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POTENCIAL FUNCIONAL E PERFIS DE COMPOSTOS FENÓLICOS, CAROTENÓIDES E FIBRAS DE CULTIVARES DE LARANJAS E TANGERINAS E CONSERVAÇÃO PÓS-COLHEITA DA TANGERINA 'DANCY' DA CITRICULTURA FAMILIAR

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CERTIFICADO DE APROVAÇÃO

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ANTONIO FERNANDO DA SILVA

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RESUMO GERAL

Os citros fazem parte da alimentação, estando entre as frutíferas mais cultivadas no mundo. O Brasil é um dos maiores produtores e exportadores de citros, concetrando a produção no Sudeste e Nordeste do país. O Território da Borborema-PB produz laranjas e tangerinas que suprem o mercado regional, que exige estudos aprofundados para valorizar e inserir estes frutos no mercado global. É necessário definir padrões de identidade e qualidade, perfis de compostos funcionais do suco e cascas, além de alternativas limpas para a conservação pós-colheita, a exemplo de recobrimentos biodegradáveis. Este trabalho avaliou os citros produzidos no Território da Borborema mediante três experimentos: **I –** Caracterização dos compostos bioativos, fibras e atividade antioxidante da casca e polpa de cultivares de laranjas e tangerinas; **II** - Conservação pós-colheita de tangerina com recobrimento de amido adicionado de solvente natural eutético profundo (NADES) e **III** - Qualidade dos frutos de laranjeiras durante a maturação. **Principais resultados: Experimento I,** Avaliou-se no suco e cascas de frutos de 10 cultivares de laranjas (Baianinha, Salustiana, Rubi, Westin, Sincorá, Lima, Baia, Comum, Mimo, Pera) e 6 de tangerinas (Piemonte, Clemenules, Page, Dancy, Ponkan e Murcot), os perfis de fibras, minerais, fenólicos livres e ligados, carotenoides das cascas; além da atividade antioxidante (AAT) pelos métodos dos radicais ABTS, DPPH e ORAC. Cascas dos citros apresentam altos teores de fibra dietética e minerais, são ricas em polifenóis, destacando-se a Dancy em fenolicos livres (1,46 g GAE. $100g^{-1}$ MS) e ligados (0,95 g GAE. $100g^{-1}$ MS) hesperidina (239,99 $mg.100g^{-1}$), tangeritina (46,94 $mg.100g^{-1}$), ácido clorogenico (118,05 $mg.100g^{-1}$) e ácido cafeico (86,76 $mg.100g^{-1}$). Polpa e cascas de tangerina possuem teores elevados de carotenoides. AAT de fénólicos livres e ligados por ABTS, DPPH e ORAC confirmam o potencial funcional das cascas de citrus. A tangerina 'Dancy' possui maior AAT P-ligados. **Experimento II**: Avaliou-se a conservação pós-colheita da tangerina 'Dancy', com amido de mandioca e jaca, associado à glicerol ou a solvente Natural eutético profundo (NADES), em DIC, fatorial 5 x 6 (recobrimento x periodos de avaliação), AM+NADES, AM+GLI, AJ+NADES, AJ+GLI e controle, com três repetições de quatro frutos cada. Os recobrimentos mantiveram a qualidade dos frutos durante o armazenamento. **Experimento III:** Utilizou-se um DIC, fatorial 3x3 para avaliar a qualidade de três cultivares de laranja ('Baía', 'Comum' e 'Mimo-do-Céu'), três estádios de maturação (verde; verde amarelado e amarelo), avaliado-se o albedo e suco. A qualidade das laranjas 'Baia' e 'Mimo-do-Céu' insere-se nos padrões do CEAGESP. A 'Mimo-do-Céu' possui maior ácido ascórbico (50,26 $mg.100g^{-1}$). Durante a maturação a firmeza diminuiu e os sólidos solúveis, PET e a AAT do suco e do albedo aumentam. Em geral, o albedo apresenta teor de PET cerca de oito vezes superiores ao do suco, que reflete em AAT bem superior nesta porção que, portanto, se destacou pelo elevado potencial funcional, principalmente na laranja 'Baía'. Cultivares de laranjas e tangerinas deste estudo possuem altos teores de fibras e compostos bioativos essenciais para a alimentação humana e os recobrimentos testados permitem maior conservação, mantenção da qualidade, que permite o transito mercados competitivos distantes.

Palavras-chave: *Citrus.* Padrões de qualidade. Tecnologia limpa. NADES. Conservação pós-colheita. Hispiridina. Fenólicos ligados. Tangeritina. β-caroteno.

GENERAL ABSTRACT

Citrus are part of the diet, being among the most cultivated fruit trees in the world. Brazil is one of the largest citrus' producers and exporters, concentrating its production in the Southeast and Northeast of the country. The Borborema Territory-PB produces oranges and tangerines that supply the regional market, which requires in-depth studies to value and insert these fruits in the global market. It is need to define identity and quality standards, functional compound profiles of juice and peels, as well as clean alternatives for postharvest conservation, such as biodegradable coatings. This work evaluated the citrus produced in the Borborema Territory through three main experiments: **I** - Characterization of bioactive compoundand fiber profile and antioxidant activity of the peel and pulp of orange and tangerine cultivars; **II** - Postharvest preservation of tangerine with starch coating added with natural deep eutectic solvent (NADES); and **III** - Quality of orange fruits during ripening. **Main results: Experiment I**, It was evaluated on the juice and peel of fruits of 10 orange cultivars (Baianinha, Salustiana, Rubi, Westin, Sincorá, Lima, Baia, Common, Mimo, Pera) and 6 tangerines (Piemonte, Clemenules, Page, Dancy, Ponkan and Murcot), the fiber, mineral, free and bonded phenolic, carotenoid profiles in the peels; in addition to the antioxidant activity (TAA) by the radicals ABTS, DPPH and ORAC methods. Citrus peels have high levels of dietary fiber and minerals, are rich in polyphenols, with Dancy standing out in free phenolics (1.46 g GAE. $100g^{-1}$ DM) and bonded (0.95 g GAE. $100g^{-1}$ DM) hesperidin (239.99 $mg.100g^{-1}$), mandarin (46.94 $mg.100g^{-1}$), chlorogenic acid (118.05 $mg.100g^{-1}$) and caffeic acid (86.76 $mg.100g^{-1}$). Tangerine's pulp and peels have high levels of carotenoids. TAA of free and bound phenols by ABTS, DPPH and ORAC confirm a higher functional potential of citrus peels. The 'Dancy' tangerine had the highest bouns phenolics TAA. **Experiment II:** Postharvest conservation of 'Dancy' tangerine was evaluated, with cassava and jackfruit starch, associated with glycerol or a deep eutectic natural solvent (NADES), in DIC, 5 x 6 factorial (coating x evaluation periods), AM + NADES, AM + GLI, AJ + NADES, AJ + GLI and control, with three replications of 4 fruits each. The coatings maintained the quality of the fruits during storage. **Experiment III:** A 3x3 factorial DIC was used to evaluate the quality of three orange cultivars ('Baía', 'Comum' and 'Mimo-do-Céu'), three maturation stages (green; yellowish green and yellow), albedo and juice were evaluated. The quality of 'Baia' and 'Mimo-do-Céu' oranges is in line with CEAGESP standards. 'Mimo-do-Céu' has a higher ascorbic acid (50.26 $mg.100g^{-1}$). During maturation, firmness decreased and soluble solids, PET and the TAA of juice and albedo increased. In general, albedo has a poliphenol content about eight-fold higher than that of juice, which reflects in TAA much higher in this portion, which, therefore, stood out for its high functional potential, especially in the 'Baia' orange. Cultivars of oranges and tangerines in this study have high levels of fibers and bioactive compounds essential for human consumption and the tested coatings allow for greater conservation, maintaining quality, which allows transit to much distant competitive markets.

Keywords: *Citrus.* Quality standards. Clean technology. NADES. Postharvest conservation. Hispiridine. Bound phenolics. Tangeritin, β-carotene.

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1 INTRODUÇÃO GERAL

O mundo inteiro tem apresentado crescimento contínuo na produção e consumo de frutas frescas, com destaque para China, Índia e Brasil, que respondem por mais de 40% do total mundial de frutas frescas produzidas. O Brasil é o terceiro maior produtor de frutas com mais de 44 milhões de toneladas e a citricultura destaca-se como uma das principais frutíferas, colocando o país como maior exportador de suco de laranja (Satari e Karimi, 2018; Sharma et al. 2017; Ruviaro, Barbosa & Macedo, 2018).

A região Sudeste, estado de São Paulo concentra aproximadamente 80% da produção de citros; no entanto, o Nordeste apresenta-se como a segunda maior região produtora do país, contribuindo com 11% da produção nacional (Ibraf, 2018; CNA, 2018). No Território da Borborema, estado da Paraíba, a citricultura concentra-se na produção familiar, sendo esta uma importante atividade, geradora de emprego, renda e desenvolvimento na região (MDA, 2010), na qual se predomina o cultivo de importantes cultivaresde tangerinas e laranjas, buscando atender as demandas do mercado regional de frutos frescos.

Os frutos de tangerinas, que correspondem a classe de citros mais explorada na região, são vulneráveis a infecções por microrganismos, distúrbios fisiológicos e lesões físicas, resultando em uma enorme perda de frutas frescas a cada ano (Xu, Qin & Ren 2018). E sabe-se que para reduzir os altos níveis de perdas pós-colheita dos frutos, são necessárias tecnologias eficientes e sustentáveis para manter a qualidade do produto (Cissé et al., 2015), como revestimentos biodegradáveis obtidos de fontes de amidos vegetais amplamente encontradas na natureza adicionados de plastificantes que dão maior estabilidade aos recobrimentos.

O uso de recobrimentos biodegradáveis na conservação pós-colheita de frutos, não somente melhora a aparência, mas prolongar a vida útil pós-colheita possibilitando novos mercados mais distantes da região produtora (Malgarim et al., 2007; Valencia-Chamorro et al., 2009).

É necessário criar estratégias para valorização da qualidade dos produtos da citricultura familiar visando seu fortalecimento, expansão e geração de desenvolvimento sustentável regional. Neste sentido, a avaliação da qualidade de novas cultivares inseridas nestas áreas é de extrema importância, além disso definir estratégias para utilização das porções até então não aproveitadas, visto que, tanto para o consumo fresco quanto no processamento para a obtenção do suco, 50% do fruto é descartado, o que corresponde majoritariamente a casca

(albedo + flavedo); no entanto o teor de compostos fenólicos totais é mais elevado na casca que no suco. Além disso estas porções são muito ricas em fibras e substancias pécticas (Escobedo-Avellaneda et al., 2014).

O consumo regular de fibras alimentares tem sido uma das mais constantes recomendações feitas por nutricionistas e órgãos oficiais para a prevenção de doenças do trato gastrointestinal, cardiovasculares, prevenção ou tratamento de diabetes e obesidade (Bortoluzzi e Marangoni, 2006). Neste contexto o consumo de frutos vem despontando como alternativa na complementação de uma dieta saudável, rica em compostos funcionais, o que levou a mudanças nos hábitos alimentares dos consumidores, aumentando a demanda por alimentos mais saudáveis e funcionais (Crizel et al., 2013).

A casca de laranja é uma rica fonte de fibras dietética e possuem outros compostos de interesse para a saúde como Flavonoides, polifenóis, carotenoides, ácido ascórbico (Crizel et al, 2013). Além disso alguns peptídeos que foram encontrados isoladamente no albedo e flavedo de laranja e tangerina (Sass-Kiss e SASS, 2000), os quais podem inclusive, apresentarem benefícios para a saúde humana como atividade anti-hipertensiva (Coscueta et al, 2016).

Os ácidos orgânicos e os compostos fenólicos estão entre os principais compostos presentes nas frutas cítricas, sendo importantes fatores de sua qualidade, uma vez que os compostos fenólicos conferem propriedades antioxidantes e os açúcares, os atributos de sabor. Os ácidos cítrico e málico são os principais ácidos orgânicos componentes das frutas cítricas (Bush, 2014).

Além de ter propriedades antioxidantes, polifenóis têm várias outras ações biológicas específicas na prevenção e/ou tratamento de doenças. Com base em dados epidemiológicos, os efeitos preventivos destes metabólitos secundários são inferidos em termos de doenças cardiovasculares, neurodegenerativas e câncer (Arts e Hollman, 2005; Cole et al., 2005). Alguns destes efeitos promotores de saúde são atribuídos aos compostos fenólicos e aos carotenoides. As partes não comestíveis da laranja e tangerina (cascas) são muito ricas nessas moléculas (Sharma et al., 2017).

Os compostos fenólicos possuem larga aplicação na indústria, podendo ser utilizados como corantes e conservantes naturais para alimentos, na produção de tintas, e como ingredientes na cosmética e na farmácia (Ignat et al. 2011). Diante disso, a identificação e quantificação destes compostos são de extrema importância para a valorização e caracterização de usos potencias dos subprodutos da laranja.

Então, a casca, considerada subproduto e resíduo poluente da agroindústria, têm despontado como uma promissora fonte de compostos funcionais, muito superior à quantidade observada no suco (Ademosun et al., 2016). Adicionalmente o uso de resíduos de frutas, podem diminuir os impactos ambientais e contribuir com a economia das regiões onde estão instaladas pequenas agroindústrias. Neste sentido, esse material pode vir a agregar valor ao fruto com a comprovação da presença de compostos que contribuem para a saúde humana como já foram comprovados em resíduos de Soja (Glycine max), Romã (Punica granatum) (Coscueta et al., 2016; Gullon et al., 2015), podendo inclusive ser de interesse para a indústria nutracêutica e farmacêutica (Mirabella, Castellani, Sala, 2014).

Dessa forma, há uma grande necessidade de se estudar as características funcionais das variedades de laranjas e tangerinas produzidas no território da Borborema paraibana, bem como desenvolver tecnologias de conservação pós-colheita, devido a sua importância econômica, e o potencial de seus subprodutos, como fonte de fitocompostos que agem comprovadamente no combate do estresse oxidativo no organismo, podendo vir a servir de ingredientes na composição de alimentos e fármacos industrializados.

2 OBJETIVOS

2.1 Objetivo Geral

Avaliar as laranjas e tangerinas produzidas pela agricultura familiar do Território da Borborema, estado da Paraíba, Brasil, buscando sua valorização no mercado de frutos frescos, bem como o aproveitamento dos subprodutos da indústria de processamento.

2.2 Objetivos Específicos

- Avaliar a qualidade, compostos bioativos e atividade antioxidante das cultivares de laranja Baía, Comum e Mimo-do-Céu em três estádios de maturação;
- Avaliar as mudanças fisiológicas e qualidade de tangerina ‘Dancy’ armazenada com recobrimento a base de amidos de mandioca e semente de jaca, utilizando NADES como plastificante;
- Detalhar o perfil de fenólicos, carotenoides e a atividade antioxidante no suco de dez cultivares de laranjas e seis de tangerinas;
- Avaliar o perfil de fenólicos, fibras e minerais do albedo de cultivares de laranjas;
- Estabelecer um perfil detalhado de carotenóides, compostos fenólicos, fibras e minerais de cascas de seis cultivares tangerina e dez de laranjas e atribuir o potencial

funcional relativo para uso na nutrição humana como fonte de fibra alimentar e fitocompostos.

3 REVISÃO DE LITERATURA

3.1 Citros

Os citros são amplamente cultivados e consumidos em todo o mundo. A laranja corresponde ao fruto cítrico mais importante comercialmente e seu principal produto é o suco, que é consumido em diversos países de diferentes culturas e hábitos alimentares variados (Neves et al., 2010).

O Brasil é mundialmente conhecido como um dos maiores produtores e exportadores de citros. A região sudeste, com destaque para o estado de São Paulo, concentra a maior produção nacional de citrus, principalmente laranja, no entanto a região nordeste do país também possue uma produção expressiva, principalmente com aprodução de citros de mesa, laranjas e tangerinas que suprem a demanda local destes frutos, e que corresponde a uma importante fonte de renda principalmente na economia familiar (Lopes et al.,2011).

Os citros produzidos na região da Borborema paraibana são destinados ao consumo in natura e de sucos frescos. As laranjeiras de baixa acidez por exemplo, dentre elas a lima e a Mimo-do-Céu, são bastante apreciadas e demandadas, principalmente por idosos, grávidas, crianças, lactantes, e pessoas com problemas gástricos e/ou intestinais (Silva et al., 2016). A tangerina Dancy é a cultivar mais produzida e comercializada nesta região, sendo os municípios de Matinhas, Alagoa Nova e São Sebastião de Lagoa de Roça os municipios que mais produzem a fruta no estado (SILVA et al., 2014).

As laranjas de mesa são bastante demandadas por mercados de frutas frescas, algumas amplamente produzidas em todo o país, como por exemplo as laranjas Pera e Baía, entretanto outras são produzidas em pequena escala na região da Borborema como a laranja Comum por exemplo (LOPES et al., 2011). Estes frutos possuem particularides que merecem ser investigadas.

Bastante apreciado devido ao sabor e aroma natural característicos da fruta; o suco de laranja desempenha papel importante na dieta, se destacando por ser fonte de vitamina C, flavonoides e carotenoides, e por conter ácido fólico, potássio e fibras (Stella et al., 2011; Stinco et al., 2012).

Além do suco que é extraído da polpa, a laranja ainda possui albedo e flavedo que

compõem a casca e são muito ricos em compostos funcionais que podem ser utilizados como ingredientes na formulação de produtos alimentares. A laranja se enquadra como alimento funcional porque todas as suas porções comestíveis fornecem benefícios à saúde, além de proverem nutrientes essenciais (Ramírez,2011).

Além da sua funcionalidade e nutrição, subprodutos do processamento de laranja também tem a vantagem de ser sem glúten e lactose, tornando-os ingredientes potencialmente ideal para uma gama de produtos por exemplo, produtos de panificação, doces, bebidas e confeitaria. Atualmente, tornou-se uma tendência a cada vez mais comum os consumidores procurarem produtos que contenham ingredientes naturais que contribuam para uma boa funcionalidade do organismo (O'Shea et al., 2015). Cerca de 45% a 60% da laranja, na indústria de sucos cítricos, acaba não sendo utilizada e sendo esse material, muito rico em fibras e outros compostos funcionais.

3.2 Fibras

As fibras desempenham um papel importante em várias funções fisiológicas humanas e pode ser utilizada na prevenção e tratamento de algumas doenças. Em razão disso, pesquisadores das áreas de alimentos vem procurando desenvolver produtos alimentícios com maiores teores de fibra alimentar, e para tal, buscam fontes alternativas de fibras dietéticas que podem promover benefícios a saúde. (Lamothe et al., 2015).

A fibra alimentar é um componente essencial para uma dieta saudável, seus benefícios não se limitam a saúde digestiva, estudos têm demonstrado que a fibra saudável pode também baixar o colesterol, promover os níveis saudáveis de açúcar no sangue, reduzir o risco de doença cardiovascular, e ajudar as pessoas a perder peso ou manter um peso saudável e redução do risco de algumas formas de câncer (Kaczmarczyk et al., 2012; Mann e Cummings, 2009).

As propriedades físico-químicas significativas de fibra alimentar incluem a solubilidade, viscosidade, capacidade de retenção de água e de fermentação. Com base na solubilidade em água, as fibras dietéticas são de dois tipos: solúvel (pectina, gomas, mucilagem e algumas hemiceluloses) e insolúvel (celulose, e lignina). (Elleuch et al., 2011 e Mudgil e Barak, 2013).

A casca de laranja é uma fonte de fibra dietética que pode ser utilizado como ingrediente alimentar (Lario et al., 2004). As fibras cítricas são uma alternativa melhor que fibras de cereais devido ao conteúdo de fibra dietética solúvel e compostos bioativos associados (Balasundram et al, 2006; Crizel et al, 2013).

3.3 Compostos fenólicos

Os fenólicos são largamente encontrados nos vegetais e correspondem a um grupo muito diversificado de fitoquímicos derivados de fenilalanina e tirosina. São essenciais no crescimento e reprodução das plantas, além de atuarem como agente antipatogênico e contribuírem na pigmentação, sendo sintetizados em condições de estresse como, doenças, cortes, radiações UV, dentre outros. Em alimentos, são responsáveis pela cor, adstringência, aroma e estabilidade oxidativa (Naczk; Shahidi, 2004; Giada; Mancini Filho, 2006). O grupo dos compostos fenólicos engloba uma série de compostos que vêm sendo bastante estudados, por apresentarem influência na qualidade dos alimentos (Balasundram et al., 2006; Hossain e Rahman, 2011).

As principais fontes de compostos fenólicos são frutas cítricas, como limão, laranja e tangerina, além de outras frutas à exemplo da cereja, uva, ameixa, pêra, maçã e mamão, sendo encontrados em maiores quantidades na casca que no suco da fruta (Pimentel, 2005). Os compostos fenólicos mais comum em frutas cítricas são os ácidos fenólicos e flavonóides (Stinco et al., 2013). Os principais ácidos fenólicos são os ácidos hidroxicinâmicos (ácidos ferúlico, caféico, clorogênico, p-cumárico e sinápico), e os hidroxibenzóicos (ácidos gálico, vanílico e p–hidroxibenzóico) (Kelebek & Selli, 2011). Quanto aos flavonóides ocorrem na forma de flavanonas agliconas e glicosiladas, flavonas polimetoxiladas e flavonóis glicosídeos (Escobedo-Avellaneda et al., 2014).

O conteúdo de fenolicos totais varia dependendo da variedade e porção da laranja, (Magda et al., 2008). Extratos fenólicos de casca de laranja aparenta ser uma alternativa interessante, por apresentarem propriedades anti-cancerígenas, anti-inflamatórios, anti-oxidantes e anti-aterogénicas (Ghasemi et al., 2009). O Flavedo, parte externa da casca da laranja, possui maior teor de flavonas polymethoxyladas e também de carotenóides, encontrada principalmente como epoxycarotenoids. Já o albedo é uma importante fonte de compostos fenólicos totais, com um teor médio semelhante ao flavedo e mais elevados do que suco. Informações da distribuição destes compostos em fracções de laranja é muito importante para a sua extração comercial e na formulação de alimentos funcionais (Escobedo-Avellaneda et al., 2014).

Os compostos fenólicos presentes em frutos de laranja apresentam grande importância para a saúde humana, uma vez que contribuem significativamente para o potencial antioxidante dos alimentos (Oliveira et al., 2009). Diversas pesquisas vem mostrando que compostos como antocianinas, flavonoides e carotenoides apresentam

correlação com a atividade antioxidante de frutos (Alothman et al., 2009; Silva et al., 2012). Dessa forma, embora já exista alguns estudos relatando a presença de composto fenólicos em citros, ainda são necessários estudos aprofundados acerca da quantificação e composição estrutural de compostos fenólicos de frutos das cultivares de laranja de mesa: Baianinha, Salustiana, Rubi, Westin, Sincorá, Lima (Sucory), Baía, Comum, Mimo do Céu, e das cultivares de tangerinas: Dancy, Piemonte, Clemenules, Page, Ponkan e Murcot.

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Artigo 1. Phenolic, carotenoids, mineral, proteins and fibers profiles and functional potential of tangerine peels flour from different cultivars

Directed to: Food Chemistry

Abstract

Citriculture is one of the most produced crops in the world. Tangerine is an important fruit crop of family farming in the Territory of Borborema, Paraíba, Brazil. The tangerine peels are discarded both by the juice industry and when consumed fresh, which represent about 50% of the fruits. Alternatives for using these by-products are necessary to reduce damage to the environment, enhance production and diversify the food industry. Here, the peel of six tangerine cultivars were evaluated for the first time to identify the profiles of minerals, proteins and peptides, polyphenols, fibre and functional potential for use in human food. The modulation of polyphenols throughout the gastrointestinal tract was also studied for the best cultivar. The tangerine peels showed a large content of free and bound phenolic compounds associated to a high content of dietary fibers. The soluble fraction of the tangerine peel showed characteristic peaks of proteins and peptides with molecular weights of 900 KDa, 105 KDa and between 1 and 0.5 KDa. Some phenolic compounds present, mainly caffeic acid are gradually released in the phases of the gastrointestinal tract. The Dancy tangerine was highlighted by its greater contents of the phenolics chlorogenic acid, caffeic acid, hesperidin and tangeritine and higher antioxidant activity in the extracts of the bound phenolic. Altogether, tangerine peels contain a large number of bioactive compounds with potentially health-promoting properties.

1 INTRODUCTION

More than 80% of citrus fruits produced in the world are used to manufacture processed items, such as juices, nectars and jellies. The waste generated by both agribusinesses and fresh

tangerine consumption, corresponds to around 50% of the total weight of fresh fruit and these residues add up to around 40 million tons worldwide (Sharma et al. 2017; Ruviaro, Barbosa & Macedo, 2018). This volume of waste has been causing concern in the field, because when these residues are disposed incorrectly, they end up polluting nature and damaging the ecosystem, which results in serious environmental problems (Rezzadori, Benedetti & Amante, 2012).

The circular economy aims to solve the socio-environmental problems of this magnitude, using the fruit in an integral way in the production of useful and essential products to the population, generating financial and socio-environmental benefits, making the entire productive chain sustainable. In this sense, tangerine peels can be converted into high-value products and may be used in a variety of applications. This alternative would make possible to diversify revenue fluxes in food processing and reduce the cost of production, generate natural ingredients to replace synthetic chemicals and at the same time prevent the environment pollution (Sharma et al. 2017; Redcorn, Fatemi, Engelberth, 2018).

Citrus by-products are considered renewable economic resources for the extraction of bioactive molecules and valuable compounds such as (poly)phenolics that are natural antioxidants and can be used in the pharmaceutical, nutraceutical, cosmetic and food industries as natural additives or ingredients for functional foods (Delgado & Fleuri, 2016; Mahato et al. 2018; Ruviaro, Barbosa & Macedo, 2018).

Phenolic compounds are among the main compounds present in citrus fruits, which are important factors of their quality, since they confer antioxidant properties, in addition to having several other specific biological actions in the prevention and / or control of diseases. Based on epidemiological data, the preventive effects of these secondary metabolites are inferred in terms of cardiovascular, neurodegenerative and cancer diseases (Delgado & Fleuri, 2016).

The peel of citrus fruits as the most external part of fruit is the tissue with the highest phenolic content and the greatest capacity for scavenging free radicals compared to the other parts of the fruit, a natural protection of the plant (Zhang, Yang & Zhou, 2018; Wang et al 2016). Phenolic compounds have wide application in the industry and can be used as natural colorants and preservatives in food, and with other relevant applications in cosmetics and pharmaceutical industries (Ignat et al. 2011). The fiber in the tangerine peel is also rich in dietary fiber, which helps the intestine to function properly besides other benefitial properties (Oduntan & Arueya, 2019). However, studies on antioxidant fibers from fruit, in particular Tangerine peels are still scarce. In view of this, the identification and quantification of these compounds is extremely important for adding value and definition of potential uses of the tangerine peels produced in the region of Borborema, Northeast Brazil by the familiar farming.

Therefore, the peels of citrus fruits, considered by-products and still polluting residues of the agroindustries, have emerged as a promising source of functional compounds, much higher than the amount observed in the juice (Ademosun et al., 2016). In addition, the use of tangerines byproducts can reduce and even eliminate the risks of environmental impacts and contribute to the economy of the regions producing tangerines, where agroindustries that process these fruits are installed (Hoang et al., 2015). In this sense, the tangerine peel can be a good source of phytocompounds and nutrients such as fibre and its use as a functional ingredient/food may add value to the fruit, specially through the evidence of the presence of compounds that contribute to human health, as already been proven for other plant species (Coscueta et al., 2016; Gullon et al., 2015). In addition, to the food sector, it may also be of interest to the nutraceutical, pharmaceutical and cosmetic industry (Mirabella, Castellani, Sala, 2014).

The objective of this work was to establish, for the first time, a detailed profile, of

carotenoid, phenolic compounds, fibers and minerals from tangerine peels from 6 different cultivares and attribute them the relative functional potential for use in human nutrition as a source of dietary fiber and phytocompounds.

2 MATERIALS E METHODS

The Tangerine fruits (Citrus reticulata) of the cultivars Piemonte, Clemenules, Page, Dancy, Ponkan e Murcot; they were harvested randomly in a commercial orchard, between 6 and 8 am, at the C3 maturation stage - predominantly orange in color, according to the CEAGESP (2011), that also included the absence of pests, diseases and apparent injuries. The commercial orchard it is located in the municipality of Alagoa Nova, region of Borborema Territory, Northeast Brazil andhas predominant soil of the type ARGISSOLO RED Eutrophic abrupt (EMBRAPA, 2016). Production is carried out without any type of irrigation.

The fruits were packed in high density polyethylene boxes, protected with bubble wraped and transported to the post-harvest biology and technology laboratory. The fruit were processed through a squeezer Arno Citrus Power PA 32 to remove the juice, and the skins were separated and left to dry in a forced circulation oven at 50 °C brand new model 1513D. After drying, the peel were macerated with mortar and pistil and ground in an electric processor until it became a fine flour that was vacuum-packed and stored at room temperature until to be analyzed.

The experiment was carried out in a completely randomized design (DIC) with peels of six tangerine cultivars and three replicates for each cultivar, where each fruit represents a repetition.

2.1 Free Phenolics

The extracts for phenolic compounds determination were made with aqueous-methanolic mixture (80%). 20 mL were added to 1 gram of dry tangerine peel flour; they

were homogenized with ultra-turrax for 2 min (IKA Ultra-turrax T18, Wilmington , USA). The mixture was centrifuged (5000 rpm for 20 min, 4 °C). The supernatant was analyzed for antioxidant activity, total phenolics by spectrophotometry and the phenolic profile was analyzed by HPLC-DAD.

2.2 Bound Phenolics

Bound phenolics were extracted according to Xie et al. (2015) with some modifications. After washing the tengerine peels with aqueous methanol (80%), the bound phenolics were released, hydrolyzing the remaining solid residue with 20 ml of NaOH 4 M at room temperature and stirred on an orbital shaker at 250 rpm for 4 horas.

The hydrolyzate was acidified to pH 1.5-2.0 by the gradual addition of 6 M HCl. After centrifugation at 5 000 rpm for 20 min, the supernatant was extracted five times with 30 ml of the ethyl acetate. The fraction of ethyl acetate was dried using a rotary evaporator vacuum at 30 °C. The resulting residue was then dissolved in 100% methanol to 5 ml. The obtained extract was stored at -20 °C until used.

2.3 Antioxidant capacity - DPPH, ABTS and ORAC tests

The methodology of DPPH was based on method described by Bobo-Garcia et al. (2014) with some modifications. Briefly, 20 μL of extract were added to a 96-well plate containing 180 μL of DPPH solution (2,2-diphenyl-1-picryl-hydrazil; Sigma Chemical Company, St. Louis, MO, USA) (150 μM). The absorbance was measured at 515 nm on a microplate reader (spectrophotometer Multiskan GO microplate, Thermo Scientific, Thermo Fisher Scientific Inc., USA) after 40 min in the dark. TE (Sigma Chemical Company, St. Louis, MO, USA) standards (0 to 500 μM) were prepared and analyzed in the same way to obtain the calibration curve. The percentage of inhibition was determined and the extracts were expressed in mg equivalent of Trolox (TE) / g DW.

The ABTS test was also performed on a 96-well microplate, following the method described by Gonçalves et al. (2009) with some modifications. To 20 µL of the sample or Trolox or solvent were added 180 µL of ABTS•+ working solution. The mixture was incubated for 5 min at 30 ° C, and the absorbance at 734 nm was measured in a Multidetection plate reader (Synergy H1, Vermont, USA).

The oxygen radical absorbance capacity (ORAC) was performed on a 96 well black microplate (Nunc, Denmark), following the method described by Dávalos et al. (2004), with some modifications. The reaction was done with 75 mM phosphate buffer (pH 7.4), and the final reaction mixture was 200 µL. The antioxidants (20 µL) and fluorescein (120 µL; 70 µM, final concentration) were placed in the microplate well. A blank (FL + AAPH) using phosphate buffer instead of the antioxidant solution and eight calibration solutions using Trolox (1-8 µM, final concentration) as antioxidant also was be performed in each assay. The mixture was pre-incubated for 10 min at 37 °C. The AAPH solution (60 µL; 12 mM , final concentration) was added quickly using a multichannel pipette. The microplate was immediately placed in the reader and the fluorescence was recorded at 1 min intervals over a 140 min period.

The test was also performed with a multiple detection plate reader (Synergy H1, Vermont, USA). The excitation wavelength is fixed at 485 nm and the emission wavelength is 528 nm (Ubeda et al., 2011). The microplate was automatically agitated before each reading.

2.4 Carotenoids

For the carotenoid extracts the methodology of Oliveira et al (2016) with small modifications was followed, 1 g of dry sample, plus 1 g of sodium carbonate were used, which was homogenized with 4 ml of distilled water and 1 ml of cold ethanol (4ºC) for 1 min with an ultra-turrax. Hexane (8 mL) was added and the resulting paste was centrifuged, after

centrifugation the upper layer was removed into the polypropylene tube. The extraction was repeated twice with 2.88 ml of hexane. Both resulting hexane fractions were combined and used for saponification with 10% methanolic KOH overnight on an orbital shaker. The mixture was washed with 25 ml of 10% NaCl and three washes with deionized water.

2.5 Quantitative colorimetric analysis of the total carotenoid content

The carotenoid content in the tangerine peel extracts was determined in triplicates, using a spectrophotometric analysis at 454 nm. □-carotene (6.3 × 10-5 - 4.0 × 10^{-3} mg/mL) was used as a standard curve. The total carotenoid content was expressed based on mg of β-carotene equivalents/g of biomass.

The carotenoids were eluted using a mobile phase consisting of acetonitrile (55%), methanol (22%), dichloromethane (11.5%) and hexane (11.5%). It was added ammonium acetate 0.02% to stabilize carotenoids under isocratic conditions at a flow rate of 1.0 ml/min for 20 min at 25 °C with an injection volume of 50 μL. The calibration curve was constructed with pure β-carotene, zeaxanthin, lutein, β-cryptoxanthin standards and expressed in micrograms per gram. All analyzes of each extract were done in triplicate.

2.6 Quantitative analysis of carotenoids by HPLC with DAD detector

The carotenoid extracts were concentrated in Speed Vacuum Thermo Fisher Scientific. A 50 μL aliquot of tangerine peel hydrophilic extract was injected into a Waters Alliance high pressure liquid chromatograph (Waters Series 600, Mildford MA, USA), equipped with a Waters 996 PDA detector. The chromatograms were recorded using a detector diode array (Waters, Mildford MA, USA) at wavelengths ranging from 200 to 600 nm in 2 nm intervals. The absorbance was measured at 454 nm.

The carotenoids were eluted using a mobile phase consisting of acetonitrile (55%), methanol (22%), dichloromethane (11.5%) and hexane (11.5%). It was added ammonium

acetate 0.02% to stabilize carotenoids under conditions isocratic at a flow rate of 1.0 ml/min for 20 min at 25 °C with an injection volume of 50 µl. The calibration curve was prepared with pure standards of α-carotene, β-carotene, zeaxanthin, lutein, β-cryptoxanthin and violaxanthin and expressed in micrograms per gram. All analyzes of each extract were done in triplicate.

2.7 Quantification and characterization of fibers

2.7.1 Determination of dietary fiber

One gram of each sample was used to determine dietary fiber; procedures were performed to remove fat, with 25 ml of N- Hexane and sugars with 20 ml of 80% ethanol to avoid interfering with the enzymatic treatment process for separating the fibers.

40 ml buffer was added (MES/Tris, 0.05 M), pH 8.2 and the samples were placed in a bath at 100 °C with 50 □L of α- amylase with constant agitation for 30 min; after this stage, the temperature was lowered to 60 ºC and 10 ml of deionized water and 100 µL were added and left at 60 ºC for 30 min; after that step, 5 ml of 0.56 N HCL was added and the pH value was adjusted between 4.1 and 4.8 with 5% NaOH or 5% HCL; immediately after 200 µL of amyloglycosidase was added and maintained at 60 ºC for 30 min.

The material was filtered through cadinhos Gooch the porous plate, the liquid fraction containing soluble fiber was lyophilized to quantitate the soluble fiber as insoluble fiber that was taken to the drying oven at 50 °C.

2.7.2 Characterization of the molecular weight of the soluble fiber

The molecular weight distribution (MW) of the soluble fiber was determined by gel filtration chromatography using the AKTA Pure 25 FPLC system (Fast Protein Liquid Ghromatography by gel filtration) coupled to two gel filtration columns: Superdex 200 raise 10/300 GL and Superdex peptide, 10/300 GL. The eluent used was the 0.05 M phosphate

buffer, pH 7.0 containing 0.15 M sodium chloride and 0.2 g L^{-1} of sodium azide at a flow rate of 0.5 ml min^{-1}.

2.8 Minerals

200 mg of each sample were mixed with 5 mL of 65% Nitric Acid and placed to be digested in a microwave digestor Berghof Speedwave MWS-3+ for 47 min. The profile of minerals were determined in an spectrômetro of emission Optics Perkin Elmer Optima 7000 DV, all samples in triplicate.

2.9 In vitro simulated gastrointestinal digestion

The simulated gastrointestinal digestion study was conducted with samples of tangerine peel Dancy (the variety identified with best compositional performance), according to the method developed by Madureira et al. (2011). Three replicates were performed with 1g homogenized with 20 mL of deionized water. An initial 2 mL aliquot was collected before starting digestion. To simulate the mouth stage, the pH of the samples was adjusted to values between 5.6 and 6.9, using HCL 1M. Artificial saliva was simulated using α- amylase (Sigma-Aldrich Chemistry, St. Louis, Missouri, USA) at 100 U/ml and added at a rate of 0.6 ml/min digestion, and incubated in a 1 minute s at 37 °C at 200 rpm. After this process a 2 mL aliquot was removed.

The pH was adjusted to 2.0 using HCL 1 M; and gastric juice was simulated by the addition of Pepsina (Sigma-Aldrich Chemistry, St. Louis, Missouri, USA) 25 mg/ml and added at a rate of 0.05 mL/mL sample and were then incubated at 60 min at 37 ˚C at 130 rpm. After this procedure, another 2 mL aliquot was removed.

Intestinal digestion was simulated by adjusting the pH of the samples to 6.0 using 1 M NaHCO3. Was added 2 g/L P ancreatin the (Sigma-Aldrich Chemistry, St. Louis, Missouri, USA) and 12 g/L bile salts (Oxoid ™, Hampshire, UK) at a proportion 0.25 ml/ml of sample;

to the samples were incubated for 120 minutes at 37 to 45 rpm. Once again, a 2 mL aliquot was collected to analyze the phenolic profile in HPLC.

2.10 Statistical analysis

The evaluation data were subjected to analysis of variance (ANOVA) and the cultivar averages were subjected to the Tukey test up to 5% probability. The data was analysed using the Emmeans package of software R version 5.1 (Lenth, 2018) was used.

3 RESULTS AND DISCUSSION

Herein, the peel of six tangerine cultivars were evaluated for the first time to establish the profiles of minerals, proteins and peptides, polyphenols, fiber and functional potential for use as human food. These data may descriminate unique quality features for the flour of these peels.

3.1 Free and bond total extractable polyphenols (TEP)

Polyphenols are present in plants cells in a free form and entrapped in the structural fiber and proteins (Jakobek 2015). The free extractable and bond polyphenols from tangerine peel of 6 cultivars are depicted in Figure 1. As observed the tangerine peel has a high content of total free extractable polyphenols, with a mean of 1.33 g.GAE.100g^{-1}, close to the content reported by Wang et al (2016) in *Citrus reticulata* Blanco peel cv Chachiensis peels that ranged from 1.4 to 1.9 g.GAE.100 g^{-1} DW depending on the maturation stage. In here, the fruits that stood out were ‘Piemonte’ with 1.49 g.GAE.100g^{-1} and ‘Dancy’ with 1.46 g.GAE.100g^{-1}. These results are inferior, however, to those reported by Zhang, Yang & Zhou (2018) that evaluated 19 citrus genotypes from a Chinese germplasm bank and obtained an average of TPC content of 2.72 g.GAE.100g-1. It is also lower than to those reported by Ruviaro, Barbosa & Macedo (2018) (2.7 g.GAE.100g-1) using enzymes for the extraction of phenolics from citrus residues, which was expected since the enzymes permitted to release

part of the entrapped bond phenolics. Wang et al (2016) stated that the content of phenolics in fruit might vary according to characteristics of each cultivar, cultivation conditions, in addition to the various techniques and solvents used for extraction.

The peels of the Ponkan cultivar, on the other hand, had a lower content of free (1.07 g.GAE.100g^{-1}) and bound (0.55 g.GAE.100g^{-1}) polyphenols, thus pointing out that the peel of this cultivar has the lowest polyphenol content among the tangerines evaluated herein.

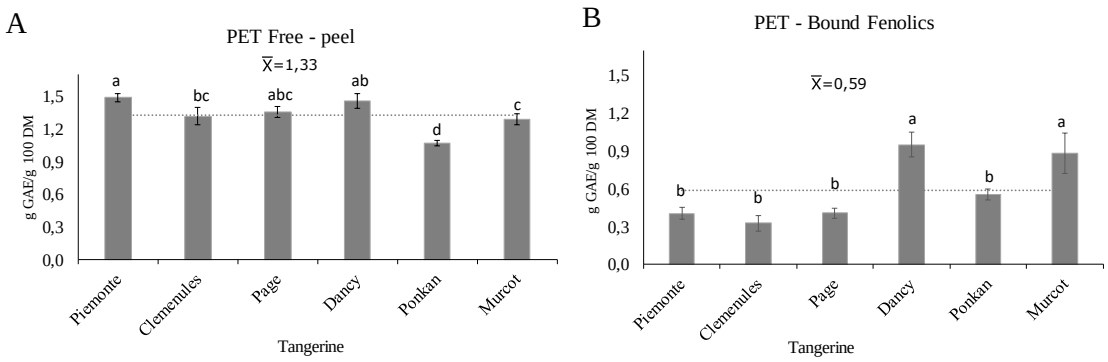

Figura 1. Contents of Total Free Extractable Polyphenols (TEP Free) (A) and Total Bound Phenolics (TBP), from the peel of 6 different ripe tangerine cultivars. Bars with the same letters do not differ by the Tukey test at 5% ($p \leq 0.05$).

The total bound phenolics (TBP) from the the different tangerine peels, released by basic hydrolysis, showed an average value of 0.59 g.GAE.100g^{-1}, which corresponds to less than half of those TEP free determined by methanol extraction, yet it represents a significant content that is adhered to the fibers, proteins and other components of the tangerine peels, even after the extraction process with 80% methanol. These type of polyphenols indicate the presence of antioxidant fiber in the residual peels flour, which can be used as functional ingredient as source of fibre and at same time assuming a role of preservative as either antioxidant or potential antimicrobial (Saura-Calixto, 1998). These higher contents appear with emphasis in the peels of 'Dancy' and 'Murcot', which presented contents of 0.95 and 0.88 g.GAE.100g^{-1}, respectively, which differed ($p < 0.05$) from the the peel of other cultivars evaluated.

The results herein for tangerine cultivars were superior to those reported by Obohh &

Ademosun (2012) in orange peels, with 0.68 g.GAE.100g^{-1}. Overall, our results also corroborate those authors who stated that there is a greater content of free than bound phenolics in citrus peels (Obohh & Ademosun, 2012)

It is evident that Dancy tangerine peel stands out from the peels of other cultivars analyzed in this research, due to the larger content of phenolic compounds, either free or bound. This is a promising result that shows these peels may provide a source for the extraction of antioxidant compounds or antioxidant fibre, both to be used as a functional food ingredient for human consumption, as already proven, in study evaluating other citrus industry residues (Garcia-Amezquita et al., 2018). This would give support in the formulation of new functional products and would allow the use the waste that is normally discarded from fruit/vegetables processing (Ruviaro, Barbosa & Macedo, 2018).

3.2 Profile of phenolic compounds (HPLC-DAD)

The phenolic compound present in tangerine peels are shown in Table 1. It has to be pointed out that the peels of the Dancy cultivar have the highest content of total free phenolics (645.53 mg.100g^{-1}), which, by adding up all the phenolics identified, is about 2-fold as high as the content of Page (393.6 mg.100g^{-1}), Murcot (373.36 mg.100g^{-1}) and Ponkan (321.41 mg.100g^{-1}) cultivars and almost 3-fold higher than Piedmont (260.06 mg.100g^{-1}) and Clemenules (235.13 mg.100g^{-1}), which, in turn, exhibited the lowest sum for all identified phenolics.

The hesperidin was the most abundant (181.94 mg.100g^{-1}), except for Clemenules and Murcot cultivars where it was not detected. The 'Dancy' tangerine was once again the cultivar that noteworthy from the others evaluated, presenting the largest content, with 239.99 mg.100g^{-1}, differing statistically ($p<0,05$) from all other cultivars analyzed. Previous studies

also reported hesperidin as the main phenolic compound present in tangerines (Wang et al. 2008; Khan et al. 2010).

Tabela 1. Profile of free phenolic compounds (mg.100g^{-1} DW) in the peel of ripe tangerine cultivars.

Cultivar	Gallic Acid	Chlorogenic Acid	Caffeic Acid	Isoferulic Acid	Hesperidin	Neohesperidin	Quercetin	Tangeretin	Totals
Piemonte	31.81 ± 0.7 f	61.74 ± 1.3 d	24.09 ± 0.1 cd	Nd	141.00 ± 2.8 d	nd	nd	1.42 ± 0.4 f	**260.06d**
Clemenules	36.24 ± 0.1 e	68.67 ± 0.4 c	31.88 ± 0.8 c	30.81 ± 0.0 d	nd	62.61 ± 0.1 b	nd	4.92 ± 0.0 d	**235.13e**
Page	56.14 ± 0.8 c	13.58 ± 0.0 f	51.47 ± 0.1 b	Nd	167.91 ± 0.4 c	100.75 ± 0.7 a	nd	3.75 ± 0.0 e	**393.6b**
Dancy	68.74 ± 0.0 b	118.05 ± 0.9 a	86.76 ± 0.4 a	85.05 ± 0.6 b	239.99 ± 0.3 a	nd	nd	46.94 ± 0.0 a	**645.53a**
Ponkan	48.37 ± 0.0 d	21.20 ± 0.2 e	19.77 ± 5.3 cd	35.13 ± 2.0 c	178.86 ± 6.4 b	nd	nd	18.08 ± 0.0 b	**321.41c**
Murcot	69.98 ± 0.4 a	103.59 ± 0.3 b	57.42 ± 9.3 b	93.70 ± 0.6 a	nd	nd	31.76 ± 0.0	16.91 ± 0.1 c	**373.36b**
Mean	**51.88**	**64.47**	**45.23**	**61.17**	**181.94**	**81.68**	**31.76**	**15.34**	

Means followed by the same lower case letter in the column did not differ by the Tukey test at 5% ($p \leq 0.05$). nd= not detected.

Hesperidin is an important flavonoid for the pharmaceutical industry because it has gastroprotective activities. A recent study reported that hesperidin has gastric healing activity in the ulcerated mucosa, an effect that is favored by the reduction of oxidative damage to the mucosa, due to the reduction of neutrophil migration and by strengthening protection (Silva et al. 2019).

Four phenolic acids were quantified in the tangerine peels, with chlorogenic acid showing the highest content (64.47 mg.100g^{-1}), followed by isoferrulic acid (61.17), which was not detected in the 'Piemonte' and 'Page'; and gallic and caffeic acids with 51.88 and 45.23 mg.100g^{-1}, in this order.

Quercetin was quantified only in the 'Murcot' (31.76 mg.100g^{-1}). Wang et al (2016) stated that variations in citrus fruit phenolics may be due to the characteristics of different cultivars, cultivation conditions and extraction solvents, among others.

Tangeritin was the flavonoid that was quantified at the lowest content in the peels of the evaluated tangerine cultivars (15.34 mg.100g^{-1}), yet the 'Dancy' was the one with the highest level of tangeritin (46.94 mg.100g^{-1}), which differed stastistically ($p<0.05$) from all other cultivars evaluated herein. The main free phenolic acid in methanol found in the tangerine peel was chlorogenic acid (64.47 mg.100g^{-1}) followed by isoferulic (61.17 mg.100g^{-1}), gallic (51.88 mg.100g^{-1}) and caffeic acids (45.23 mg.100g^{-1}).

Several studies confirm that the flavonoids present in citrus peels have bioactivity in several diseases, including anti-hyperlipidemia, anti-obesity, antivirus, anti-cancer, anti-inflammation, anti-allergen and analgesia (Guo et al., 2016, Mallick & Khan, 2016, Mannucci et al., 2017). In addition, recently published research claims that citrus flavonoids are also promising in the treatment of metabolic dysregulation and liver steatosis also known as liver fat (Jiang et al. 2019).

Herein, is important to point out that 'Dancy' tangerine presents the highest contents of

chlorogenic and caffeic acids; and the flavonoids hesperidin and tangeritin. Furthermore, it also has a higher content of gallic and isoferrulic acid than the other cultivars.

3.3 Bound Phenolics Profile (HPLC-DAD)

Results of bound phenolic profile of the tangerine peels by HPLC-DAD are reported in Table 2. As observed it was found relevant amounts of caffeic (22.33 $mg.100g^{-1}$), isoferulic (135.23 $mg.100g^{-1}$) and *p*-coumaric (34.18 $mg.100g^{-1}$) acids, as well as of flavonoids such as hesperidin (649.87 $mg.100g^{-1}$) and narigenin (8.60 $mg.100g^{-1}$). When compared between cultivars, the narigenin was only found in the 'Ponkan'; Similarly, tangeritin was also restricted to three cultivars, in the 'Dancy' (12.57 $mg.100g^{-1}$), 'Ponkan' (3.38 $mg.100g^{-1}$) and 'Murcot' (5.13 $mg.100g^{-1}$), which differed statistically ($p<0,05$), and was the least significant flavonoid.

Tabela 2. Profile and contents of bound phenolics (mg.100g^{-1} DW) in the peel of ripe tangerine cultivars.

Cultivar	Caffeic Acid	Isoferulic Acid	p-Coumaric Acid	Hesperidin	Narangenin	Tangeretin	Totals
Piemonte	10.78 ± 0.01^{d}	80.93 ± 0.05^{d}	26.39 ± 0.00^{d}	506.22 ± 0.07^{e}	nd	nd	624.32
Clemenules	2.38 ± 0.02^{e}	45.32 ± 0.03^{e}	17.96 ± 0.01^{f}	310.61 ± 0.03^{f}	nd	nd	376.27
Page	0.45 ± 0.01^{f}	80.69 ± 0.01^{d}	33.81 ± 0.01^{c}	696.34 ± 0.04^{b}	nd	nd	811.29
Dancy	93.09 ± 0.10^{a}	343.12 ± 0.89^{a}	60.03 ± 0.03^{a}	1066.22 ± 3.22^{a}	nd	12.57 ± 0.16^{a}	1575.03
Ponkan	14.42 ± 0.06^{b}	107.66 ± 0.02^{c}	22.55 ± 0.09^{e}	686.82 ± 0.53^{c}	8.60 ± 0.06	3.38 ± 0.02^{c}	843.33
Murcot	12.84 ± 0.05^{c}	153.63 ± 0.23^{b}	44.34 ± 0.01^{b}	633.03 ± 1.68^{d}	nd	5.13 ± 0.46^{b}	848.97
Mean	**22.33**	**135.23**	**34.18**	**649.87**	**8.60**	**7.03**	

Means followed by the same lower case letter in the column did not differ by the Tukey test at 5% ($p \leq 0.05$). nd= not detected.

Although the tangerine peels present a greater variety and higher Total Free Extractable Polyphenols (TEP Free) than Total Bound Phenolics (TBP) (see Figure 1), measured by spectrophotometer Folin & Ciocalteau method, herein, based on the sum of individual phenolics, it was observed an opposite trend. It is important to highlight that Folin & Ciocalteau method has different interferences, namely sugars and proteins that me be present in the extract used to measure free polyphenols, which could lead to an increase Total Free Extractable Polyphenols abtained.

Scarce information has been reported on bound phenolics in citrus peels; the study of Bocco et al (1998) reported the presence of phenolic acids such as caffeic acid (1.1 $mg.100g^{-1}$), p-coumaric acid (19.3 $mg.100g^{-1}$), ferulic acid (158 $mg.100g^{-1}$) and sinapinic acid (95.4 $mg.100g^{-1}$) in orange peels.

Some of those polyphenols have only been quantified in the bound form, namely the case of the p-coumaric, synaptic acids and the flavanone naringenin. Furthermore, others phenolic acids such as isoferulic and the flavonoid hesperidin were present in greater contents, however the last is the most representative among all, showing 3 times more than the total sum of the other polyphenols, showing the great relevance of this bound polyphenol in this byproducts. Hesperidin is the main phenolic in the tangerine peels evaluated herein; its content in the bound phenolic extract is about 3-fold higher than in the extract of free phenolics.

Recent study by Liu, Li & Feng (2019) found that the citrus flavonoids hesperidin and naringina alleviate the effects of alcohol damage on embryonic development and neurological behavior. According to Peleg et al. (1991) ferulic acid in citrus fruits occurs mainly in bound form. In this study, these phenolic acids and bound flavonoids were released satisfactorily after alkaline hydrolysis.

Once again, the ‘Dancy’ tangerine peel showed the highest content of bound phenolics,

since the total bound phenolics are two times higher that the others, which standing out from the other cultivars evaluated in here. The sequence, in decreasing order, of the cultivars based on the bound phenolic levels was similar to that of the free phenolics, with the highest content Dancy (1575.03 mg.100g^{-1}) followed by Murcot (848.97 mg.100g-1), Ponkan (843.33 mg. 100g^{-1}), Page (811.29 mg.100g^{-1}), Piedmont (624.32 mg.100g^{-1}) and Clemenules (376.27 mg.100g^{-1}), with the lowest content.

These results place the 'Dancy' tangerine in an important position in relation to the others evaluated, because in addition to be a fruit widely cultivated in the Borborema Territory, Northeast Brazil, it is very much appreciated for fresh consumption. Thus, this fruit has the potential to be used also by the juice industry and its peels can be used for the extraction of these phenolic compounds to be used in the food, pharmaceutical and cosmetics industry.

3.5 Total and individual carotenoids

The average, total carotenoids (Table 3) in the tangerine peels analysed showed to be 43.57 mg.100g^{-1}, and the Clemenules stood out from the others cultivars with 73.17 mg.100g^{-1} and Piemonte occupied the second position with 51.87 mg.100g^{-1}. In a recent study Giuffrida et al (2019) suggests that the carotenoid profile, besides varying among species and varieties, is also dependent on the maturity stage, processing conditions and storage steps, which significantly affect them.

Tabela 3. Individual by HPLC-DAD and total carotenoids by spectrophotometry (mg, $100g^{-1}$) in the peel of ripe tangerine cultivars.

Cultivar	Total carotenoids	α-carotene	β-carotene	β-cryptoxanthin	Lutein	Violaxanthin	Zeaxanthin
Piemonte	51.87 ± 6.65^{b}	nd	0.25 ± 0.54^{d}	3.16 ± 0.17^{a}	2.68 ± 0.14^{a}	125.00 ± 7.44^{b}	1.15 ± 0.33^{ab}
Clemenules	73.17 ± 7.10^{a}	nd	4.65 ± 1.74^{c}	4.21 ± 1.17^{a}	2.60 ± 0.35^{a}	169.34 ± 2.69^{a}	1.44 ± 0.09^{a}
Page	36.78 ± 1.25^{cd}	nd	10.30 ± 2.35^{ab}	nd	0.69 ± 0.27^{b}	61.55 ± 2.97^{c}	0.89 ± 0.14^{c}
Dancy	41.64 ± 3.07^{bc}	9.43 ± 1.03	6.97 ± 0.47^{bc}	nd	0.85 ± 0.02^{b}	16.74 ± 0.79^{cd}	1.19 ± 0.08^{ab}
Ponkan	28.68 ± 1.71^{d}	nd	Nd	nd	nd	3.91 ± 0.53^{d}	1.30 ± 0.02^{ab}
Murcot	29.29 ± 1.92^{d}	nd	11.67 ± 0.64^{a}	nd	nd	5.25 ± 0.46^{d}	0.90 ± 0.02^{c}
Mean	**43.57**	**9.43**	**6.77**	**3.69**	**1.70**	**63.56**	**1.15**

Means followed by the same lower case letter in the column did not differ by the Tukey test at 5% ($p \leq 0.05$). nd= not detected.

In the carotenoid profile evaluated by HPLC, the Piemonte and Clemenules stood out from the other cultivars mainly by the higher contents of violaxanthin with 125,00 and 169,34 mg.100g-1 (ca. 10 times the sum concentration of other carotenoids), differing statistically ($p < 0.05$) from each other and from the other cultivars evaluated, followed by β-cryptoxanthin, with 3.16 and 4.21 $mg.100g^{-1}$ and lutein with 2.68 and 2.60 $mg.100g^{-1}$, respectively. The violaxanthin was the carotenoid present in greater contents in all the peels of the evaluated tangerine cultivars (excepted by the Murcot), which was probably the one that most influenced the total carotenoids, since the Piemonte and Clemenules presented much higher contents of this carotenoid than the other cultivars.

Violaxanthin is the precursor of zeaxanthin, whose cycle is an important mechanism of photo protection of higher plants (Jahns et al., 2009; Nichelmann et al., 2016; Goss et al., 2017). According to Lux et al. (2019) violaxanthin esters prevailed in the flavedo of ripe orange, while α- and β-carotenes were not detected; they also suggested that the interconversion of chloroplasts into chromoplasts in the flavedo of ripe oranges is responsible for the accumulation of violaxanthin concomitantly with decreasing concentrations of the other mentioned carotenoids. This may explain the large content of violaxanthin found in the peel of the tangerine cultivars herein, since the fruits evaluated were harvested ripe.

Carotenoids are responsible for the yellow, orange and red pigmentation of the ripe tangerine peels and contribute to the antioxidant activity of citrus (Rodrigo et al. 2013; Yoo & Moon, 2016). As the fruits ripe, there is an accumulation of carotenoids, which are mainly responsible for the coloring of the tangerine peels (Peng et al. 2013). The carotenoids can be classified into two types based on the functional groups; xanthophylls, containing oxygen as a functional group, including lutein and zeaxanthin, and carotenes, which contain only the original hydrocarbon chain with no functional group, such as α-carotene, β-carotene and lycopene (Saini, Nile & Park 2015).

In general, β-xanthophylls, particularly violaxanthin and β-cryptoxanthin, and their esters, are common to citrus (Gross, 1987). β-cryptoxanthin is responsible for the most intense orange coloration of tangerine fruits (Rodrigo et al. 2013). This explains why the peels of the Piemonte and Clamenules have a more intense orange color than the other cultivars evaluated herein.

The peel of the Dancy cultivar was the unique in which α-carotene (9.43 mg, $100g^{-1}$) was detected, which could be a good marker of cultivar traceability and authenticity in the market. Recent studies indicate that the consumption of α-carotene can decrease the risk of chronic diseases associated with weight gain, as well as decrease the risk of death from cardiovascular disease and cancer (Li et al. 2011; Nuss et al. 2017).

Citrus fruits have a considerable natural diversity in carotenoid pigments, which provide aesthetic value and attract consumer attention (Zheng et al., 2019). The carotenoids present in citrus fruit have good bioaccessibility and bring functional potential and then health benefits when ingested (Rodrigo et al., 2015)

3.4 Antioxidant Capacity

In the peels of tangerine cultivars, the antioxidant capacity of the free phenolics extracts evaluated by the ABTS method (Figure 3 A) showed an overall mean of 27.14 μM Trolox.g^{-1} and the bound phenolics 25.37 μM Trolox.g^{-1}. By the DPPH method (Figure 3 B) the free phenolics were 7.23 μM Trolox.g^{-1} and the bound 6.06 μM Trolox.g^{-1}. This makes evident that the potential antioxidant activity correlates directly with total polyphenols, as expected. In turn, by the ORAC method (Figure 3 C) the extracts showed the highest antioxidant capacity, both in the free (363.73 μM Trolox.g^{-1}) and bound (149.59 μM Trolox.g^{-1}) forms, however it is clear that the free polyphenols are more prone (2 times) to react with the oxidation system (H_20_2, resembling more directly the biological system)

Obohh & Ademosun (2012) also reported a higher antioxidant activity in free phenolics

extracts (1.7 mg.ml^{-1}) when compared with those bound (1.0 mg.ml^{-1}) using the DPPH method. These same authors evaluating the antioxidant capacity by the ABTS method found a difference between free and bound phenolics depending on the cultivar evaluated, as well as in the present study.

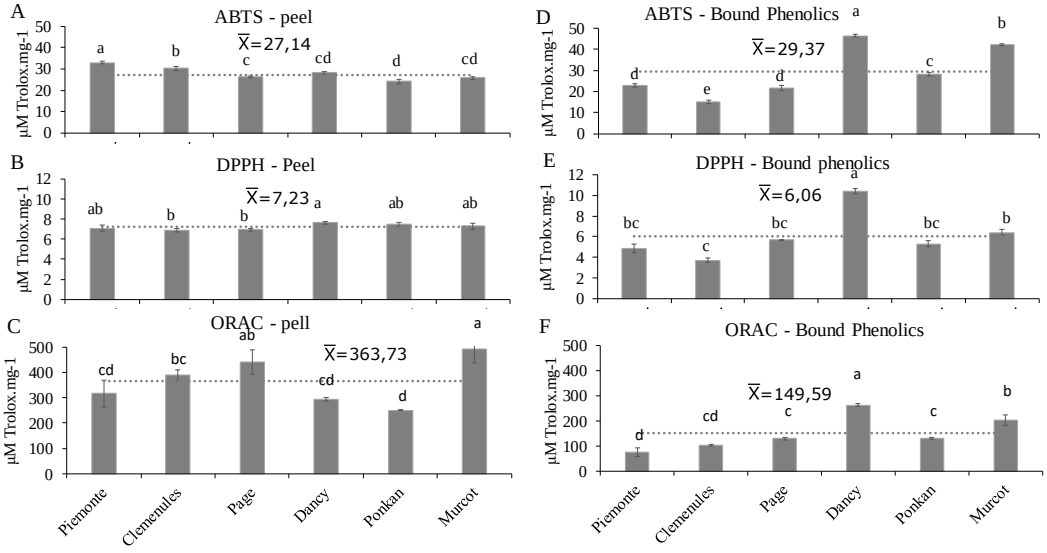

Figura 2. Antioxidant activity of free phenolics by ABTS (A), DPPH (B), ORAC (C) and bound phenolic by ABTS (D), DPPH (E), ORAC (F) methods in the peel of ripe tangerine cultivars. Bars with the same lower case letters do not differ by the 5% tukey test ($p \leq 0.05$).

Again, the 'Dancy' tangerine stands out from the others evaluated in here due to the higher antioxidant capacity exhibited by the extract of the bound phenolics, which was even almost two-fold higher than the free ones by the ABTS and DPPH methods. These results are corroborated by those shown for TEPs, confirming the premise that TEP contents are the main responsible for the antioxidant activity of these fruits.

In this study, the test for antioxidant activity by the ORAC method has a value of 5 to 15 times higher than by the ABTS and DPPH methods. According to Khan et al. (2010) the ORAC assay is more suitable than DPPH for the evaluation of antioxidant activity in citrus, due to the fact that the main orange flavanones naringenin and hesperedin are relatively weak antioxidants, as they do not exhibit a catechol group (1,2-di-hydroxybenzene), which is the critical structural determinant of strong phenolic antioxidants. As a consequence, they react

very slowly with the DPPH radical. Thus, much more reactive radicals are needed, such as the peroxyl radicals provided in the ORAC test.

3.6 Dietary Fiber

The results in here (Figure 5) demonstrate that the peel of tangerines are great sources of soluble (21.5 g.100g^{-1}) and insoluble (33.97 g.100g^{-1}) and a good ingredient to convey a high level of total fibers (55.47 g .100g^{-1}) content to a food products and related nutritional and health claims. These values are close to those reported by Crizel et al (2019) in orange (Citrus sinensis L.) peels from a Brazilian juice industry that found values of 15.6 g.100g^{-1} for soluble fiber, 48.2 g.100g^{-1} for insoluble fiber and 63.7 g.100g^{-1} for total fiber.

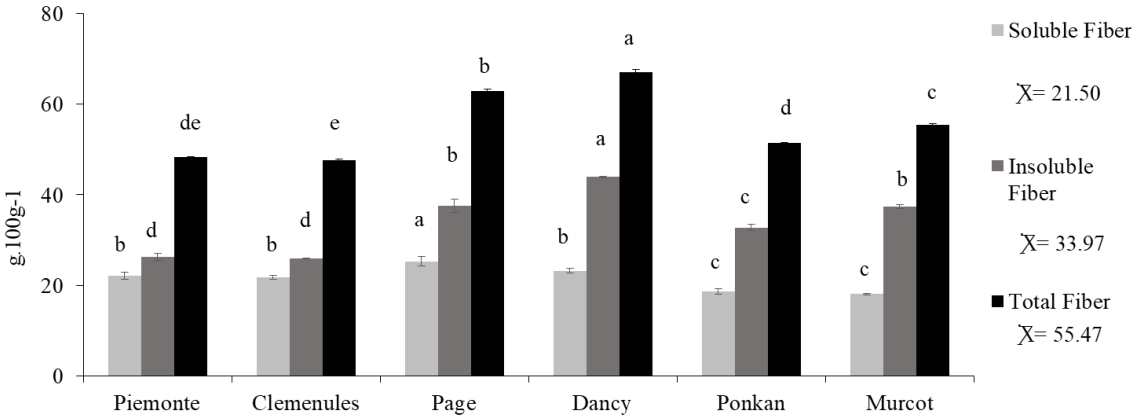

Figura 3. Soluble, insoluble and total fiber contents in the peel of ripe tangerine cultivars. Bars with the same lower case letters do not differ by the 5% tukey test ($p \leq 0.05$).

Recurrent studies have shown that the technological and nutraceutical functionalities of dietary fibers depend on their composition and, specifically, on its proportion of fractions of soluble and insoluble fibers and that these can be increased depending on the extraction technology used (Garcia-Amezquita et al. 2018; Garcia-Amezquita et al. 2013). Thus, the tangerine peels can be fully used to obtain specialized ingredients to increase functional value in different food products. In this way, it would allow reducing the disposal of polluting material, as well as increase the possibilities of adding value and increasing revenue both for

the industry and producers.

The ‘Dancy’ tangerine peel once again stands out from the others, differing statistically ($p < 0.05$), as it has a greater content of total fibers (67.13 $g.100g^{-1}$), which corresponds to the sum of the soluble (23.23 $g.100g^{-1}$) and insoluble (43.91 $g.100g^{-1}$) fibers that also clearly differed from the other cultivars evaluated. However, the peel of the ‘Page’ showed more soluble fibers (25.25 $g.100g^{-1}$) also differing statistically ($p < 0.05$), than the others and is second after ‘Dancy’ in insoluble (37.63 $g.100g^{-1}$) and total (62.88 $g.100g^{-1}$) fibers.

The peels of the Murcot and Ponkan cultivars have the lowest proportion of soluble fiber with 18.07 and 18.66 $g.100g^{-1}$, respectively, differing statistically from the others ($p < 0.05$), however did not differ among them. It has to be pointed out, however, that these values are higher than those found by Wang et al (2015) in Ponkan tangerine peels, which was 13.02 $g.100g^{-1}$. Still in Ponkan tangerine herein, it were obtained contents of insoluble and total fiber of the order of 32.77 and 51.44 $g.100g^{-1}$, respectively, corroborating with Wang et al (2015) that obtained 49.61 and 62.87 $g.100g^{-1}$ for insoluble and total fibers, respectively, close to these values.

Dietary fiber is an essential component in food, so its high content provides an attractive technological functionality with huge potential uses for the formulation of new food products (Garcia-Amezquita et al. 2018), such as wholesome, rich in fiber, for meet some daily nutritional demands of human food (Oduntan & Arueya, 2019). Furthermore, a good relationship between fibers obtained from by-products is extremely important for the regulation of the intestine and in the prevention of various diseases, as they can be used as a tool for selective modulation of the intestinal microbiota (Crizel et al., 2013; Souza et al., 2019).

Few studies have evaluated dietary fibers in the peel of tangerines, however the data herein reveals a great potential of these by-products in all evaluated cultivars.

3.7 Peptide size profile by gel filtration chromatography

Table 4 shows the molecular weight with the respective elution volumes, corresponding to the peaks expressed through the size exclusion chromatography, by Fast Protein Liquid Chromatography (FPLC) of the soluble fiber from peel of the tangerine cultivars.

Gel filtration chromatography separates peptides based on size. Therefore, the fraction with the shortest retention time has the highest molecular weight (Zhang et al., 2012). The cultivars Page and Dancy showed peaks corresponding to higher molecular weight proteins with ca. 900 KDa. In addition to these, all cultivars showed a peak ca. 105 KDa; comparing the percentage of area, Piemonte and Clemenules cultivars showed the lowest values, which represents 9.02 and 9.47% of the total area respectively, and the highest were attributed to Dancy and Ponkan cultivars with 24.95 and 27.79% of the total area. Studies by FPLC analysis in kiwi fruit, allowed isolating a protein named actinchinin, with 25 KDa, which demonstrated an antifungal action (Xia & Ng, 2004). In soy residues, Voss et al (2019) identified several new peptide sequences.

Proteins and peptides with biological activities occur naturally in plant foods, however in most cases, bioactive peptides are found as part of a protein sequence and need to be released to ensure its activity (Garcia et al., 2013).

The peels of all cultivars evaluated exhibited a peptide with a molecular weight between 1 and 0.5 KDa, with emphasis on the Piemonte and Ponkan with 62.65 and 59.63% of total area. According to García et al (2013) peptides with molecular weight between 0.5 and 1 kDa exhibit better antioxidant activity than higher molecular weight peptides. The area percentage of the peptides smaller than 0.5 KDa varied from 28.34 in the Piemonte to 43.28 in Murcot peel cultivars.

Some peptides were isolated from peels of citrus varieties such as the cyclic peptide citrusin XI, isolated from *Citrus unshiu*, which revealed anti-inflammatory activity (Noh et al.

2015).

There is a large amount of bioactive peptides hidden among the chemical structure of proteins present in food matrices from plant sources. Those activities range from antimicrobial, antithrombotic, antihypertensive, opioid, immunomodulatory, mineral and antioxidant bonds. Due to these reasons, bioactive peptides play a significant role in human health and are considered the new generation of biologically active regulators. They can prevent oxidation and microbial degradation in food and also improve the treatment of various diseases and disorders, thus increasing the quality of life (Sánchez & Vázquez, 2017).

Tabela 4. Molecular weight (KDa) and relative % of the peaks areas expressed by FPLC size exclusion chromatography of the soluble fiber of the peel of ripe tangerine cultivars

Cultivar	Kda	Elution Vol (mL)	Area	Peak área (%)
Piemonte	~105	21	18.54	9.02
	1 – 0.5	34	128.80	62.65
	∑ < 0.5	> 35	58.27	28.34
Clemenules	105	21	25.77	9.47
	1 – 0.5	34	142.70	52.43
	∑ < 0.5	> 35	103.69	38.11
Page	900	16	2.00	1.06
	105	21	28.97	15.40
	1 – 0.5	34	76.28	40.54
	∑ < 0.5	> 35	80.90	43.00
Dancy	900	16	3.37	1.56
	105	21	54.08	24.95
	1 – 0.5	34	82.21	37.94
	∑ < 0.5	> 35	158.22	35.56
Ponkan	105	21	67.84	27.79
	1 – 0.5	34	145.6	59.63
	∑ < 0.5	> 35	30.73	12.59
Murcot	105	21	32.57	16.04
	1 – 0.5	34	82.60	40.68
	∑ < 0.5	> 35	87.88	43.28

nd: not detected. ∑ < 0.5: Somatorio dos picos menores que 0.5 KDa

3.8 Minerals

As showed in table Table 5, tangerine peels are a great source of essential minerals to the human diet such as calcium (417.54 mg.100g^{-1}), iron (0.57 mg.100g^{-}1), potassium (1032.51 mg. 100g^{-1}), magnesium (89.38 mg.100g^{-1}) and phosphorus (52.60 mg.100g^{-1}). The Brazilian National Health Surveillance Agency (ANVISA) (Brazil 2005) recommends daily consumption of 5000 mg of calcium, 14 mg of iron, 260 mg of magnesium and 700 mg of phosphorus. Therefore, the consumption of flour from the tangerine peels can significantly help to supply part of the daily needs of these minerals for human food.

Once again, the Dancy stood out in the content of calcium (628.19 mg.100g^{-1}), potassium (1259.06 mg.100g^{-1}) and phosphorus (71.59 mg.100g^{-1}), with the highest contents of these minerals than other cultivars. Czech et al (2019) reported means of 166.37 mg.100g^{-1} for calcium, 1.48 mg.100g^{-1} for iron, 632.29 mg.100g^{-1} for potassium, 57.85 mg.100g^{-1} magnesium and 89.24 mg.100g^{-1} of phosphorus in fresh tangerine peel (*Citrus Reticulata* Blanco). In peel of fresh 'Ponkan' tangerin, Barros et al (2012) found 373.36 mg.100g^{-1} of calcium, 1.40 mg.100g^{-1} of iron, 659.39 mg.100g^{-1} of potassium and 58.52 mg.100g^{-1} of magnesium. These contents are close to those found in same cariety, but others cultivars evaluated in this research showed higher contents and emonstrating to be a relevant source of minerals.

The Brazilian table of food composition (TACO) (NEPA, 2011) informs that the tangerine has 130 mg of calcium and 80 mg of Magnesium, for each 100 g of its dry matter edible portion. It is important to note that TACO does not consider peels as an edible part in this evaluation; however, the data herein confirm that tangerine peels are far richer in essential compounds to health mainly calcium and potassium, and can be incorporated into the human diet, guaranteeing that the safety isses are assured.

Tabela 5. Mineral contents (mg.100g^{-1}) in the peel of ripe tangerine cultivars

Cultivar	Calcium	Iron	Potassium	Magnesium	Phosphorus	Aluminum	Boron	Sodium	Zinc
Piemonte	335.38 ± 0.35^{cd}	0.21 ± 0.14^{b}	692.56 ± 28.73^{e}	95.64 ± 2.16^{b}	27.75 ± 0.32^{b}	2.03 ± 0.07^{c}	1.69 ± 0.01^{ab}	63.36 ± 0.50^{a}	0.00 ± 0.00^{c}
Clemenules	295.21 ± 1.55^{d}	1.79 ± 0.46^{a}	1050.50 ± 14.16^{c}	102.04 ± 0.38^{b}	33.78 ± 0.37^{b}	1.67 ± 0.09^{c}	1.50 ± 0.03^{ab}	39.25 ± 0.99^{b}	0.03 ± 0.01^{c}
Page	493.71 ± 7.79^{b}	0.44 ± 0.06^{b}	1079.10 ± 23.26^{bc}	128.89 ± 3.59^{a}	31.78 ± 1.15^{b}	3.52 ± 0.12^{b}	2.12 ± 0.02^{a}	53.56 ± 2.70^{ab}	0.17 ± 0.00^{b}
Dancy	628.19 ± 8.86^{a}	0.17 ± 0.24^{b}	1259.06 ± 51.22^{a}	71.65 ± 0.15^{c}	71.59 ± 5.43^{a}	5.09 ± 0.01^{a}	1.58 ± 0.32^{ab}	47.85 ± 2.09^{ab}	0.22 ± 0.05^{ab}
Ponkan	405.85 ± 0.01^{c}	0.38 ± 0.08^{b}	925.20 ± 7.11^{d}	58.60 ± 0.86^{d}	75.69 ± 0.42^{a}	2.92 ± 0.02^{b}	1.15 ± 0.27^{b}	21.85 ± 1.82^{c}	0.28 ± 0.03^{a}
Murcot	346.88 ± 48.46^{cd}	0.46 ± 0.33^{b}	1188.63 ± 32.88^{ab}	79.47 ± 4.10^{c}	75.01 ± 2.88^{a}	2.08 ± 0.38^{c}	1.18 ± 0.34^{b}	57.39 ± 8.7^{a}	0.05 ± 0.03^{c}
Mean	**417.54**	**0.57**	**1032.51**	**89.38**	**52.60**	**2.88**	**1.54**	**47.21**	**0.12**
DRI (mg)	**50000**	**14**	**3500***	**260**	**700**	**ND**	**ND**	**2000****	**7**

Means followed by the same lower case letter in the column did not differ by the Tukey test at 5% ($p \leq 0,05$). DRI: Daily recommended daily intake by ANVISA. *Minimum daily intake recommendation according to WHO. **Maximum daily intake recommendation according to WHO. ND: not defined.

3.9 Phenolic Profile in Gastrointestinal Simulation

Based on the highest content and distinct profiles of bioactive compounds with significant health benefit claims, a simulation of the gastro intestinal tract (table 6) was performed for the best nutrional profile tangerine peels the 'Dancy' cultivar. The sample of this tangerine peels was submitted to simulated gastrointestinal tract, and samples from each key stage were collected to understand the evolution of phenolic compounds considering their stability when submitted to the environmental conditions – pH, enzymes and bile salts. The release of gallic, chlorogenic, caffeic and isoferrulic acids and the flavonoids hesperidin and tangeritin was quantified in each stage of the tract.The results showed that some of the phenolics were gradually released throughout the tract.

Tabela 6. Evolution of polyphenols (mg.100g^{-1}) under simulated gastrointestinal digestion of the peel of ripe Dancy cultivar.

Phase	**Gallic Acid**	**Clorogenic Acid**	**Caffeic Acid**	**Isoferulic Acid**	**Hesperidin**	**Tangeritin**
Initial	57.83^{a}	117.85^{a}	14.34^{c}	74.63^{a}	118.18^{a}	7.18^{c}
Oral	44.77^{b}	110.26^{ab}	56.09^{b}	58.99^{b}	85.66^{b}	29.10^{a}
Gastric	59.55^{a}	85.27^{c}	85.86^{a}	51.47^{b}	75.26^{b}	12.69^{b}
Intestinal	39.52^{b}	103.78^{b}	74.81^{a}	54.42^{b}	58.11^{c}	0.73^{d}

Means followed by the same lower case letter in the column did not differ by the Tukey test at 5% ($p \leq 0,05$). n=4.

Gallic and caffeic acids were released in greater quantity in the gastric phase 59.55 mg.100g^{-1} and 85.86 mg.100g^{-1}, respectively; whereas chlorogenic, isoferrulic acid, hesperidin and tangeritin, 110.26 mg.100g^{-1}, 58.99 mg.100g^{-1}, 85.66 mg.100g^{-1}, 29.10

mg.100g^{-1}, respectively, were made more bioacessible in the oral phase, which simulates the mouth, afterwhich they were slightly decreased due to their susceptibility to pH conditions and enzymes in the different stages.

In a study of the digestibility of citrus pectin, Ferreira-Lazarte et al. (2019) reported that these products can be used as prebiotics in human food. Another study showed that orange pomace showed a prebiotic effect in an *in vitro* model of the colon and is efficient in stimulating a microbial community, mainly *Ruminococcus*, *Lachnospira*, *Roseburia*, *Dialister* and *Bacteroides* (Souza et al. 2019). However, no study have reported until now how the polyphenols present in tangerine flour were affected by gastrointestinal digestion.

In vitro gastrointestinal simulation is a valuable tool for investigating the effect of digestion on polyphenol bioaccessibility to obtain essential data to support claims of biological relevance of food to human health (Haas et al., 2019).

The polyphenols present in food are usually bound to carbohydrates, fibers and proteins; during gastrointestinal digestion. Polyphenols stand out from these, making them more bioaccessible (Jakobek 2015; Thakur et al., 2020).

With the exception of caffeic acid and tangeritine, all other phenolic compounds were quantified with a higher content in the initial phase, before the action of any enzyme. Evaluating olive byproducts solid fraction during gastrointestinal simulation, Ribeiro et al (2020) reported a greater antioxidant activity in the initial phase, which the authors related to the polyphenols releasing .

The gallic acid content increased in the gastric phase releasing 59.55 mg.100g^{-1}, differing statistically from the other phases ($p \leq 0.05$) and decreased in the intestine (39.52 mg.100g^{-1}). For chlorogenic acid, the oral phase (110.26 mg.100g^{-1}) decreased as related the gastric phase (85.27 mg.100g^{-1}) ($p \leq 0.05$) and increased again in the intestine (103.78 mg.100g^{-1}).

For caffeic acid, a progressive increase from the initial phase (14.34 mg.100g-1) to the gastric phase (85.86 mg.100g-1) and intestinal phase (74.81 mg.100g-1) was observed; these last two did not differ statistically by the Tukey test ($p \leq 0.05$). Similar pattern was observed by Ribeiro et al (2020) evaluating the intestinal simulation of olive residues, which found a greater release of caffeic acid in the gastric phase.

The levels of isoferrulic acid did not differ statistically between the oral (58.99 $mg.100g^{-1}$), gastric (51.47 $mg.100g^{-1}$) and intestinal (54.42 $mg.100g^{-1}$) phases. While the hesperidin content suffered a reduction from the initial phase (118.18 $mg.100g^{-1}$) to the intestinal phase (58.11 $mg.100g^{-1}$), differing statistically by the Tukey test at 5% probability ($p \leq 0.05$). The tangeritin level also showed a great reduction in the oral phase (29.10 $mg.100g^{-1}$) for the gastric phase (12.69 $mg.100g^{-1}$) as well as for the intestinal phase (0.73 $mg.100g^{-1}$), clearly indicating that these two last compounds are degraded with the acidic conditions of the gastrointestinal tract (Gayoso et al., 2016). However, in the case of hesperidin, about 50% of the initial phase is still bioavailable in the colon to be used by microorganisms.

3.10 Pearson's correlation

Pearson's correlation analysis (Figure 6A) of the antioxidant activity and the bioactive compounds revealed that in the evaluated tangerine cultivars the antioxidant activity by the ABTS method had a strong positive correlation with β-cryptoxanthin, lutein, violaxanthin and with the total carotenoids. While using the DPPH radical method, there was a strong positive correlation with tangeritin and moderate with gallic acid, isoferrulic acid and hesperidin. In turn, using the ORAC method, there was a strong positive correlation with β-carotene and a moderate one with quercitin and neohesperidin.

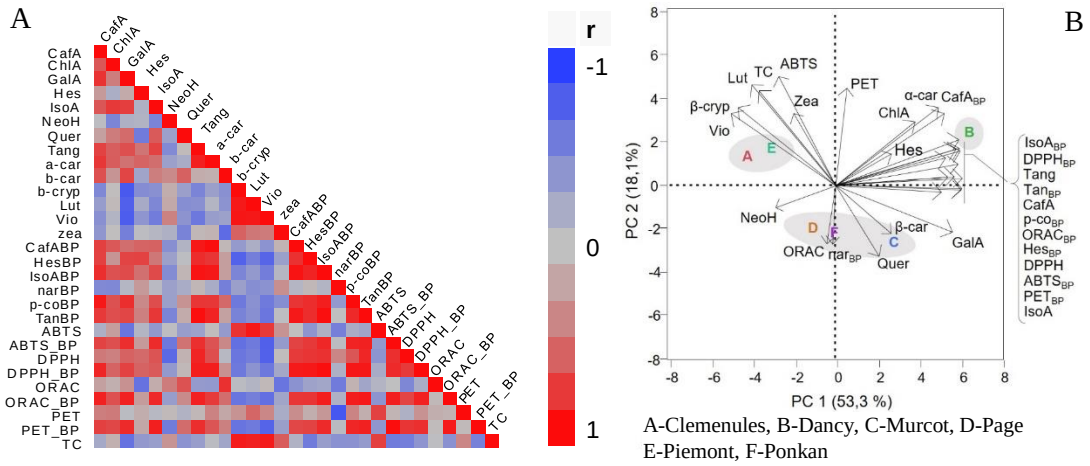

Figura 4. Pearson's correlation (A) and Principal Components (B) analyses of the bioactive compounds and functional capacity of the peels of tangerine cultivars.

CafA: Cafeic Acid; **ChlA:** Clorogenic Acid; **GalA:** Galic Acid; **Hesp:** hesperidin; **IsoA:** Isoferulic Acid; **NeoH:** Neohesperidin; **Quer:** Quercitin; **Tang:** Tangeritin; **a-car:** Alfa-Caroteno; **b-car:** Beta-Caroteno;
b-cryp: Beta-Cryptoxantin; **Lut:** Lutein; **Vio:** Violaxantin; **Zea:** Zeaxantina; **CafABP:** Cafeic Acid, Bound phenolics extract; **HespBP:** hesperidin, Bound phenolics extract; **IsoABP:** Isoferulic Acid, Bound phenolics extract; **narBP:** Narangenin, Bound phenolics extract; **PcoBP:** p-Coumaric Acid, Bound phenolics extract; **TanBP:** Tangeritin, Bound phenolics extract; **ABTS:** Atividade antioxidante pelo método do radical ABTS; **ABTS_BP:** Atividade antioxidante pelo método do radical ABTS, Bound Phenolics Extract; **DPPH:** Atividade antioxidante pelo método do radical DPPH; **DPPH_BP:** Atividade antioxidante pelo método do radical DPPH, Bound Phenolics Extract; **ORAC:** Atividade antioxidante pelo método ORAC; **ORAC_BP:** Atividade antioxidante pelo método ORAC, Bound Phenolics Extract; **PET:** Polifenóis extraíveis totais livres; **PET_BP:** Polifenóis extraíveis totais, Bound Phenolics; **TC:** Carotenoides Totais.

In turn, when evaluating the antioxidant activity of the bound phenolics, the obtained results from the three methods (ABTS, DPPH and ORAC) presented a strong positive correlation with the caffeic, isoferrulic and p-coumaric acids and with the hesperidin and the tangeritin, which clearly means that the presence of these compounds ensures a high antioxidant activity in the tangerine peels.

The total free extractable polyphenols showed a strong positive correlation with antioxidant activity by the ABTS method. The total bound extractable polyphenols showed a strong positive correlation with caffeic acid, isoferrulic acid, p-coumaric acid, hesperidin, tangeritin and with antioxidant activities by the three evaluated methods (ABTS, DPPH and

ORAC). Several studies have found a direct correlation between antioxidant activity and phenolic compounds, which has been considered for several plant matrices the group of compounds that most contribute to the antioxidant capacity (Clerici and Carvalho-Silva, 2011; Gullon et al., 2015; Dutra et al., 2017; Neri-Numa et al., 2018; Haas et al., 2019; Dantas et al., 2019).

Total carotenoids correlated positively with β-cryptoxanthin, lutein, violaxanthin, zeaxanthin and with the antioxidant activity measured by the ABTS method.

In the analysis of principal components (Figure 6B), two components were responsible for an accumulated variance of 71.4%. The caffeic, gallic and isoferrulic acids and tangeritin among the free phenolics, in addition to α-carotene, β-cryptoxanthin and violaxanthin carotenoids contributed to form the principal component 1 (PC 1), along with the bound phenolics and the antioxidant activities of the bound phenolic extracts. The principal component 2 (PC 2) was formed by lutein, free antioxidant activity by the method of the radical ABTS and total extractable polyphenols (TEP).

By the correlation matrix, it was possible to observe the formation of three groups in which the cultivars were grouped by similarity. The cultivars Clemenules and Piemonte formed the Group 1; Murcot, Page and Ponkan made up the Group 2 and the cultivar Dancy isolated formed the Group 3. This separation of cultivar Dancy in a separate group from all other cultivars, confirms that it is highlighted by the phenolics content, both free and bound, which are much higher than for the other cultivars evaluated in this study. This cultivar was highlighted by the higher contents of the total phenolic compounds, hesperidin, tangeritin, caffeic acid, isoferrulic acid, free and bound, in addition to chlorogenic acid, α-carotene and the antioxidant activity of free phenolics (DPPH) and bound (ABTS, DPPH and ORAC).

4. CONCLUSIONS

This is the most detailed study of the functional potential of tangerine peels and that revealed

the potential of this by-product grown in the Northeast region of Brazil.

The 'Dancy' tangerine peel showed a great functional potential due to the high content of free and bound phenolic compounds, mainly caffeic acid and the flavanols hesperidin, and tangeritin. Furthermore, it also exhibited to be a good source of minerals and high fiber contents, which can help in the proper functioning of the human intestine when included in human food.

The soluble fraction of the tangerine peel showed the presence of relevant amounts of low molecular weight peptides and free aminoacids. Future research, however, is necessary to evaluate whether these peptides have bioactive and antihypertensive activity.

The profile of phenolics in the course of the *in vitro* gastrointestinal tract reveals that a some phenolics are released throughout the phases of the tract mainly from bound phenolic linked to the fibre through digestive enzymes, making them available for absorption by the human body.

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Artigo2. Functional potential and profiles of phenolic compound, carotenoid and fiber in the peel of orange cultivars

Directed to: Journal of Functional Foods

Abstract

Orange is one of the most consumed fruits in the world and its peel, about 50% of the fruit, is discarded. These by-products can be used as ingredients of an alternative diet, as long as they have their proven potential. Thus, the objective of this work was to define the food and functional potential present in the peel of ten orange cultivars produced by family farming in Northeast Brazil, aiming to identify the mineral, peptide, polyphenol, fiber and carotenoid profiles, in addition to the digestibility of phenolics. The orange peel has a wide profile of free and bound polyphenols, in addition to carotenoids and minerals, which associated with a high proportion of soluble (20.74 $g.100g^{-1}$) and insoluble (36.13 $g.100g^{-1}$) dietary fibers, constitute a low cost valuable alternative of functional food. The soluble fiber has bioactive peptides with molecular weights between 1 and 0.5 KDa. Furthermore, the phenolic compounds present are gradually released in the phases of the gastrointestinal tract, guaranteeing a wide functional and bioactive potential to the human diet of these orange peels. By the PC analisys, the peel of the Baia, Mimo e Comum cultivars formed a separated group due to its superior fuctonal quality.

Key words: Fruit waste, hesperidina, neohespiridina, violaxanthin, β-Carotene, dietary fibers, simulated digestion

1. INTRODUCTION

Orange is one of the most cultivated and commercialized fruits in the world, either as fresh fruit due to its recognized nutritional quality, its attractive color, in addition to its pleasant taste and aroma or processed by the citrus juice industry, which is very appreciated by consumers since it is rich in vitamins and minerals (Zou et al., 2016; Ruviaro, Barbosa &

Macedo 2019; Czech et al., 2019). Brazil is the first orange producer in the world, which in 2019 produced approximately 18 million tons (IBGE, 2020). Most of this production was processed and exported to other countries as juice, an important Brazilian product sold and consumed worldwide (Mendes et al., 2019).

Citriculture is a representative activity of family farming in the Borborema Territory, state of Paraiba, Northeastern Brazil. Among the orange varieties produced in the region, the vast majority are Creole, some with international recognition as the case of 'Baia' orange and others little known, such as those of low acidity, inserted in the group of sweet oranges, such as 'Mimo' and those with higher acidity, such as the 'Common' orange. These varieties have high antioxidant activity in the albedo and juice and also high quality standard for fresh consumption and processing, in addition to meeting the citrus marketing standards required in the country (Silva et al., 2019a).

During processing or even in the fresh consumption, about 50% of the fruit is wasted, this part corresponds almost entirely to peels that are most often discarded by the agroindustry and consumers, causing damage to the environment due to the large volume of residues (Sharma et al., 2017; Ruviaro, Barbosa & Macedo 2019; Singh et al., 2020). However, the peel contains a wide variety of secondary metabolites with high antioxidant activity compared to other parts of the fruit (Czech et al., 2019).

The World Health Organization (WHO) and healthy eating guides encourage the consumption of fruits and foods in general, rich in non-protein components, such as dietary fibers, minerals, vitamins, carotenoids and polyphenols, to maintain a healthy life (September Malaterre, Remize & Poucheret, 2018). In this sense, citrus fruits have in addition to essential nutrients, a large amount of biologically active phytochemicals, which are of interest to health such as ascorbic acid, carotenoids, which are provitamin A and flavonoids, all recognized as powerful antioxidants (BARROS et al. , 2012; CRIZEL et al, 2013; ZOU et al., 2016;

SMERIGLIO et al., 2019).

In addition to ascorbic acid, phenolic compounds also have antioxidant activity and are among the main compounds present in citrus fruits, especially in the peels (ZHANG, YANG and ZHOU, 2018; SINGH et al., 2020). Polyphenols have several other specific biological actions such as antimicrobial, antioxidant, anticancer and anti-inflammatory, antimutagenic and antiallergic properties (SINGH et al., 2016, SRIDHARAN et al., 2016, FERREIRA et al., 2018).

In addition, orange peels have high fiber content, which act in conjunction with phenolics, benefiting consumers with properties that bring benefits to human health (González-Aguilar, Blancas-Benítez, and Sáyago-Ayerdi 2017). Simple phenolic acids and flavonoids are the most common phenolic compounds and are present in free soluble, esterified and bound or insoluble soluble forms (Gulsunoglu et al., 2019). Therefore, orange peels can be considered as a promising source of free and bound functional compounds, far superior to the contents found in the juice (ADEMOSUN et al., 2016).

In this sense, orange peels can be used to extract bioactive molecules and valuable compounds such as phenolics that are natural antioxidants and can be used in the pharmaceutical, nutraceutical, cosmetic, beverage and food industries as natural additives for functional foods, as well as included in the meal as a flour to accompany meals (Zou et al., 2016; Delgado & Fleuri, 2016; Mahato et al. 2018; Ruviaro, Barbosa & Macedo, 2019; Sharma et al. 2017).

In view of all these statements, the identification and quantification of these compounds in fruits from small producer orchards is extremely important for the valorization and definition of potentials for the peel of orange cultivars produced by the family in the Borborema Territory, Paraíba state, Northeast of Brazil. In this sense, the objective of this work was to define the compounds of importance for human consumption and the functional

potential present in the peel of ten orange cultivars.

2 MATERIAL AND METHODS

Oranges (Citrus Sinensis L. Osbeck) of the cultivars Baianinha, Salustiana, Rubi, Westin, Sincorá, Lima, Baía, Comum, Mimo and Pera were randomly harvested, between 6 and 8 AM, at the C3 maturity stage - predominantly yellow, according to the CEAGESP Standards (2011). The fruits were from a commercial family farming orchard located in the municipality of Alagoa Nova, Borborema Territory, Paraiba state, Northeast of Brazil. The rural property has predominant Argissolo Red Eutrophic Abrupt type of soil (EMBRAPA, 2016).

The fruits were packed in high density polyethylene boxes with a capacity of 18 Kg, protected with bubble wrap and transported to the Laboratory of Biology and Technology Post-harvest. The fruits were processed using a citrus fruit squeezer (Arno Citrus Power PA 32) and the peels were manually separated from the albedo using a sharp knife and left to dry overnight in a forced circulation oven at 50 °C until reach a constant weight (Tecnal, model 1513D). After drying, the peel were macerated using mortar and pistil and ground in an electric processor until it became a fine flour, which was vacuum-packed and stored in a refrigerator until analysis.

Experiment was carried out in a completely randomized design (CRD) with peels of ten orange cultivars and four replicates for each cultivar.

2.1 Free Phenolics

The extracts for free phenolic compounds were prepared with aqueous ethanol (800 mL/L), which was added to 1 gram of dry orange peel flour. It was homogenized using a Ultra-Turrax (IKA Ultra-Turrax T18, WilmingtonThe, USA) for 2 min. The mixture was centrifuged (5000 rpm for 20 min, at 4 °C). The supernatant was evaluated for antioxidant activity, total phenolics by spectrophotometry and the phenolic profile by HPLC-DAD.

2.2 Bound Phenolics

Bound phenolics were extracted according to Xie et al. (2015) with some modifications. After washing the flour of orange peels with aqueous methanol (800 mL/L), the bound phenolic were released. The remaining solid residue was hydrolyzed with 20 mL of NaOH 4 M at room conditions and stirred in an orbital shaker at 250 rpm for 4 hours. The hydrolyzate was acidified to pH 1.5-2.0 by the gradual addition of 6M HCl. After centrifugation at 5 000 rpm for 20 min, the supernatant was extracted five times with 30 mL of ethyl acetate. The fraction ethyl acetate was dried, using a rotary vacuum evaporator at 30 °C. The resulting residue was then dissolved in 5 mL of 100% methanol. The obtained extract was stored at -20 °C until used.

2.3 Antioxidant capacity - DPPH, ABTS and ORAC assyss

The methodology for DPPH was based on Bobo-Garcia et al. (2014) with some modifications. Briefly, 20 μL of extract was added to a 96-well plate containing 180 μL of DPPH solution (2,2-diphenyl-1-picryl-hydrazil; Sigma Chemical Company, St. Louis, MO, USA) (150 μM) . The absorbance was measured at 515 nm on microplate reader (Spectrophotometer Multiskan GO microplate, Thermo Scientific, Thermo Fisher Scientific Inc., USA) after 40 minutes in the dark. Trolox (Sigma Chemical Company, St. Louis, MO, USA) standards (0 to 500 μM) were prepared and analyzed in the same way to obtain the calibration curve. The percentage of inhibition was determined and the extracts were expressed in mg equivalent of Trolox (TE)/g DW.

The ABTS elimination test was also performed on a 96-well microplate, following the method described by Gonçalves et al. (2009) with some modifications. To 20 μL of the sample or Trolox or solvent were added 180 μL of ABTS $^{\bullet+}$ working solution. The mixture was incubated for 5 min at 30 ° C, and the absorbance at 734 nm was measured with the Multidetection plate reader (Synergy H1, Vermont, USA).

The oxygen radical absorbance capacity (ORAC) was performed on a 96 well black microplate (Nunc, Denmark), following the method described by Dávalos et al. (2004) with some modifications. The reaction was done with 75 mM phosphate buffer (pH 7.4), and the final reaction mixture was 200 µL. The antioxidants (20 µL) and fluorescein (120 µL; 70 nM , final concentration) were placed in the microplate well. A blank (FL + AAPH) using phosphate buffer instead of the antioxidant solution and eight calibration solutions using Trolox (1-8 uM, final concentration) as antioxidant also was performed in each assay. The mixture was pre- incubated for 10 min at 37 °C. The AAPH solution (60 µL; 12 mM, final concentration) was added quickly using a multichannel pipette. The microplate was immediately placed in the reader and the fluorescence was recorded at 1 min intervals over a 140 min period.

The test was also performed with a multiple detection plate reader (Synergy H1, Vermont, USA). The excitation wavelength was fixed at 485 nm and the emission wavelength was 528 nm (Ubeda et al., 2011). The microplate was automatically agitated before each reading.

2.4 Carotenoids

For the carotenoid extracts it was followed the methodology of Oliveira et al (2016) with small modifications, 1 g of dry sample, plus 1 g of sodium carbonate were used, which was homogenized with 4 mL of distilled water and 1 mL of cold ethanol (4ºC) for 1 min using an Ultra-Turrax. Hexane (8 mL) was added and the resulting paste was centrifuged, after centrifugation the upper layer was removed into a polypropylene tube. The extraction was repeated twice with 2.88 mL of hexane. Both resulting hexane fractions were combined and used for saponification with 10% methanolic KOH overnight on an orbital shaker. The mixture was washed with 25 mL of 10% NaCl and three washes with deionized water.

2.5 Quantitative colorimetric analysis of the total carotenoid content

The carotenoid content in the orange peel extracts was estimated in triplicates, using a spectrophotometric analysis at 454 nm. β-carotene (6.3 × 10-5 - 4.0 × 10^{-3} mg/mL) was used as a standard curve. The total carotenoid content was expressed based on mg of β-carotene equivalents/g of biomass.

2.6 Quantitative analysis of carotenoids by HPLC with DAD detector

The carotenoid extracts were concentrated in Speed Vacuum Thermo Fisher Scientific. A 50 μL aliquot of orange peel hydrophilic extract was injected into a Waters Alliance high pressure liquid chromatograph (Waters Series 600, Mildford MA, USA), equipped with a Waters 996 PDA detector. The chromatograms were recorded using a detector diode array (Waters, Mildford MA, USA) at wavelengths ranging from 200 to 600 nm in 2 nm intervals. The absorbance was measured at 454 nm.

The carotenoids were eluted using a mobile phase consisting of acetonitrile (55%), methanol (22%), dichloromethane (11.5%) and hexane (11.5%). It was added ammonium acetate 0.02% to stabilize carotenoids under conditions isocratic at a flow rate of 1.0 ml/min for 20 min at 25 °C with an injection volume of 50 μl. The calibration curve was constructed with pure standards of α-carotene, β-carotene, zeaxanthin , lutein, β-cryptoxanthin and violaxanthin and expressed in micrograms per gram. All analyzes of each extract were done in triplicates.

2.7 Fiber Profile

One gram of each sample was used to determine dietary fiber; procedures were performed to remove fat, with 25 mL of N- Hexane and sugars with 20 mL of 80% ethanol to avoid interfering with the enzymatic treatment process for separating the fibers.

40 mL buffer was added (MES/Tris, 0.05 M), pH 8.2 and the samples were placed in a

water bath at 100 °C with 50 uL of α-amylase with constant agitation for 30 minutes. After this stage, the temperature was lowered to 60 ºC and 10 mL of deionized water and 100 µL were added and left at 60ºC for 30 minutes. Following that step, 5 mL of 0.56 N HCl was added and the pH was adjusted between 4.1 and 4.8 with 5% NaOH or 5% HCL; immediately after 200 µL of amyloglycosidase was added and maintained at 60ºC for 30 minutes.

The material was filtered through Gooch's crucible porous plate, the liquid fraction containing soluble fiber was lyophilized to quantitate the soluble fiber and insoluble fiber that was taken to the drying oven at 50 °C.

2.8 Total Proteins

The protein was analyzed using the Kjeldahl method (AOAC, 2002). 0.5 g of flour peel from each dry orange cultivar were used, 1 g of catalyst (Bromocresol green) and 4 mL of sulfuric acid were added. Placed in digesting tubes, the samples were put to heat to 420 °C in the protein digester until a colorless solution was obtained. After cooling, 20 mL of H_2O was added and the sample was placed in the distillation apparatus and a 10N NaOH discharge was made in each sample, with an Erlenmeyer containing 25 mL of 4% boric acid at the other end of the distiller (after the condenser). After distillation, the solution was titrated with HCl 0.2N until the volume necessary to reach the final point (color change) was recorded. The nitrogen-protein conversion factor (6.25) was used for the calculation to obtain the crude protein content. The results were expressed in g / 100g DM.

2.9 Determination of the molecular weight distribution

The molecular weight distribution (MW) of the soluble fiber was determined by gel filtration chromatography using the AKTA Pure 25 FPLC system (Fast Protein Gel Chromatography by gel filtration) coupled to two gel filtration columns: Superdex 200 raise 10/300 GL and Superdex peptide, 10/300 GL. The eluent used was the 0.05M phosphate

buffer, pH 7.0 containing 0.15M sodium chloride and 0.2 $g.L^{-1}$ of sodium azide at a flow rate of 0.5 ml min^{-1}.

2.10 Minerals

It was used 200 mg of each sample, added of 5 mL of 65% nitric acid and placed to be digested in a microwave digestor (Berghof Speedwave MWS-3+) for 47 minutes. The profile of minerals was determined in a spectrometer of emission (Optics Perkin Elmer Optima 7000 DV), all samples in triplicate.

2.11 In vitro simulated gastrointestinal digestion

A simulated gastrointestinal digestion study was conducted with samples of the peel of 'Mimo' orange, according to the method developed by Madureira et al. (2011). Three replications of 1g were homogenized with 20 mL of deionized water. An initial 2 mL aliquot was collected before starting digestion. To simulate the mouth stage, the pH of the samples was adjusted to values between 5.6 and 6.9, using HCl 1M. Artificial saliva was simulated using α-amylase (Sigma- Aldrich Chemistry, St. Louis, Missouri, USA) at 100 U/mL and added at a rate of 0.6 mL/min digestion, and incubated for a 1 minute at 37 °C at 200rpm. After this process, a 2 mL aliquot was removed.

The pH was adjusted to 2.0 using HCl 1M; and gastric juice was simulated by the addition of pepsina (Sigma-Aldrich Chemistry, St. Louis, Missouri, USA) 25 mg/mL and added at a rate of 0.05 mL/mL sample and were then incubated at 60 minutes at 37 ˚C at 130 rpm. After this procedure, another 2 mL aliquot was removed.

Intestinal digestion was simulated by adjusting the pH of the samples to 6.0 using 1M $NaHCO_3$. It was added 2 g/L Pancreatin the (Sigma-Aldrich Chemistry, St. Louis, Missouri, USA) and 12 g/L bile salts (Oxoid ™, Hampshire, UK) at a proportion 0.25 mL/mL of sample; the samples were incubated for 120 minutes at 37 to 45 rpm. Once again, a 2 mL

aliquot was collected to analyze the phenolic profile in HPLC.

The evaluation data were subjected to analysis of variance (ANOVA) and the means of peel cultivars were subjected to the Tukey test up to 5% probability. To carry out these analyzes, the Emmeans package of software R version 5.1 (Lenth, 2018) was used.

3 RESULTS AND DISCUSSION

In this study, the peels of ten orange cultivars produced by family farmers in the Territory of Borborema, state of Paraíba, northeastern Brazil were evaluated for the profiles of minerals, proteins, polyphenols, fibers and functional potential for use as human food. The results found suggest that orange peel flour has different dietary characteristics that make it a valued food source.

3.1 Free and bound Extratable Polyphenols (EP)

For the free total extractable polyphenols from the peel of orange cultivars (Figure 1A), an overall mean content of 1.22 g.GAE.100g^{-1} in dry matter (DM) was found, and the highest content was that of 'Common' orange, with 1.42 g.GAE.100g^{-1}, which differed statistically from the others ($p \leq 0.05$) with the exception of the cultivar 'Mimo' (1.38 g.GAE.100g^{-1}), 'Sincorá' (1. 33 g.GAE.100g^{-1}) and 'Baía' (1.32 g.GAE.100g^{-1}). This latter was also the cultivar that stood out with the highest content of bound phenolics in the extracts (Figure 1 B) with 0.68 g.GAE.100g^{-1} ($p \leq 0.05$) whose overall mean content was 0.47 g.GAE. 100g^{-}1.

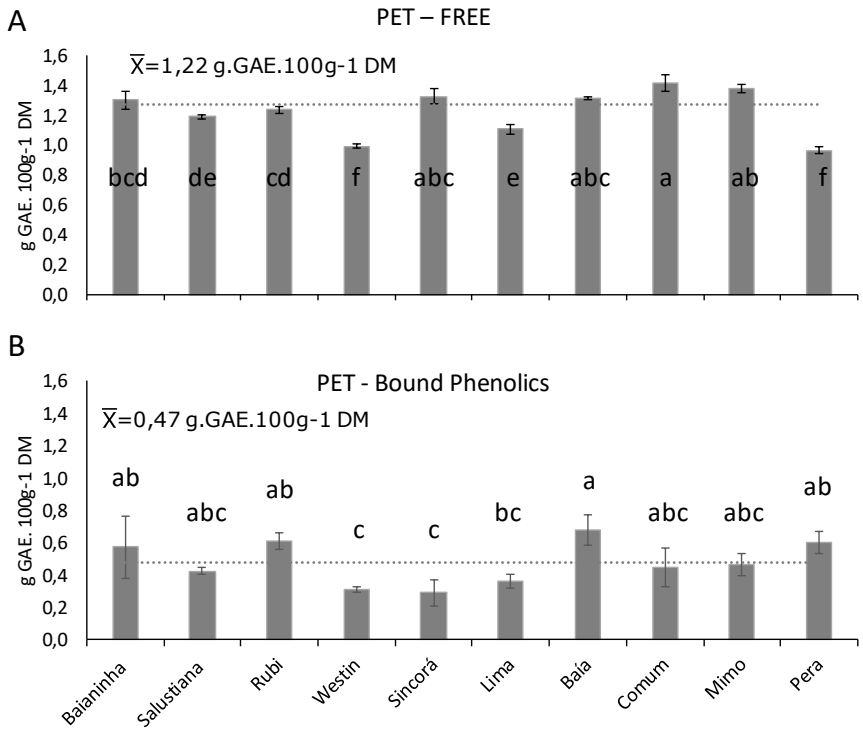

Figura 1. Free Total Extractable Polyphenols (TEP) (A) and bound phenolics (B) in the peel of orange cultivars. Bars with the same lowercase letters do not differ by Tukey's test at 5% probability ($p \leq 0.05$).

The free total phenolic content of the orange peels evaluated herein is higher than that reported by Chen, Yang & Liu (2011) and also by Ruviaro, Barbosa & Macedo (2019), which were 0.65 and 0.7 g.GAE.100g^{-1}, respectively. However, they are similar to those found by Nayak et al (2015) using different microwave powers and solvent concentrations, whose polyphenol contents varied from 0.83 to 1.21 g.GAE.100g^{-1} depending on the microwave power and solvent concentration used.

According to Wang et al (2016) the variations in the phenolic contents, can be attributed to the specific characteristics of each cultivar, cultivation conditions, in addition to the different techniques and solvents used in the extraction.

It should be noted in this study that, in the case of bound phenolics, the overall mean among the orange cultivars evaluated (0.47 g.GAE.100g^{-1}) was less than 50% of the free phenolics measured in the methanol extract, which means that the extraction with methanol

80% extracted the vast majority of phenolics from orange peels.

However, in view of the results presented herein, it can be said that to exhaust and quantify all the phenolic compounds in these materials, it is necessary to perform basic hydrolysis, since a significant amount of phenolics are bound to fibers, proteins and other structures of the peel, especially in 'Baía' (0.68 g.GAE.100g-1), 'Pera' (0.60 g.GAE.100g^{-1}), 'Rubi' and 'Baianinha' cultivars, which presented contents above the overall mean. Obohh and Ademosun (2012) also found 0.68 g.GAE.100g^{-1} of bound phenolics in orange peels. These results confirm, therefore, that agro-industrial residues are rich in phenolics in both free-soluble, and bound- insoluble forms (Gulsunoglu et al., 2019).

3.2 Profile of free phenolic compounds (HPLC-DAD)

In general, the main phenolic compounds present in orange peels evaluated (Table 1), are hesperidin and neohesperidine with 135.23 and 183.47 mg.100g^{-1}, respectively. However, the only cultivar in which neohesperidine was not detect was Mimo. In turn, excepted by Mimo, ‘Westin’ and ‘Lima’ cultivars, the peels of the other cultivars evaluated in this study have a higher content of neohesperidine than hesperidin, which is herein considered as the main compound in orange peels.

The peel of 'Baianinha' orange has the highest content of neohesperidine (398.06 mg.100g^{-1}), differing statistically from all other cultivars ($p \leq 0.05$), and gallic (30.55 mg.100g^{-1}) and isoferrulic acids (61.14 mg.100g^{-1}) ($p \leq 0.05$). In turn, the 'Common' has the highest content of caffeic acid (64.65 mg.100g^{-1}) and tageritin (19.55 mg.100g^{-1}) also differing statistically ($p \leq 0.05$) from the other cultivars studied, while 'Mimo' showed statistical difference ($p \leq 0.05$) from the others, due to its higher content of chlorogenic acid (95.68 mg.100g^{-1}) and hesperidin (261.75 mg.100g^{-1}).

Recent studies highlighted that both neohesperidine and hesperidin found in abundance in the peel of orange cultivars evaluated herein, are important for the treatment and prevention of

Tabela 1. Profile of free phenolic compounds in the peel of orange cultivars measured by HPLC-DAD (mg.100g^{-1})

Cultivar	Caffeic Acid	Chlorogenic Acid	Gallic Acid	Isoferulic Acid	Hesperidin	Neo-hesperidin	Hesperitin	Tangeretin	Totals
Baianinha	15.13 ± 0.2^{de}	48.66 ± 0.8^{d}	30.55 ± 6.9^{a}	61.14 ± 0.0^{a}	189.27 ± 0.5^{b}	398.06 ± 0.4^{a}	nd	6.31 ± 0.0^{g}	749.12
Salustiana	1.55 ± 0.0^{f}	42.80 ± 0.1^{f}	20.38 ± 0.0^{cd}	29.61 ± 0.1^{c}	116.29 ± 0.0^{cd}	236.67 ± 0.3^{c}	nd	9.22 ± 0.0^{d}	456.52
Rubi	13.69 ± 0.5^{e}	45.91 ± 1.1^{e}	28.05 ± 0.9^{ab}	27.89 ± 1.2^{d}	126.48 ± 5.7^{cd}	250.16 ± 4.5^{b}	nd	7.43 ± 0.0^{e}	499.61
Westin	24.36 ± 0.2^{cd}	66.93 ± 0.4^{c}	27.29 ± 1.5^{ab}	20.25 ± 0.2^{f}	118.41 ± 1.3^{cd}	102.00 ± 1.1^{f}	nd	6.59 ± 0.0^{f}	365.83
Sincorá	29.04 ± 0.1^{c}	67.84 ± 0.1^{c}	25.18 ± 0.1^{abc}	23.19 ± 0.0^{e}	75.51 ± 7.9^{f}	101.25 ± 3.6^{f}	nd	0.78 ± 0.0^{j}	322.79
Lima	28.81 ± 1.3^{c}	Nd	24.37 ± 0.3^{abc}	Nd	110.45 ± 0.1^{de}	77.20 ± 0.1^{g}	nd	1.99 ± 0.0^{h}	242.82
Baia	44.75 ± 7^{b}	86.72 ± 0.9^{b}	26.16 ± 0.0^{abc}	26.78 ± 0.5^{d}	89.10 ± 1.3^{ef}	137.60 ± 1.0^{e}	nd	14.44 ± 0.2^{b}	425.55
Comum	64.65 ± 0.7^{a}	Nd	25.97 ± 0.3^{abc}	Nd	128.73 ± 1.8^{cd}	170.90 ± 2.4^{d}	nd	19.55 ± 0.1^{a}	409.8
Mimo	45.00 ± 0.1^{b}	95.68 ± 0.2^{a}	22.44 ± 0.4^{bcd}	35.57 ± 1.2^{b}	261.75 ± 6.1^{a}	Nd	nd	12.39 ± 0.0^{c}	472.83
Pera	26.40 ± 10.1^{c}	33.50 ± 0.1^{g}	16.13 ± 0.5^{d}	Nd	136.33 ± 1.5^{c}	177.38 ± 0.2^{d}	43.41 ± 1.1	1.51 ± 0.0^{i}	434.66
Mean	**29.34**	**61.00**	**24.65**	**32.06**	**135.23**	**183.47**	**43.41**	**8.02**	

Means followed by the same lower case letter in the column did not differ by the Tukey test at 5% ($p \leq 0,05$). nd= not detected.

various diseases such as osteoporosis, fibrosis and pulmonary dysfunction and colorectal tumors and ulcers (Tan et al. 2017 ; Guo et al. 2019; Gong et al. 2019; Silva et al. 2019b). In this sense, orange peel can be more widely explored, in order to be introduced into human food to provide such benefits or used by the pharmaceutical industry to obtain these compounds (Mahato, Sharma, Sinha, & Cho, 2018). 'Mimo' was the only orange evaluated in which neohesperidine was not detected in the peel, probable due to it be grouped as a sweet orange (Silva et al., 2019a).

Tageritin was another flavonoid quantified in the peels of all orange cultivars, however with a lower content (8.02 $mg.100g^{-1}$). The main phenolic acids in orange peels, free in aqueous methanol, are chlorogenic acid (61.00 $mg.100g^{-1}$) followed by isoferulic (32.06 $mg.100g^{-1}$), caffeic (29.34 $mg.100g^{-1}$) and gallic (24.65 $mg.100g^{-1}$) acids. It is interesting that hesperitin was quantified only in the peel of the 'Pera' with 43.41 $mg.100g^{-1}$. However, these differences in the phenolic profile can be attributed to the conditions of extraction and analysis, mainly the solvents and fruit cultivars used in the analysis (Wang et al., 2016; Gulsunoglu et al., 2019).

Several previous studies have shown that the flavonoids present in citrus peels, have anti-cancerigenigena, anti-hyperlipidemic, anti-obesity, antivirus, anti-inflammatory, anti-allergic, analgesia and anti-diabetic effects in addition to protecting and helping in the treatment of hepatic steatosis also known as fat in the liver (Oboh & Ademosun, 2011; Guo et al., 2016; Mallick and Khan, 2016; Mannucci et al., 2017; Jianping et al., 2019). These statements, coupled with the results of polyphenols obtained in the study carried out in here, which allow us to state that the orange peel flour can provide health benefits.

3.3 Bound Phenolics Profile (HPLC-DAD)

In the extract of the bound phenolic compounds, analyzed by HPLC-DAD (Table 2), the presence of isoferulic (92.89 $mg.100g^{-1}$), *p*-coumaric (32.90 $mg.100g^{-1}$) and synapic

(17.45 mg.100g^{-1}) acids was verified, this latter found only in 'Mimo' (14.27 mg.100g^{-1}) and 'Pera' (20.64 mg.100g^{-1}) peels ($p \leq 0$ .05). In addition, naringenin (16.32 mg.100g^{-1}) and a large content of hesperidin (511.21 mg.100g^{-1}) were also quantified, this latter present in the peel of all cultivars evaluated.

Although the free phenolics of orange peels have been identified and quantified in greater abundance, there are some phenolics compounds that have only been detected in the bound form, such as the *p*-coumaric (32.90 mg.100g^{-1}) and synaptic (17.45 mg.100g^{-1}) phenolic acids and also the flavanone naringenin (16.32 mg.100g^{-1}).

Narangenin is an important bioactive compound with recognized biological and pharmacological activities, such as antioxidants and anti-inflammatory, among others (Singh 2016; Sun et al. 2019; Mendes et al 2019). In a recent study Liu, Li and Feng (2019) reported that hesperidin and naringin alleviate the effects of alcohol damage on embryonic development and neurological behavior.

In some industries, citrus wastes, including peels, are used for pectin extraction. However, even after the extraction of this product, many phenolic compounds remain that can be extracted (Barbosa, Ruviaro & Macedo 2018). These are possibly the linked phenolics, which were quantified in this work, which represent a large part of the total phenolics existing in orange peels. In addition, Oboh & Ademosun (2011) stated that both free phenolic extracts, as well as those bound to Citrus Maxima peels, can be used as nutraceuticals for the treatment of hypertension and type 2 diabetes.

Thus, it can be stated that in order to extract a greater amount of phenolics from orange peels, in addition to aqueous methanol, an alkaline hydrolysis is necessary to extract both free and bound phenolics and, consequently, provide an adequate valuation of orange peels. In this context, according to Pérez-Jiménez and Saura-Calixto (2015), in some fruits, the linked phenolics correspond to 50% of the total polyphenol content.

Tabela 2. Bound phenolics profile in peels of orange cultivars evaluated by HPLC-DAD (mg.100g^{-1}).

Cultivar	Isoferulic Acid	*p*-Coumarico	Sinapic Acid	Hesperidin	Narangenin	Totals
Baianinha	85.37 ± 0.24 e	32.60 ± 0.06 d	Nd	1353.25 ± 5.39 a	40.68 ± 0.14 a	1511.9
Salustiana	64.80 ± 0.07 h	22.17 ± 0.01 i	Nd	400.19 ± 0.39 e	8.98 ± 0.07 f	496.14
Rubi	55.72 ± 0.28 i	22.80 ± 0.02 h	Nd	511.70 ± 0.43 c	Nd	590.22
Westin	77.60 ± 0.20 g	29.34 ± 0.04 f	Nd	313.67 ± 0.89 i	11.32 ± 0.12 d	431.93
Sincorá	95.62 ± 1.19 c	30.73 ± 0.28 e	Nd	376.92 ± 4.00 f	16.58 ± 0.17 c	519.85
Lima	81.28 ± 0.70 f	26.24 ± 0.22 g	Nd	368.03 ± 3.61 g	20.33 ± 0.17 b	495.88
Baia	162.95 ± 0.11 a	54.21 ± 0.09 a	Nd	446.66 ± 0.55 d	10.32 ± 0.02 e	674.14
Comum	Nd	nd	Nd	372.55 ± 0.18 fg	Nd	372.55
Mimo	88.29 ± 0.83 d	37.67 ± 0.36 c	14.27 ± 0.06 b	324.65 ± 3.78 h	6.05 ± 0.18 g	470.93
Pera	124.38 ± 0.00 b	40.37 ± 0.07 b	20.64 ± 0.03 a	644.51 ± 0.50 b	Nd	829.9
Mean	**92.89**	**32.90**	**17.45**	**511.21**	**16.32**	

Means followed by the same lower case letter in the column did not differ by the Tukey test at 5% of probability ($p \leq 0,05$). nd= not detected.

3.4 Total Carotenoids and Carotenoid Profile

The total carotenoids in the peels of orange cultivars (Table 6) showed an overall mean of 15.3 mg.100g^{-1}. The 'Pera' (22.46 mg.100g^{-1}), 'Common' (21.82 mg.100g^{-1}) and Rubi (19.90 mg.100g^{-1}) cultivars were those with the highest levels of total carotenoids, however, they did not differ statistically ($p \leq 0.05$).

Orange peels are rich in carotenoids, such as violaxanthin (12.88 mg.100g^{-1}), which was the one with the highest content in the peels of all varieties studied, whose means in this study were β-carotene (8.26 mg .100g^{-1}), α-carotene (7.37 mg.100g^{-1}), and zeaxanthin (0.28 mg.100g^{-1}). Previous studies have also reported that the main carotenoid in oranges is violaxanthin (Agócs et al., 2007; Petry et al., 2019). In this sense, the content of violaxanthin corresponded to about 33.8% of the total orange peel carotenoids according to Agócs et al (2007).

The 'Pera' orange had a higher content of α- and β-carotene, with 12.10 mg.100g^{-1} and 13.00 mg.100g^{-1}, however, it was the one that presented less violaxanthin (5.79 mg.100g^{-1}), which has been reported as the main carotenoid of sweet oranges. In turn, the highest content found for violaxanthin was in the 'Sincorá' (20.00 mg.100g^{-1}) and zeaxanthin in the 'Common' (0.37 mg.100g^{-1}).

For β-carotene, Pera and Rubi cultivars presented the highest levels with 13.00 and 15.60 mg.100g^{-1}, respectively, which did not differ between them ($p \leq 0.05$). Notably, the levels of β-carotene in these cultivars are much higher than those of Wang et al (2008) in orange peels Liucheng (Citrus sinensis (L.) Osbeck) produced in Taiwan (5.02 mg.100g^{-1}). However, the content reported by those authors was close to the levels of β-carotene in the peel of most cultivars evaluated in this study, such as Sincorá, Common and Mimo, with, in this order, 5.86; 6.36; 6.59 mg.100g^{-1}.

Tabela 3. Total carotenoids by spectrophotometry and HPLC-DAD profile of the peels of orange cultivars (mg.100g^{-1})

Cultivar	Total carotenoids	α-Carotene	β-Carotene	Violaxanthin	Zeaxanthin	Totals
Baianinha	14,70 ± 2,10^{cd}	4,98 ± 0,73^{e}	9,24 ± 4,83^{b}	16,96 ± 3,04^{abc}	0,23 ± 0,04^{c}	46.11
Salustiana	13,92 ± 0,88^{cde}	nd	9,58 ± 0,90^{b}	15,34 ± 0,99^{abcd}	0,28 ± 0,02^{bc}	39.12
Rubi	19,90 ± 2,34^{ab}	nd	15,60 ± 1,46^{a}	17,83 ± 2,22^{ab}	0,33 ± 0,03^{ab}	53.66
Westin	10,17 ± 1,09^{de}	6,10 ± 0,29^{e}	3,89 ± 0,3^{d}	11,19 ± 0,45^{def}	0,23 ± 0,02^{c}	31.58
Sincorá	13,34 ± 1,95^{cde}	nd	5,86 ± 0,71^{c}	20,00 ± 2,05^{a}	0,29 ± 0,03^{abc}	39.49
Lima	11,29 ± 2,12^{de}	nd	8,92 ± 2,18^{b}	13,42 ± 1,84^{bcd}	0,23 ± 0,02^{c}	33.86
Baia	9,89 ± 0,87^{e}	5,97 ± 0,34^{e}	3,61 ± 0,40^{d}	8,41 ± 1,53^{efg}	0,25 ± 0,02^{c}	28.13
Comum	21,82 ± 1,51^{a}	8,17 ± 0,30^{c}	6,36 ± 1,76^{c}	12,82 ± 1,34^{cde}	0,37 ± 0,04^{a}	49.54
Mimo	16,39 ± 0,68^{bc}	6,89 ± 0,59^{d}	6,59 ± 0,67^{c}	7,00 ± 0,28^{fg}	0,29 ± 0,01^{abc}	37.16
Pera	22,46 ± 1,22^{a}	12,10 ± 0,63^{a}	13,00 ± 1,40^{a}	5,79 ± 0,79^{g}	0,25 ± 0,02^{c}	53.6
Mean	**15.34**	**7.37**	**8.26**	**12.88**	**0,28**	

Means followed by the same lower case letter in the column did not differ by the Tukey test at 5% ($p \leq 0,05$). nd= not detected.

Carotenoids are the main pigments in the citrus peel (Rodrigo et al., 2013). Furthermore, in human food, carotenoids have important functions such as nutritional and biological activity, such as, for example, their recognized protective effects against the development of chronic and degenerative diseases by capturing free radicals; in addition to the activity of provitamin A exhibited by some carotenoids (Serdula et al., 1996; Maiani et al., 2009; Meyers et al., 2014). Accumulation of β, β-xanthohylls (9-Z-violaxanthina and β-cryptoxanthin) and apocarotenoids C30 carotenoids, in specific suborganellar storage structures, is also a common feature in the peel of ripe oranges and tangerines (Rodrigo et al ., 2013).

3.5 Antioxidant activity

The antioxidant activity, by the ABTS method, of the peel's free phenolic extracts of the evaluated orange cultivars (Figure 2 A) showed an overall mean of 26.63 μM Trolox.g^{-1} and those of bound phenolics 23.94 μM Trolox.g^{-1} (Figure 2 D). By the DPPH method (Figure 2 B), the antioxidant activity of the free phenolics was 6.99 μM Trolox.g^{-1} and the bound ones 4.59 μM Trolox.g^{-1} (Figure 2 E). In turn, using the ORAC method (Figure 2 C), greater antioxidant activity was detected in the extracts of both, free (370.86 μM Trolox.g^{-1}) and bound (137.65 μM Trolox.g^{-1}) phenolic forms (Figure 2 F).

A Pearson correlation was performed between the antioxidant activity methods, and it was observed that ABTS and DPPH (r^2 = 0.96) had a higher correlation than DPPH and ORAC (r^2 0.66) and than ORAC and ABTS (r^2 0.66). In general, ABTS values were higher than DPPH, and ORAC values were much higher than those obtained by DPPH and ABTS. A similar pattern was reported by Ribeiro et al. (2020) in olive residues. Moreover, the correlation between the antioxidant activities of free and bound phenolic extracts, determined by the ABTS, DPPH and ORAC methods, were very low (r^2 = 0.06), (r^2 = - 0.12) and (r^2 = 0.44) respectively.

It is interesting to note that the peels of 'Common' orange showed the highest antioxidant activity of the free phenolic extracts by the three evaluated methods, ($p \leq 0.05$) ABTS (30.87 μM Trolox.g^{-1}), DPPH (7.67 μM Trolox. g^{-1}) and ORAC (483 μM Trolox.g^{-1}). In turn, for the bound phenolic extracts, the highest ABTS value was for Baianinha (34.98 μM Trolox.g^{-1}) which differed statistically from the others ($p \leq 0.05$). This cultivar was followed by Bahia, whose antioxidant activity of bound phenolics also stood out from the other cultivars by the methods of DPPH (5.64 μM Trolox.g^{-1}) and ORAC (230 μM Trolox.g^{-1}) ($p \leq 0.05$).

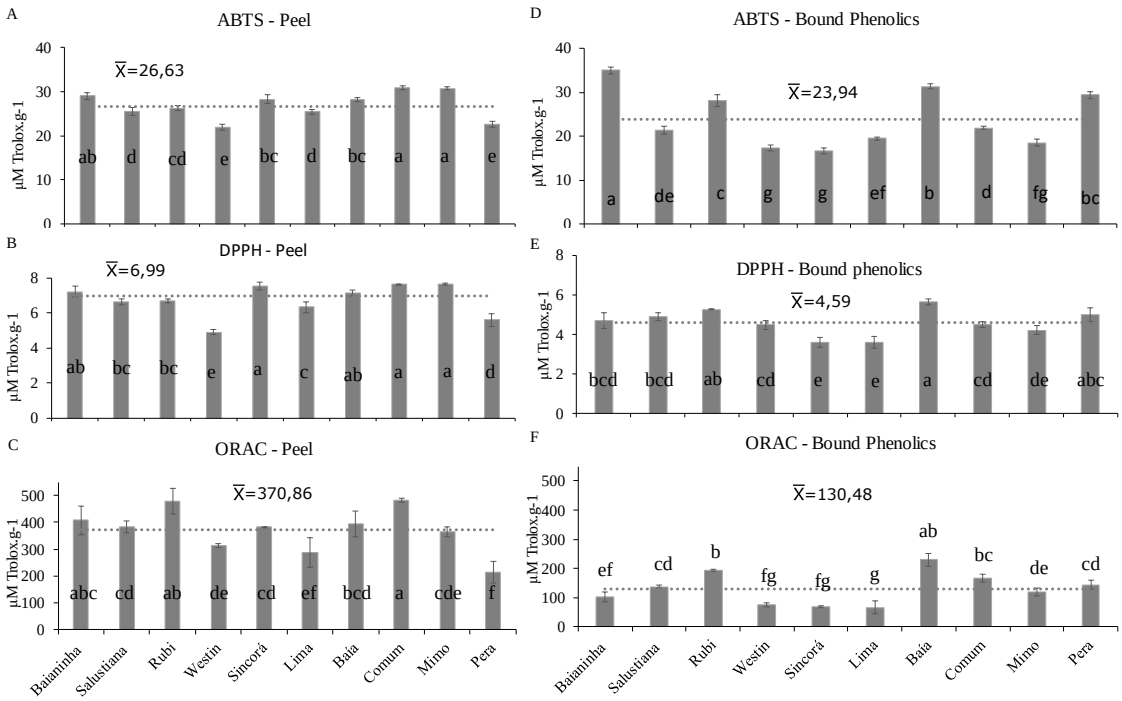

Figura 2. Antioxidant activity of phenolic extracts, in the free, by ABTS (A), DPPH (B), ORAC (C), and bound forms, by ABTS (D), DPPH (E), ORAC (F) methods from the peels of orange cultivars.
Bars with the same lowercase letters do not differ by Tukey's test at 5% ($p \leq 0.05$).

There is still no single, definitive analysis method for assessing the antioxidant activity of citrus fruits (Zou et al., 2016). For this reason, in this work three methods were used to determine antioxidant activity in the peel of orange cultivars. However, Khan et al. (2010)

stated that the ORAC assay is more suitable for the evaluation of antioxidant activity in citrus due to the fact that the main orange flavanones, naringenin and hesperetin, are relatively weak antioxidants, as they do not exhibit a catechol group (1,2-di -hydroxybenzene), which is the critical structural determinant of strong phenolic antioxidants. This is probably why the antioxidant activity of the peels by the ORAC method has shown a great difference in relation to the ABTS and DPPH methods.

3.6 Total proteins

The overall mean content of total protein in the peel (Figure 4 A) of the oranges evaluated herein was 5.98 $g.100g^{-1}$. The Pera had the highest total protein content (7.82 $g.100g^{-1}$) that differed statistically from the other cultivars ($p \leq 0.05$), followed by Westin and Lima with contents of 6.91 and 6.34 $g.100g^{-1}$, respectively. On the other hand, Common and Baia cultivars had the lowest protein content in the peel (4.53 $g.100g^{-1}$) that differed statistically from the others ($p \leq 0.05$).

The levels of total proteins found in the peels of the oranges evaluated herein are close to those reported by Matsuo et al (2019) in peels of *Citrus natsudaidai* grown in Japan (5.30 $g.100g^{-1}$). The National Health Surveillance Agency (ANVISA) (Brazil 2005) recommends the daily consumption of 50 g of protein for adults. However, it is necessary to evaluate the profile of the amino acids present to verify the effective nutritional contribution to the diet.

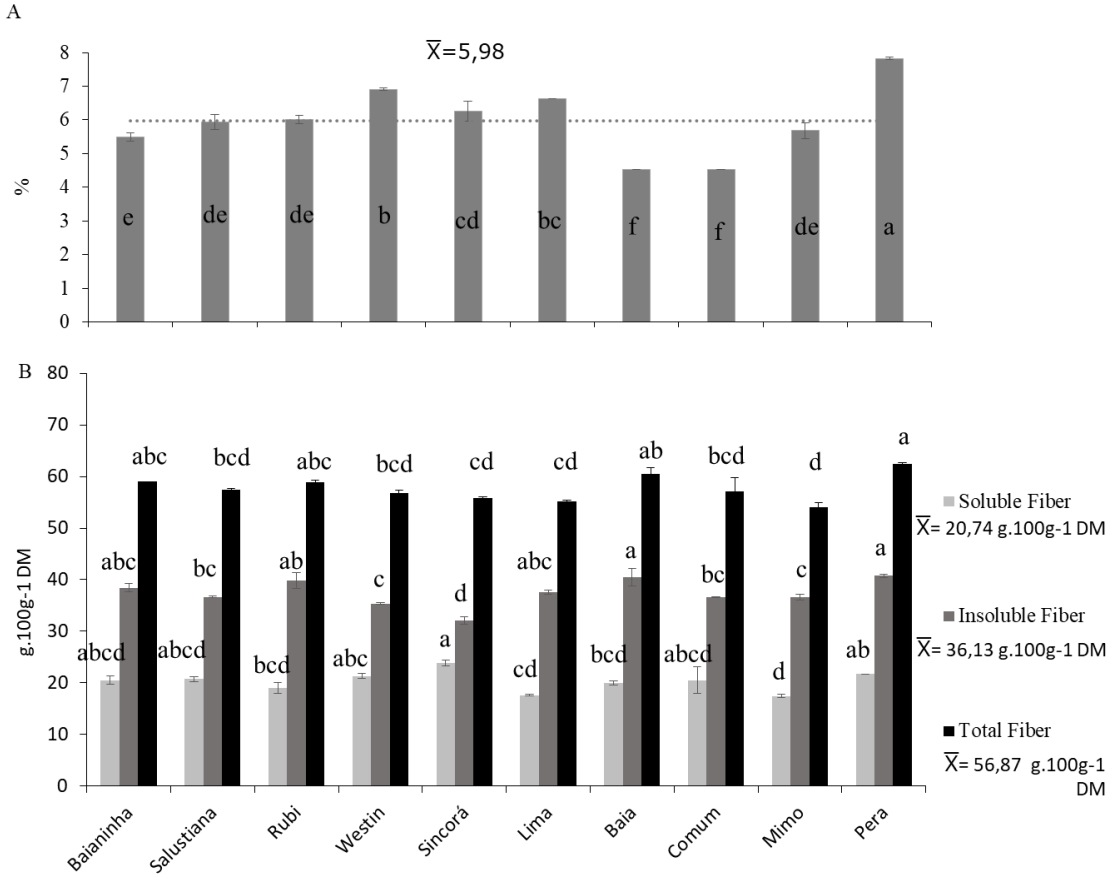

Figura 3. Proteins (A) and fibers (B) from the peel of orange cultivars produced in Northeast Brazil. Bars with the same lowercase letters do not differ by the Tukey test at 5% ($p \leq 0.05$).

3.7 Fibers

The orange peel is rich in dietary fibers (figure 4 B), with the mean contents of 20.74 $g.100g^{-1}$ of soluble fibers; 36.13 $g.100g^{-1}$ of insoluble fibers and 56.87 $g.100g^{-1}$ of total fibers per gram. The technological and nutraceutical functionalities of dietary fibers depend on their composition and, specifically, on the proportion of fractions of soluble and insoluble dietary fibers (Garcia-Amezquita et al., 2018).

For soluble fiber, there was no marked difference between cultivars, since 'Sincorá' had the highest content 23.82 g.100g-1, which was statistically equal ($p \leq 0.05$) to Pera (21.72 $g.100g^{-1}$), Westin (21.34 $g.100g^{-1}$), Salustiana (20.74 $g.100g^{-1}$), Baianinha (20.54 $g.100g^{-1}$)

and Common (20.51 g.100g^{-1}) cultivars. The contents of soluble fibers found in the peels of different cultivars in this reseaach are close to those of Garcia-Amezquita et al (2019) in orange peels, using different conditions for the extrusion of these by-products, which varied from 6.3 to 23.8 g.100g^{-1}, depending on the extraction procedure.

Soluble dietary fiber effectively reduces serum cholesterol and increases glucose metabolism and insulin response, due to the delayed gastric emptying and decreased absorption of macronutrients. Soluble fibers become viscous in water in the small intestine (Lattimer & Haub, 2010; Oduntan & Arueya, 2019). Thus, it is believed that the increase in viscosity prevents the movement of cholesterol, bile acids and other lipids and hinders the formation of micelles, thus reducing the absorption of cholesterol and, therefore, promoting its excretion by the body (Carr & Jesch, 2006).

In the same sense, without major differences, Baia (40.46 g.100g^{-1}), Pera (40.74 g.100g^{-1}), Rubi (39.82 g.100g^{-1}), Baianinha (38.41 g.100g^{-1}) and Lima (37.57 g.100g^{-1}) cultivars showed the highest levels of insoluble fiber but did not differ statistically from each other ($p \leq 0.05$). Regarding total fibers, which corresponds to the sum of soluble and insoluble fibers, the Baia (60.44 g.100g^{-1}), Pera (62.46 g.100g^{-1}), Rubi (58.90 g.100g^{-1}) and Baianinha (58.96 g.100g^{-1}) cultivars also did not differ statistically from each other ($p \leq 0.05$), although they were those with higher levels.

Garcia-Amezquita et al (2019) also found insoluble fibers with levels close to those of the cultivars studied here, with a minimum of 23.3 g.100g^{-1} and a maximum of 41.8 g.100g^{-1}. As for the total fiber content, those autors reported a minimum of 44.6 g.100g^{-1} and a maximum of 51.9 g.100g^{-1} lower than that found in this research, since the mean content for insoluble fibers was 56.87 g.100g^{-1}, indicating the superiorities of the oranges studied herein.

Studies carried out to investigate the effect of fibers on human health suggest that increased consumption of soluble and insoluble dietary fibers reduces the risk of developing

cardiovascular diseases, combating risk factors such as high levels of LDL cholesterol (Chau, Huang, & Lin , 2004; Kendall, Esfahani and Jenkins, 2010), which points out the importance of adding orange peels in the diet.

3.8 Bioactive peptides by gel filtration chromatography

Through gel filtration chromatography, proteins, peptides and amino acids of different molecular weights were observed in the peels of orange cultivars (Table 7). The Baianinha, Sincorá, Baia and Comum cultivars presented peaks equivalent to 1026 KDa, 924 kDa, 8802 kDa, 913 kDa, respectively, however the percentage of area equivalent to these peaks was very low, 0.56%, 1.71%, 0.59% and 0.39%, respectively. In addition to these peaks, all cultivars showed a peak of approximately 100 kDa, which is equivalent to a larger percentage of area, reaching 14.84% for the cultivar Baianinha, while Lima had the lowest area percentage for the peak in this molar mass range, with 6.16%.

The peptides represented by the peaks that correspond to the range with less than 1 kDa were those that presented a higher percentage of area in the peels of all cultivars evaluated. In the range between 1 - 0.5 kDa, the Common cultivar presented the largest area with 72.02% followed by Pera and Mimo with 59.13 and 58.49%, respectively. In the sum of peaks lower than 0.5 kDa, the Rubi and Lima cultivars presented the highest percentage of area with 49.72 and 45.36%, respectively. To our knowledge, the fractionation of peptides from the peel of orange cultivars has not yet been evaluated.

Previous research, however, claims that peptides with molecular weights lower than 1.5 kDa and higher than 0.5 kDa have greater antioxidant action when compared to those lower than 0.5 kDa and greater than 1.5 kDa (Li, Han, Chen, 2008; Ajibola et al., 2011).

Tabela 7. Molecular weight and elution volume of the peaks expressed by FPLC size exclusion chromatography of the soluble fiber of the orange cultivars

Cultivar	KDa	Area	Peak area (%)
Baianinha	1026	1.46	0.56
	100	38.85	14.84
	1 - 0.5	108	41.29
	∑ < 0.5	113	43.31
Salustiana	103	30.32	10.13
	1 – 0.5	139	46.44
	∑ < 0.5	129	43.43
Rubi	105	24.58	9.49
	1 – 0.5	105.6	40.79
	∑ < 0.5	128.7	49.72
Westin	107	15.9	9.94
	1 – 0.5	73.5	45.95
	∑ < 0.5	70.5	44.11
Sincorá	924.3	4.87	1.71
	100.39	30.76	10.80
	1 - 0.5	134.2	47.11
	∑ < 0.5	115	40.39
Lima	108	19.79	6.16
	1 - 0.5	155.9	48.49
	∑ < 0.5	145.8	45.36
Baia	880	1.88	0.59
	109	33.28	10.55
	1 - 0.5	140.5	44.55
	∑ < 0.5	126.8	40.21
Comum	913	1.34	0.39
	110	37.96	10.45
	1 - 0.5	261.5	72.02
	∑ < 0.5	62.2	17.12
Mimo	109	43.35	11.25
	1 – 0.5	225.3	58.49
	∑ < 0.5	116.5	30.26
Pera	109.84	33.91	16.82
	1 – 0.5	119.2	59.13
	∑ < 0.5	48.47	24.05

Bioactive peptides have several biological functions that have been proven in previous research, including antihypertensive, antioxidant, anti-inflammatory, immunomodulatory, hypolipidemic, anti-diabetic, anti-cancer and anti-adhesive activities (Sun and Wu, 2017, Udenigwe & Fogliano, 2017, Xu et al., 2019). Then it is possible to state that bioactive peptides, alone or in combination with other compounds, can be used in the formulation of efficient functional foods for the prevention and treatment of diseases (Sun et al. 2020), pointing to the enormous potential of orange peels to compose as part of a functional diet.

However, these biological activities depend mainly on the structural properties of the peptide, such as amino acid composition, sequence, molecular size, surface and hydrophobicity, liquid charge and spatial conformation (Udenigwe & Fogliano, 2017).

According to Grootaert et al (2017), the presence of digestive enzymes and the food matrix strongly affect the integrity of the intestinal bioactive peptide. In this study, however,

it is possible that the enzymes used in obtaining the soluble fibers may have interfered with the composition of the peptides.

3.9 Minerals

The orange peels are representative sources of minerals (Table 8) such as calcium (466.07 mg.100g^{-1}), potassium (1472 mg.100g^{-1}), magnesium (76.26 mg.100g^{-1}) and phosphorus (55.01 mg.100g^{-1}), essentials in the human diet. The two oranges that stood out the most were Bahia and Common with the highest content of calcium (594.99 and 553.03 mg.100g^{-1}) and magnesium (122.83 and 121.92 mg.100g^{-1}, in that order) . The 'Pera' peel showed greater prominence in iron (2.07 mg.100g^{-1}), potassium (1472.67 mg.100g^{-1}), phosphorus (95.43 mg.100g^{-1}) and zinc (0,67 mg.100g^{-1}), which indicates the potential of the flour of orange peels for supplying essential minerals to the diet.

The overall mean of minerals present in the peels studied in here is close to the levels reported by Barros et al (2012) who reported 488.89 and 495.21 mg.100g^{-1} of calcium, 3.40 and 2.19 mg.100g^{-1} of iron, 871.0 and 796.41 mg.100g^{-1} of potassium and 80.13 and 83.23 mg.100g^{-1} of magnesium in the peel of Lima and Pera oranges, respectively. However, the mineral contents of this work are higher than those reported by Czech et al (2019) who reported 131.35 mg.100g^{-1} for calcium, 1.60 mg.100g^{-1} for iron, 482.76 mg.100g^{-1} for potassium, 41.38 mg .100g^{-1} of magnesium and 79.31 mg.100g^{-1} of phosphorus in the Navelina orange peel.

Tabela 8. Mineral contents (mg.100g^{-1}) in the peel of ripe orange cultivars

	Calcium	**Iron**	**Potassium**	**Magnesium**	**Phosphorus**	**Aluminum**	**Boron**	**Sodium**	**Zinc**
Baianinha	359,98 ± 24,81^{f}	1,00 ± 0,05^{bc}	798,18 ± 14,70^{e}	109,07 ± 1,45^{bc}	38,37 ± 3,15^{d}	2,32 ± 0,23^{f}	2,51 ± 0,55^{a}	43,39 ± 6,56^{bc}	0,04 ± 0,00^{c}
Salustiana	389,24 ± 1,85^{ef}	0,48 ± 0,19^{bc}	853,13 ± 16,97^{cde}	100,08 ± 2,09^{cd}	44,66 ± 1,44^{cd}	2,66 ± 0,07^{ef}	2,43 ± 0,25^{a}	95,11 ± 1,74^{a}	0,01 ± 0,00^{c}
Rubi	467,00 ± 8,40^{cd}	0,78 ± 0,09^{bc}	792,42 ± 10,29^{e}	107,71 ± 1,56^{bc}	38,32 ± 2,27^{d}	3,40 ± 0,01^{cd}	2,26 ± 0,34^{a}	48,14 ± 3,57^{b}	0,14 ± 0,01^{bc}
Westin	382,85 ± 3,60^{ef}	1,28 ± 0,24^{ab}	920,17 ± 16,81^{c}	104,30 ± 1,21^{cd}	49,56 ± 1,6^{c}	2,51 ± 0,00^{ef}	2,48 ± 0,14^{a}	33,25 ± 1,05^{cd}	0,19 ± 0,02^{b}
Sincorá	485,46 ± 11,45^{cd}	2,00 ± 0,37^{a}	696,55 ± 0,01^{f}	109,62 ± 1,33^{bc}	38,23 ± 2,12^{d}	3,53 ± 0,12^{cd}	2,16 ± 0,18^{a}	29,09 ± 2,74^{de}	0,09 ± 0,02^{bc}
Lima	492,36 ± 22,92^{bc}	0,35 ± 0,19^{c}	814,58 ± 32,55^{de}	115,31 ± 3,43^{ab}	35,59 ± 3,18^{d}	3,84 ± 0,22^{bc}	2,08 ± 0,18^{a}	25,02 ± 2,55^{def}	0,05 ± 0,00^{c}
Baia	594,99 ± 2,21^{a}	0,89 ± 0,08b^{c}	905,98 ± 22,45^{cd}	122,83 ± 1,5^{a}	71,49 ± 2,05^{b}	4,61 ± 0,02^{a}	1,78 ± 0,21^{a}	16,50 ± 0,90^{f}	0,09 ± 0,07^{bc}
Comum	553,03 ± 11,22^{ab}	0,17 ± 0,1^{c}	1115,91 ± 20,79^{b}	121,92 ± 1,38^{a}	51,26 ± 3,54^{c}	4,31 ± 0,04^{ab}	1,65 ± 0,33^{a}	28,07 ± 2,59^{def}	0,07 ± 0,00^{bc}
Mimo	509,76 ± 27,96^{bc}	0,29 ± 0,39^{c}	1094,95 ± 48,62^{b}	94,75 ± 4,18^{d}	87,24 ± 2,44^{a}	3,90 ± 0,40^{bc}	1,61 ± 0,36^{a}	18,11 ± 1,63^{ef}	0,14 ± 0,04^{bc}
Pera	426,06 ± 22,01^{de}	2,07 ± 0,30^{a}	1472,67 ± 13,03^{a}	76,26 ± 3,56^{e}	95,43 ± 1,91^{a}	3,01 ± 0,14^{de}	1,90 ± 0,27^{a}	17,36 ± 1,24^{f}	0,67 ± 0,04^{a}
Mean	**466,07**	**0,93**	**946,45**	**106,18**	**55,01**	**3,41**	**2,09**	**35,40**	**0,15**
DRI (mg)	**50000**	**14**	**3500***	**260**	**700**	**ND**	**ND**	**200****	**7**

Means followed by the same lower case letter in the column did not differ by the Tukey test at 5% ($p \leq 0,05$). DRI: Daily recommended daily intake by ANVISA. *Minimum daily intake recommendation according to WHO. **Maximum daily intake recommendation according to WHO. ND: not determined.

With all this mineral richness of orange peels, it can be said that their consumption can significantly help to meet the needs of human food for these minerals, since the National Health Surveillance Agency (ANVISA) (Brazil 2005) recommends consumption 5000 mg of calcium, 14 mg of iron, 260 mg of magnesium and 700 mg of phosphorus.

In the future, the orange peel flour can be used as ingredients for the development of functional foods rich in fibers, minerals and natural antioxidants (Barbosa, Ruviaro, & Macedo 2018). Furthermore, in addition to having a large content of fibers and minerals that are essential for the human diet, they also have important phenolic compounds for the proper functioning of human metabolism.

3.9. Phenolic Profile of Gastrointestinal Simulation

The gastrointestinal process was evaluated only for the Mimo cultivar since it had the highest content of chlorogenic acid and hesperidin (main phenolic of citrus) in the extract of free phenolics. Through an *in vitro* simulation, it was possible to quantify six phenolic compounds, cafeic, chlorogenic and gallic acids, hesperidin, synapic acid and tangeritin (Table 6).

Tabela 9. Phenolic compounds in the simulation of gastrointestinal digestion in the peel of Mimo' orange ($mg.100g^{-1}$)

Fase	Caffeic Acid	Clorogenic Acid	Gallic Acid	Hesperidin	Sinapic Acid	Tangeritin
Initial	$38,18 \pm 0,75^{a}$	$81,11 \pm 2,52^{a}$	nd	$183,90 \pm 5,48^{a}$	$5,33 \pm 2,91^{b}$	$0,44 \pm 0,2^{a}$
Oral	$47,15 \pm 2,44^{a}$	$71,24 \pm 3,56^{ab}$	$17,84 \pm 0,46^{ab}$	$142,49 \pm 11,99^{ab}$	$0,92 \pm 0,8^{c}$	$0,60 \pm 0,10^{a}$
Gastric	$35,25 \pm 9,43^{a}$	$53,64 \pm 11,86^{c}$	$21,78 \pm 3,36^{a}$	$132,75 \pm 24,23^{b}$	$6,51 \pm 0,35^{ab}$	$1,01 \pm 0,03^{a}$
Intestinal	$39,85 \pm 3,86^{a}$	$62,98 \pm 2,55^{bc}$	$16,08 \pm 0,64^{b}$	$84,24 \pm 23,33^{c}$	$10,41 \pm 0,21^{a}$	$2,82 \pm 3,22^{a}$

Means followed by the same lower case letter in the column did not differ by the Tukey test at 5% ($p \leq 0,05$). n=4. nd= not detected.

The content of caffeic acid did not change significantly during the gastrointestinal simulation phases and, therefore, did not differ statistically ($p \leq 0.05$). However, chlorogenic acid was found in greater content in the initial phase (81.11 mg.100g^{-1}), without addition of enzymes and in the oral phase (71.24 mg.100g^{-1}) after the action of α-amylase. Hesperidin also followed the same pattern, with greater content being released in the initial phase (183.90 mg.100g^{-1}) followed by oral (142.49 mg.100g^{-1}).

Gallic acid was not present in the initial fraction and the highest contents were found in the oral (17.84 mg.100g^{-1}) and gastric (21.78 mg.100g-1) phases. In turn, synapic acid was mostly quantified in the intestinal (10.41 mg.100g^{-1}) and gastric (6.51 mg.100g-1) phases.

According to Sun et al. (2020) the phytochemicals present in the extracts do not always reflect the content available to be absorbed and metabolized by the human body, so it is important to understand the bioaccessibility of phytochemicals in citrus fruits and develop a more biologically relevant approach to assess their potential.

In vitro gastrointestinal simulation is a valuable tool for investigating the effect of digestion on polyphenols bioaccessibility to obtain essential data and support the claims of biological relevance of food for human health (Haas et al., 2019). According to Sun et al. (2020) the antioxidant activity of samples of digested citrus fruits is greater than that quantified in samples of fresh fruit before digestion and this fact is attributed to a greater release of some phenolic and carotenoid compounds. This is possibly due to the fact that the polyphenols present in food are generally linked to carbohydrates, fibers and proteins and during gastrointestinal digestion these compounds stand out and become more bioaccessible (Jakobek 2015; Thakur et al., 2020).

3.10 Pearson's Correlation

Through Pearson's correlation it was observed that the antioxidant activity by the three evaluated methods (ABTS, DPPH and ORAC), presented a strong positive correlation with

the total extractable polyphenols (PET), which also correlate in a positive and moderate way, with the tangeritin, and this last, in turn, also correlates positively with PET.

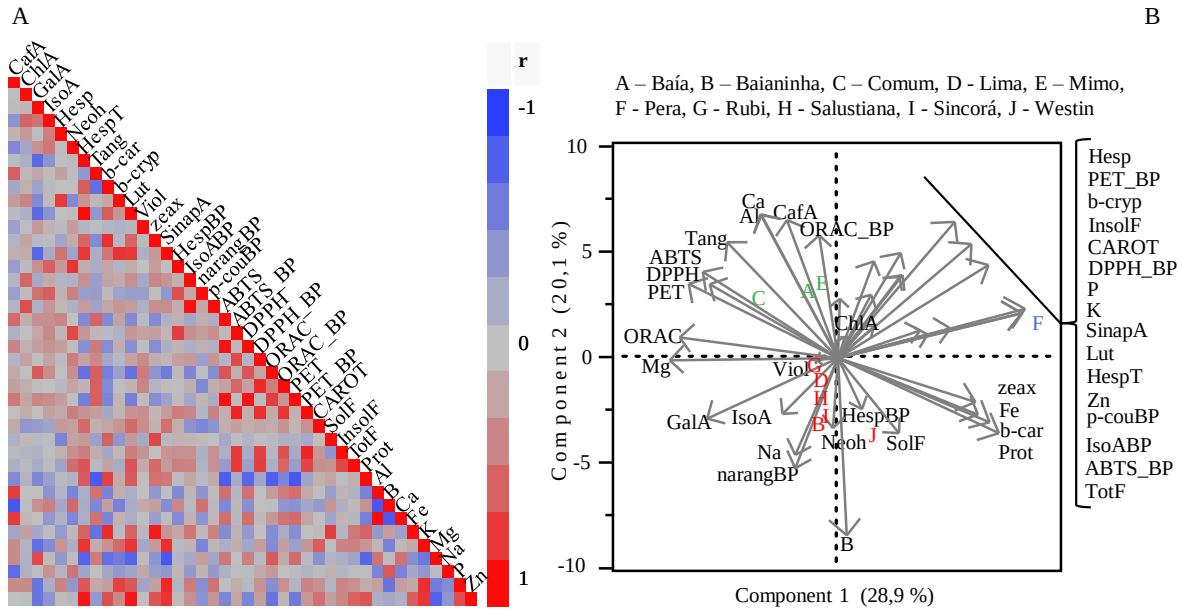

Figura 4. Pearson correlation (A) and Principal Components Analysis (B) of orange peel cultivars.

CafA: Cafeic Acid; **ChlA:** Clorogenic Acid; **GalA:** Galic Acid; **IsoA:** Isoferulic Acid; **Hesp:** hesperidin; **Neoh:** Neohesperidin; **HespT:** hesperitin; **Tang:** Tangeritin; **b-car:** Beta-Carotene; **b-cryp:** Beta-Cryptoxantin; **Lut:** Lutein; **Viol:** Violaxantin; **Zeax:** Zeaxantin; **SinapA:** Sinapic Acid; **HespBP:** hesperidin, Bound phenolics extract; **IsoABP:** Isoferulic Acid, Bound phenolics extract; **narangBP:** Narangenin, Bound phenolics extract; **P-couBP:** p-Coumaric Acid, Bound phenolics extract; **ABTS:** Antioxidant activity radical method ABTS; **ABTS_BP:** Antioxidant activity radical method ABTS, Bound Phenolics Extract; **DPPH:** Antioxidant activity radical method DPPH; **DPPH_BP:** Antioxidant activity radical method DPPH, Bound Phenolics Extract; **ORAC:** Antioxidant activity method ORAC; **ORAC_BP:** Antioxidant activity method ORAC, Bound Phenolics Extract; **PET:** Total free extractable polyphenols in methanol; **PET_BP:** Total extractable polyphenols, Bound Phenolics; **CAROT:** Total Carotenoids, **SolF:** Soluble Fiber; **InsolF:** Insoluble Fiber; **TotF:** Total Fiber; **Prot:** Proteinas; **Al:** Aluminum; **B:** Boro; **Ca:** calcium; **Fe:** Iron; **K:** Potassium; **Mg:** Magnesium; **Na:** Sodium; **P:** phosphorus; **Zn:** Zinc

The same pattern was observed for the extract of the bound phenolics, in which the antioxidant activity by the three evaluated methods was positively correlated with the total extractable polyphenols of the bound phenolics (PET_BP).

Other outstanding results are the insoluble and total fibers, which were positively correlated, from moderate to strong, with the antioxidant activity and with the total extractable polyphenols. This means that many phenolic compounds are retained in the insoluble fibers and that the peel s of orange cultivars, which have a higher percentage of soluble fibers are able to provide more free phenolics.

Iron had a strong positive correlation with soluble fibers and with two carotenoids, zeaxanthin and β-carotene, in addition to a moderate positive correlation with lutein. Another mineral element that also was strongly positively correlated with lutein was potassium. In addition, potassium had a strong positive correlation with synapic acid and moderate with β-cryptoxanthin and total carotenoids.

In the principal component analysis (PC) three components were responsible for explaining an accumulated variance of 65% (Figure 6B), TEP, caffeic acid, tangeritin, in addition to the antioxidant activity contributed to form CP1. In turn, CP2 was formed by violaxanthin, gallic and isoferrulic acids, neohesperidine, soluble fibers, boron, sodium, in addition to the bound phenolics hesperidin and narangenin. CP3 was formed by the other carotenoids, in addition to the bound phenolics, minerals and other compounds evaluated.

Through the covariance matrix it was possible to observe the formation of three groups in which the cultivars were grouped by similarity. The peels of the cultivars Baía, Mimo and Comum formed the Group 1. Baianinha, Lima, Rubi, Salustiana, Sincorá and Westim formed the Group 2 and the cultivar Pera alone formed the Group 3. Group 1 corresponds to the Criole oranges produced in the Borborema Territory, this separation indicates that the peel of

these oranges have significant differences in the phenolic and carotenoids contents and in the antioxidant activity, presenting a much higher functional potential than the other cultivars evaluated herein.

4 CONCLUSIONS

Flour from orange peels can serve as a functional food, as it provides fibers, minerals, bioactive peptides and phenolic compounds for human consumption. Furthermore, the peels of oranges can be used to extract phenolic compounds for future application as ingredients for the development of functional foods and extraction of natural antioxidants that prevent oxidative changes, to be added to foods, replacing synthetic antioxidants.

The peel of oranges is rich in phenolic compounds and has high antioxidant activity. Basd on that, the cultivars Baía, Comum and Mimo formed an isolated group from the other varieties in the principal components analysis due to its superior fuctional quality.

The soluble fiber of the orange peels has peptides with high molecular weights that can be of importance for a functional diet.

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Artigo 3. Perfis de fenólicos, fibras e minerais do albedo de variedades de laranjas

Directed to: Food Research International

Resumo

O albedo, porção esponjosa e de coloração branca do interior da casca da laranja, que é geralmente descartado no consumo da fruta fresca ou na indústria de suco, tem potencial de ser utilizado para consumo direto ou aproveitado na indústria de alimentos e farmaceutica. O objetivo deste trabalho foi avaliar os perfis de fenólicos, fibras e minerais do albedo de dez cultivares de laranjas produzidas no Território da Borborema, Nordeste do Brasil. Farinha seca de albedo foi utilizada para investigar os polifenóis extraíveis totais, os perfis de fenólicos, minerais e fibras, além da atividade antioxidante (AAT) pelos métodos de ABTS, DPPH e ORAC. Em matéria seca, o albedo possui em média 1.0 g.GAE.100g^{-1} de polifenóis extraíveis totais, a hesperidina, presente em todos, é o flavonoide majoritário (264.82 mg.100g^{-1}) na 'Baianinha', 'Pera' e 'Comum', nesta ordem, seguido pela neohesperidina (66.29 mg.100g^{-1}), na 'Pera', os quais podem ser utilizados como marcadores de autenticidade. Pelo método do ORAC o albedo exibiu maior AAT (273.98 μM Trolox.mg^{-1}). É composto de 62.93% de fibras, sendo 23.63% de fibras solúveis e 39.30% insolúveis. Além disso, apresenta 4.22 mg.100g^{-1} de proteínas, 802.0 mg.100g^{-1} de potássio, 411.71 mg.100g^{-1} de cálcio, 88 mg.100g^{-1} de magnésio e 40.27 mg.100g^{-1} de potássio. O albedo de cultivares de laranjas cultivadas noo Território da Borborema é fonte de fibras dietéticas, minerais e flavonoides importantes para a saúde humana, podendo ser utilizado como ingrediente de dietas funcionais.

Palavras-chave: Resíduos de frutas, hesperidina, neohespiridina, fibra solúvel, fibras insolúveis, potássio.

1 INTRODUÇÃO

O grande volume de processamento de suco de laranja gera uma quantidade significativa de resíduos depositada anualmente no meio ambiente e principalmente nas regiões mais produtoras. A ausência de tecnologias aproveitamento e de informações a respeito do potencial deste subproduto leva a consideráveis perdas econômicas além de prejuízos ambientais (Silva et al., 2019A). Para minimizar os custos produção e evitar danos ambientais, buscam-se métodos de recuperação de resíduos que contribuam para o crescimento da bioeconomia e minimizem os efeitos ambientais negativos relacionados à agroindústria, gerando produtos voltados para as indústrias alimentícia, cosmética e farmacêutica (Sharma et al., 2017, Zema et al., 2018).

A casca de laranja corresponde a maior parte dos resíduos descartados pelas indústrias e é composta de flavedo (exocarpo) e albedo (mesocarpo). O albedo é um tecido celulósico branco, esponjoso, logo abaixo da casca e é rico em fibras de melhor qualidade do que outras fontes de fibras alimentares devido à presença de compostos bioativos associados com propriedades antioxidantes, que podem fornecer efeitos adicionais de promoção da saúde (Marín et al., 2007; Sharma et al., 2017).

Além disso, a relação entre consumo regular de fibras e saúde é bastante reconhecido por esta desempenhar um papel fundamental na prevenção de doenças, como constipação, hemorróidas, hipercolesterolemia e câncer colorretal (Fernández-López et al., 2004; Marín et al., 2007). Assim as fibras alimentares são recomendadas para manter a saúde digestiva adequada e consequentemente promover uma boa qualidade de vida diária (Rao & Quartarone, 2019). Além disso, estudo recente por Ramin et al (2020) sugere que a ingestão de fibras minimiza o risco de sintomas depressivos e promove uma melhor qualidade de vida em saúde mental.

Nas laranjas, o albedo é a fonte mais importante de fenólicos totais, além do maior

conteúdo de flavanonas glicosiladas e maior atividade antioxidante (Escobedo-Avellaneda et al., 2014). Dentre as variedades de laranja produzidas por agricultores familiares do Território da Borborema, estado da Paraíba, Nordeste do Brasil, algumas especificamente possuem maior espessura do albedo como o caso das laranjas Baia, Baianinha e Comum. Estas variedades além de possuírem alto padrão de qualidade para consumo fresco e processamento, seu albedo também possuem altos teores de polifenois totais e alta atividade antioxidante (Silva et al., 2019B). No entanto, para nosso conhecimento não existem informações mais detalhadas de perfil de compostos fenólicos, nem tampouco de fibras e minerais do albedo destas variedades.

Assim, o objetivo deste trabalho foi avaliar o perfil de fenólicos, fibras e minerais do albedo de dez cultivares de laranjas produzidas no Território da Borborema, estado da Paraíba.

2 MATERIAIS E MÉTODOS

Laranjas (Citrus sinensis) das cultivares Baianinha, Salustiana, Rubi, Westin, Sincorá, Lima, Baía, Comum, Mimo e Pera foram colhidas ao acaso, entre 6 e 8h, na fase de maturação C3 - predominantemente amarela, segundo o CEAGESP Standards (2011), de um pomar comercial da agricultura familiar localizado no município de Alagoa Nova, Território da Borborema, Nordeste do Brasil. A propriedade rural possui solo predominante do tipo Argissolo Vermelho Eutrófico Abrupto (EMBRAPA, 2016).

Os frutos foram acondicionados em caixas de polietileno de alta densidade com capacidade para 18 Kg, protegidas com plástico bolha e transportados para o laboratório. Os frutos foram processados em espremedor de frutas cítricas (Arno Citrus Power PA 32) e o albedo separado da casca manualmente com faca afiada e deixado secar durante a noite em estufa de circulação forçada a 50 ° C até atingir peso constante (Tecnal, modelo 1513D).

Após a secagem, as cascas foram maceradas em almofariz e pistilo e moídas em processador elétrico até a formação de uma farinha fina, a qual foi embalada a vácuo e armazenada em geladeira até a análise.

O experimento foi conduzido em delineamento inteiramente casualizado (DIC) com albedo de dez cultivares de laranja e quatro repetições para cada cultivar.

2.1 Fenólicos Livres

Os extratos dos compostos fenólicos foram feitos com etanol aquoso (800 ml / L), que foi adicionado a 1 grama de albedo seco. Foi homogeneizado em Ultra-Turrax (IKA Ultra-Turrax T18, Wilmington, EUA) por 2 min. A mistura foi centrifugada (5000 rpm durante 20 min, a 4 ° C). O sobrenadante foi avaliado quanto à atividade antioxidante, fenólicos totais por espectrofotometria e o perfil fenólico por HPLC-DAD.

2.2 Capacidade antioxidante - testes DPPH, ABTS e ORAC

A metodologia para DPPH foi baseada em Bobo-Garcia et al. (2014) com algumas modificações. Resumidamente, 20 μL de extrato foram adicionados a uma placa de 96 poços contendo 180 μL de solução DPPH (2,2-difenil-1-picril-hidrazil; Sigma Chemical Company, St. Louis, MO, EUA) (150 μM). A absorvância foi medida a 515 nm em leitor de microplaca (microplaca Spectrophotometer Multiskan GO, Thermo Scientific, Thermo Fisher Scientific Inc., EUA) após 40 minutos no escuro. Os padrões Trolox (Sigma Chemical Company, St. Louis, MO, EUA) (0 a 500 μM) foram preparados e analisados da mesma forma para a obtenção da curva de calibração. A porcentagem de inibição foi determinada e os extratos expressos em mg equivalente de Trolox (TE) / g DW.

O teste de eliminação de ABTS também foi realizado em microplaca de 96 poços, seguindo o método descrito por Gonçalves et al. (2009) com algumas modificações. A 20 μL da amostra ou Trolox ou solvente foram adicionados 180 μL de ABTS • + solução de trabalho. A mistura foi incubada durante 5 min a 30 ° C e a absorvância a 734 nm foi medida

com o leitor de placas Multidetection (Synergy H1, Vermont, EUA).

A capacidade de absorção do radical de oxigênio (ORAC) foi realizada em microplaca preta de 96 poços (Nunc, Dinamarca), seguindo o método descrito por Dávalos et al. (2004) com algumas modificações. A reação foi realizada com tampão fosfato 75 mM (pH 7,4) e a mistura de reação final foi de 200 µL. Os antioxidantes (20 µL) e a fluoresceína (120 µL; 70 nM, concentração final) foram colocados no poço da microplaca. Um branco (FL + AAPH) usando tampão fosfato em vez da solução antioxidante e oito soluções de calibração usando Trolox (1-8 uM, concentração final) como antioxidante também foi realizado em cada ensaio. A mistura foi pré-incubada por 10 min a 37 ° C. A solução AAPH (60 µL; 12 mM, concentração final) foi adicionada rapidamente usando uma pipeta multicanal. A microplaca foi imediatamente colocada no leitor e a fluorescência foi registrada em intervalos de 1 minuto ao longo de um período de 140 minutos.

O teste também foi realizado com um leitor de placas de detecção múltipla (Synergy H1, Vermont, EUA). O comprimento de onda de excitação foi fixado em 485 nm e o comprimento de onda de emissão em 528 nm (Ubeda et al., 2011). A microplaca foi agitada automaticamente antes de cada leitura.

2.3 Perfil de Fibra

Um grama de cada amostra foi usado para determinar a fibra alimentar. Os procedimentos foram realizados para retirada de gordura, com 25 mL de N-hexano e açúcares com 20 mL de etanol 80%, para não interferir no processo de tratamento enzimático de separação das fibras.

Adicionou-se 40 mL de tampão (MES / Tris, 0,05 M), pH 8,2 e as amostras foram colocadas em banho-maria a 100 ° C com 50 uL de α-amilase com agitação constante por 30 minutos. Após esta etapa, a temperatura foi baixada para 60 ºC e foram adicionados 10 mL de água deionizada e 100 µL e deixados a 60 ºC por 30 minutos. Seguindo essa etapa, 5 mL de

0,56 N HCl foram adicionados e o pH foi ajustado entre 4,1 e 4,8 com 5% NaOH ou 5% HCl; imediatamente após a adição de 200 µL de amiloglicosidase e a manutenção a 60ºC por 30 minutos.

O material foi filtrado através da placa porosa do cadinho de Gooch, a fração líquida contendo fibra solúvel foi liofilizada para quantificar a fibra solúvel e a fibra insolúvel que foi levada ao forno de secagem

3 RESULTADOS E DISCUSSÃO

Neste trabalho o albedo de dez cultivares de laranjas foram extraídos e avaliados quanto aos perfis de fenólicos, fibras, minerais e potencial antioxidante. Os resultados sugerem que o albedo possui importantes compostos funcionais que podem contribuir para uma dieta alimentar saudável, agregando valor para a citricultura familiar do Território da Borborema.

3.1 Polifenóis extraíveis totais (PET)

O albedo das cultivares de laranjas (figura 1) possui uma quantidade de polifenóis extraíveis totais em média de 1.00 g.GAE.100g^{-1} por matéria seca (DM) e os maiores teores foram encontrados no albedo das cultivares ‘Rubi’ 1,26 g.GAE.100g^{-1} e ‘Baianinha’ 1.22 g.GAE.100g^{-1} as quais diferiram estatisticamente das demais ($p \leq 0,05$). Além destas o albedo das cultivares Salustiana, Baia e Lima também a possuem teores acima da média geral com 1.12; 1.06 e 1.02 g.GAE.100g^{-1} respectivamente.

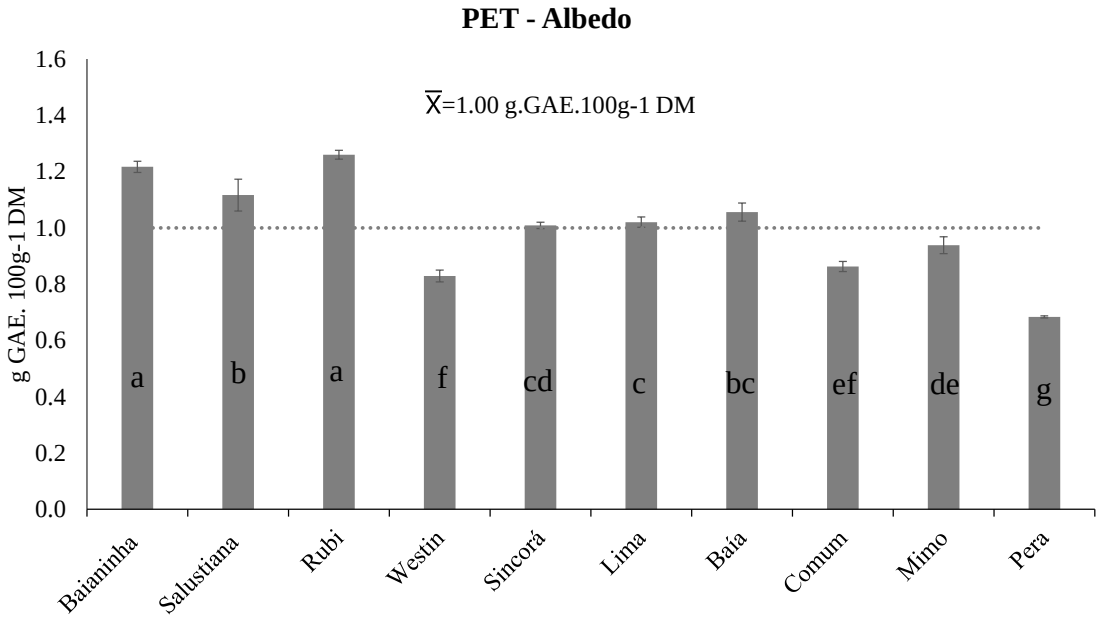

Figura 1. Polifenois Extraíveis Totais (PET) do albedo de cultivares de laranjas produzidas no Nordeste do Brasil em base seca. Barras com letras iguais não diferem estatisticamente pelo teste de Tukey a 5% ($p \leq 0,05$). n=4

Nossos resultados são aproximados aos de Nayak et al (2015) em cascas de laranja que encontrou teores de polifenois variando de 0.8 a 1.2 g.GAE.100g^{-1} de matéria seca (DM). E um pouco inferior ao encontrado por Escobedo-Avellaneda et al. (2014) em albedo de laranja 'Valência' que variou de 1.37 a 2,28 g.GAE.100g^{-1} DM.

Variações na quantidade de fenólicos nos frutos, podem ser atribuídas as características intrínsecas das diferentes cultivares, além das varias condições de cultivo e das diferenças nos solventes utilizados para a extração (Wang et al., 2016).

3.2 Perfil de compostos fenólicos (HPLC-DAD)

A composição fenólica do albedo da laranja é muito abundante (Tabela 1), quantificamos os ácidos Cafeico (17,00 mg.100g^{-1}), Clorogenico (31,46 mg.100g^{-1}), Galico (26,95 mg.100g^{-1}) e o ácido Sinapico (17,67 mg.100g^{-1}) encontrado apenas no albedo das cultivares 'Baía'(16,21 mg.100g^{-1}), 'Comum' (17,73 mg.100g^{-1}) e 'Mimo' (19,07 mg.100g^{-1}), além dos flavonoides Hesperidina (264,82 mg.100g^{-1}), Neohesperidina (66,29 mg.100g^{-1}).

Tabela 10. Perfil de compostos fenólicos livres do albedo de cultivares de cultivares de laranjas produzidas no Nordeste do Brasil (mg.100g^{-1}) em base seca

Cultivar	Caffeic Acid	Clorogenic Acid	Gallic Acid	Sinapic Acid	Hesperidin	Neohesperidin
Baianinha	9,37 ± 0,00^{f}	16,09 ± 0,24^{d}	40,46 ± 7,6^{a}	nd	478,04 ± 0,46^{a}	65,61 ± 0,34^{e}
Salustiana	25,43 ± 0,00^{b}	51,78 ± 4,53^{a}	29,98 ± 0,11^{b}	nd	330,30 ± 1,71^{d}	80,98 ± 0,17^{b}
Rubi	22,38 ± 0,20^{c}	40,26 ± 0,08^{b}	40,11 ± 0,7^{a}	nd	261,53 ± 0,13^{e}	56,96 ± 0,08^{h}
Westin	25,63 ± 0,28^{b}	56,09 ± 4,77^{a}	25,53 ± 0,13^{bc}	nd	218,28 ± 0,93^{g}	63,17 ± 0,76^{f}
Sincorá	15,47 ± 0,10^{d}	26,67 ± 0,08^{c}	27,66 ± 0,02^{bc}	nd	160,77 ± 0,1^{h}	47,62 ± 0,02^{j}
Lima	30,28 ± 0,07^{a}	44,76 ± 0,01^{b}	24,49 ± 0,00^{bc}	nd	222,12 ± 0,23^{f}	54,11 ± 0,11^{i}
Baia	4,63 ± 0,08^{g}	7,57 ± 0,01^{e}	22,00 ± 0,01^{c}	16,21 ± 0,01^{c}	150,93 ± 0,10^{i}	60,44 ± 0,01^{g}
Comum	12,28 ± 0,00^{e}	21,06 ± 0,00^{cd}	25,50 ± 0,00^{bc}	17,73 ± 0,09^{b}	346,90 ± 0,09^{c}	74,21 ± 0,2^{c}
Mimo	22,51 ± 0,01^{c}	43,31 ± 0,01^{b}	20,98 ± 0,01^{c}	19,07 ± 0,52^{a}	97,59 ± 0,14^{j}	66,92 ± 0,03^{d}
Pera	2,07 ± 0,06^{h}	7,02 ± 0,13^{e}	12,78 ± 0,13^{d}	nd	381,74 ± 3,30^{b}	92,82 ± 1,01^{a}
Mean	17.00	31.46	26.95	17.67	264.82	66.29

Médias com letras iguais na coluna, não diferem estatisticamente pelo teste de Scott-Knott a 5% ($p \leq 0,05$). nd= não determinado.

Hesperidina foi predominante no albedo das laranjas avaliadas, um flavonoide de importância para a indústria farmacêutica, que tem sido foco de diversos estudos pelos efeitos benéficos à saúde, dentre eles, a prevenção contra doenças cardiovasculares, proteção contra câncer e supressão do estresse oxidativo (Sahu et al. 2013; Kaltalioglu et al. 2019; Celano et al. 2019; Sánchez-Recillas et al. 2019), além também apresentar atividades gastroprotetora que pode inclusive curar ulcéras (SILVA et al. 2019A).

O albedo da cultivar Baianinha apresenta o maior teor de hesperidina (478,04 $mg.100g^{-1}$) diferindo estatisticamente das demais cultivares aqui avaliadas ($p \leq 0,05$), seguido das cultivares Pera (381.74 $mg.100g^{-1}$) e Comum (346 $mg.100g^{-1}$).

A neohesperidina foi o segundo maior flavonoide presente no albedo das laranjas, com destaque para a 'Pera' (92.82 $mg.100g^{-1}$) que apresentou maior teor, com diferença estatística das demais ($p \leq 0,05$) seguidos das cultivares Salustiana (80.98 $mg.100g^{-1}$) e Comum (74.21 $mg.100g^{-1}$). Este flavonoide, um glicosídeo flavanona, é encontrado em frutas cítricas, que proporciona um forte sabor amargo (Zhang 2012). Recentemente, Tan et al. (2017) descobriram que a neoesperidina inibe o desenvolvimento da osteoporose, enquanto Gong et al (2019) que inibe o aparecimento de tumores colorretal por meio de alterações na microbiota intestinal. Neste sentido, pela primeira vez, um amplo espectro do potencial do albedo destas cultivares e laranjas produzidas na região é aqui apresentado. O impacto do potencial destes flavonoides no albedo de laranjas é fortemente destacado com base nos resultados de Guo et al (2019) que demonstraram que a neohesperidina inibe a sinalização do TGF-β1/Smad3 e alivia fibrose pulmonar idiopática, que, muito recentemente, foi considerada por Guan et al (2020) como uma das possíveis sequelas deixadas pela infecção em humanos afetados pelo vírus da Covid-19, o qual vem assolando todos os continentes.

O teor de ácido gálico foi maior para o albedo das cultivares Baianinha (40.46 $mg.100g^{-1}$) e Rubi (40.11 $mg.100g^{-1}$) os quais diferiram estatisticamente entre as demais, mas foram

iguais entre si ($p \leq 0,05$).

O acido clorogenico está presente em maiores teores no albedo das cultivares Westin (56.09 $mg.100g^{-1}$) e Salustiana (51.78 $mg.100g^{-1}$) as quais diferiram estatisticamente das demais ($p \leq 0,05$). Já para o acido cafeico o maior teor foi quantificado na cultivar Lima (30.28 $mg.100g^{-1}$) a qual diferiu estatisticamente das demais avaliadas aqui ($p \leq 0,05$). E o acido sinapico foi quantificado apenas no albedo das cultivares crioulas Mimo (19.07 $mg.100g^{-1}$), Comum (17.73 $mg.100g^{-1}$) e Baia (16.21 $mg.100g^{-1}$) ($p \leq 0,05$).

Diversos estudos confirmam que os flavonoides e ácidos fenólicos presentes nos citrus, possuem bioatividade contra diversas enfermidades, incluindo anti-hiperlipidemia, anti-obesidade, antivírus, anti-câncer, anti-inflamação, anti-alérgeno e analgesia (Guo et al. al., 2016 , Mallick e Khan, 2016 , Mannucci et al., 2017). Pesquisa recente afirma que os flavonóides cítricos, também são promissores no tratamento da desregulação metabólica e da esteatose hepática (gordura no fígado) (Jiang et al. 2019).

Portanto podemos afirmar que o consumo do albedo de laranja regularmente, pode trazer benefícios a saúde humana, diminuindo inclusive os efeitos de doenças degenerativas devido as novas perspectivas sobre o efeito quimiopreventivo de alguns flavonoides encontrados aqui (Gong et al., 2019).

3.3 Atividade Antioxidante do albedo de laranjas pelos métodos de ABTS, DPPH e ORAC

O albedo apresentou em media geral 28,85 μM $Trolox.mg^{-1}$ de atividade antioxidante pelo método de ABTS (figura 2). As cultivares Baianinha e Rubi possuem os maiores teores com 94.67 μM $Trolox.mg^{-1}$ e 32.74 μM $Trolox.mg^{-1}$ respectivamente as quais diferiram estatisticamente das demais, entretanto não apresentaram diferença significativa entre si ($p \leq 0,05$). A cultivares Salustiana (30.59 μM $Trolox.mg^{-1}$) e Baia (30.00 μM $Trolox.mg^{-1}$) também possuem teores acima da média geral.

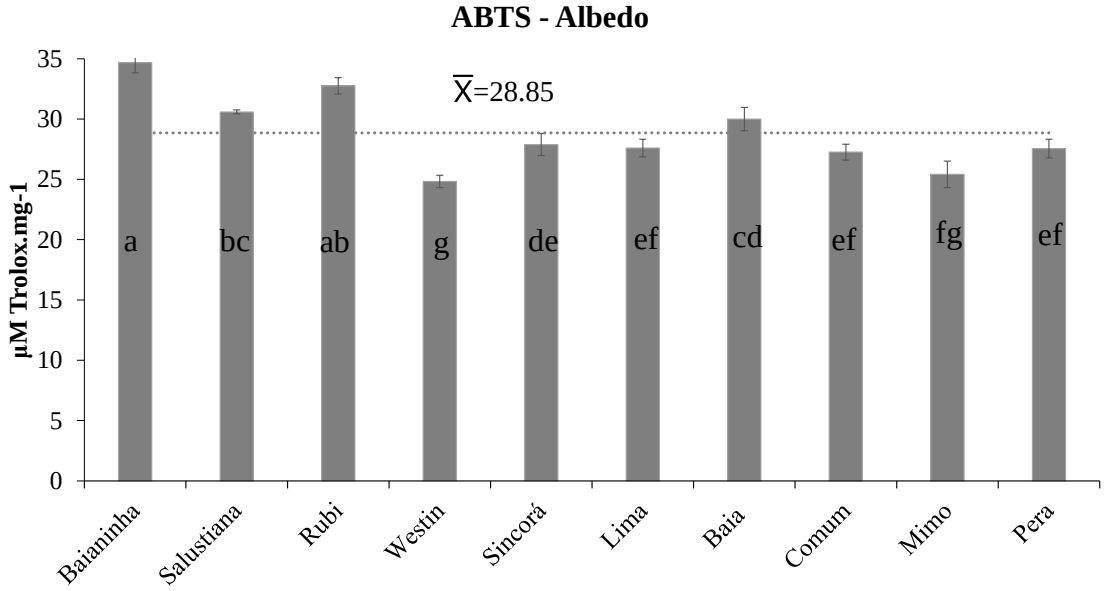

Figura 5. Atividade antioxidante pelos métodos do albedo de cultivares de laranjas produzidas no Nordeste do Brasil pelo método de ABTS. Barras com letras iguais não diferem estatisticamente pelo teste de Scott-Knott a 5% ($p \leq 0,05$).

Pelo método do DPPH (figura 3) a média geral encontrada para a atividade antioxidante foi de 5,95 µM Trolox.mg^{-1} e a cultivar Salustiana diferiu estatisticamente ($p \leq 0,05$) das demais por apresentar o maior teor com 7.28 µM Trolox.mg^{-1}, seguido das cultivares Rubi (6.54 µM Trolox.mg^{-1}), Lima (6.45 µM Trolox.mg^{-1}), Mimo (6.36 µM Trolox.mg^{-1}), Comum (6.36 µM Trolox.mg^{-1}), Baianinha (6.28 µM Trolox.mg^{-1}), Sincorá (6.20 µM Trolox.mg^{-1}) que também possuem teores acima da média geral.

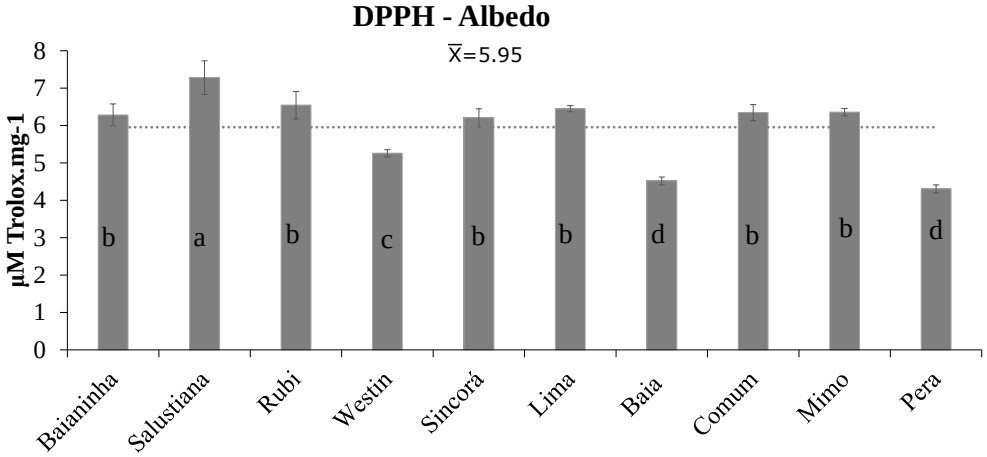

Figura 6. Atividade antioxidante pelos métodos do albedo de cultivares de laranjas produzidas no Nordeste do Brasil pelo método de DPPH. Barras com letras iguais não diferem estatisticamente pelo teste de Scott-Knott a 5% ($p \leq 0,05$).

Através do método ORAC (figura 4) foi quantificada a maior atividade antioxidante do albedo de laranja 273,98 μM Trolox.mg^{-1}, a cultivar Rubi é a que possui maior teor com 335.25 μM Trolox.mg^{-1} a qual diferiu estatisticamente das demais avaliadas neste estudo. As cultivares Salustiana (290.81 μM Trolox.mg^{-1}), Comum (289.69 μM Trolox.mg^{-1}), Sincorá (283.90 μM Trolox.mg^{-1}) e Baianinha (279.79 μM Trolox.mg^{-1}) também possuem teores acima da média Geral.

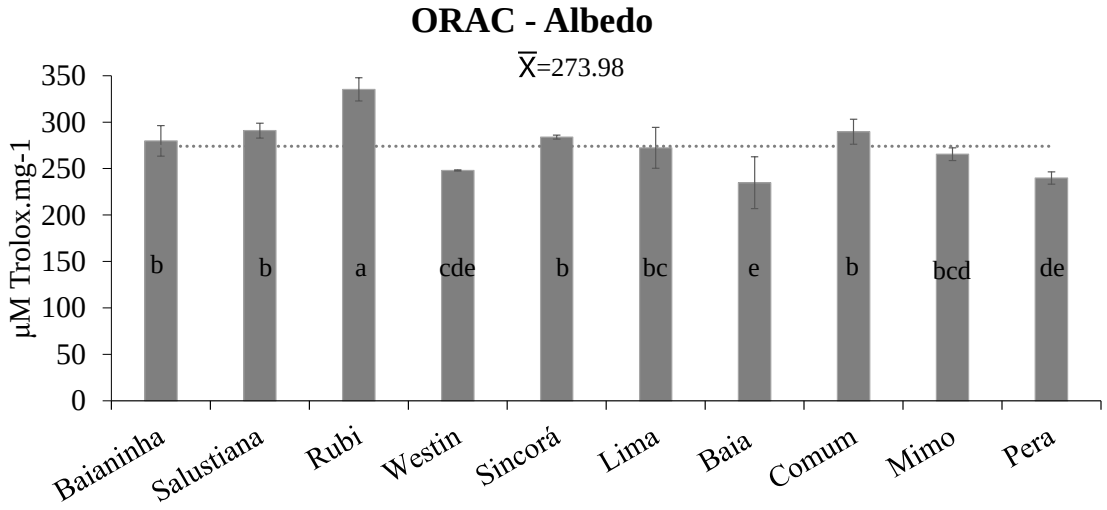

Figura 7. Atividade antioxidante pelos métodos do albedo de cultivares de laranjas produzidas no Nordeste do Brasil pelo método de ORAC. Barras com letras iguais não diferem estatisticamente pelo teste de Scott-Knott a 5% ($p<0,05$).

A atividade antioxidante encontrada no albedo das cultivares avaliadas em nossa pesquisa é um pouco inferior ao reportado por Escobedo-Avellaneda et al. (2014) em albedo de laranja 'Valência' que variou de 373 a 483 μM Trolox.mg^{-1} DM (dados originas convertidos para massa seca). No entanto as variações na capacidade antioxidante pode se dar devido diferenças observadas também nos Polifenois extraíveis totais que são os principais compostos responsáveis pela atividade antioxidante os quais tiveram uma correlação positiva ($r^2 \geq 0.64$). Neste sentido estas variações também podem ser atribuídas diferenças de cada cultivar, condições de cultivo e condições de extração (Wang et al., 2016).

Assim como vimos nesse estudo que no albedo de laranja a atividade antioxidante pelos

método de ORAC é maior, Khan et al. (2010) afirma que o ensaio de atividade antioxidante in vitro pelo método de ORAC é o mais adequado para avaliar a atividade antioxidante em tecidos dos citros.

3.4 Fibras dietéticas

Avaliando as fibras dietéticas encontramos altos teores de fibras com média geral de fibra solúvel 23.63 %, insolúvel (39.30 %) e total (62.93 %) no albedo das laranjas analisadas. O albedo de todas as cultivares não apresentaram grandes diferenças no teor de fibra solúvel, no entanto a cultivar Rubi apresentou o maior teor com 27.80 % e a Mimo o menor 17.54 % ($p < 0.05$).

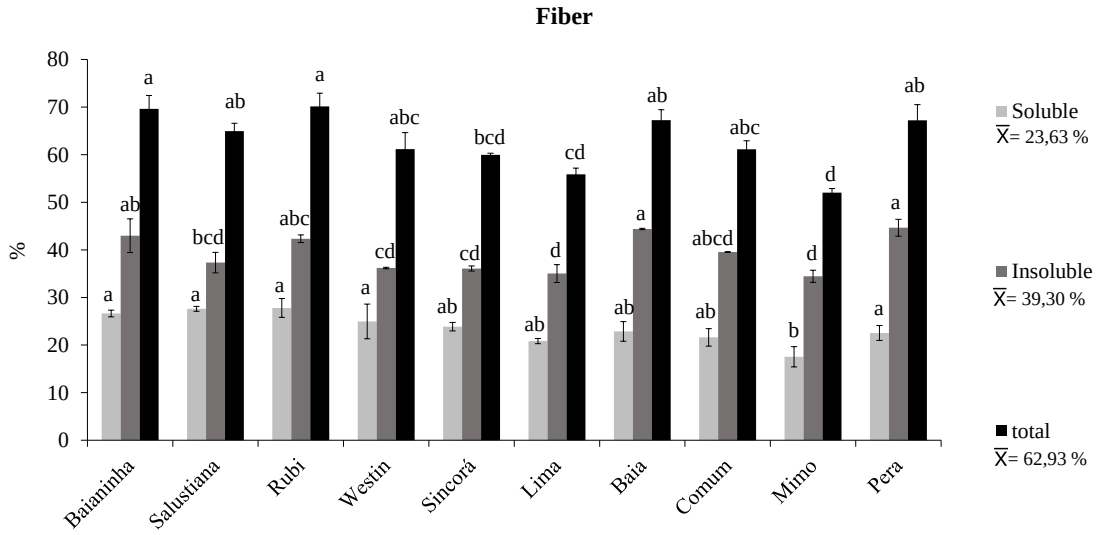

Figura 8. Fibras dietéticas do albedo de cultivares de laranjas produzidas no Nordeste do Brasil. Barras com letras iguais não diferem estatisticamente pelo teste de Scott-Knott a 5% ($p<0,05$).

As cultivares Pera e Baia apresentaram maiores teores de fibra insolúvel com 44.70 % e 44.39 % respectivamente, entretanto não diferiram estatisticamente ($p < 0.05$) das cultivares Baianinha (42.97 %), Rubi (42.34 %) e Comum (39.53 %). E para o teor de fibras totais também não houve grandes diferenças estatísticas, onde as cultivares Rubi e Baianinha apresentaram os maiores teores 70.14 e 69.61 % respectivamente, e o menor teor foi

registrado no albedo da cultivar Mimo 52.0 %.

Nossos resultados estão são aproximados aos encontrados por Silva et al (2017) analisando resíduos de laranja que encontrou 27.88 % de fibras Solúveis e 34.26 % de fibras insolúveis. Segundo Marin et al (2007) a composição final das fibras é mais dependente do processo industrial de extração do suco do que do tipo de citros.

O albedo é um dos tecidos que compõe a casca de laranja, é rico em fibras de melhor qualidade do que outras fontes de fibras alimentares. As fibras dietéticas funcionais e os antioxidantes extraídos de subprodutos cítricos podem ser utilizados como ingredientes em vários processos alimentares para obter produtos mais saudáveis (Sharma et al., 2017).

Estudos realizados anteriormente sugerem que o aumento do consumo de fibras alimentares reduz o risco de desenvolvimento de doenças cardiovasculares, combatendo fatores de risco como altos níveis de colesterol LDL (Chau, Huang, & Lin, 2004; Kendall, Esfahani e Jenkins, 2010). Neste sentido devemos destacar a importância da utilização do albedo de laranja na alimentação humana, frente ao grande potencial de fibras do albedo e o bom efeito das fibras na saúde das pessoas.

3.5 Proteínas totais

A média geral de proteínas totais no albedo das cultivares de laranjas é 4.22 $g.100g^{-1}$. As cultivares Pera, Westin, Rubi, e Baianinha apresentaram teores superiores à média com 5.20; 5.06; 4.45 e 4.35 $g.100g^{-1}$ respectivamente. Por sua vez, a cultivar que apresentou o menor teor foi a Comum com 3.13 $g.100g^{-1}$ ($p < 0.05$).

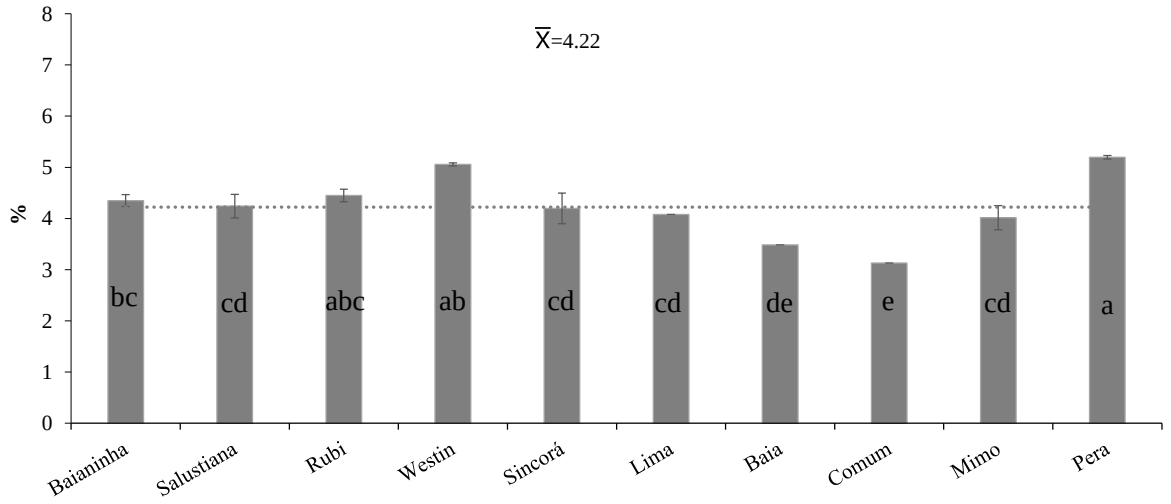

Figura 9. Proteínas totais do albedo de laranjas produzidas no nordeste do Brasil. Barras com letras iguais não diferem estatisticamente pelo teste de Scott-Knott a 5% (p<0,05).

Os teores mais altos encontrados em nossa pesquisa estão aproximados dos encontrados por Silva et al (2017) que encontrou cerca de 6 g.100g^{-1} de proteínas em resíduos de laranja. A Agência Nacional de Vigilância Sanitária (ANVISA) (Brasil 2005) recomenda o consumo diário de 50 g de proteína para adultos. No entanto, é necessário avaliar o perfil dos aminoácidos presentes para verificar a efetiva contribuição nutricional para a dieta.

3.6 Perfil de Minerais

O potássio é o mineral mais abundante no albedo da laranja com média geral de 802 mg.100g^{-1} e destaque para a cultivar Mimo que apresentou 1152.48 mg.100g^{-1} diferindo estatisticamente das demais analisadas neste estudo (p<0.05). Em seguida a cultivar Comum que também possui elevado teor de Potássio 1030.21 mg.100g^{-1} diferindo também das demais cultivares (p<0.05). Cálcio foi o segundo maior elemento mineral encontrado no albedo de laranja com 411.71 mg.100g^{-1} e a cultivar Baia apresentou o maior teor (488.43 mg.100g^{-1}) enquanto que a cultivar Westin o menor (328.62 mg.100g^{-1}).

Tabela 11. Minerais do Albedo (mg.100g^{-1})

Cultivar	Calcium	Iron	Potassium	Magnesium	Phosphorus	Aluminum	Boron	Sodium	Zinc
Baianinha	351.95 ± 8.09^{cd}	3.06 ± 0.54^{a}	652.72 ± 2.54^{e}	85.00 ± 1.41^{bc}	29.09 ± 3.49^{c}	2.35 ± 0.04^{cde}	1.53 ± 0.26^{a}	40.91 ± 2.04^{bc}	0.28 ± 0.06^{cd}
Salustiana	336.07 ± 15.01^{d}	0.79 ± 0.33^{b}	803.06 ± 19.77^{cd}	81.62 ± 1.07^{bc}	35.37 ± 3.85^{c}	2.13 ± 0.16^{de}	1.71 ± 0.2^{a}	96.33 ± 0.82^{a}	0.22 ± 0.01^{cde}
Rubi	423.26 ± 26.76^{abc}	0.61 ± 0.56^{b}	717.61 ± 24.16^{de}	94.68 ± 0.02^{abc}	30.30 ± 5.63^{c}	3.01 ± 0.24^{abc}	1.68 ± 0.31^{a}	44.30 ± 3.26^{b}	0.29 ± 0.04^{cd}
Westin	328.62 ± 39.58^{d}	1.32 ± 0.60^{b}	829.50 ± 72.48^{c}	88.17 ± 0.28^{abc}	39.71 ± 6.65^{c}	1.92 ± 0.39^{e}	1.70 ± 0.47^{a}	32.52 ± 4.80^{cd}	0.36 ± 0.02^{c}
Sincorá	411.59 ± 4.27^{abcd}	0.12 ± 0.17^{b}	648.30 ± 15.66^{e}	84.27 ± 2.44^{bc}	28.04 ± 4.24^{c}	2.92 ± 0.07^{abc}	1.45 ± 0.24^{a}	27.64 ± 1.67^{de}	0.24 ± 0.01^{cde}
Lima	399.39 ± 5.81^{bcd}	0.99 ± 0.49^{b}	823.76 ± 20.65^{cd}	101.54 ± 0.45^{a}	26.24 ± 0.42^{c}	2.68 ± 0.00^{bcd}	1.25 ± 0.01^{a}	23.21 ± 0.02^{e}	0.13 ± 0.02^{e}
Baia	488.43 ± 4.92^{a}	0.57 ± 0.17^{b}	723.91 ± 13.72^{cde}	62.66 ± 0.31^{d}	57.59 ± 0.60^{a}	3.64 ± 0.10^{a}	1.29 ± 0.24^{a}	14.27 ± 1.05^{f}	0.30 ± 0.02^{cd}
Comum	481.01 ± 29.88^{ab}	0.33 ± 0.43^{b}	1030.21 ± 20.48^{b}	98.51 ± 0.64^{ab}	40.97 ± 4.23^{bc}	3.49 ± 0.18^{a}	1.25 ± 0.22^{a}	29.85 ± 1.92^{de}	0.18 ± 0.04^{de}
Mimo	432.40 ± 3.40^{abc}	1.53 ± 0.04^{ab}	1152.48 ± 3.51^{a}	78.25 ± 0.35^{c}	59.46 ± 0.65^{a}	3.41 ± 0.01^{ab}	1.22 ± 0.03^{a}	30.55 ± 0.78^{de}	1.14 ± 0.06^{a}
Pera	464.38 ± 34.64^{ab}	0.65 ± 0.30^{b}	643.46 ± 11.70^{e}	80.53 ± 0.01^{c}	55.90 ± 1.62^{ab}	3.54 ± 0.27^{a}	1.40 ± 0.03^{a}	11.85 ± 1.69^{f}	0.59 ± 0.05^{b}
Mean	**411.71**	**0.99**	**802.0**	**88.0**	**40.27**	**2.91**	**1.45**	**35.14**	**0.37**
DRI (mg)	**50000**	**14**	**3500***	**260**	**700**	**ND**	**ND**	**200****	**7**

Médias com letras iguais na coluna, não diferem estatisticamente pelo teste de Scott-Knott a 5% ($p \leq 0,05$). nd= não determinado.

O albedo também é rico em Magnesio e fosforo, com medias gerais de 88.0 e 40.27 $mg.100g^{-1}$, além de possuir Ferro (0.99 $mg.100g^{-1}$), alumínio (2.91 $mg.100g^{-1}$), Boro (1.45 $mg.100g^{-1}$), Sódio (35.14 $mg.100g^{-1}$) e Zinco (0.37 $mg.100g^{-1}$).

Silva et al (2017) estudou a bioacessibilidade de cálcio, ferro e magnésio em resíduos de citros e revelou que o magnésio é o mineral mais bioacessível em resíduos de citros, seguidos por ferro e cálcio.

Os nossos resultados para minerais no albedo das diferentes cultivares de laranja, são aproximados aos teores encontrados por Barros et al (2012) em cascas das laranjas Lima e Pera, com 488.89 e 495.21 $mg.100g^{-1}$ de cálcio, 3.40 e 2.19 $mg.100g^{-1}$ de ferro, 871.0 e 796.41 $mg.100g^{-1}$ de potássio e 80.13 e 83.23 $mg.100g^{-1}$ de magnésio respectivamente.

3.7 Correlação de Pearson e Componentes principais

Através da analise de correlação de Pearson (figura 7A) foi possível descriminar que os polifenois extraíveis totais do albedo de cultivares de laranjas apresentam correlação forte e positiva com a atividade antioxidante (AAT) pelo método de ABTS e moderada com a AAT pelos métodos de DPPH e ORAC. Além disso, o ácido gálico foi o fenólico que apresentou correlação mais forte com os polifenóis, bem como com a AAT pelos três métodos.

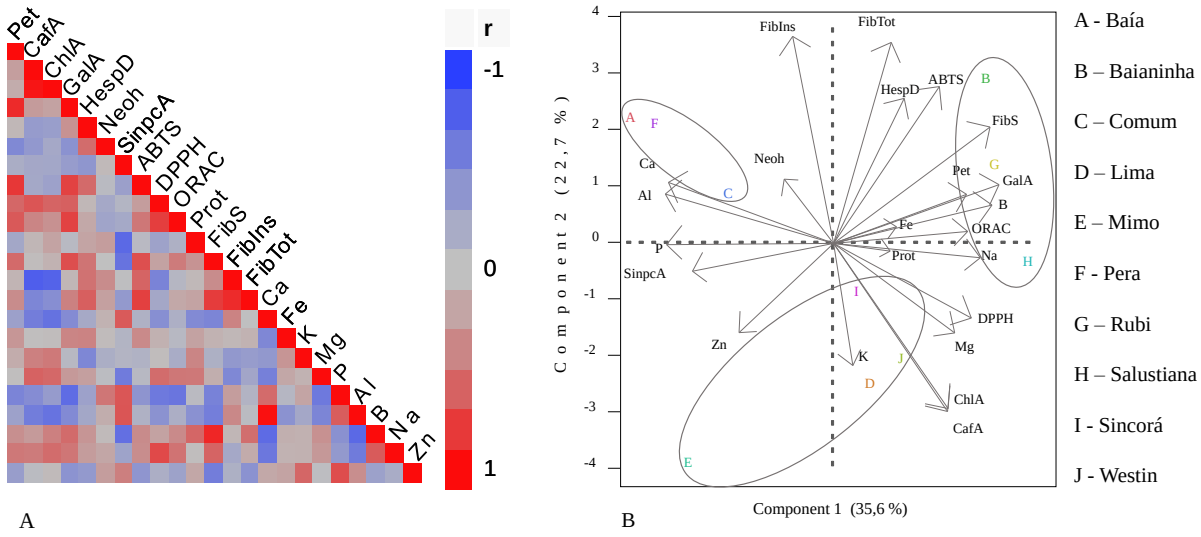

Figura 10. Correlação de Pearson (A) e Componentes principais (B) do albedo de cultivares de laranjas do Nordeste do Brasil.

PET: Total free extractable polyphenols in methanol; **CafA:** Cafeic Acid; **ChlA:** Clorogenic Acid; **GalA:** Galic Acid; **HespD:** hesperidin; **Neoh:** Neohesperidin; **SinpcA:** Sinapic Acid; **ABTS:** Antioxidant activity radical method ABTS; **DPPH:** Antioxidant activity radical method DPPH; **ORAC:** Antioxidant activity method ORAC; **Prot:** Proteinas; **FibS:** Soluble Fiber; **FibIns:** Insoluble Fiber; **FibTot:** Total Fiber; **Ca:** calcium; **Fe:** Iron; **K:** Potassium; **Mg:** Magnesium; **P:** phosphorus; **Al:** Aluminum; **B:** Boro; **Na:** Sodium; **Zn:** Zinc

A atividade antioxidante pelo método do ABTS também se correlacionou fortemente com as fibras solúveis e totais. No mesmo sentido, a fibra solúvel também se correlacionou com o ácido clorogênico. Estes resultados indicam que no albedo, grande parte dos compostos fenólicos são ligados às fibras e quanto maior o percentual de fibra solúvel, maior o teor de fenólicos livres e maior atividade antioxidante.

Na análise dos componentes principais (Figura 7B), três componentes foram responsáveis por explicar 72,1 % da variabilidade total. Os ácidos caféico e clorogenico, além dos minerais zinco, potássio e magnésio e atividade antioxidante pelo método do DPPH contribuíram para formar o CP 1. Contribuíram para a formação do componente principal 2 a neohesperidina, o ácido sinápico e os minerais cálcio, fosforo e alumínio também contribuíram para a formação do CP 3.

Desta forma, as cultivares se agruparam por semelhança nos teores de compostos em

três grupos diferentes. As cultivares Baia, Comum e Pera compõe o Grupo 1, as quais têm em comum altos teores dos minerais cálcio, alumínio e fosforo. Baianinha, Rubi e Salustiana formam o Grupo 2 e tem em comum maior teor de polifenois extraíveis totais, fibras solúveis e ácido gálico. E as cultivares Lima, Mimo, Sincorá e Westin formaram o Grupo 3 devido a menor semelhança aos demais, além das cultivares Mimo e Lima fazem parte de um grupo caracterizados por serem laranjas de baixa acidez.

4 CONCLUSÕES

O albedo de cultivares de laranjas cultivadas no Território da Borborema é uma fonte de fibra dietética de excelente qualidade, ampla disponibilidade de minerais, além de ser uma excelente fonte de compostos antioxidantes como flavonoides e ácidos fenólicos. Neste sentido, quando utilizado na alimentação humana, pode trazer benefícios para todo o sistema digestivo por apresentar boa proporção de fibras solúvel e insolúvel, além de compostos antioxidantes ligados que proporcionam elevada atividade antioxidante que protege de danos oxidativos. O albedo é fonte significativa de hesperidina (cultivares Baianinha, Rubi e Comum) e neohesperina (Pera, Salustriana e Comum).

A neohesperidina do albedo de laranjas é um flavonoide reconhecido por aliviar a fibrose pulmonar,uma das possíveis sequelas deixadas pela infecção em humanos afetados pelo vírus da Covid-19. Assim o consumo do albedo fornece elementos que nutrem e ao mesmo tempo promovem o bom funcionamento dos organismos.

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Artigo 4. Perfis de fenólicos, carotenoides e potencial funcional de suco de laranjas e tangerinas

Resumo

Suco de laranjas e tangerinas são amplamente consumidas em todo o mundo, sendo a agricultura familiar do Território da Borborema, estado da Paraíba, Nordeste do Brasil produtor de variedades muito apreciadas. No entanto, informações dos perfis de compostos fenólicos e carotenoides e atividade antioxidante destas variedades ainda são limitadas. Diante disto este estudo teve o objetivo de avaliar o perfil de fenólicos, carotenoides e atividade antioxidante do suco de laranjas e tangerinas produzidas no Território da Borborema. Dez cultivares de laranja e seis de tangerinas foram investigadas quanto aos polifenóis extraíveis totais (PET), perfil de fenólicos, carotenoides totais (CT), perfil de carotenoides, e atividade antioxidante (AAT) pelos métodos de ABTS, DPPH e ORAC. Em base seca, o suco das laranjas e tangerinas possui em média 0.41 g.GAE.100g^{-1} de PET, cujos principais fenólicos são a neohesperidina, hesperidina e ácido isoferrulico, nesta ordem. O suco de tangerinas possui teores superiores de CT bem como de carotenoides individuais. Foram quantificados β-caroteno, β-cryptoxantina, luteina, violaxantina e zeaxantina. A AAT pelo método de ORAC foi cerca de 4.5 vezes superior às obtidas pelo DPPH e ABTS. A zeaxantina foi o caratenoide presente em todas as laranjas e tangerinas (em teores dez vezes superior), que pode ser apontado como um marcador de autenticidade no suco dos citros aqui avaliados.

Key words: *Citrus sinensis, Citrus reticulata*, neohesperidina, hesperidina, β-caroteno, β-cryptoxantina, violaxantina,

1 INTRODUÇÃO

A citricultura é amplamente produzida no mundo, com uma produção anual aproximada

de 125 milhões de toneladas (FAOSTAT, 2020), sendo o Brasil o maior produtor. Os citrus correspondem também as frutas mais consumidas no mundo, como fruta fresca ou processada (Satari e Karimi, 2018). Do total de citrus produzido, as laranjas representaram 52%, seguidos das tangerinas com 33% (Rosas-Mendoza et al., 2020).

Além dos nutrientes essenciais, as frutas cítricas são uma boa fonte de fitoquímicos biologicamente ativos, como carotenoides, que são provitamina A, e flavonoides (Barros et al., 2012, Zou et al., 2016; Smeriglio et al., 2019).

Algumas cultivares de laranjas são fontes reconhecidas de compostos bioativos, como vitamina C, carotenóides e polifenóis (principalmente flavanonas como hesperidina), que podem atuar em conjunto fornecendo antioxidantes e anti-inflamatórios e outras propriedades relacionadas à saúde (Gironés-Vilaplana et al., 2014, Zou et al., 2016), tais como redução do risco de doenças cardiovasculares, aterosclerose, alguns tipos de câncer, diabetes e diminuir os níveis de lipídios no sangue (Liu et al., 2017, Lv et al., 2015).

Entretanto, o suco das principaiss variedades de citrus cultivadas no Território da Borborema, Nordeste do Brasil e comercializadas regionalmente, ainda necesitam de estudos aprofundados quanto aos perfis de fenolicos, bem como de carotenoides e como estes estão correlacionados com a atividade antioxidante. Diante disto, a identificação e quantificação destes compostos é de extrema importância para a identificação de marcadores de autenticidades, buscando a agregação de valor das laranjas e tangerinas produzidas pela agricultura familiar da região.

Neste sentido, o objetivo deste trabalho foi avaliar o perfil de fenólicos, carotenoides e a atividade antioxidante do suco de cultivares de laranjas e tangerinas produzidas no Território da Borborema, estado da Paraíba, Brasil.

2 MATERIAIS E MÉTODOS

Os frutos foram colhidos em pomar comercial localizado no município de Alagoa Nova,

região do Território da Borborema, Nordeste do Brasil. A propriedade rural possui solo predominante do tipo ARGISSOLO VERMELHO Eutrófico abrúptico (Embrapa, 2016).

Laranjas (*Citrus sinensis*) das cultivares Baianinha, Salustiana, Rubi, Westin, Sincorá, Lima, Baía, Comum, Mimo, Pera e tangerinas (*Citrus reticulata*) das cultivares Piemonte, Clemenules, Page, Dancy, Ponkan e Murcot foram colhidas aleatoriamente do pomar, entre 6 e 8 horas da manhã, no estádio de maturação C3 – de cor predominantemente laranja, de acordo com as Normas CEAGESP (2011), bem como pela ausência de pragas, doenças e lesões aparentes. Na ocasião da colheita os frutos foram acondicionados em caixas de polietileno de alta densidade, protegidas com plástico bolha e transportados ao laboratório de biologia e tecnologia Pós-colheita.

No laboratório de Biologia e Tecnologia Pós-Colheita, os frutos foram processados em espremedor de frutos Arno Citrus Power PA 32, após processados o suco foi armazenados sobre refrigeração (- 4 ^{0}C) até ser liofilizado. Logo após a liofilização o suco foi embalado a vácuo e armazenados em temperatura ambiente até o dia das analises.

O experimento foi realizado em delineamento inteiramente casualizado (DIC) com suco de 10 cultivares de laranjas e 6 cultivares de tangerinas com quatro repetições por cultivar.

2.1 Fenólicos Livres

Os extratos para compostos fenólicos foram feitos com metanol aquoso (80%), o qual foi adicionado a 1 g do suco liofilizado; eles foram homogeneizados com ultra-turrax por 2 min (IKA Ultra-turrax T18, Wilmington, EUA). A mistura foi centrifugada (5000 rpm por 20 min, 4 ° C). O sobrenadante foi analisado quanto à atividade antioxidante, fenólicos totais por espectrofotometria e o perfil de fenólicos foi analisado por HPLC-DAD.

2.3 Capacidade antioxidante – ensaios DPPH, ABTS e ORAC

A metodologia seguida para DPPH foi baseada na descrita por Bobo-García et al.

(2014) com algumas modificações. Resumidamente, 20 µL de extrato foram adicionados a uma placa de 96 poços contendo 180 µL de solução de DPPH (2,2-difenil-1-picril-hidrazil; Sigma Chemical Company, St. Louis, MO, EUA) (150 µM). A absorvância foi medida a 515 nm no leitor de microplacas (Espectrofotômetro Multiskan GO Microplate, Thermo Scientific, Thermo Fisher Scientific Inc., EUA) após 40 minutos no escuro. Os padrões TE (Sigma Chemical Company, St. Louis, MO, EUA) (0 a 500 µM) foram preparados e analisados da mesma forma para obter a curva de calibração. A porcentagem de inibição foi determinada e os extratos foram expressos em mg equivalentes de Trolox (TE)/g DW.

O teste de eliminação do ABTS também foi realizado em uma microplaca de 96 poços, seguindo o método descrito por Gonçalves et al. (2009) com algumas modificações. A 20 µL da amostra ou Trolox ou solvente são adicionados 180 µL de ABTS • + solução de trabalho. A mistura é incubada por 5 min a 30 ° C, e a absorbância a 734 nm é medida com o leitor de placas Multidetection (Synergy H1, Vermont, EUA).

A capacidade de absorbância do radical de oxigênio (ORAC) foi realizado em uma microplaca preta de 96 poços (Nunc, Dinamarca), seguindo o método descrito por Dávalos et al. (2004) com algumas modificações. A reação foi feita com tampão fosfato 75 mM (pH 7,4), e a mistura de reação final foi de 200 µL. Antioxidantes (20 µL) e fluoresceína (120 µL; 70 nM, concentração final) foram colocadas no poço da microplaca. Um ensaio em branco (FL + AAPH) usando tampão fosfato em vez da solução antioxidante e oito soluções de calibração usando Trolox (1-8 µM, concentração final) como antioxidante também forão realizados em cada ensaio. A mistura foi pré-incubada por 10 min a 37 °C. A solução AAPH (60 µL; 12 mM, concentração final) foi adicionada rapidamente usando uma pipeta multicanal. A microplaca foi imediatamente colocada no leitor e a fluorescência é registrada em intervalos de 1 min durante um período de 140 min.

O ensaio também foi realizado com um leitor de placas de detecção múltipla (Synergy

H1, Vermont, EUA). O comprimento de onda de excitação é fixado em 485 nm e o comprimento de onda de emissão em 528 nm (Ubeda et al., 2011). A microplaca foi agitada automaticamente antes de cada leitura.

2.4 Carotenoides

Para os extratos de carotenoides seguimos a metodologia de Oliveira et al (2016), foram utilizados 1g de suco liofilizado, o qual foi homogeneizado com 4 mL de água destilada e 1 mL de etanol gelado (4ºC) por 1 min com um ultra-turrax. Foi adicionado hexano (8 mL) e a pasta resultante foi centrifugada, após a centrifugação a camada superior foi removida para o tubo de polipropileno. A extração foi repetida duas vezes com 2,88 mL de hexano. Ambas as frações de hexano resultantes foram combinadas e usadas para saponificação com KOH metanólico 10% durante a noite em agitador orbital. A mistura foi lavada com 25 mL de NaCl 10% e três lavagens com água desionizada.

2.5 Carotenóides Totais

O teor de carotenoides totais foi estimado em triplicatas, utilizando análise espectrofotométrica a 454 nm. O β-caroteno (6,3 × 10-5 - 4,0 × 10^{-3} mg/mL) foi utilizado como curva padrão. O conteúdo total de carotenóides foi expresso com base em mg de equivalentes de β-caroteno/g de biomassa (Oliveira et al., 2016).

2.6 Análise quantitativa de carotenóides por HPLC com detector DAD

Os extratos de carotenoides foram concentrados em Speed Vacuum Thermo Fisher Scientific. Uma alíquota de 50 µL de extrato hidrofílico foi injetada em um cromatógrafo líquido de alta pressão Waters Alliance (Waters Series 600, Mildford MA, EUA), equipado com um detector PDA Waters 996. Os cromatogramas foram registados através de um detector de arranjo de diodos (Waters, Mildford MA, EUA) em comprimentos de onda variando de 200 a 600 nm em intervalos de 2 nm. A absorvância foi medida a 454 nm.

Os carotenóides foram eluídos utilizando a fase móvel composta por acetonitrila (55%), metanol (22%), diclorometano (11,5%) e hexano (11,5%). Foi adicionado acetato de amônio a 0,02% para estabilizar os carotenóides em condições isocráticas a uma taxa de fluxo de 1,0 mL / min durante 20 min, a 25 ° C com um volume de injeção de 50 μL. A curva de calibração foi construída com padrões puros de α-Caroteno, β-caroteno, zeaxantina, luteína, β-criptoxantina e violaxantina e expressa em microgramas por grama. Todas as análises, de cada extrato, foram feitas em triplicatas.

2.7 Análise estatística

Os dados foram submetidos à análise de variância (ANOVA) e as médias das cultivares foram submetidas ao teste de Scott-Knott até 5% de probabilidade ($p<0.05$). Os dados foram analisados utilizando o pacote Emmeans do software R, versão 5.1 (Lenth, 2018).

3 RESULTADOS E DISCUSSÃO

O perfil de fenólicos e carotenoides, além da atividade antioxidante do suco de dez cultivares de laranja e seis cultivares de tangerinas produzidas no Território da Borborema, Nordeste do Brasil apresentam potencais funcionais diferenciados, conforme dados apresentados a seguir.

3.1 Polifenóis livres extraíveis totais (PET)

O suco de laranjas e tangerinas (Figura 1) apresentam em média 0,41 g.GAE.100g^{-1} de PET. As cultivares de laranja Salustiana e Rubi apresentaram os maiores teores de PET com 0,48 e 0,50 g.GAE.100g^{-1}, respectivamente, as quais diferiram estatisticamente das demais cultivares aqui avaliadas ($p<0.05$). Em contrapartida, as cultivares de laranja Baía e Lima as de tangerinas Clemenules e Dancy apresentaram os menores teores com 0.34, 0.36, 0.34 e 0.37 g.GAE.100g^{-1} respectivamente, diferindo também das demais avaliadas ($p<0.05$).

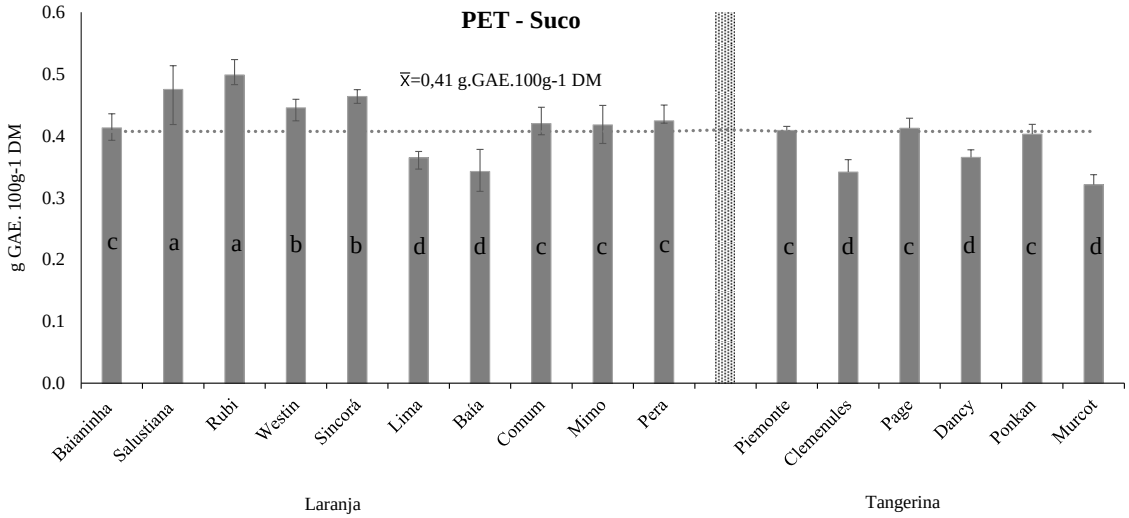

Figura 11. Polifenois Extraíveis Totais (PET) em base seca, no suco de cultivares de laranjas e tangerinas do Território da Borborema. Barras com letras iguais não diferem estatisticamente pelo teste de Scott-Knott a 5% ($p \leq 0,05$)

Os teores médios de PET das cultivares de laranjas e tangerinas estudadas nesta pesquisa, são aproximados aos encontrados por Wang et al (2016) que foi de 0,42 g.GAE.100g^{-1} em polpa de laranja madura. Assim como foi observado aqui, diversos outros estudos afirmam que os citros são boas fontes de polifenóis (Wang et al. 2016; Zhang, Yang e Zhou, 2018).

3.2 Perfil de compostos fenólicos (HPLC-DAD)

No suco de laranja liofilizado foi possível identificar a presença do ácido isoferulico (15,71 mg.100g^{-1}), hesperidina (75,65 mg.100g^{-1}) e neohesperidina (128,03 mg.100g^{-1}). Nas tangerinas foi quantificado o ácido galico (11,34 mg.100g^{-1}), apenas no suco das tangerinas 'Page' e 'Dancy', ácido isoferulico (13,97 mg.100g^{-1}), hesperidina (87,83 mg.100g^{-1}) e a neohesperidina (117,53 mg.100g^{-1}). Os teores de fenólicos em frutos e hortaliças variam dependendo da espécie, genética, fatores ambientais, tratos agronômicos (Lado; Gambetta; Zacarias, 2018).

Tabela 12. Perfil de compostos fenólicos (mg.100g^{-1} DM) do suco de cultivares de laranja e tangerinas da agricultura familiar do Território da Borborema

	Cultivar	Gallic Acid	Isoferulic Acid	Hesperidin	Neohesperidin
Laranja	Baianinha	nd	14,34 ± 0,04 h	91,18 ± 0,48 f	110,49 ± 0,06 j
	Salustiana	nd	15,30 ± 0,00 e	72,77 ± 0,00 i	128,17 ± 0,00 g
	Rubi	nd	16,84 ± 0,15 b	98,47 ± 0,45 e	131,25 ± 0,21 f
	Westin	nd	16,15 ± 0,05 c	65,72 ± 0,12 j	110,32 ± 0,01 k
	Sincorá	nd	17,63 ± 0,00 a	90,19 ± 0,03 g	125,31 ± 0,12 h
	Lima	nd	16,17 ± 0,01 c	64,71 ± 0,59 k	135,36 ± 0,22 c
	Baia	nd	nd	105,79 ± 0,22 d	134,31 ± 0,01 d
	Comum	nd	14,75 ± 0,07 f	51,25 ± 0,09 l	109,47 ± 0,11 l
	Mimo	nd	16,01 ± 0,02 d	41,55 ± 0,02 m	117,61 ± 0,02 i
	Pera	nd	14,20 ± 0,00 i	74,89 ± 0,28 h	178,01 ± 0,05 a
	Mean	**nd**	**15.71**	**75.65**	**128.03**
Tangerina	Piemonte	nd	13,95 ± 0,03 j	208,11 ± 0,00 a	nd
	Clemenules	nd	nd	13,39 ± 0,00 p	76,05 ± 0,13 o
	Page	12,08 ± 0,17 a	13,41 ± 0,06 k	115,20 ± 0,01 c	133,78 ± 0,02 e
	Dancy	10,60 ± 0,07 b	nd	18,81 ± 0,02 o	108,47 ± 0,04 m
	Ponkan	nd	nd	29,02 ± 0,07 n	97,77 ± 0,03 n
	Murcot	nd	14,55 ± 0,01 g	141,65 ± 0,06 b	171,57 ± 0,16 b
	Mean	**11.34**	**13.97**	**87.83**	**117.53**

Mean followed by the same lowercase letter in the column do not differ statistically by the Scott-Knott test at 5% ($p \leq 0,05$). nd= not detected.

Pesquisa recente afirma que os flavonóides cítricos, também são promissores no tratamento da desregulação metabólica e da esteatose hepática também conhecida como gordura no fígado (Jianping et al. 2019). A hesperidina é um dos flavonóides predominante no suco de laranjas e tangerinas aqui avaliadas, que tem sido apontado em diversos estudos que este composto apresenta diversos efeitos benéficos à saúde dentre eles, a prevenção contra doenças cardiovasculares, proteção contra câncer e supressão do estresse oxidativo (Sahu et al. 2013; Kaltalioglu et al. 2019; Celano et al. 2019; Sánchez-Recillas et al. 2019). Além destas, a hesperidiana apresenta também atividade gastroprotetora, visto que estudo recente confirma inclusive que a hesperidina ajuda a curar ulceras na mucosa, efeito favorecido pela redução dos danos oxidativos da mucosa, devido à redução da migração de neutrófilos e por fortalecer a proteção (Silva et al. 2019).

A neohesperidina, o flavonoide glicosilado encontrado em maior conteúdo no suco de laranjas e tangerinas aqui avaliadas, é reconhecida por auxiliar no tratamento e prevenção nas frutas cítricas pode ser útil no tratamento e prevenção de diversas doenças. Tan et. Al. (2017) descobriram que a Neoesperidina inibe o desenvolvimento de osteoporose. Guo et al (2019) mostraram que a neohesperidina reduziu fibrose e disfunção pulmonar em testes preliminares com camundongos e portanto pode ser útil no tratamento dessas doenças também em humanos. E Gong et. Al. (2019) revelou que a neohesperidina inibi o aparecimento de tumores colorretal, que pode ser mediada por alterações da microbiota intestinal, indicando novas perspectivas sobre o efeito quimiopreventivo deste flavonoide, valorizando o suco de laranjas e tangerinas do Território da Borborema.

Os compostos fenólicos presentes em frutos e hortaliças exercem reconhecidos efeitos benéficos na prevenção de doenças degenerativas (Zou et al., 2016; Cirmi et al., 2016; Del Rio et al., 2013). Neste contexto, dados comprobatórios apontam para os efeitos benéficos à saúde proporcionados pelos flavonoides, aqui quantificados em altos teores na polpa das

cultivares de laranjas e tangerinas produzidas no Território da Borborema, Nordeste do Brasil. Podemos afirmar que o consumo destes frutos regularmente, leva aos consumidores diversas ações protetoras a determinados órgãos, inclusive proteção de danos oxidativos que podem ser causadas por radicais livres.

3.3 Carotenoides totais

Em base seca, os carotenoides totais foram bem superiores no suco das tangerinas avaliadas (Figura 3), que diferiram significativamente dos teores das laranjas. O suco de tangerina possui um teor médio cerca de onze vezes superior ao suco de laranja, 85,23 e 7,68 mg.100g^{-1}, respectivamente.

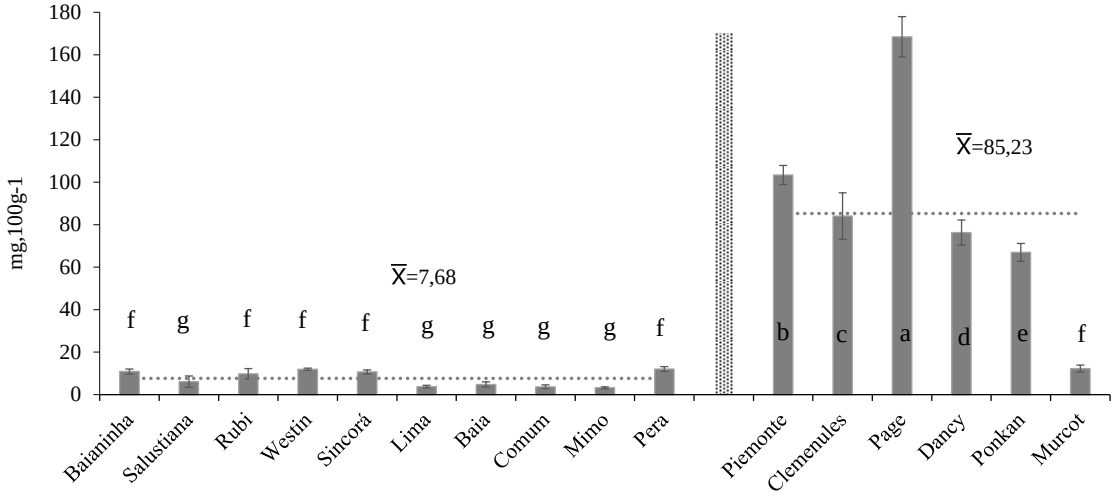

Figura 12. Carotenoides totais da polpa em base seca, no suco de cultivares de laranjas e tangerinas do Território da Borborema. Barras com letras iguais não diferem estatisticamente pelo teste de Scott-Knott a 5% ($p<0.05$)

A tangerina da cultivar Page possui o maior teor de carotenoides totais, destacando-se estatisticamente ($p<0.05$) das demais com teor de 168.47 mg,100g^{-1}, sendo o dobro da média geral das tangerinas. O segundo maior teor foi o da tangerina Piemonte o qual também foi superior à média geral deste grupo de citrus, com 103.37 mg,100g^{-1} com diferença estatística de todas as outras cultivares avaliadas neste estudo ($p<0.05$). Por sua vez, o menor teor nas cultivares de tangerina foi encontrado para na Murcote com 12.20 mg,100g^{-1}.

As cultivares de laranja Salustiana, Lima, Baia, Comum e Mimo possuem os menores teores de carotenoides totais 6.08; 3.71; 4.82; 3.66; e 3.20 mg,100g^{-1}, respectivamente, estas diferiram estatisticamente das demais laranjas avaliadas aqui.

3.4 Perfil de Carotenoides por HPLC-DAD

Assim como nos carotenoides totais, para o perfil de carotenoides analisado por HPLC (Tabela 5), em base seca, também observou-se que o suco das tangerinas apresenta conteúdos bem superiores aos das laranjas, com teores médios de β-caroteno (1.85 e 1.14 mg,100g^{-1}), o β-cryptoxantina (2.62 e 7.44, Luteina (0.29 e 0.35 mg,100g^{-1}), violaxantina (3.89 e 13.01 mg,100g^{-1}) e zeaxantina (0.32 e 3.18 mg,100g^{-1}), respectivamente. β-carotene não foi detectado na laranja Baia nem nas tangerinas Dancy e Ponkan. Por sua vez, foi detectada apenas nas laranjas Mimo e Pera e na tangerina Murcot. Lutein foi detectada apenas na laranja Pera e na tangerina Murcot, esta última que apresentou todos os carotenoides avaliados. Interessante é que a zeaxantina foi o caratenoide presente em todas as laranjas e tangerinas avaliadas, mas ainda cerca de 10 vezes superior nas tangerinas, que pode ser apontado como um marcador de autenticidade no suco dos citros aqui avaliados.

Em base seca, o suco das tangerinas Clemenules e Piemonte apresentaram os maiores teores de β-caroteno com 10.0 e 9.68 mg,100g^{-1}, respectivamente, as quais diferiram estatisticamente das demais cultivares ($p<0.05$). A laranja 'Mino' diferiu das demais com maior teor de β-cryptoxantina 2.56 mg.100g^{-1} ($p<0.05$); e para a violaxantina e zeaxantina a Piemonte também diferiu de todas as outras ($p<0.05$) com teores de 19.55 e 4.15 mg.100g^{-1}, respectivamente.

Os carotenoides podem ser classificados em dois grandes grupos, com base em grupamentos funcionais; as xantofilas, que contêm oxigênio como grupo funcional, incluindo luteína e zeaxantina, e os carotenos, que contêm apenas a cadeia de hidrocarboneto original sem nenhum grupo funcional, como α-caroteno, β-caroteno e licopeno (Saini, Nile & Park,

2015).

Tabela 13. Carotenoides **em base seca, no suco de cultivares de laranjas e tangerinas do Território da Borborema** por HPLC-DAD (mg,100g^{-1})

	Cultivar	β-carotene	β-cryptoxanthin	Lutein	Violaxanthin	Zeaxanthin
Laranja	Baianinha	2,90 ± 0,59^{c}	nd	nd	nd	0,36 ± 0,03^{c}
	Salustiana	0,85 ± 0,23^{d}	nd	nd	nd	0,19 ± 0,02^{c}
	Rubi	1,68 ± 0,12^{c}	nd	nd	3,89 ± 0,22^{d}	0,30 ± 0,02^{c}
	Westin	4,15 ± 0,41^{b}	nd	nd	nd	0,44 ± 0,04^{c}
	Sincorá	4,55 ± 0,43^{b}	nd	nd	nd	0,43 ± 0,03^{c}
	Lima	1,07 ± 0,26^{c}	nd	nd	nd	0,18 ± 0,01^{c}
	Baia	nd	nd	nd	nd	0,32 ± 0,03^{c}
	Comum	0,73 ± 0,82^{d}	nd	nd	nd	0,24 ± 0,03^{c}
	Mimo	1,97 ± 0,76^{c}	2,56 ± 0,08^{a}	nd	nd	0,20 ± 0,03^{c}
	Pera	5,71 ± 0,94^{b}	1,13 ± 0,05^{b}	0,29 ± 0,09^{a}	nd	0,51 ± 0,05^{c}
	Mean	**2.62 (9)**	**1.85 (2)**	**0.29 (1)**	**3.89 (1)**	**0.32 (10)**
Tangerina	Piemonte	9,68 ± 0,41^{a}	nd	nd	19,55 ± 3,78^{a}	4,15 ± 0,50^{a}
	Clemenules	5,03 ± 0,51^{b}	nd	nd	19,18 ± 3,04^{a}	3,63 ± 0,53^{b}
	Page	10,0 ± 0,96^{a}	nd	nd	12,7 ± 2,35^{b}	3,69 ± 0,40^{b}
	Dancy	nd	nd	nd	nd	3,65 ± 0,37^{b}
	Ponkan	nd	nd	nd	9,42 ± 0,70^{c}	3,50 ± 0,51^{b}
	Murcot	5,03 ± 0,35^{b}	1,14 ± 0,10^{b}	0,40 ± 0,19^{a}	4,20 ± 0,31^{d}	0,48 ± 0,04^{c}
	Mean	**7.44 (4)**	**1.14 (1)**	**0.35 (1)**	**13.01 (5)**	**3.18 (6)**

Médias com letras iguais na coluna, não diferem estatisticamente pelo teste de Scott-Knott a 5% ($p<0.05$). nd= não detectado. Between branckets, de number of data used for the mean calculation.

Os carotenoides são responsáveis pela pigmentação amarelo e laranja de citros e também contribuem para as suas atividades antioxidantes (Rodrigo et al. 2013; Yoo & Moon, 2016). Com o amadurecimento dos frutos cítricos, ocorre o acúmulo de carotenoides, principais responsáveis pela coloração (Peng et al., 2013).

3.3 Atividade Antioxidante (AAT)

Pelo método de ABTS (figura 2A) a média geral da atividade antioxidante foi de 16.33 μM Trolox.g^{-1} e as laranjas Salustiana, Rubi, Sincorá e a tangerina Piemonte, apresentaram os valoress se destacando estatisticamente das demais deste estudo ($p<0.05$) com 19.80; 20.16; 19.95 e 20.72 μM Trolox.g^{-1}, respectivamente.

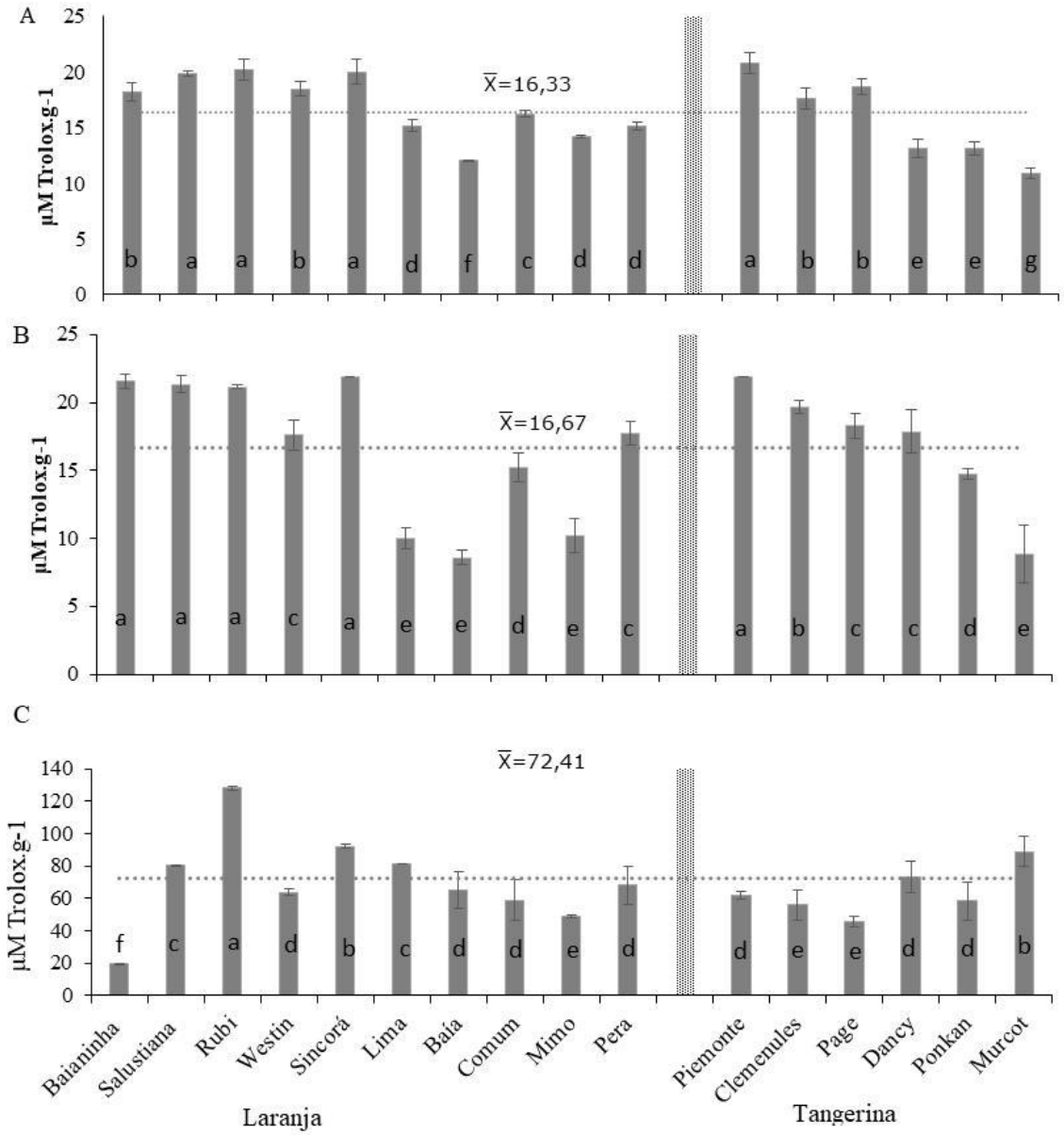

Figura 13. Atividade antioxidante dos fenólicos livres do suco de cultivares de laranjas e tangerinas do Território da Borborema, pelos métodos do ABTS (A), DPPH (B), ORAC (C). Barras com letras iguais não diferem estatisticamente pelo teste de Scott-Knott a 5% ($p<0,05$)

Resultados próximos foram também aqui verificados pelo método do DPPH (Figura 2B) com média geral de 16.67 μM Trolox.g^{-1}, com as laranjas Salustiana, Rubi, Sincorá, Baianinha e a tangerina Piemonte apresentando os maiores valores 21.33; 21.14; 21.90; 21.58 e 21.90 μM Trolox.g^{-1}, respectivamente, destacando-se estatisticamente das demais ($p<0.05$).

Por sua vez, a AAT pelo método do ORAC (Figura 2C) foram encontrados valores cerca de 4,5 vezes mais elevados que pelos métodos de ABTS e DPPH, uma média geral de 72.41 μM Trolox.g^{-1}. Pelo ORAC, a AAT da cultivar Rubi destacou-se estatisticamente ($p<0.05$) de todas as outras, com o teor de 128.18 μM Trolox.g^{-1}.

De acordo com Khan et al. (2010) o ensaio ORAC é o método mais adequado para a avaliação da atividade antioxidante em citros devido ao fato de que as principais flavanonas presentes neste material vegetal são antioxidantes relativamente fracos, pois não exibem um grupo catecol (1,2-di-hidroxibenzeno), que é o determinante estrutural crítico de fortes antioxidantes fenólicos. Como consequência, eles reagem muito lentamente com o radical DPPH. Assim, são necessários a presença no método de radicais muito mais reativos, como é o caso dos peroxils fornecidos no teste ORAC.

4 CONCLUSÕES

No suco das laranjas e tangerinas do Território da Borborema foram detectados em conteúdos elevados compostos fenólicos importantes para a saúde humana, dentre eles a neohesperidina que foi quantificado em maior teor e possui efeitos positivo comprovados contra enfermidades importantes que afetam os humanos.

Tangerinas regionais possuem 11 vezes mais carotenoides totais em seu suco do que o suco das laranjas. O carotenoide presente em maior conteúdo foi a violaxantina, com teor bem superior no suco das tangerinas, enquanto que entre as laranjas este foi quantificado apenas na cultivar Rubi.

A zeaxantina foi o caratenoide presente em todas as laranjas e tangerinas, mas com teores 10

vezes superior nas tangerinas, que pode ser apontado como um marcador de autenticidade no suco dos citros aqui avaliados. A atividade antioxidante pelo método do ORAC é 4.5 vezes maior que nos outros métodos avaliados.

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Artigo 5. Fisiologia e qualidade de tangerina ‘Dancy’ sob recobrimentos de fontes de amido com Solvente Eutetico Natural Profundo

Resumo

A tangerina Dancy é a principal cultivar produzida pela agricultura familiar do Território da Borborema, sob manejo orgânico, caracterizando-se como o maior produtor desta cultivar no Brasil. Entretanto, estes frutos demandam estratégias viáveis para a conservação pós-colheita. Neste sentido, recobrimento biodegradáveis a base de amido de mandioca e semente de jaca, plastificados com glicerol ou com solventes eutéticos naturais profundos (NADES) para avaliar a taxa metabólica e a qualidade pós-colheita de tangerina Dancy. Os tratamentos utilizados foram: fécula de jaca mais glicerol (FJ+G), FJ+NADES, fécula de mandioca mais G (FM+G) e FM+NADES e o controle (C), frutos sem recobrimentos. O uso de recobrimentos reduziu a taxa metabólica com relação ao controle, de modo a reduzir a produção de CO_2 e a evolução da coloração, sem implicações nos sólidos solúveis e na acidez titulável.

Key words: Fruit waste, respiratory activity, NADES, glycerol, color evolution.

1 INTRODUÇÃO

Os frutos cítricos pertencem à família Rutaceae e são consideradas uma das maiores espécies de plantas, composta por 40 espécies diferentes, amplamente dispersas nas regiões tropicais, subtropicais e temperadas (Singh et al., 2020). São as frutas mais cultivadas em todo o mundo e contem varios fitoquímicos benéficos valiosos, com a heperidina, Neohesperidina e o acido Isoferrulico (Hou et al., 2019, Satari e Karimi, 2018). Muitas variedades e híbridos diferentes de citros foram produzidos como resultado do cruzamento natural ou artificial. Laranjas, toranjas, tangerinas, limões e limas são populares por valores nutricionais e correspondem as principais culturas cítricas industrializadas (Satari & Karimi, 2018).

A tangerina apresenta teores relativamente elevados de compostos fenólicos como flavanonas, flavonas, ácidos fenólicos e carotenoides (Wang et al., 2007; Xu et al., 2009) com considerável atividade antioxidante (Couto et al., 2010). Contudo, a disponibilidade desses compostos depende de diversos fatores como tratos culturais, maturação na colheita e condições de armazenamento.

Os frutos de tangerinas são vulneráveis a infecções por microrganismos, distúrbios fisiológicos e lesões físicas, resultando em uma enorme perda de frutas frescas a cada ano (Xu, Qin & Ren 2018). Assim, altos níveis de perdas pós-colheita de frutos requerem tecnologias eficientes e sustentáveis para manter a qualidade (Cissé et al., 2015), como revestimentos biodegradáveis obtidos de fontes de amidos vegetais amplamente encontradas na natureza adicionados de plastificantes que dão maior estabilidade aos recobrimentos.

O uso de recobrimentos biodegradáveis na conservação pós-colheita de frutos, visa não só melhorar a aparência, mas prolongar a vida útil pós-colheita, reduzindo a atividade respiratória e diminuindo a deterioração do produto (Malgarim et al., 2007; Valencia-Chamorro et al., 2009). Um recobrimento adequado deve permitir trocas gasosas adequadas

entre a fruta e o meio ambiente, evitando assim o risco de fermentação, mas satisfatoriamente (Liang et al., 2015), reduzindo as taxas metabólicas e, portanto, a taxa respiratória, a fim de aumentar sua vida útil (Aquino et al., 2015).

O amido de mandioca é uma fonte biodegradável com alto potencial de uso em recobrimentos (Guimarães et al., 2017). Entretanto, fontes não convencionais de amido, como o de sementes de jaca (*Artocarpus heterophyllus* Lam.), que corresponde a um amplo descarte deste fruto, têm sido exploradas com relação às propriedades de formação de filmes e na conservação pós-colheita de frutos, como a jaca (Rodrigues et al., 2018). Entretanto**,** estas matrizes exigem a adição de um agente plastificante. O glicerol é o mais utilizado como plastificante em filmes comestíveis. Entretanto, sua adição diminui a resistência à tração, promovendo o alongamento dos filmes, no entanto, a permeabilidade ao oxigênio aumenta com concentrações crescentes de glicerol (Laohakunjit & Noomhorm, 2004). Outro ponto negativo do glicerol é que ele tende a migrar para materiais amiláceos, o que pode levar à retrogradação do amido após períodos mais longos de armazenamento (Zdanowicz, 2020). Nesse contexto, um novo e amplo grupo de meios denominados solventes eutéticos profundos - DES vem sendo testado como agentes plastificantes de amido (Zdanowicz, Wilpiszewska & Spychaj, 2018). Ácido oxálico é reconhecido pelos seus efeitos no controle de doenças na pós-colheita. Entretanto, a sua aplicação direta pode resultar em desordens fisiológicas. A aplicação de ácido oxálico na pós-colheita de frutos e hortaliças tem se mostrado eficiente em retardar o amadurecimento (Huang et al., 2013; Öz; Kafkas; Bozdoğan, 2016; Razzaq et al., 2015), induzido a resistência contra fungos (Ruíz-Jiménez et al., 2014), por estimular o metabolismo secundário e a síntese de fenólicos (Li et al., 2014; Razavi; Razzaq et al., 2015), além de aliviar a incidência de desordens fisiológicas (Li et al., 2016; Ruíz-Jiménez et al., 2014), o que lhe confere caráter funcional. Além disso, o ácido oxálico pode ser usado na formulação de NADES com o cloreto de colina (Martínez et al., 2016), o que evita a liberação

direta na superfície do fruto. A utilização de NADES como plastificante em filmes de amido é, portanto, uma alternativa sustentável e inovadora nos recobrimentos a base de amido, sendo perfeitamente aplicável em pequenos empreendimentos da agricultura familiar, como a citricultura do Território da Borborema.

Neste sentido o objetivo deste estudo foi avaliar as mudanças fisiológicas e da a qualidade e de frutos de tangerina Dancy, armazenados com recobrimento a base de amidos de mandioca e semente de jaca, utilizando NADES como plastificante.

2 MATERIAL E MÉTODOS

2.1 Material vegetal

As tangerinas foram colhidas em pomar comercial no localizado no município de Alagoa nova e transportadas para o Laboratório de Biologia e Tecnologia Pós-Colheita do CCA/UFPB, localizado em Areia - PB. No laboratório, os frutos foram selecionados quanto à maturação, tamanho, ausência de doenças e danos aparentes e tiveram o pedúnculo cortado a 0,5 cm, com faca de aço inox sanificada. Os frutos foram lavados com água corrente para remoção de sujidades e colocados a secar ao ar.

2.2 Elaboração do NADES

O solvente eutetico profundo natural (NADES) foi obtido da combinação de 1 mol de ácido oxálico com 2 mols cloreto de colina. Esses componentes foram colocados em balão de vidro selado, aquecidos e agitados até a formação de uma mistura eutetica líquida e translucida (Abbott et al., 2004).

2.3 Recobrimento de amido de jaca

O amido das sementes de jaca (*Artocarpus heterophyllus* Lam.) Foi preparado após a colheita dos frutos maduros e as sementes foram removidas da polpa e colocadas para secar à

sombra. Após a secagem, o revestimento das sementes foi removido manualmente das sementes e higienizado com 50 ppm de cloro livre por 3 min. As sementes foram trituradas três vezes com água bidestilada, seguida de filtração, e o resíduo foi triturado novamente em um multiprocessador por 3 minutos. Após as filtrações, o produto coletado foi decantado por 12 horas. No final da decantação, a água foi descartada e o amido foi seco no forno a 40 ° C por 10 horas até atingir uma umidade residual de 17% (Rodrigues et al., 2018).

O amido de jaca (2 e 4%) foi gelificado com água destilada a 70 ° C (Lima et al., 2012). Cada dispersão foi combinada com a de 2% de amido, além da dispersão de amido isolada, de modo que após a homogeneização, a concentração dos componentes nas matrizes era de 4%.

2.4 Recobrimento de amido de mandioca

A solução de fécula foi preparada utilizando-se de mandioca de mesa (*Manihot esculenta* Crantz), oriunda de cultivos da agricultura familiar. As raízes foram descascadas, trituradas, adicionada água suficiente para cobrir a massa formada e deixada em repouso por 24 horas. Em seguida, a fécula foi drenada e colocada a secar em estufa a 70 ºC. Depois de seca, a umidade foi determinada e preparou-se solução equivalente a 3,0%, utilizando-se de água destilada fervente, obtendo-se uma solução geleificada que, em seguida, foi resfriada. Após o recobrimento, mangas de todos os tratamentos foram armazenadas (Lima et al., 2012).

2.5 Adição dos plastificantes

A partir de testes preliminares, 1% de glicerol e 0,5% de NADES foram adicionados às matrizes sob constante agitação, quando as soluções atingiram 60 °C. Assim, foram obtidas as formulações FJ+G; FJ+NADES; FM+G e FM+NADES, (FJ: fécula de jaca, G: Glicerol, NADES: Natural Deep Eutectic Solventes, FM: Fécula de mandioca) a serem aplicadas nas tangerinas em comparação a frutos controle – C, sem recobrimento.

2.6 Aplicação dos recobrimentos

As tangerinas foram imersas em cada recobrimento à base de amido de semente de jaca e de mandioca. por 1 min sob rotação suave dos frutos para melhor aderência à dispersão. Em seguida, as frutas revestidas foram mantidas em condições ambientes até a drenagem completa. Posteriormente, os frutos foram colocados em bandejas de isopor e armazenados em condições ambientes (21 ± 1 ° C e 90 ± 5% UR) por 27 dias.

2.7 Delineamento experimental e Análise Estatística

O experimento foi conduzido em delineamento inteiramente casualisado em esquema fatorial 5×6, combinando 5 recobrimentos (FJ+G; FJ+NADES; FM+G e FM+NADES e C) e seis períodos de avaliação (0, 6, 12, 18, 24 e 27 dias), utilizando três repetições, composto por três frutos cada.

Os dados obtidos foram submetidos à análise de variância pelo teste F ($p \leq 0,05$). Para o fator período de armazenamento (dias), foi aplicada análise de regressão polinomial até o segundo grau para as médias dos tratamentos nos dias. As características que não apresentaram ajuste significativo aos modelos com base na significância de seus coeficientes apenas foram apresentadas as médias, comparando-se pelo teste de Tukey ($p \leq 0,05$).

2.8 Avaliações

2.8.1 Produção de Etileno e de CO_2

A avaliação da produção de etileno e CO_2 em tangerina Dancy sob os diferentes recobrimentos foi realizada na condição ambiente (25 ± 2°C) em três repetições de três frutos cada (± 330 g/repetição). Os frutos recobertos foram colocados em potes herméticos sob sistema de fluxo contínuo de oxigênio. As leituras foram iniciadas após os frutos estarem 24 horas sob sistema de fluxo de O_2 e foram realizadas a cada 12 horas durante 13 dias. Foi

injetado 0,3 ml dos gases do fluxo em analisador de CO2 (Sable Systems PA-10a) (DANTAS et al., 2016). A produção de CO_2 foi expressa em CO_2 $ml.h^{-1}.Kg^{-1}$.

As demais avaliações realizadas foram: massa fresca, determinada em balança semi-analítica; firmeza (N) determinada em dois pontos distintos na região equatorial de cada fruto íntegro com penetrômetro (Magness Taylor Pressure Tester, CANADÁ). Com base na AOAC (2006), a acidez titulavel (AT - % de ácido cítrico) foi determinada por titulometria; sólidos solúveis (SS - %) com refratômetro digital (Atago DR A1, Japão); relação SS/AT pelo quociente entre os valores de SS e AT; pH utilizando-se de potenciômetro com eletrodo de membrana de vidro; e teor de ácido ascórbico por titulação com DFI (AOAC, 2006).

3 RESULTADOS E DISCUSSÃO

3.1 Respiração

A tangerina apresentou um padrão de liberação de CO_2 típico de frutos NÃO climatérico (Figura 1). Os recobrimentos influenciaram na taxa respiratória, representada pela liberação de CO_2. Tangerinas do controle apresentaram maior taxa respiratória (3.48 mL $CO_2.Kg^{-1}.h^{-1}$) do inicio do armazenamento até as 156 horas o que corresponde a 6.5 dias de armazenamento, enquanto que o tangerinas com ASJ apresentou a menor taxa respiratória (2.28 mL $CO_2.Kg^{-1}.h^{-1}$) durante o mesmo período. Estes resultados indicam que a aplicação de recobrimentos reduz a taxa metabólica de tangerina Dancy.

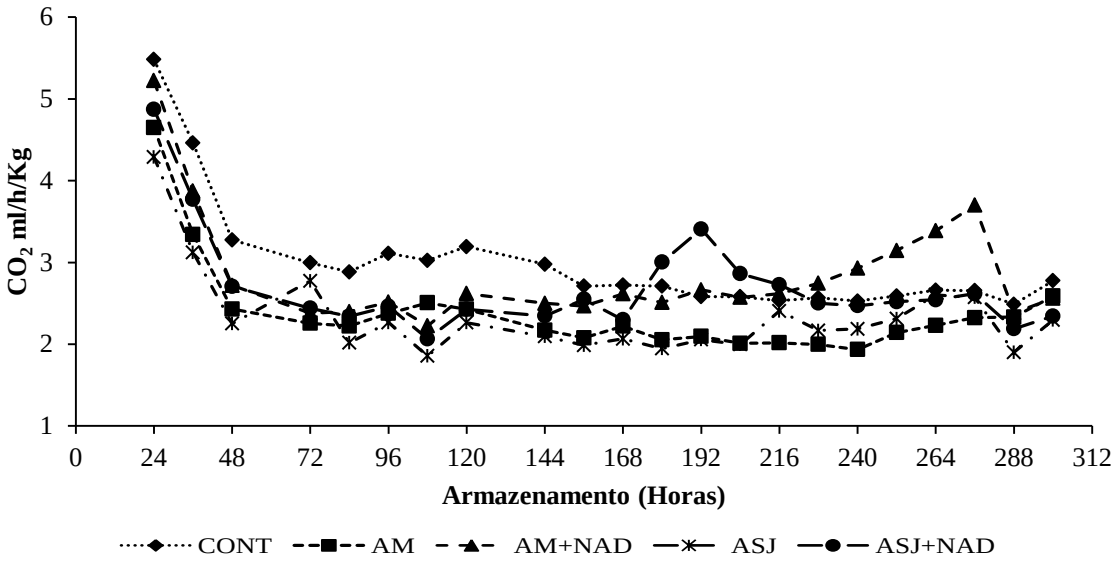


Figura 14. Taxa respiratória (mL $CO_2.Kg^{-1}.h^{-1}$)de tangerina Dancy armazenada com recobrimento biodegradável mantidos sob a condição ambiente (21±1°C e 90±% UR). CONT (Controle), AM (Amido de mandioca + Glicerol), AM+NAD (Amido de Mandioca + Natural deep eutectic solventes), ASJ (Amido de semente de Jaca + Glicerol), ASJ+NAD (Amido de semente de Jaca + Natural deep eutectic solventes).

Esse comportamento e resultados são semelhantes aos resportados por Khalid et al., (2017) em frutos de tangerina 'Kinnow', onde foi verificado um queda busca na produção de CO_2 no inicio da avaliação e depois os com pequenas oscilações entre 2.5 e 4.5 $ml.h^{-1}.Kg^{-1}$ durante o período 7 dias de armazenamento.

Os frutos citricos são classificados como não climatológicos, de acordo com seus padrões de respiração e produção de etileno; sua taxa de respiração diminuiu constantemente após a colheita (Sudheer e Indira, 2007; Khalid et al., 2017; Morales et al., 2020). O quanto menor for a taxa respiratória de um fruto, maior é a sua vida útil, decorrente da menor utilização dos substratos armazenados, devido ao baixo processo oxidativo relacionados a menor atividade das enzimas da cadeia respiratória (Siddiqui, 2018). E Como as frutas citricas pertences ao grupo não climatérico, os quais não possuem grandes reservas de carboidratos, o amadurecimento ocorre enquanto ligado à planta (Hui et al., 2004).

3.1 Perda de Massa e Firmeza

Foi observada interação significativa para perda de massa dos frutos de tangerina Dancy armazenados sob condições ambientes (figura 1A), ouve um comportamento semelhante para todos os tratamentos e a perda de massa total foi entre 10 e 13 % ao final do período de 27 dias de armazenamento.

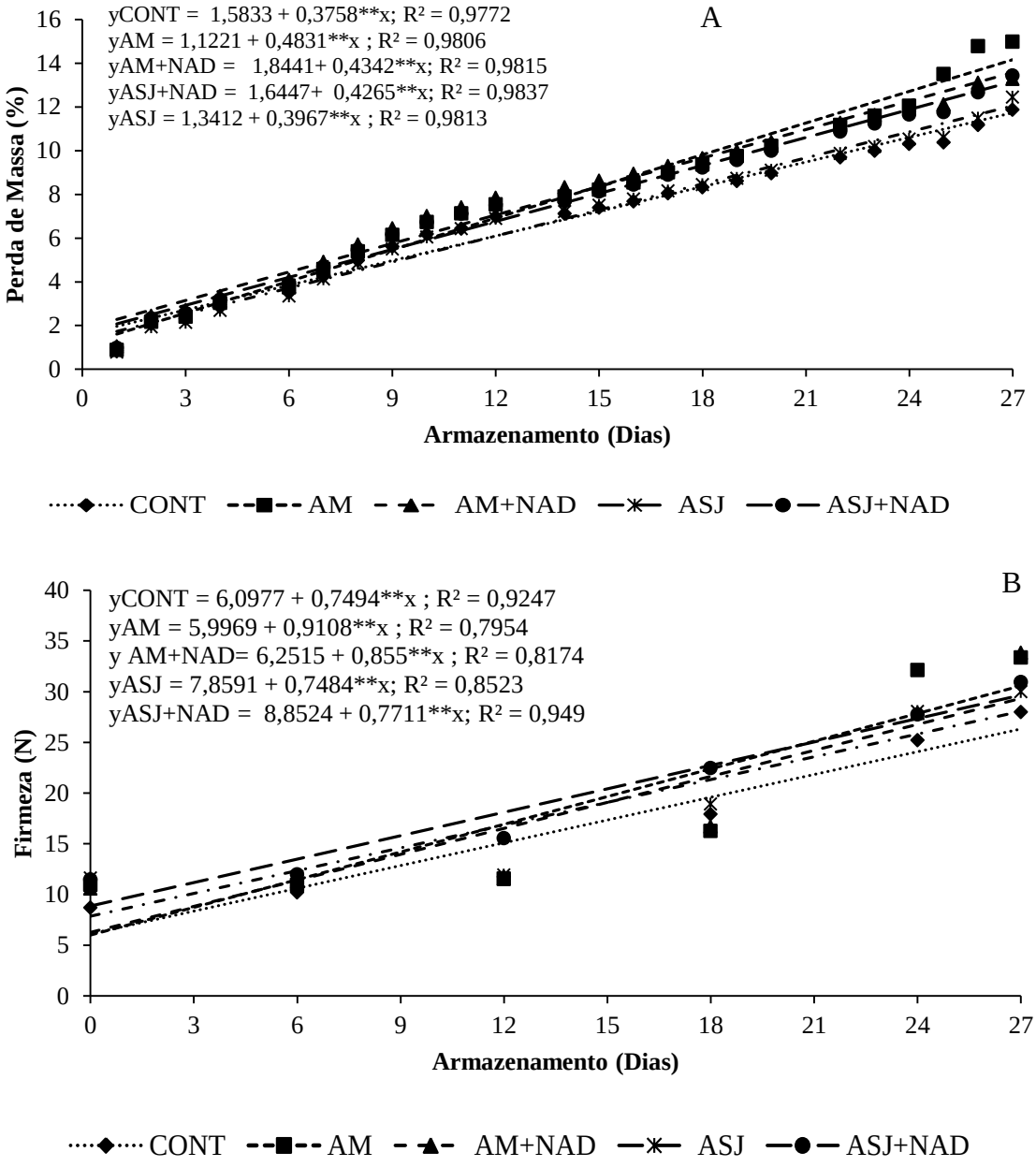


Figura 15. Perda de massa fresca (A) e Firmeza (B) de tangerina Dancy armazenada com recobrimento biodegradável mantidos sob a condição ambiente (21±1°C e 90±% UR). CONT (Controle), AM (Amido de mandioca + Glicerol), AM+NAD (Amido de Mandioca + Natural deep eutectic solventes), ASJ (Amido de semente de Jaca + Glicerol), ASJ+NAD (Amido de semente de Jaca + Natural deep eutectic solventes).

Em frutos de tangerineira Ponkan recobertos com filmes armazenados sob condições ambiente, Dantas et al. (2018) verificou uma perda de massa fresca foi de 14,86% ao final dos 18 dias de armazenamento.

Condições de armazenamento como Temperatura, umidade relativa do ar, bem como as embalagens ou recobrimentos aplicados nos frutos são fatores que influencia na perda de massa de frutos cítricos (Ladaniya & Ladaniya, 2010; Rodrigues et al 2018).

A firmeza da tangerina 'Dancy' também teve aumento linear ao longo dos 27 dias de armazenamento. Na grande maioria dos frutos ocorre uma redução na firmeza ao longo do período de armazenamento. Esta característica é muito relativa, e especifica para cada tipo de casca que o fruto possui, pois a casca sofre com a perda de água para o ambiente causando o ressecamento da mesma, assim a casca pode se tornar desidratada necessitando de uma maior pressão para penetrá-la (Ladaniya, 2008). Dantas et al, (2018) também encontraram maior firmeza no final do armazenamento de tangerina Ponkan a 24 ºC, segundo ele os frutos apresentavam aspecto desidratado e por isso o firmeza era maior que a inicial.

A manutenção da firmeza é um dos fatores importantes para se conseguir transportar os frutos a longas distancias além de ajudar a definir a quantidade de frutos por caixa, e os tipos de embalagens que podem ser utilizadas no armazenamento e transporte até o consumidor final (Pareek, 2016). A firmeza dos frutos é um importante indicativo de qualidade e vida útil mais longa, sendo relevante na aceitação pelo consumidor (Aquino et al., 2015).

3.2 Luminosidade e coloração da casca

Para luminosidade ao longo do tempo ouve uma variação de 30.89 no inicio a 36.49 no final do armazenamento (figura 3A). Por outro lado, os recobrimentos diferiram entre si, considerando o efeito isolado dos tratamentos (figura 3B). Os frutos recobertos com ASJ + NADES apresentaram a maior luminosidade 33.57, diferindo dos demais tratamentos com exceção do controle que foi de 34.59. Os tratamentos AM e ASJ possuem as menores médias

de luminosidade com 32.28 e 32.68 respectivamente.

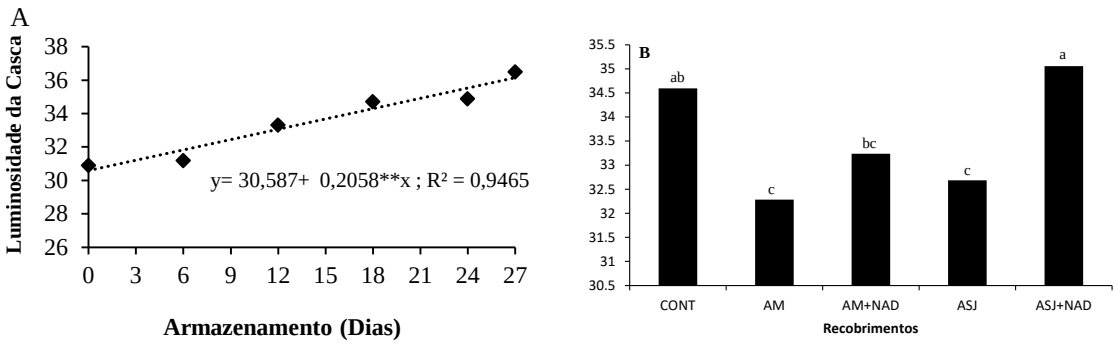


Figura 16. Luminosidade de tangerina Dancy armazenada com recobrimento biodegradável mantidos sob a condição ambiente (21±1°C e 90±% UR). CONT (Controle), AM (Amido de mandioca + Glicerol), AM+NAD (Amido de Mandioca + Natural deep eutectic solventes), ASJ (Amido de semente de Jaca + Glicerol), ASJ+NAD (Amido de semente de Jaca + Natural deep eutectic solventes).

Em frutos de tangerina Ponkan, Dantas et al. (2018) encontraram maior luminosidade nos frutos do controle do que aqueles com recobrimento, armazenados à temperatura ambiente (24 ºC), nos frutos de Dancy a variação média foi de 43,6 a 44,7.

Quanto ao parâmetro de colocaração a* os tratamentos controle, AM, AM+NAD e ASJ+NAD apresentaram um aumento linear ao longo do armazenamento, enquanto que a ASJ ajustou-se ao modelo quadrático. O tratamento ASJ+NAD tinha o maior valor de a* ao final do armazenamento 22.76, seguidos do controle com 19.09 e ASJ com 17.91; isso significa que estes recobrimentos não interferem na evolução natural da coloração da casca, visto que o parâmetro a* varia de verde (-60) a vermelho (+60).

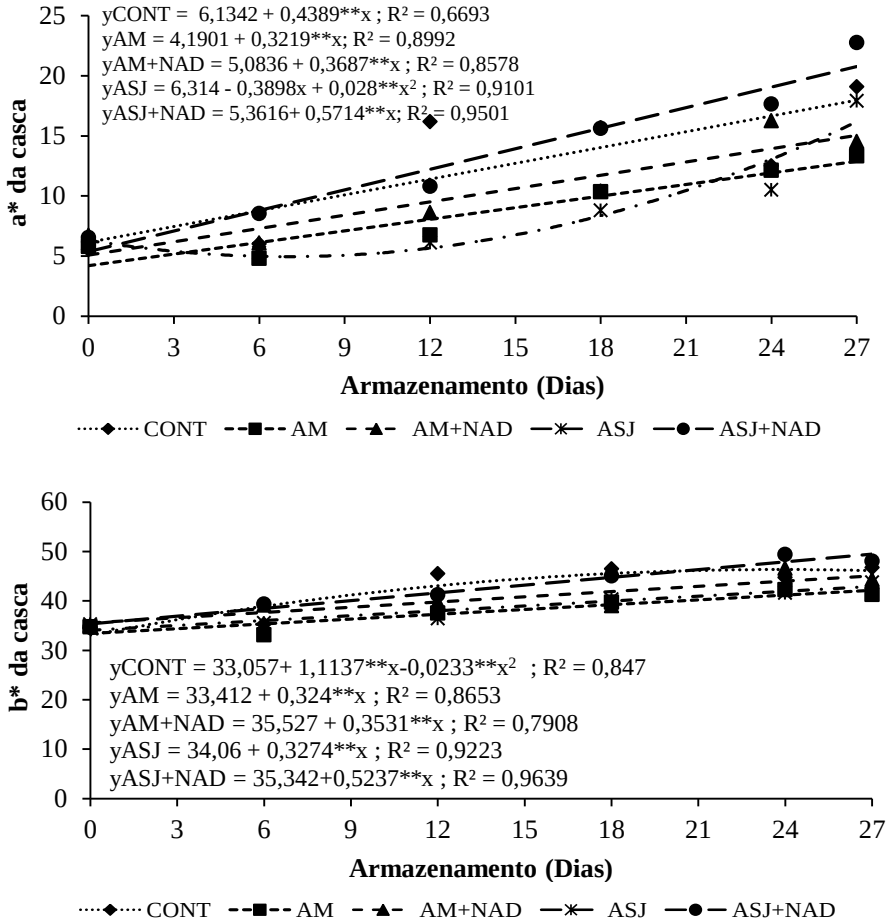


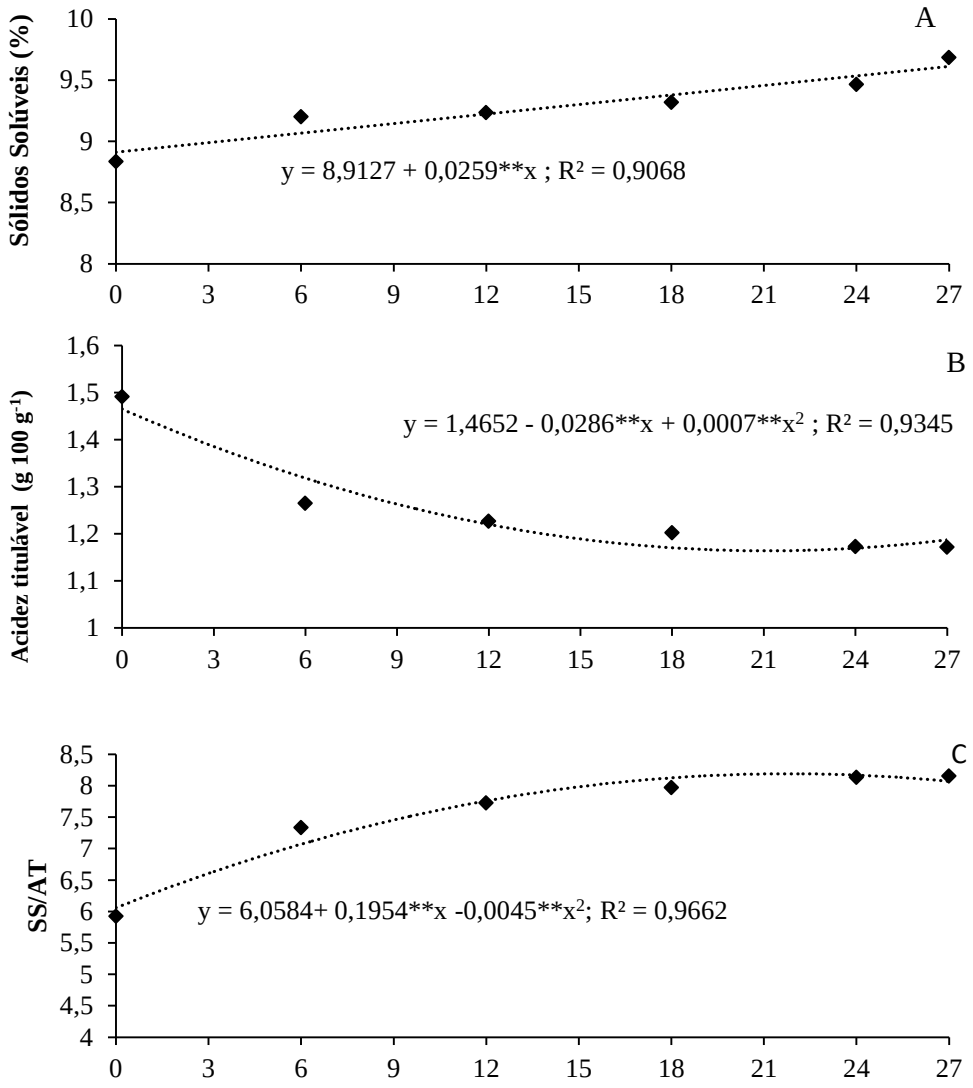
Figura 17. Parametros de coloração a* e b* da casca de tangerina Dancy armazenada com recobrimento biodegradável mantidos sob a condição ambiente (21±1°C e 90±% UR). CONT (Controle), AM (Amido de mandioca + Glicerol), AM+NAD (Amido de Mandioca + Natural deep eutectic solventes), ASJ (Amido de semente de Jaca + Glicerol), ASJ+NAD (Amido de semente de Jaca + Natural deep eutectic solventes).

Com exceção do controle, houve um acréscimo linear ao longo do armazenamento para o parâmetro b*, o qual varia do Azul (-60) ao amarelo (+60). O recobrimento com ASJ+NAD apresentou a maior evolução da coloração neste parametro variando do 34.68 ao 48.01.

Tanto para o parâmetro a* quanto no b* o recobrimento de amido de mandioca com glicerol (AM) apresentou a menor evolução ao longo do armazenamento, isso significa que este recobrimento interfere no desenvolvimento da coloração da casca dos frutos de tangerina Dancy.

3.2 Físico-químicas

O teor de sólidos solúveis (figura 4A) evoluiu linearmente ao longo do armazenamento de 8.83 % no inicio do armazenamento para 9.68 % aos 27 dias. Não ouve interação significativa entre os tratamentos. Estes resultado é aproximado ao encontrado por Dantas et al. (2018) em tangerina Ponkan armazenada em temperatura ambiente que variaram entre 8,5 e 9,3%. Belo et al. (2018) caracterizando 26 variedades de tangerinas encontraram valores que variam de 7.52 a 13.23 % de sólidos solúveis nos frutos maduros.



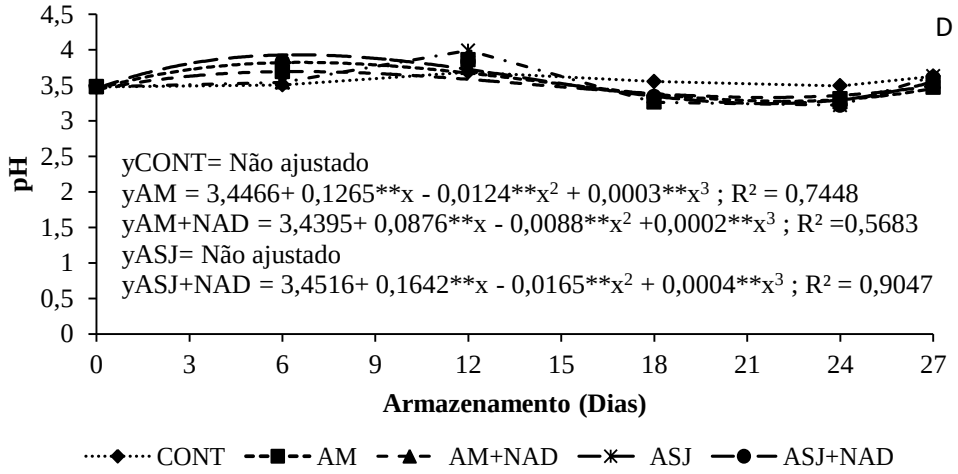


Figura 18. Sólidos solúveis (A), Acidez titulável (B), Relação sólidos solúveis (SS/AT) (C), acidez titulável (D) de tangerina Dancy armazenada com recobrimento biodegradável mantidos sob a condição ambiente (21±1°C e 90±% UR). CONT (Controle), AM (Amido de mandioca + Glicerol), AM+NAD (Amido de Mandioca + Natural deep eutectic solventes), ASJ (Amido de semente de Jaca + Glicerol), ASJ+NAD (Amido de semente de Jaca + Natural deep eutectic solventes).

Sacarose, glicose e frutose são os principais açúcares solúveis presentes no suco de tangerina, conforme reportado por Zhu et al. (2011). A manutenção do conteúdo de sólidos solúveis durante o armazenamento foi reportado por Damiani et al. (2008) para a tangerina minimamente processada.

A acidez titulável (figura 4B) decresceu ao longo do armazenamento de 1.49 g 100 g^{-1} no inicio do armazenamento para 1.17 g 100 g^{-1} no final. Em tangerina Ponkan armazenadas em temperatura ambiente durante um período de 18 dias Dantas et al. (2018) encontraram uma variação de acidez titulável entre 0,46 e 0,59 g.100g^{-1}. O teor de acidez titulável varia de cultivar para cultivar e é natural uma alteração ao longo do armazenamento. Belo et al. (2018) caracterizando 26 variedades de tangerinas reportou uma variação de acidez titulável de 0.52 a 2.19 g.100g^{-1} do ácido cítrico.

As tangerinas são preferencialmente consumidas quando a acidez é reduzida ao ponto em que seu suco se torna agradável ao paladar dos consumidores, e o ácido cítrico é seu principal ácido orgânico (Couto & Canniatti-Brazaca, 2010).

A relação SS/AT (figura 4C) teve um aumento quadrático, partindo de 5.93 a 8.16 no

final do armazenamento. Este comportamento foi provocado pelo aumento dos sólidos solúveis aliados a redução na acidez. Para a maioria dos frutos cítricos, a relação SS/AT é um índice utilizado para monitorar a maturidade, determinando o balanço do sabor doce com a finalidade de consumo na forma fresca (Couto & Canniatti-Brazaca, 2010; Dantas et al. 2018).

Para o pH ouve interação tempo x recobrimento para os tratamentos AM, AM+NAD e ASJ+NAD no entanto os tratamentos controle e ASJ não se ajustaram ao modelo. No inico do armazenamento as tangerinas tinha um pH de 3.48 e aumentaram no de 12 ficando entre 3.69 para o controle e 3.99 pra ASJ, a partir daí todos os tratamento apresentaram um decréscimo e no final do armazenamento o pH voltou a aumentar. Segundo Belo et al. (2018) os valores médios de pH da tangerina foi 3,49 variando de 2,99 a 3,96 em estudo com 26 variedades.

4 CONCLUSÕES

Os recobrimentos reduziram a taxa metabólica, mantiveram a qualidade pós-colheita por um maior período de tempo, reduziu a intensidade dos processos fisiológicos, manteve os conteúdos sólidos solúveis, acidez titulável e ratio, os quais são os atributos de sabor. Ao mesmo tempo, os frutos recobertos com os amidos tanto da mandioca quanto da semente de jaca, permitiram a evolução da coloração da casca, ao tempo que retardaram o amadurecimento, o qual é determinante para a comercialização.

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Artigo 6. QUALITY, BIOACTIVE COMPOUNDS AND ANTIOXIDANT ACTIVITY DURING MATURATION OF ORANGES PRODUCED IN THE BORBOREMA TERRITORY[1]

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ABSTRACT - The family farming from the Borborema Territory-PB, Brazil, produces sweet oranges that supply the regional market. In this context, it is necessary to define standards of identity and quality, as well as to quantify the bioactive compounds in the fruit, in view of adding value and creating more competitive markets. This work sought to evaluate the quality, bioactive compounds and total antioxidant activity (TAA) of oranges from family farming. A 3×3 factorial completely randomized design was used, with three cultivars (Baía, Comum, Mimo-do-Céu) and three maturity stages (predominantly green, green/yellow, yellow), with 60 replications of 1 fruit for the physical evaluations, and 4 of 15 fruit for the others. The whole fruits were evaluated by color index, length, diameter, fresh weight and firmness. The juice was assessed for yield, pH, soluble solids (SS), titratable acidity (TA), SS/AT ratio, and ascorbic acid. Total extractable polyphenols (TEP) and $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ total antioxidant activity (TAA) were measured in the juice and albedo. The 'Baía' and 'Mimo-do-Céu' oranges presented quality parameters aligned with the CEAGESP standards. On average, the ascorbic acid content was higher than 45 mg 100 g^{-1}, with 'Mimo-do-Céu' presenting the highest content (50.26 mg 100 g^{-1}). During maturation, the firmness decreased, and the SS, TEP and TAA of the juice and the albedo increased. In general, the TEP content was about eight-fold higher in the albedo than juice, corresponding to the much higher TAA in this portion, thereby highlighting its higher functional potential, especially for 'Baía' orange.

Keywords: *Citrus sinensis.* Quality standards. Albedo. Functional potential. Adding value.

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QUALIDADE, COMPOSTOS BIOATIVOS E ATIVIDADE ANTIOXIDANTE DURANTE A MATURAÇÃO DE LARANJAS PRODUZIDAS NO TERRITÓRIO DA BORBOREMA

RESUMO - A agricultura familiar do Território da Borborema-PB, produz laranjas doces que supre o mercado regional. Nesse contexto, se faz necessária a definição de padrões de identidade e qualidade, bem como quantificar os compostos bioativos nas porções do fruto buscando a agregação de valor, visando mercados mais competitivos. O objetivo deste trabalho foi avaliar a qualidade, compostos bioativos e atividade antioxidante de laranjas da citricultura familiar. Utilizou-se um DIC, fatorial 3x3, com três cultivares (‘Baía’, ‘Comum’ e ‘Mimo-do-Céu’), três estádios de maturação C1 (predominantemente verde); C2 (verde amarelado) e C3 (amarelo), com 60 repetições de um fruto para as avaliações físicas e 4 de 15 frutos para as demais. As avaliações no fruto inteiro foram: índice de coloração, comprimento, diâmetro, massa fresca e firmeza. No suco foram avaliados: rendimento, pH, sólidos solúveis, acidez titulável, SS/AT e ácido ascórbico. Os polifenóis extraíveis totais (PET) e atividade antioxidante total (AAT) foram determinados pelos métodos $ABTS^{\bullet+}$ e $DPPH^{\bullet}$ no suco e albedo. As laranjas ‘Baia’ e ‘Mimo-do-Céu’ apresentam parâmetros de qualidade que se enquadram nas Normas do CEAGESP. Em média o teor de ácido ascórbico foi superior a 45 $mg.100g^{-1}$, sendo a ‘Mimo–do-Céu’ com maior teor (50,26 $mg.100g^{-1}$). Durante a maturação a firmeza diminuiu e os sólidos solúveis, PET e a AAT do suco e do albedo aumentaram. Em geral, o albedo apresentou teor de PET cerca de oito vezes superior ao do suco, que refletiu em AAT bem superior nesta porção que, portanto, se destacou pelo elevado potencial funcional, principalmente na laranja ‘Baia’.

Palavras-chave: *Citrus sinensis.* Padrões de qualidade. Albedo. Potencial funcional. Agregação de valor.

1 INTRODUCTION

The orange [*Citrus sinensis* (L.) Osbeck] belongs to the Rutaceae family and is one of the most cultivated fruit trees. Its fruit are among the most commercialized and consumed, and Brazil is the largest producer in the world (SOUSA et al., 2016). Approximately 80% of the national citrus production is concentrated in the southeast region, in the state of São Paulo. However, the northeast is the second largest producing region of the country, contributing to 11% of the national production (CNA, 2018; IBGE, 2018). In the Borborema Territory, state of Paraíba, citriculture is the major activity of the family farming, generating employment, income and development for the region (BRASIL, 2010), by producing table oranges to meet the demands of the regional market, with particular attention focused on Baía, Comum and Mimo-do-Céu cultivars (LOPES et al., 2007).

However, it is necessary to establish standards of identity and quality for the classification of fruits intended for the local market, allowing to add value to the product and improve profits for the producers. Furthermore, it is necessary to evaluate the content of the bioactive compounds and the antioxidant activity of each edible portion of the fruit, seeking to add value not only to the juice, but also to other edible portions of the fruit that are usually discarded (LADO; GAMBETTA; ZACARIAS, 2018; RODRIGO et al., 2013).

Oranges are recognized as a source of vitamin C, carotenoids and phenolic compounds that have high antioxidant capacity both in the juice and its byproducts, such as the albedo (mesocarp), which can be consumed fresh associated with the pulp (endocarp) or used in the preparation of products with functional properties for human consumption (YOO; MOON, 2016). The antioxidant substances present in the albedo can inhibit reactive free radicals and, consequently, protect other molecules against oxidation, generating health-promoting effects in the prevention of degenerative diseases, including cancer (CIRMI et al., 2016; YOO et al.,

2004).

In this sense, the albedo of the citrus, usually discarded, also contains dietary fiber in higher quantity and quality than cereal fibers, in addition to bioactive compounds (flavonoids, carotenoids and vitamin C), which provide additional health benefits (LADO; GAMBETTA; ZACARIAS, 2018). The citrus albedo has a higher content of total dietary fiber due to a higher soluble fiber fraction than the peeled pulp. Certain water-soluble fibers, such as pectin, reduce the postprandial serum-cholesterol and the blood glucose in humans. Insoluble fibers (lignin, cellulose and some hemicelluloses) serve almost exclusively as bulking agents that promote less intestinal transit time and increase the fecal mass, thereby improving the efficiency of the intestine and colon, and reducing the risk of colorectal cancer (BILGIÇLI; AKTAŞ; LEVENT, 2014).

Maturation induces changes that are influenced by environmental factors and agronomic practices, and the study of these changes is fundamental to define the maturity and quality indices of fruits (DANTAS et al., 2016; WANG et al., 2016). Regardless of whether the orange is intended for the industry or fresh consumption, some physical aspects, such as coloring, fresh weight, pulp yield, besides the functional potential, should be deeply considered for standardization, classification and adding value to the product. In this way, differential criteria for quality can be assigned, which will support the practice of fair prices while enabling more competitive markets (LADO; GAMBETTA; ZACARIAS, 2018).

Based on the above, this study aimed to evaluate the quality, bioactive compounds and antioxidant activity of the sweet orange cultivars Baía, Comum and Mimo-do-Céu, produced in the Borborema Territory-PB, harvested at three distinct stages of maturity.

2 MATERIAL AND METHODS

The fruits were harvested from a commercial orchard of the family farming, conducted under usual cultural practices of cleaning, pruning, organic fertilization and rescue irrigation,

located in the municipality of Alagoa Nova, Borborema Territory region, Paraiba state, Brazil. The rural property has predominant soil type ARGISSOLO RED Eutrophic abrupt (EMBRAPA, 2016). The predominant tropical climate is "As", according to the classification of Köppene Geiger, with annual average temperature of 22.2 °C and average annual rainfall of 1317 mm, with higher rainfall intensities in the winter (ALVARES, 2014).

Oranges of the cultivars Baía, Comum and Mimo-do-Céu were randomly harvested from the orchard, between 6 and 8 A.M., in 3 maturity stages by selection of peel coloration, corresponding to the Subclasses: C1 - predominantly green; C2 - green/yellow; C3 - yellow peel (Figure 1), according to CEAGESP (2011) standards, as well as the absence of pests, diseases and apparent injuries. At the time of harvest the fruits were packed in high density polyethylene boxes, protected with bubble wrap and transported to the laboratory for the analysis.

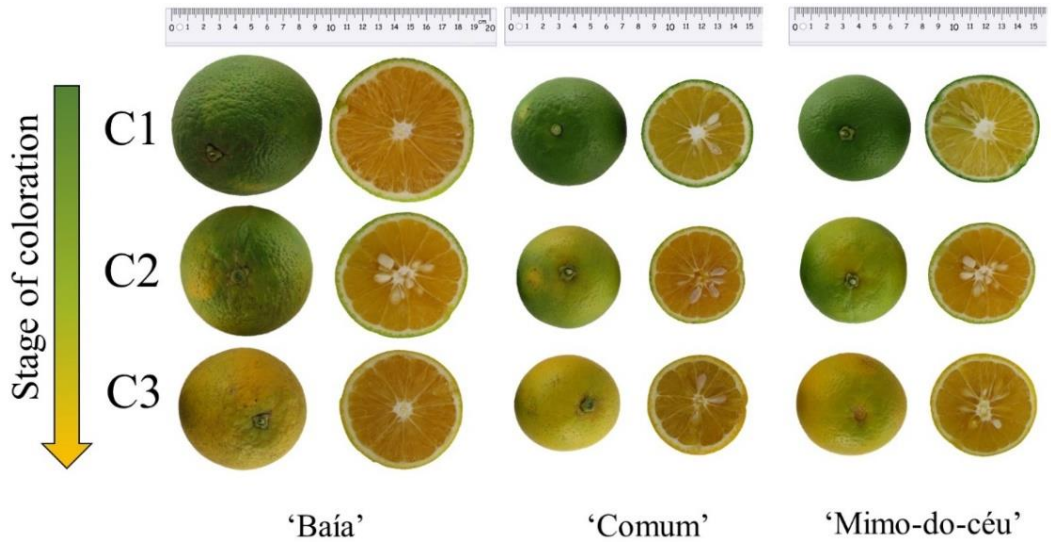

Figura 1. Appearance of whole and halved fruits of 'Baía', 'Comum' and 'Mimo-do-Céu' oranges harvested in three maturity stages (C1 - predominantly green; C2 - green/yellow; C3 – yellow), based on the color of the peel, from the Territory of Borborema – PB, Brazil.

The experiment was conducted in a completely randomized design (CRD) and arranged in a simple 3x3 factorial scheme, with three orange cultivars (Baía, Common and Mimo do Céu) and three stages of peel coloration (C1, C2 and C3). For the physical characteristics, 60

fruits per maturity stage of each cultivar were used, each fruit being considered a replication, totaling, therefore, 60 replications per maturity stage and 180 fruit per cultivar. For the physicochemical characteristics, bioactive compounds, and antioxidant activity, 4 replications of 15 fruit per maturity stage were used for each cultivar.

In the laboratory, the physical characteristics evaluated for fruits of each cultivar were: fresh mass (g), obtained with the help of a semi analytical scale; longitudinal and transverse diameters (mm) measured with the aid of a caliper; juice yield, determined by the ratio between the total fresh fruit mass and the mass of the peel, seeds and pulp residue (%); firmness (N), determined in two points in the equatorial region of each fruit with a bench penetrometer, and the evolution of the peel color through an objective evaluation, using a Minolta digital calorimeter, which expresses the color in the parameters: L* (corresponds to the clarity/brightness); a* (defines the transition from the green color (-a*) to the red color (+ a*) and b* (represents the transition from the blue color (-b*) to the yellow color (+ b*), and the farther from the center (= 0), the more saturated is the color. Afterwards, these parameters (L, a *, and b *) were used to calculate the color index (CI), which indicates the degree of green/yellow variation of the fruits, according to the equation proposed by Camelo and Gomes (2004), $IC=2000^{*}(a^{*})/L^{*}\sqrt{(a^{*})^{2}+(b^{*})^{2}}$.

For the physicochemical evaluations, bioactive compounds and antioxidant activity, the fruits were separated into four replicates of 15 fruits. The evaluations after extraction and homogenization of the juice were: pH; soluble solids (SS%); titratable acidity (AT%) and SS/AT ratio by the quotient between SS and TA, according to the methodologies described by Silva et al. (2014), and ascorbic acid content (AA mg.100 g^{-1}) as measured by titration with DFI (STROHECKER; HENNING, 1967).

The total extractable polyphenols content (Galic Acid Equivalent – GAE) and the antioxidant activities by the sequestration capacities of the 2,2'-azino-bis-3-

ethylbenzothiazoline-6-sulfonic acid radical cation ($ABTS^{+}$) and the stable free radical 2,2-diphenyl-1-picrylhydrazil (DPPH) were evaluated in the juice and albedo, according to methodology described by Dantas et al. (2015) and the results were expressed as μM Trolox (Trolox Equivalent - TE) g^{-1} fresh weight. The antioxidant activity was determined by the DPPH method, according to Rufino et al. (2010) and its antioxidant capacity was expressed as the antioxidant concentration required to reduce the amount of free radicals by 50% (EC50), with the values expressed in g fruit/g DPPH.

The data of the evaluations were submitted to analysis of variance (ANOVA) and the means of the varieties, the stages, and the unfolding of the interaction x variety were submitted to the Tukey test up to 5% probability. In order to perform these analyzes, the Emmeans software package R version 5.1 was used (LENTH, 2018).

3 RESULTS AND DISCUSSION

The physical changes occurring during the maturation of the orange cultivars evaluated are presented in Table 1. Notably, the color index (CI) of the fruits evolved significantly with the advance of maturation, indicating that the coloration transformed from green to completely yellow. However, this evolution was less pronounced in cultivar Baía, whose CI increased from -6.87 for fruit harvested in the C1 stage (green coloration) to 3.23 in the C3 stage (yellow). For Comum orange, the CI varied from -10.45 (at C1) to 3.83 (at C3). In turn, the most pronounced transformation in the coloration was observed for Mimo-do-Céu, with a CI spanning -14.93 (at C1) to 4.85 (at C3). These changes correspond to the transition of the coloration of the peel, from green to yellow/orange, due to the degradation of chlorophyll and synthesis of carotenoid pigments (DING et al., 2015). During maturation, changes in fruit color are expected, and there may be an expressive increase in the CI from the intermediate phase of ripening (DANTAS et al., 2015).

Table 5. Physical characteristics of Baía, Comum e Mimo-do-Céu orange fruit cultivars harvested in three maturity stages, based on the color of the peel

		Maturity Stage	Color index	Length (mm)	Diameter (mm)	Fresh weight (g)	Firmness (N)
Cultivar	Baía	C1	-6.87 cA	89.41 aA	86.63 aA	363.23 aA	49.14 aA
		C2	-2.17 bA	81.72 bA	80.03 bA	283.41 bA	40.15 aA
		C3	3.23 aA	79.91 bA	78.69 bA	263.13 bA	27.09 bA
		Mean	-1.94	83.68	81.78	303.26	38.79
		CV %	31.28	10.14	9.68	17.34	45.64
	Comum	C1	-10.45 cB	73.79 aB	77.55 aB	143.71 aB	27.12 aB
		C2	-4.02 bB	59.02 bB	61.61 bB	125.03 aB	20.7 abB
		C3	3.83 aA	57.63 bC	60.63 bC	113.86 aC	16.18 bB
		Mean	-3.54	63.48	66.60	127.53	21.33
		CV %	36.25	8.09	8.61	18.12	44.7
	Mimo-do-Céu	C1	-14.93 cC	60.84 aC	64.06 aC	129.53 aB	44.97 aA
		C2	-4.68 bB	62.44 aB	65.13 aB	144.07 aB	19.88 bB
		C3	4.85 aA	62.81 aB	66.69 aB	148.62 aB	25.53 bA
		Mean	-4.92	62.03	65.29	140.74	30.12
		CV %	38.41	6.66	5.91	17.69	47.54

In the column, means followed by the same lowercase letter within each cultivar among maturity stages, and by the same capital letter for each maturity stage among cultivars, do not differ, by the Tukey test: $p \leq 0.05$. $n = 60$.

For ‘Baía’ and ‘Comum’ oranges, the largest fruit, based on length (89.41 and 73.79 mm) and diameter (86.63 and 77.55 mm), were observed in the first maturity stage (C1). For ‘Mimo-do-Céu’, the length and diameter did not differ among the three maturity stages evaluated. However, according to the CEAGESP classification standards (2011), the oranges of these three cultivars from the Borborema Territory are classified as medium-sized, and, under this criteria, meet the demands of more competitive markets for quality. Size is one of the characteristics used by the CEAGESP to classify fruits by caliber and, thus, define the marketing prices.

The overall mean fresh weight of the fruits was 303.26 g, and the highest was found for ‘Baía’ orange, with 363.23 g in the C1 stage (Table 1). ‘Baía’ fruit presented a reduction in fresh weight during maturation. Conversely, the fresh weight of oranges of the cultivars Comum and Mimo-do-Céu did not differ between maturity stages, with mean values of 127.53 and 140.74 g, respectively. Growing conditions, soil, water availability and the supply of the essential nutrients for the crop influence the fresh weight, size and general quality of the fruits (LADO; GAMBETTA; ZACARIAS, 2018).

Regarding the firmness, oranges harvested in the C1 stage were firmer, mainly for ‘Baía’ (49.14 N) and ‘Mimo do Céu’ (44.97 N), and the less firm was ‘Comum’ in the C1 (27.12 N) and C3 (16.18 N) maturity stages. Possibly, these differences in firmness among cultivars are associated with the different thicknesses and polymeric structure of the peel (LADO; GAMBETTA; ZACARIAS, 2018), among other factors. For ‘Ponkan’ mandarin at maturity stage C3, Silva et al. (2014) reported a mean firmness of 11.78 N. In the present study, the orange firmness declined as maturity advanced. This effect can be justified because the firmness is directly related to the solubilization of pectic substances present in the orange albedo (RODRIGO et al., 2013) and so the firmer the fruit, the less these polymers are solubilized (DING et al., 2015). Firmness is one of the most important factors in defining the

amount of fruit per box during packaging operations, in addition to the types of packaging that can be used for the transport and storage (PAREEK, 2016).

The juice yield (Table 2) did not differ among maturity stages within the cultivars. However, Baía orange showed the lowest juice yield (43%), and the highest yield was derived from Mimo-do-Céu (66.83%) in the C3 stage. The percentage of juice yields for ‘Baía’ and ‘Comum’ oranges are close to the pulp yields of 48.6 to 53.8%, reported by Emmanouilidou and Kyriacou (2017) for ‘Delta’ orange from different rootstocks, at different maturity stages. In comparison, 'Mimo-do-Céu' orange, herein, was far superior regarding this characteristic. In addition, for these three cultivars from the Borborema Territory, evaluated at the three maturity stages, the juice yield was higher than that established by the CEAGESP (2011) in the Classification Standards for Table Citrus. A high juice yield in oranges is extremely important, both for the juice industry and for fresh consumption, and may even add value to the fruit (PAREEK, 2016), as a result of this desirable characteristic for consumption, which impacts positively on the yields of the industrialized product and consumer satisfaction of fresh fruit.

Table 6. Physicochemical characteristics of Baía, Comum e Mimo-do-Céu orange fruit cultivars harvested in three maturity stages, based on the color of the peel

		Maturity Stage	Juice (%)	pH	Soluble Solids (%)	Titratable Acidity (g.100g¹)	SS/TA
Cultivar	Baía	C1	37.44 aB	3.73 aB	9.31 cA	0.52 bB	17.90 aB
		C2	46.96 aB	3.59 abB	10.94 bA	0.62 abB	17.65 aB
		C3	45.10 aB	3.52 bB	12.31 aAB	0.65 aB	18.93 aB
		Mean	43.17	3.61	10.85	0.60	18.16
		CV %	9.29	2.74	3.28	6.05	6.90
	Comum	C1	52.98 aA	3.28 aC	8.75 cAB	1.07 aA	8.18 aB
		C2	50.83 aB	3.36 aC	9.94 bB	0.83 bA	11.98 aB
		C3	50.97 aB	3.27 aC	12.00 aB	1.04 aA	11.54 aB
		Mean	51.59	3.30	10.23	0.98	10.57
		CV %	8.81	1.40	9.95	9.34	7.80
	Mimo-do-Céu	C1	57.95 aA	5.88 aA	8.06 bC	0.07 aC	115.14 bA
		C2	62.13 aA	5.76 aA	11.43 bA	0.07 aC	163.29 aA
		C3	66.83 aA	5.85 aA	13.13 aA	0.10 aC	131.30 bA
		Mean	62.30	5.83	10.88	0.08	136.58
		CV %	8.98	1.60	5.76	21.78	7.21

In the column, means followed by the same lowercase letter within each cultivar among maturity stages, and by the same capital letter for each maturity stage among cultivars, do not differ, by the Tukey test: $p \leq 0.05$. $n = 4$.

The pH of the ‘Baía’ orange decreased during maturation from 3.73 (at C1) to 3.52 (at C3), but did not differ among the maturity stages for 'Common' and 'Mimo-do-Céu', with mean pH values of 3.30 and 5.83, respectively. Considering that the lower the pH value, the more acidic the orange, ‘Comum’ was the most acidic while ‘Mimo-do-Céu’ was the less acidic orange. ‘Mimo-do-Céu’ is classified as a orange of the group, characterized by low acidity (SOUSA et al., 2016), which explains why its pH is much higher than those of the other evaluated cultivars.

The soluble solids (SS%) content, an indirect indicator of the sugar level of the fruit, increased significantly as maturation advanced. For ‘Baía’ orange, the SS content increased

from 9.31 to 12.31%, ‘Comum’ from 8.75 to 12%, and ‘Mimo-do-Céu’ from 8.06 to 13.13%, respectively. The CEAGESP (2011) Standards for Table Oranges establish a minimum SS of 10% for commercialization. Thus, at the more advanced maturity stages (C2 and C3), orange cultivars Baía and Mimo-do-Céu from the Borborema Territory exceed the CEAGESP standards in these criteria, whereas, for Comum, only the C3 stage complied with this regulation, which characterizes this cultivar as acid and not sweet, and, in turn, explains its lower SS/TA ratio, and, consequently, lower perceived sweetness. In this sense, according to Ding et al. (2015); and Pareek (2016), the SS content may vary depending on several factors, such as the cultivar, the climate, the soil and the growing region.

The TA of ‘Baía’ orange increased during maturation from 0.52 g citric acid 100 g^{-1} (at C1) to 0.65 g 100 g^{-1} (at C3). ‘Comum’ recorded the highest TA. ‘Mimo-do-Céu’ presented the lowest, and the values did not differ according to the stage of maturity, ranging from 0.07 (C1) to 0.10 (C3) g citric acid 100 g^{-1}. As mentioned above, 'Mimo-do-Céu' is a sweet orange with low acidity compared with ‘Lima’ orange, which is considered the sweetest of this group (SOUSA et al., 2016). Indeed, Couto and Canniatti-Brazaca (2010); and Silva et al. (2016) reported TA values for ‘Lima’ orange of 0.23 and 0.34 g citric acid 100 g^{-1}, respectively, well above that of ‘Mimo-do-Céu’ of the Borborema Territory, evaluated in this work.

For the SS/TA ratio, Baía and Comum cultivars did not differ among the three maturity stages. On the contrary, ‘Mimo-do-Céu’ orange showed a significant increase in the SS/TA from stage C1 (119.62) to stage C2 (172.97). In ‘Ponkan’ mandarin, Silva et al. (2014) used the SS/TA ratio to describe the maturity and quality for acceptance and consumption of this citrus fruit. In this context, ‘Comum’ presented the lowest SS/TA ratio (10.60) while ‘Mimo-do-Céu’ showed a much higher SS/TA ratio (143.99), characterizing its very sweet and low acid flavor.

The ascorbic acid content (Table 3) of ‘Baía’ and ‘Comum’ oranges increased from

40.51 and 42.63 mg 100 g^{-1} at the C1 maturity stage, to 49.17 and 49.24 mg 100 g^{-1} at C3, respectively. Conversely, ‘Mimo-do-Céu’ orange differed from the others since its ascorbic acid content decreased during maturation. However, this cultivar was distinguished by having the highest mean content of vitamin C (50.26 mg 100 g^{-1}) (ascorbic acid + dehydroascorbic acid), which was more than that reported for ‘Lima’ orange (*C. sinensis* (L.) Osbeck) in the Brazilian Table of Food Composition (NEPA, 2011), of 41.3 mg 100 mL^{-1} of juice.

The ascorbic acid contents of the three cultivars examined in this study were higher than that in ‘Valencia’ orange juice (29.2 to 39.8 mg 100 g^{-1}; ESCOBEDO-AVELLANEDA et al., 2014), lower than those in ‘Pêra’, ‘Lima’, ‘Natal’, ‘Valência’ and ‘Baía’ oranges (62.50, 64.58, 84.03, 78.47 and 80.03 mg 100 g^{-1}, respectively; COUTO; CANNIATTI-BRAZACA, 2010) but close to the vitamin C content reported by Lado, Gambetta and Zacarias (2018), which ranged from 46 to 60 mg 100 g^{-1} in sweet orange varieties. These variations can be due to the region of cultivation, cultural management, climate and year of production, besides the degree of maturation of the fruit (ALÓS; RODRIGO; ZACARÍAS, 2014; COUTO; CANNIATTI-BRAZACA, 2010; YOO; MOON, 2016).

Table 7. Ascorbic acid and total extractable polyphenols (TEP) content of the juice and albedo portions of Baía, Comum e Mimo-do-Céu orange fruit cultivars harvested in three maturity stages, based on the color of the peel.

		Maturity Stage	Ascorbic acid (mg.100g¹)	TEP (mg GAE.100g^{-1})	
				Juice	Albedo
Cultivar	Baía	C1	40.51 bB	19.82 bA	138.75 bC
		C2	46.92 aA	23.37 abA	159.09 bC
		C3	49.17 aA	23.88 aB	211.80 aA
		Mean	45.53	22.35	169.88
		CV %	3.74	12.21	6.14
	Comum	C1	42.63 bB	21.41 cA	216.19 aA
		C2	44.55 abA	26.74 bA	228.99 aA
		C3	49.24 aA	31.26 aA	234.61 aA
		Mean	45.47	26.47	226.60
		CV %	4.74	5.95	9.36
	Mimo-do-Céu	C1	53.19 aA	19.84 cA	172.58 bB
		C2	46.19 bA	26.56 bA	189.43 bB
		C3	47.38 bA	31.18 aA	222.20 aA
		Mean	50.26	25.86	194.73
		CV %	9.83	7.89	5.37

In the column, means followed by the same lowercase letter within each cultivar among maturity stages, and by the same capital letter for each maturity stage among cultivars, do not differ, by the Tukey test: $p \leq 0.05$. n = 4. GAE = Galic Acid Equivalent.

The total extractable polyphenols (TEP) of the juice and albedo increased with the maturation of the evaluated cultivars (Table 3). In the juice of 'Baía', 'Comum' and 'Mimo-do-Céu' oranges, the TEP content increased from 19.82, 21.41 and 19.84 mg (at C1) to 23.88, 31.26 and 31.28 mg GAE 100 g^{-1} (at C3), respectively, denoting the increased content of compounds with claimed functional properties (CIRMI et al., 2016) during maturation.

In the fruit albedo, the TEP contents ranged from 138.75 to 211.80 mg 100 g^{-1} for 'Baia', and from 172.58 to 222.20 mg 100 g^{-1} for 'Mimo-do-Céu', respectively. The TEP content in 'Comum' orange, although not differing among maturity stages, increased from 216.19 (at C1) to 234.61 mg GAE 100 g^{-1} (at C3), which was the cultivar that showed the highest overall PET contents among the cultivars analyzed in this study. These results corroborate with Wang et al. (2016), who verified a significant increase in the TEP content in

the citrus epicarp (albedo + flavedo). These data also corroborate those detected in the epicarp of different orange cultivars, which ranged from 104.2 to 223.2 mg 100 g^{-1} (ZEFANG et al., 2016), and from 66.10 to 407.55 mg 100 g^{-1} (GHASEMI; GHASEMI; EBRAHIMZADEH, 2009). However, these amounts are lower than those indicated in 'Valencia' orange albedo that ranged from 553.1 to 730.0 mg 100 g^{-1} (ESCOBEDO-AVELLANEDA et al., 2014), yet close to that observed in 'Sweet' orange epicarp, of 288.17 mg 100 g^{-1} (CASQUETE et al., 2015).

For total antioxidant activity (TAA) evaluated by the $ABTS^{\bullet+}$ method, there was no significant difference among maturity stages in the juice of the oranges, independently of the cultivar (Table 4). However, 'Mimo-do-Céu' presented the highest value, with 3.33 μM TE g^{-1} at C1 stage, and a general mean of 2.91 μM TE g^{-1}, possibly explained by its higher content of ascorbic acid and TEP than the other cultivars. For 'Baía' and 'Comum' oranges, the mean values were 2.04 and 1.91 μM TE g^{-1}, respectively.

Given that the highest TAA by the $DPPH^{\bullet}$ assay is characterized by the lowest content of material required to capture 1 g of this radical, the TAA of the juice, although it increased with maturation, was very low (higher values), notably for cultivar 'Mimo-do-Céu'. In contrast, the juice of 'Comum' orange at the C3 maturity stage stood out as displaying the highest TAA (lower value) among the cultivars considered (Table 4).

The three major groups of compounds responsible for the beneficial effects attributed to the natural antioxidants found in fruits and vegetables are ascorbic acid, phenolics and carotenoids (ALVES et al., 2010). The phenolic compounds present in plants have recognized beneficial effects that contribute to preventing against degenerative diseases (CIRMI et al., 2016; DEL RIO et al., 2013; ZOU et al., 2016). Fruits and vegetables have phenolic contents that vary, depending on the species, genetics, environmental conditions, agronomic management and maturity stages (LADO; GAMBETTA; ZACARIAS, 2018), among other

factors. Thus, the lowest TAA by the DPPH$^{\bullet}$ method in 'Mimo-do-Céu' orange juice might be due to the low content of PET and other antioxidants.

In the albedo, 'Baia' cultivar presented the highest TAA by the ABTS$^{\bullet+}$ radical assay, followed by 'Comum' and 'Mimo-do-Céu', with 26.27, 17.76 and 16.04 μM TE g^{-1}, respectively. However, there was no significant difference in TAA between maturity stages when comparing 'Baía' with 'Comum' and 'Mimo-do-Céu' oranges. Although, the TAA values of 'Baia' albedo, in all three maturity stages, were superior to the other oranges. Casquete et al. (2015) noted 3.4 mg TE g^{-1} in sweet orange epicarp, well below that of the oranges evaluated herein, which may be due to edaphoclimatic conditions, cultural practices and other factors (LADO; GAMBETTA; ZACARIAS, 2018), indicating the ability of the Borborema Territory to produce oranges of distinct functional potential.

Table 8. Antioxidant activity of juice and albedo of orange fruit of Baía, Comum e Mimo-do-Céu orange cultivars harvested in three maturity stages, based on the color of the peel, by the ABTS$^{\bullet+}$ and DPPH$^{\bullet}$ methods

		Maturity	ABTS$^{\bullet+}$ (μM TE.g^{-1})		DPPH$^{\bullet}$ - EC_{50} (g pulp.g DPPH^{-1})	
		Stage	Juice	Albedo	Juice	Albedo
Cultivar	Baía	C1	1.78 aB	26.76 aA	5777.43 aB	1704.98 bB
		C2	2.54 aA	25.22 aA	4489.99 aB	2225.82 aAB
		C3	1.80 aA	26.84 aA	4599.09 aB	1913.04 abA
		Mean	2.04	26.27	4955.50	1947.95
		CV %	12.43	5.8	14.7	14.18
	Comum	C1	1.71 aB	15.82 bB	4393.76 aB	2387.04 abA
		C2	1.85 aA	17.21 abB	4172.96 aB	2578.69 aA
		C3	2.17 aA	20.25 aB	2907.99 aC	1976.35 bA
		Mean	1.91	17.76	3824.90	2314.03
		CV %	15.06	13.83	21.63	15.11
	Mimo-do-Céu	C1	3.33 aA	15.51 abB	13263.15 aA	2717.75 aA
		C2	2.62 aA	14.32 bB	9591.76 bA	2020.30 bB
		C3	2.76 aA	18.30 aB	6422.31 cA	1700.74 bA
		Mean	2.91	16.04	10492.40	2146.26
		CV %	12.44	12.07	11.95	8.48

In the column, means followed by the same lowercase letter within each cultivar among maturity stages, and by the same capital letter for each maturity stage among cultivars, do not differ, by the Tukey test: $p \leq 0.05$. n = 4. TE = Trolox Equivalent

According to Ferreira, Silva and Nunes (2018), the high antioxidant activity of citrus peel extracts may be explained by their phenolic composition, such as hesperidin, naringin, rutin, and caffeic and chlorogenic acids. As mentioned by Bilgiçli; Aktaş; Levent (2014); and Liew et al. (2018), the bioactive phytochemicals present in the citrus peel can be exploited for various applications, such as the extraction of natural antioxidants, food additives and dyes for the food industry. These antioxidants act to protect against the accumulation of reactive oxygen species, eliminating them from the system through irreversible dehydrogenation. For instance, ascorbic acid reacts directly with O_2^-, HOO^- and OH^-, eliminating $^1O_2$ (ZOU et al., 2016). Furthermore, recent research suggests that *C. reticulata* peel has therapeutic potential and a chemopreventive effect against breast cancer (FERREIRA; SILVA; NUNES, 2018). In this sense, the albedo of the oranges produced in the Borborema Territory could add value to the marketed product, in light of its highlighted functional potential.

By the $DPPH^•$ method, the antioxidant activity (TAA) of the juice of Baía and Comum cultivars did not differ among maturity stages, with general means of 4955.50 and 3824.90 g pulp g $DPPH^{-1}$, respectively. For Mimo-do-Céu, the EC_{50} significantly decreased as maturation progressed, presenting 13263.15 (at C1) to 6422.31 g pulp g $DPPH^{-1}$ (at C3), indicating that the TAA increased about two-fold during maturation. In general, the increase in antioxidant capacity with maturation substantiates the findings of Wang et al. (2016) that also verified an increase in the antioxidant activity as maturation evolved, as much in the juice as in the peel of citrus.

Although much higher than for the juice, the albedo of ‘Baía’ and ‘Comum’ oranges at C2 stage, presented the lowest antioxidant activity by the $DPPH^•$ radical scavenging assay, requiring 2225.82 and 2578.69 g of pulp, respectively, to eliminate 1 g of $DPPH^•$. The albedo of ‘Mimo-do-Céu’ had a comparatively lower EC_{50} value (1700.74 g pulp g $DPPH^{-1}$) and, consequently, higher antioxidant activity at the C3 stage. From literature studies, the residues

of the citrus agroindustry contained TAA values of 11035 μM TE g^{-1} (BARBOSA; RUVIARO; MACEDO, 2018), and 9188 μM TE g^{-1} (MADEIRA JR; MACEDO, 2015) whereas, in citrus peels, the $DPPH^{\bullet}$ radical scavenging activity ranged from 8.35 to 18.20 mg TE g^{-1} (LIEW et al., 2018), depending on the extraction technique. These differences may be a result of the different analytical protocols of the extraction method, production region, cultivar and maturation stage (FERREIRA; SILVA; NUNES, 2018).

Antioxidants act on free radicals through two main mechanisms: (i) transfer of hydrogen atoms, wherein the antioxidant donates hydrogen atoms to stabilize the free radical species, preventing them from progressing in the reactions, and (ii) by transferring electrons, so that free radicals are reduced by the donation of an electron from antioxidant compounds (CRAFT et al., 2012).

The antioxidant activity in orange depends on the cultivar and maturity stage of the fruit. In addition, the change in antioxidant activity is related to the metabolism of polyphenols, ascorbic acid, flavonoids and carotenoids (BARBOSA; RUVIARO; MACEDO, 2018; WANG et al., 2016). As Zou et al. (2016) stated, pre-/postharvest and processing factors influence the chemical structure and antioxidant capacity of citrus fruits. This activity further depends on the composition of the extract, as well as on the conditions and mechanisms of the test used (LIEW et al., 2018).

4 CONCLUSION

‘Baía’ and ‘Mimo-do-Céu’ oranges presented quality parameters that fit into the CEAGESP standards. During maturation, the firmness decreased, and the SS, TEP and TAA of the juice and albedo increased. ‘Baía’ orange had the largest size and fresh weight. However, it presented the lowest juice yield, due to the high thickness of the peel, leading to a greater amount of residue that could be used for the extraction of bioactive compounds since

the albedo presented much higher functional potential. In general, the albedo presented about eight times as much TEP than the juice, and this meant a higher TAA in that portion, which was the most functional aspect of the fruit, especially for ‘Baía’ orange. These results highlight the potential of the Borborema Territory in producing differentiated oranges with high functional potential.

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