *Musa* spp. germplasm (e.g., carotenoids) (Borges et al., 2018) did not demonstrate a clear separation by genomic group or consumption group. We found a negative correlation with C* from the pulps of the fruit, mainly the flavonoids, quercetin (r = - 0.73, p ≤ 0,05 and catechin (r = - 0.70, p ≤ 0,05), indicating that the fruit with more light-colored pulps tend to present higher flavonoids contents, differently from what was found in other antioxidant compounds (i.e., carotenoids, pro-vitamins) in *Musa* spp. (Borges et al., 2018).

According to Pearson correlation analysis, the flavonoids were the compounds that presented strong and significant correlation with the antioxidant activity. Catechin and quercetin presented the higher correlation values, regardless of the antioxidant method used (FRAP: r = 0.92 and 0.85; DPPH: r = 0.85 and 0.80; ABTS: r = 0.78 and 0.77, p ≤ 0,05, respectively). Among the acids, the gallic acid also presented positive correlation and significant to the antioxidant activity (DPPH: 0.70; ABTS: 0.67: FRAP: 0.65, p ≤ 0.05), however, inferior to the correlation detected for the flavonoids (e.g., catechin and quercetin). It is worth to point out that the phenolic compounds are

considered the most important antioxidants present in plants. The antioxidant activity of these compounds is based on its capacity of donating atoms of hydrogen to free radicals, inhibiting or interrupting the chain propagation of the oxidative reactions, converting the free radicals in less harmed molecules and repairing the oxidative damages in the human cells (Du, Li, Ma, & Liang, 2009).

Different reports are found in the literature regarding the antioxidant capacity of the phenolic compounds. Some authors suggest a correlation between these compounds with the antioxidant activity present in plants and others do not describe a relation with the total phenolic (Demiray, Pintado, & Castro, 2009). These differences can be attributed to the type and quantity of phenolic compounds in the plant cell, as verified in the present study, thus, the detection of specific compounds is essential in the analyzed samples. It is possible to observe, for example, that hydroxybenzoic acid (FRAP: 0.07; DPPH: 0.16; ABTS: 0.24) and epigallocatechin (FRAP: 0.32; FRAP: 0.22; ABTS: 0.15) presented low correlation with the antioxidant activity in *Musa* spp. fruit, regardless of the method used, differently from the other analyzed phenolic compounds.

*4.3.5 Impact of the thermal processing on the phenolic compounds content in cooking banana and plantain*

With the principal components analysis (PCA), we verified the profile of the phenolic compounds in the different types of thermal processing in the studied genotypes (plantains and cooking bananas). The retention study of bioactive compounds after the thermal processing has been one of the main preoccupations both in the industrial process and in the domestic preparations, which can generate great losses of these nutrients, depending on the used method of preparation (Borges et al., 2018; Tsamo et al., 2015).

The dispersion of varieties according to the PC1 and PC2 axis are shown in the Figure 4. PC1 and PC2 explained 92.64% of the data variance. The PC2 axis represents 14.82% of the total data variance and was responsible for the separation of the genotypes, in function of the different chemical compounds observed.
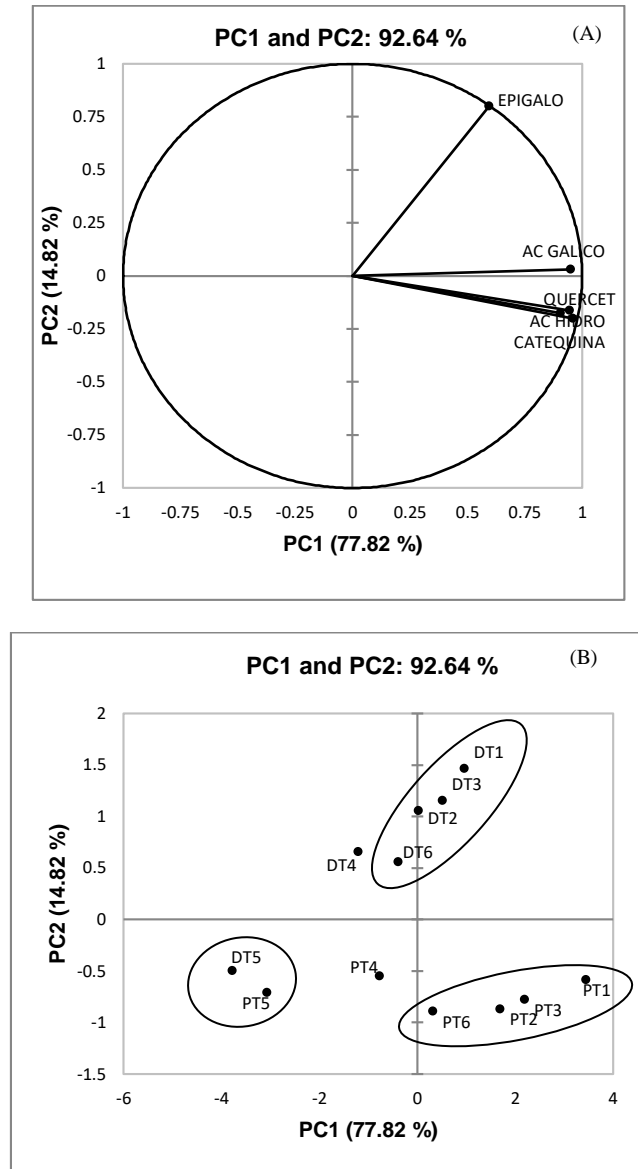
*T1: boiling with peel; T2: boiling without peel; T3: microwaving with peel; T4: microwaving without peel; T5: styr-frying; T6: *in natura.*

**Fig. 4.** Two-dimensional projections (A) and scores (B) of phenolic compounds (gallic acid, hydroxybenzoic acid, epigallocatechin and quercetin) in the first two principal components among the plantain 'D'Angola' (D) and the cooking banana 'Pelipita' (P) submitted to the different types of thermal processing.

The 'D'Angola' plantain presented the highest contents of epigallocaechin grouping in the PC2+ (Fig. 4 and Table 6). However, when the fruit of this genotype were submitted to the frying treatment, there was a significant decrease of this flavonoid.

**Table 6.** Phenolic content via HPLC (µg/g d.w.) after cooking processes of the 'D'Angola' plantain in the 'Pelipita' cooking banana.

| Cooking processes | Phenolic acids | | | Flavonoids | | | |
|---|---|---|---|---|---|---|---|
| | Gallic acid | Hydroxybenzoic acid | Mean | Epigallocatechin | Catechin | Quercetin | Mean |
| *Pelipita* | | | | | | | |
| Boiling with peel | 160.6[a] | 97.8[a] | 129.2 | 55.9[a] | 83.5[b] | 60.0[a] | 66.5 |
| Boiling without peel | 115.7[b] | 67.2[c] | 91.5 | 36.8[c] | 79.9[c] | 49.6[b] | 55.4 |
| Microwave with peel | 91.7[c] | 93.1[b] | 92.4 | 48.7[b] | 89.3[a] | 50.4[b] | 62.8 |
| Microwave without peel | 61.6[e] | 47.8[d] | 54.7 | 25.9[d] | 64.5[d] | 16.4[d] | 35.6 |
| Stir-frying | 33.8[f] | 25.2[f] | 29.5 | 1.4[e] | 13.8[e] | 7.9[e] | 7.7 |
| *In natura* | 75.5[d] | 43.9[e] | 59.7 | 27.8[d] | 79.8[c] | 38.9[c] | 48.8 |
| *D'Angola* | | | | | | | |
| Boiling with peel | 97.0[a] | 60.1[a] | 78.6 | 92.1[a] | 59.4[a] | 30.5[a] | 60.7 |
| Boiling without peel | 84.6[c] | 40.9[c] | 62,8 | 73.3[c] | 51.9[b] | 28.2[b] | 51.1 |
| Microwave with peel | 92.5[b] | 46.1[b] | 69.3 | 80.0[b] | 60.6[a] | 29.9[a] | 56.8 |
| Microwave without peel | 47.7[e] | 30.0[d] | 38.9 | 55.2[d] | 52.2[b] | 16.3[d] | 41.2 |
| Stir-frying | 17.3[f] | 10.5[e] | 13.9 | 3.2[e] | 15.1[d] | 4.4[e] | 7.6 |
| *In natura* | 76.8[d] | 45.9[b] | 61.4 | 56.8[d] | 41.9[c] | 27.3[c] | 42.0 |

*The same lower case letters do not differ by Tukey test (5%)

The cooking banana 'Pelipita' presented the highest contents of the flavonoids catechin and quercetin, besides containing the highest content of hydroxybenzoic acid regardless of the used treatment (PC2-). Despite the 'D'Angola' plantain containing the highest quantity of gallic acid in the *in natura* fruit, when the fruit were submitted to the different types of processing, the cooking banana 'Pelipita' presented the highest contents of this phenolic acid, mainly in the ebullition cooking method (peeled and unpeeled fruit).

In the cooking methods using the peel, there were significant increases in the phenolic compounds in the pulps, mainly in the phenolic acids (Table 6). This can be explained by the high quantity of this compounds in the peels and there might have happened a migration of the phenolic compounds to the pulp (Tsamo et al., 2015). In addition, the cooking banana 'Pelipita' presented, in general, a higher availability of phenolic compounds when it was submitted to the different types of thermal processing, with superior percentages of increase in phenolic acids and flavonoids (epigallocatechin and quercetin), compared to the plantain D'Angola (Table 6). These differences between the cooking banana 'Pelipita' and the plantain 'D'Angola' found in the bioactive compounds contents under the same thermal treatment could be a result of the inherent differences among the genotypes. In general, the cooking bananas present fruit with less firmness than the plantains, which are characterize by the high fruit firmness, even in more advanced ripening stage. In fact, the cooking banana 'Pelipita' showed lower firmness (1.06 N with peel and 0.25 N without peel) in relation to the plantain 'D'Angola' (2.80 N with peel and 0.80 N without peel), in the moment of the thermal processing, which might have been a contributive factor for a better extraction of these compounds in cooking bananas.

## 4.4 Conclusion

The plantains and cooking bananas presented the highest content of starch and resistant starch and the lowest contents of phenolic compounds (i.e., stg 7). We found the highest levels of total phenolic compounds in the dessert bananas and in the cooking bananas. The pulps of the dessert bananas 'Khai' and 'Ouro da Mata' and of the cooking banana 'Pacha Nadam' stood out regarding most of the analyzed minerals. Our results also indicate that the peel have a nutritional value superior than the pulp

(e.g., phenolic compounds and minerals) and is a very important byproduct for the use in pharmaceutical and food industries. The determination of these metabolic profiles can be used to select possible crossings for genetic improvement programs for the banana tree, aiming the creation of biofortified cultivars and/or for promotion and incorporation in agricultural systems of genotypes with substantial quantities of phenolic compounds and minerals. The phenols, mainly the flavonoids catechin and quercetin, are the compound that contribute the most to the antioxidant activity of the *Musa* spp. germplasm. It is worth to stress out that the phenolic compounds content is affected by the ripening stage and superior values were found in the ripe fruit (stg. 5). In addition, the thermal process increases the functional and/or nutritional value of the *Musa* spp. fruit, mainly the ebullition cooking method (fruit with peel), which should be the preferred method in domestic preparation, regardless of the cultivar.

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# CONSIDERAÇÕES FINAIS

A banana apresenta potencial como alimento promotor de saúde, em face de suas características funcionais. Entretanto, estas características estão dispersas em vários genótipos, requerendo ações do melhoramento genético com foco no desenvolvimento de cultivares biofortificadas que contribuam ao incremento da ingestão de nutrientes em populações menos favorecidas. Por meio deste trabalho é possível concluir que a identificação de genótipos em bancos ativos de germoplasma com teores superiores de compostos bioativos e seu uso em programas de melhoramento genéticos assistidos bioquimicamente, ou diretamente na alimentação humana, constituem estratégias racionais de uso de uma importante fração da biodiversidade em proveito de populações menos favorecidas, com menor impacto ambiental e econômico, no contexto de programas de mitigação da hipovitaminoses, e.g. De fato, a identificação de cultivares ricas em compostos funcionais possibilitará a incorporação em sistemas agrícolas existentes e tradicionais, (e.g., agricultores familiares), criando um nicho diferenciado de mercado, agregando valor ao produto e, consequentemente, aumentando a renda do produtor rural. Dentro do banco ativo de germoplasma da Embrapa Mandioca e Fruticultura pode-se afirmar que há grande variabilidade de características químicas e bioquímicas, sendo que há acessos com boas características pós-colheita, que poderiam ser explorados, tanto para o consumo *in natura*, como para a indústria de alimentos, principalmente quando comparados com as cultivares mais comercializadas atualmente (Capítulo 1). Além disso, foi constatada a presença de acessos divergentes e com quantidades superiores de compostos antioxidantes e/ou funcionais (vitamina C, aminas bioativas, pro-vitamínicos A, compostos fenólicos e vitamina C) que podem ser explorados em estudos bioquímicos que visam à caracterização e seleção de acessos com características químicas diferenciadas para serem utilizados em programas de melhoramento genético vegetal da cultura, bem como para a incorporação de cultivares biofortificadas nos sistemas agrícolas existentes (Capítulo 1, 2, 3 e 4). O modo em que são consumidos estes genótipos podem afetar o valor nutricional dos frutos, sendo o cozimento em água o preferível nas preparações domésticas, independente da cultivar utilizada (Capítulo 2, 3 e 4).

Tomados em conjunto, em um futuro próximo, espera-se que os resultados encontrados possam ser usados para selecionar possíveis cruzamentos em

programas de melhoramento genético da bananeira para a criação de cultivares com características importantes pós-colheita, além de biofortificadas, aumentando assim o valor nutricional e funcional da fruta. Concomitantemente, o conhecimento do perfil destes compostos ao longo do processo de amadurecimento dos frutos e na sua forma de consumo, pode contribuir para o aumento da qualidade dos frutos na pós-colheita, bem como para a escolha da melhor maneira de consumi-los quando o objetivo da população for a promoção e/ou prevenção da saúde humana.

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