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PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA E TECNOLOGIA DE ALIMENTOS

TESE

Secagem por atomização da polpa de juçara para obtenção de produtos potencialmente funcionais

Danielle Cunha de Souza Pereira

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UNIVERSIDADE FEDERAL RURAL DO RIO DE JANEIRO
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SECAGEM POR ATOMIZAÇÃO DA POLPA DE JUÇARA PARA OBTENÇÃO DE PRODUTOS POTENCIALMENTE FUNCIONAIS

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Tese submetida como requisito parcial para obtenção do grau de **Doutora em Ciência e Tecnologia de Alimentos,** no Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos, Área de Concentração em Tecnologia de Alimentos.

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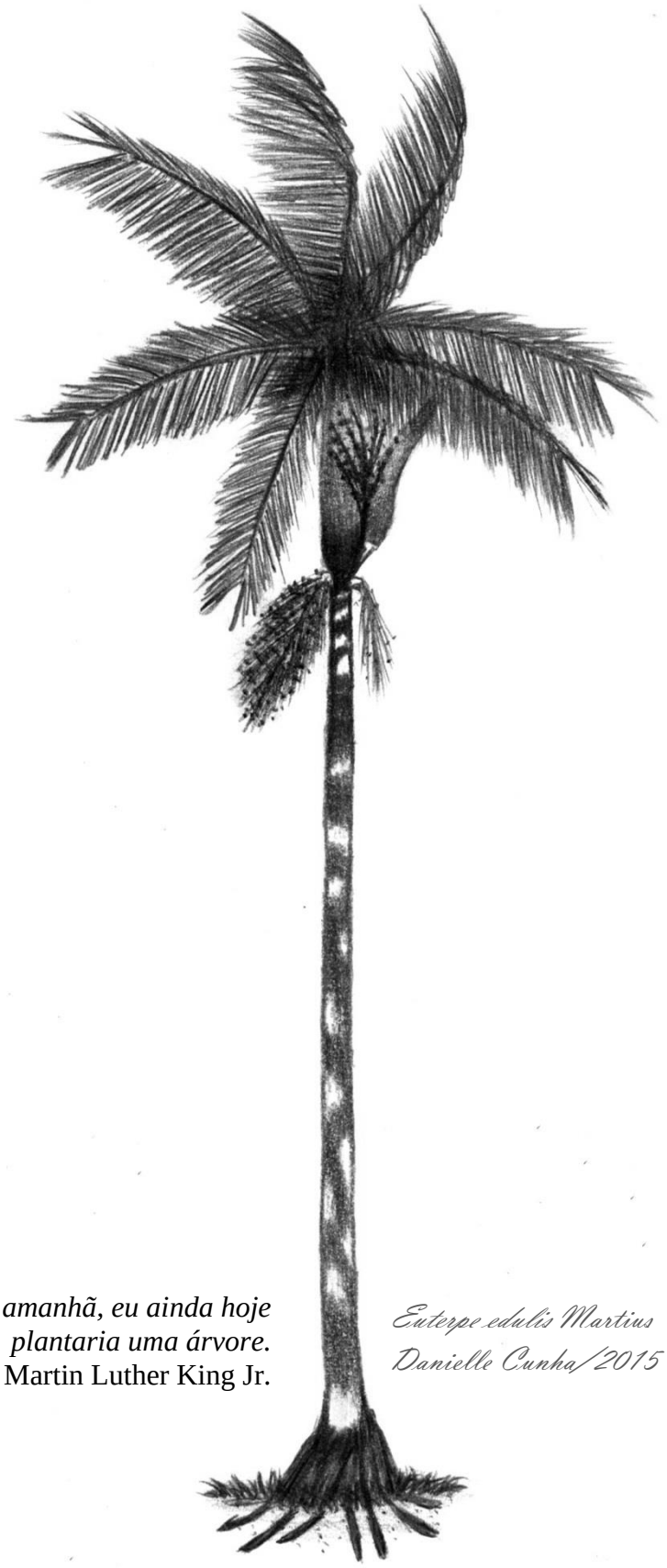
Maria Ivone Martins Jacintho Barbosa. Dra. UFRRJ

A *Eloah* por ter me concedido sabedoria
e ter conduzido minha vida com infinita
bondade e amor.

OFEREÇO

Aos meus queridos pais Valmir e Cida;
ao Jean, pelo amor que nos une;
aos meus irmãos, Jaqueline, Pricila e
Maxwell, pela amizade.
Aos meus sobrinhos Riane e Matheus Henrique
pelos momentos de alegria
e aos meus familiares e amigos que
sempre torceram por mim e que mesmo
nas horas difíceis me deram força para
continuar caminhando.

DEDICO



Se soubesse que o mundo acabaria amanhã, eu ainda hoje plantaria uma árvore.
Martin Luther King Jr.

Euterpe edulis Martius
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RESUMO

PEREIRA, Danielle Cunha de Souza. **Secagem por atomização da polpa de juçara para obtenção de produtos potencialmente funcionais**. 2020. 151f. Tese (Doutorado em Ciência e Tecnologia de Alimentos). Instituto de Tecnologia, Departamento de Tecnologia de Alimentos, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ, 2020.

O fruto da palmeira juçara (*Euterpe edulis* Martius) é um pequeno fruto tropical, não climatérico, de cor preto-violácea cuja popularidade e comercialização têm aumentado continuamente nos últimos anos. Tradicionalmente, o palmito obtido do caule é mais consumido, porém há um interesse crescente na polpa dos seus frutos, devido à interesses econômicos e de proteção à biodiversidade. A polpa de juçara é extraída dos frutos da palmeira, sendo descrito como uma fonte de compostos potencialmente bioativos, que atraiu a atenção da indústria visando a produção de alimentos funcionais. A polpa de juçara analisada tem uma acidez menor em comparação com outras frutas tropicais (0,3% de ácido cítrico), baixo teor de sólidos solúveis (4,6 ºBrix) e apresenta alto teor de antocianinas (2.928,6 mg de cianidina-3-glucosídeo/ 100g) e fenólicos totais (9.071,9 mg de ácido gálico equivalente/ 100 g), compostos que estão relacionados à elevada capacidade antioxidante (ABTS: 505,0 μmol de Trolox/ g; ORAC: 2.400,1 μmol de Trolox/ g). O fruto e a polpa de juçara são perecíveis, exigindo métodos de conservação adequados. Neste estudo, uma polpa de juçara em pó foi produzida por secagem por pulverização sem adição de agentes encapsulantes, e a estabilidade durante diferentes condições de armazenamento foi avaliada. O processo de secagem teve um rendimento de 66% e observou-se uma concentração de antocianinas de 7.079,2 mg de cianidina-3-glucosídeo/ 100g. Houve retenção de 90% dos compostos fenólicos totais (14.084,7 mg de ácido gálico equivalente/ 100g) e não foi observada alteração no potencial antioxidante (ABTS: 858,6 μmol de Trolox/ g e ORAC: 4.155,4 μmol de Trolox/ g). Os resultados físicos de solubilidade em água (72,9%), higroscopicidade (11,6%), isotermas de sorção e morfologia das partículas foram considerados adequados tecnologicamente. O teor de antocianinas e a cor da polpa em pó não sofreram alterações significativas quando armazenados a 25 ºC ou a 7 ºC na presença ou ausência de oxigênio durante 103 dias. A bioacessibilidade dos compostos fenólicos foi avaliada, houve metabolização dos compostos fenólicos totais ao longo da digestão *in vitro*, e observou-se que o processo de digestão colônica realizado pela microbiota intestinal resultou em um aumento na produção de ácidos graxos de cadeia curta (738,2% na polpa de juçara; 774,0% em pó de juçara) e na contagem de células de *Bifidobacterium* em 1 ciclo Log. Observou-se menor aumento na população de *E. coli* da microbiota exposta à polpa e ao pó de juçara, além de uma diminuição na produção de amônia de 100,8% e 127,0% para polpa e pó, respectivamente, quando comparado a um controle negativo. Esses resultados juntos, podem sugerir um efeito benéfico dos compostos fenólicos da polpa e pó de juçara na microbiota intestinal. Após o processo de digestão, determinou-se por HPLC que 26,4% do conteúdo fenólico total da polpa juçara (666,5 mg/ 100g) e 21,0% do pó juçara (787,2 mg/ 100g) atingiu o cólon, podendo apresentar uma ação *in loco* desses compostos. Na polpa de juçara, observou-se um aumento de 158,7% (ABTS) e 76,8% (ORAC) na capacidade antioxidante (1.306,4 e 4.242,9 μmol de Trolox / g, respectivamente). Enquanto, no pó de juçara, observou-se um aumento de 22,7% (ABTS) e 6,2% (ORAC) na capacidade antioxidante (1.048,9 e 4.411,0 μmol de Trolox/ g, respectivamente). Com isso, a polpa de juçara se mostrou com maior potencial antioxidante que o pó concentrado, que pode estar relacionado com uma capacidade protetora da matriz alimentícia. A juçara em pó apresentou propriedades bioativas relacionadas ao alto teor de compostos fenólicos e capacidade de modular a

microbiota intestinal, sendo uma alternativa para atuar como ingrediente alimentar. Como alternativa de aplicação, o pó de juçara foi adicionado à um suco de maçã integral. Após exposição à fermentação microbiana colônica o suco de maçã com juçara resultou em um aumento na produção de ácidos graxos de cadeia curta (1,7 mmol/ L) e na contagem de células de *Bifidobacterium* (0,7 ciclos Log). Observou-se redução na produção de amônia (28,2%), quando comparado ao controle negativo sem adição de juçara. Após a digestão, 31,3% das antocianinas (5,1 mg de cianidina-3- glucosídeo e 3,0 mg cianidina-3-rutenosídeo / 200g de suco de maçã com juçara) atingiram o cólon e um aumento de 52,9% na capacidade antioxidante (21,6 e 1.786,6 μmol de Trolox/ mL ABTS e ORAC, respectivamente) foi observado após 24 horas de fermentação. O suco de maçã suplementado com 0,5% de juçara em pó mostrou boa aceitação em estudo sensorial, onde a maioria dos consumidores (58,8%) informou que compraria o produto se este estivesse à venda, por apresentar bom gosto e promover benefícios à saúde. Assim, o suco de maçã é uma matriz viável para a adição de juçara em pó, o que permite a manutenção da bioacessibilidade dos compostos fenólicos e da capacidade antioxidante. Além disso, a juçara em pó mostrou potencial para ser utilizada como corante natural e ser classificada como alimento funcional.

Palavras-chave: bioacessibilidade, compostos bioativos, desenvolvimento de produtos, *Euterpe edulis* Martius, fermentação colônica, *spray dryer*.

ABSTRACT

PEREIRA, Danielle Cunha de Souza. **Spray drying of juçara pulp to obtain potentially functional products**. 2020. 151p. Thesis (Doctorate in Food Science and Technology). Instituto de Tecnologia, Departamento de Tecnologia de Alimentos, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ, 2020.

Juçara palm tree (*Euterpe edulis* Martius) fruit is a small, non-climacteric tropical black-violet fruit whose popularity and commercialization has steadily increased in recent years. Traditionally, the palm heart obtained from the stem is more consumed, but there is a growing interest in the fruit pulp, due to economic interests and biodiversity protection. Juçara pulp has being described as a source of potentially bioactive compounds, which attracted the attention of the industry aiming at the production of functional foods. Juçara pulp analyzed has lower acidity compared to other tropical fruits pulps (0.3% citric acid), low soluble solids (4.6 ºBrix) and high anthocyanin content (2,928.6 mg cyanidin-3-glucoside/ 100g pulp) and total phenolics (9,071.9 mg equivalent of gallic acid/ 100g), compounds that are related to high antioxidant capacity (ABTS: 505.0 μmol of Trolox/ g; ORAC: 2,400.1 μmol of Trolox/g). Juçara fruit and pulp are perishable, requiring adequate preservation methods. In this study, a powdered juçara pulp was produced by spray drying without the addition of encapsulating agents, and stability during different storage conditions was evaluated. The drying process yielded 66% and an anthocyanin concentration of 7,079.2 mg cyanidin-3-glucoside/ 100g was observed. 90% of the total phenolic compounds were retained (14,084.65 mg equivalent gallic acid/ 100g) and no change in antioxidant potential was observed (ABTS: 858.6 μmol Trolox/ g ORAC: 4,155.4 μmol Trolox/ g). Physical results of water solubility (72.9%), hygroscopicity (11.6%), sorption isotherms and particle morphology were considered technologically appropriate. The anthocyanin content and color of the pulp powder did not change significantly when stored at 25 ºC or 7 ºC in the presence or absence of oxygen for 103 days. Bioaccessibility of phenolic compounds was evaluated, there was metabolization of total phenolic compounds during *in vitro* digestion, and it was observed that the colonic digestion process performed by the intestinal microbiota resulted in an increase in short chain fatty acid production (738.2% in the juçara pulp; 774.0% juçara powder) and *Bifidobacterium* cell count in 1 Log cycle. A increase smaller was observed in the *E. coli* population of the microbiota exposed to juçara pulp and powder, and a decrease in ammonia production of 100.8% and 127.0% respectively for pulp and powder, when compared to a negative control. These results together may suggest a beneficial effect of phenolic pulp compounds of juçara pulp and powder on the intestinal microbiota. After the digestion process, it was determined by HPLC that 26.4% of the total phenolic content of juçara pulp (666.5 mg/ 100g) and 21.0% of juçara pulp (787.2 mg/ 100g) reached the colon, may present an *in loco* action of these compounds. In the juçara pulp, there was a 158.7% (ABTS) and 76.8% (ORAC) increase in antioxidant capacity (1,306.4 and 4,242.9 μmol Trolox/ g, respectively). While in juçara powder, there was a 22.7% (ABTS) and 6.2% (ORAC) increase in antioxidant capacity (1,048.9 and 4,411.0 μmol Trolox / g, respectively). Thus, juçara pulp showed higher antioxidant potential than concentrated powder, which may be related to the protective capacity of the food matrix. The juçara powder presented bioactive properties, related to the high content of phenolic compounds, and ability to modulate the intestinal microbiota, being an alternative to act as a food ingredient. As an alternative application, juçara powder was added to a whole apple juice. Following exposure to colonic microbial fermentation apple juice with juçara resulted in an increase in short chain fatty acid

production (1.7 mmol/ L) and *Bifidobacterium* cell count (0.7 Log cycles). Ammonia production decreased (28.2%) when compared to the negative control without juçara addition. After digestion, 31.3% of anthocyanins (5.1 mg cyanidin-3-glucoside and 3.0 mg cyanidin-3-rutinoside/ 200g apple juice with juçara) reached the colon and a 52.9% increase in Antioxidant capacity (21.6 and 1.786.6 μmol Trolox/ mL ABTS and ORAC, respectively) was observed after 24 hours of fermentation. Apple juice supplemented with 0.5% of juçara powder showed good acceptance in a sensory study, where most consumers (58.8%) said they would buy the product if it were for sale, due to a nice taste and because of a health benefits promotion. Thus, apple juice is a viable matrix for the addition of juçara powder. In addition, juçara powder showed potential to be used as a natural dye and characterized as functional food.

Keywords: bioaccessibility, bioactive compounds, product development, *Euterpe edulis* Martius, colonic fermentation, spray dryer.

SUMÁRIO

1 INTRODUÇÃO

Nas Américas, muitas palmeiras tropicais (Arecaceae) são naturalmente abundantes e oferecem uma série de produtos florestais não-madeireiros (VEDEL-SØRENSEN et al., 2013). *Euterpe edulis* Martius, popularmente conhecida como palmeira juçara, é uma palmeira nativa da Mata Atlântica, com distribuição geográfica litorânea do sul da Bahia até o norte do Rio Grande do Sul (REIS et al., 2000). A palavra juçara é de origem tupi – *ii'sara* e significa "coceira; comichão". Devido ao pó prurido que saía desta palmeira o nome juçara lhe foi dado (OLIVER, 2005).

O palmito produzido pela palmeira juçara é um alimento nobre e apreciado, no entanto, para sua extração é necessário o corte integral da palmeira. Devido ao seu grande consumo, o Brasil se tornou um dos maiores produtores, consumidores e já foi o maior exportador deste produto (IPEMA, 2008; EMBRAPA, 2019). Entretanto, de acordo com a Instrução Normativa nº 06 de 23 de setembro de 2008, que define a "Lista Oficial da Flora Brasileira Ameaçada de Extinção", a juçara é uma espécie ameaçada de extinção, devido ao desmatamento e a extração ilegal do palmito, o que contribui para a aceleração do processo de extinção de outras espécies, animais e vegetais, dependentes desta palmeira. Uma forma de minimizar este problema é a valorização do uso dos frutos da palmeira juçara para extração da polpa, a qual é similar à polpa de açaí, *E. oleracea*.

A preferência atual por alimentos naturais, associado a inúmeros estudos que relatam possíveis efeitos deletérios de aditivos sintéticos para a saúde, tem contribuído para a busca de novas fontes de ingredientes naturais (VIEIRA et al., 2013; KHOO et al., 2017; SIGURDSON; TANG; GIUSTI, 2017).

A procura pela polpa da juçara está em ascensão, havendo grande aceitação no Sul do Nordeste, Sudeste e Sul do Brasil, onde é usada normalmente misturada com polpas de frutas regionais, visando agregar potencialidade funcional ao produto elaborado.

Inicialmente, em 2007, devido a incentivos de projetos ambientais desenvolvidos no Brasil, os frutos da palmeira juçara eram consumidos na forma de suco e polpa, sendo agora também utilizados em centros de pesquisas como ingrediente para a fabricação de sorvetes, iogurtes, bebidas lácteas, doces, geleias e outros produtos (FELZENSZWALB et al., 2013; SCHULZ et al., 2016; PEREIRA et al., 2017), contribuindo para a conquista de espaço nos grandes centros nacionais.

Os estudos sobre a utilização dos frutos da palmeira juçara abordam predominantemente os compostos bioativos e capacidade antioxidante do fruto, como os realizados por Rufino et al. (2010), Borges et al. (2011), Borges et al. (2013), Inada et al. (2015) e Schulz et al. (2015), avaliação dos métodos de conservação e congelamento a -18 ºC, como relatado por Pereira et al. (2016 e 2017) e a extração dos seus compostos bioativos como descrito por Pereira et al. (2018) . Estes estudos demonstram o potencial de utilização dos frutos da juçara e enfatizam o seu alto teor de compostos fenólicos, contribuindo para sua aceitação pelo mercado consumidor.

Compostos fenólicos e alimentos ricos em compostos fenólicos têm sido alvo de estudos devido às suas propriedades antioxidantes. Muitos pesquisadores, e a própria indústria de alimentos, têm demonstrado um grande interesse por estes compostos, mostrando assim, a importância de manter ou adicionar tais substâncias no alimento.

Por atuarem nos radicais livres, a ação antioxidante dos compostos fenólicos presentes nos frutos, a exemplo das antocianinas, aumenta as defesas do organismo, estando associada a potentes atividades biológicas (NILE; PARK, 2014; SCHULZ al at., 2016). Apesar de na natureza estarem amplamente disseminadas em frutas e olerícolas, são poucas as fontes comercialmente utilizáveis de antocianinas (BALASUNDRAM; SUNDRAM; SAMMAN,

2006; SIMÕES et al., 2012), sendo mais utilizados produtos derivados da uva, a exemplo do corante alimentar E163, descrito por Khoo et al. (2017). Assim, a polpa de juçara vem surgindo como uma fonte promissora destes compostos (BORGES et al., 2013; PEREIRA et al., 2017; PEREIRA et al., 2018).

As antocianinas são compostos que pertencem à família dos flavonoides, responsáveis pela maioria das tonalidades de vermelho, azul e roxo observadas em plantas (CARDOSO et al., 2015; YOUSUF et al., 2016). São pigmentos solúveis em água, com potencial para substituição dos corantes artificiais em produtos alimentícios (YOUSUF et al., 2016; KHOO et al., 2017; SIGURDSON; TANG; GIUSTI, 2017).

Estes pigmentos são relativamente instáveis e apresentam maior estabilidade em baixas temperaturas e condições ácidas (SCHWARTZ; VON ELBE; GIUSTI, 2008; SIPAHLI; MOHANLALL; MELLEM, 2017). Isto é devido a mudanças na estrutura molecular das antocianinas, que tem natureza iônica (TURTURICĂ et al., 2015). Em condições ácidas (pH 1), a estrutura predominante corresponde ao cátion *flavilium* (AH^+), conferindo as cores vermelho e roxo. Elevando-se o pH (pH entre 2 e 4) ocorre a rápida perda do próton para produzir as formas quinoidais de coloração azul ou violeta. Entre pH 5 e 6, predomina a pseudobase ou carbinol e a chalcona, espécies de coloração menos intensa a incolores. Em valores de pH acima de 7, as antocianinas são degradadas, dependendo do seu grupo constituinte (CASTAÑEDA-OVANDO et al., 2009; KHOO et al., 2017). Desta forma, os métodos de processamento, conservação e armazenamento utilizados podem levar à degradação das antocianinas.

A secagem por atomização é um processo amplamente utilizado na indústria de alimentos e, em condições ideais de processamento, tem se mostrado eficaz na obtenção de produtos à base de frutas (NEDOVIC et al., 2013; SANTOS et al., 2014). Este processo destaca-se como alternativa aos processos convencionais de concentração e secagem, pois produzem produtos mais estáveis, com melhores características de aroma, composição química e nutricional (OLIVEIRA; PETROVICK, 2010; SILVA et al., 2014). Este processo pode ser utilizado concomitante à microencapsulação, método que consiste no empacotamento de substâncias em embalagens extremamente pequenas, descritas como microcápsulas, as quais podem liberar o conteúdo de forma controlada e sob condições específicas, protegendo o material encapsulado de fatores que possam vir a causar a sua deterioração, tais como pH, oxigênio, luz e umidade (ÖZKAN; BILEK, 2014; ETCHEPARE et al., 2015; LAZOU; KROKIDA, 2018).

O método de secagem por atomização utilizando o equipamento *spray dryer* é normalmente utilizado na produção de polpa de frutas em pó, por ser considerado um processo econômico e flexível (DESAI; PARK, 2005; GHARSALLAOUI, 2007).

Apesar do produto atomizado ficar pouco tempo em contato com o ar quente, a secagem por atomização pode não ser a mais adequada para a secagem de certos produtos, pois ainda assim podem ocorrer perdas (TONON; BRABET; HUBINGER, 2009; BICUDO et al., 2015). Devido à presença de açúcares e ácidos de baixo peso molecular, que apresentam baixa temperatura de transição vítrea, algumas polpas de frutas desidratadas podem apresentar características negativas como pegajosidade, aderindo às paredes do secador e proporcionando ao produto desidratado características como dificuldades de manipulação, transporte e armazenamento (WANG; LANGRISH, 2009), o que torna necessário a adição de material de parede (agente encapsulante ou carreadores). No entanto, a adição desses agentes pode promover a mudança de cor e a diluição do teor de compostos bioativos e da capacidade antioxidante. Assim, para adição de agentes carreadores devem-se levar em consideração as características finais do produto, as características físicas e químicas do líquido atomizado, o tipo e mecanismo de funcionamento do atomizador, as características do ar de secagem

(TONON; BRABET; HUBINGER, 2009) e as características físicas e químicas da polpa de fruta utilizada.

Considerando-se que a juçara apresenta naturalmente reduzido teor de carboidratos (3,89 g de carboidratos 100 g^{-1} de polpa), açúcares (3,8 g de açúcares 100 g^{-1} de polpa liofilizada) e ácidos orgânicos (0,19 g de acidez total 100 g^{-1} de polpa) (GUERGOLETTO et al., 2016; MOREIRA et al., 2017), e elevado teor de antocianinas (250-1.365,21 mg cianidina-3-glicosídeo equivalente 100 g^{-1} de fruto) (INADA et al., 2015; SCHULZ et al., 2015; PEREIRA et al., 2016; PEREIRA et al., 2018), que chega a ser duas a três vezes superior aos frutos do açaizeiro (*E. oleracea* e *E. precatória*) a qual tem similaridade sensorial (INÁCIO et al., 2013; TEIXEIRA et al., 2015; SCHULZ et al., 2016; PEREIRA et al., 2017; SIQUEIRA et al., 2018), a secagem por atomização sem a adição de agentes encapsulantes pode representar uma técnica promissora, no sentido de aumentar a estabilidade da polpa de juçara e minimizar as variáveis e custo do processo de atomização. Portanto, a produção de polpa de juçara em pó sem adição de agentes carreadores é uma alternativa promissora para o desenvolvimento de um corante natural para ser usado como ingrediente de enriquecimento potencialmente funcional. Para o nosso melhor conhecimento, é a primeira vez que a polpa de juçara é desidratada por pulverização sem a adição de agentes carreadores e a sua estabilidade, propriedades físicas e químicas, e a bioacessibilidade das antocianinas é demonstrada.

O estudo da secagem da polpa de juçara mostra-se de grande importância, uma vez que assegura sua qualidade, para que a mesma possa ser comercializada ou utilizada para o processamento de derivados de forma segura para o consumidor e indústria. Para tanto, deve-se conhecer todas as etapas envolvidas no processamento, além da estabilidade do produto desde o processamento até a sua utilização. Estas informações poderão subsidiar a proposição de um fluxograma de processamento adequado e eficiente para obtenção de alimentos potencialmente funcionais com propriedades físico-químicas, colorimétricas, microbiológicas, sensoriais e composição em compostos bioativos estáveis, e com vida útil estendida.

1.1 Estruturação do trabalho

Para facilitar a apresentação e publicação dos resultados obtidos, de acordo com os diferentes temas abordados, a tese foi dividida em capítulos, apresentados na forma de artigos.

No primeiro capítulo, há uma revisão sobre as características botânicas e formas de uso da palmeira juçara, além da composição, mercado e processamento dos seus frutos. O capítulo traz ainda um diagrama de potencialidades de uso da polpa, uma vez que o conhecimento sobre as possibilidades de uso possivelmente facilitará a inserção da polpa de juçara no setor industrial.

O segundo capítulo aborda a análise comparativa das polpas de juçara integral e da polpa utilizada para alimentar o *spray dryer* (polpa triturada-filtrada), além de descrever o processo de fabricação da polpa de juçara em pó obtida por secagem em *spray dryer* sem a adição de agentes carreadores e descrever a estabilidade da polpa de juçara em pó armazenada em diferentes condições de temperatura e oxigênio.

O terceiro capítulo apresenta a bioacessibilidade dos compostos fenólicos da polpa de juçara *in natura* e em pó submetida à digestão gastrointestinal *in vitro* até a fase de fermentação colônica. Já o quarto capítulo descreve a incorporação da juçara em pó em suco de maçã integral.

1.1.1 Artigos

1. *Towards chemical characterization and possible applications of juçara fruit: an approach to remove Euterpe edulis Martius from the extinction list*. Artigo de revisão submetido para a revista *Journal of Food Science and Technology* - ISSN 0022-1155;
2. *Spray drying of juçara pulp aiming to obtain a "pure" powdered pulp without using carrier agents*. Artigo publicado na revista *Drying Technology* - ISSN 0737-3937;

   Danielle C. de S. Pereira, Carolina Beres, Flávia dos S. Gomes, Renata V. Tonon & Lourdes M. C. Cabral (2019) *Spray drying of juçara pulp aiming to obtain a "pure" powdered pulp without using carrier agents*, ***Drying Technology***, DOI: 10.1080/07373937.2019.1625363
3. *In vitro gastrointestinal digestion influence on phenolic compounds profile of fresh and powdered juçara pulp*. Artigo submetido para a revista *Food Chemistry* - ISSN 0308-8146;
4. *Formulation of a mixed juice: suitability of whole apple juice as a vehicle for applying juçara pulp powder*. Artigo submetido para a revista *LWT - Food Science and Technology* - ISSN 0023-6438.

2 OBJETIVOS

2.1 Objetivo geral

Desenvolver um ingrediente potencialmente funcional e quimicamente estável a partir da polpa de juçara.

2.2 Objetivos específicos

- Avaliar o efeito da secagem por atomização (*spray drying*) da polpa de juçara sem utilização de agentes carreadores;
- Avaliar as características de polpa de juçara em pó armazenada sob diferentes condições de temperatura e oxigênio;
- Avaliar a bioacessibilidade dos compostos fenólicos da polpa e do pó de juçara;
- Avaliar a adequação do suco de maçã integral como veículo para aplicação da polpa de juçara em pó.

CAPÍTULO 1

Artigo submetido para a revista *Journal of Food Science and Technology* - ISSN 0022-1155

Towards chemical characterization and possible applications of juçara fruit: an approach to remove *Euterpe edulis* Martius from the extinction list

Towards chemical characterization and possible applications of juçara fruit: an approach to remove *Euterpe edulis* Martius from the extinction list

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Abstract

Juçara (*Euterpe edulis* Martius) is a palm widely distributed in the Atlantic Forest. It produces a non-climacteric, black-violet small fruit very similar to the Amazonian açaí (*Euterpe precatoria*). The fruit is known as "superfruit" because it presents chemical characteristics of great importance, the example of anthocyanins. Regarding bioactive compounds and antioxidant scavenging capacity, it presents high anthocyanin (634.26 to 2,929 mg of cyanidin-3-glucoside 100 g^{-1}) and total phenolic compounds (415.1 to 9,778.20 mg equivalents of gallic acid 100 g^{-1}) contents. The soluble solid content ranges from 3.0% to 4.9% and its pH is higher than that of other tropical fruits (4.8 to 5.6). Despite the rich bioactive compound content of juçara fruits, this plant has been traditionally used for palm heart production. The accelerated and illegal exploitation of palm heart, without the use of an adequate management has led to the risk of extinction of this species. In order to prevent this species from vanishing, several studies have valued the health characteristics of juçara fruit chemical composition. An economical approach has been the production of juçara pulp described as a source of bioactive compounds, which has attracted the attention of the industry aimed at the production of functional foods, foodstuff, cosmetics and pharmaceutical products. A full botanical and chemical characterization of juçara tree and fruit is presented in this paper, as well as suggestions to increase the use of this tropical fruit and derivates.

Keywords: *Euterpe edulis* Martius / juçara / Brazil / health benefits / market potential / application

1. Introduction

The Brazilian Atlantic Forest is one of the most biologically diverse and threatened regions on the planet (Maier et al. 2019). Vanishing of *E. edulis* poses a danger to the Atlantic Forest fauna. More than 48 bird and 20 mammals species have juçara seeds and fruits as their main source of nutrients. Toucans, guans, thrushes and bellbirds are the main seed-dispersal agents, while agoutis, tapirs, peccaries, squirrels and other animals benefit from its nutritive fruits (Moon 2017). In such a scenario, juçara palm has been described as one of the most important species for the maintenance of the Atlantic Forest biome in Brazil, which covers from the state of Bahia (10º00'S) to northern Rio Grande do Sul (30º00'S), with a coastal geographic distribution, as well as the riparian forests of the states of Minas Gerais, Goiás, Mato Grosso do Sul, São Paulo and Paraná (Fig. 1) (Reis et al. 2000).

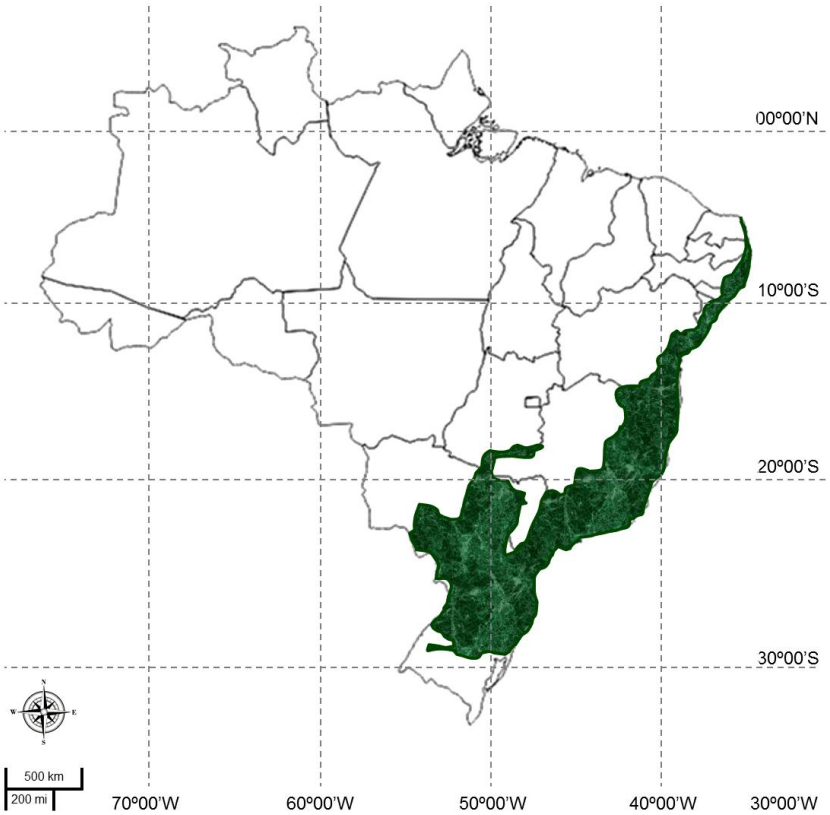

Fig. 1 Original Atlantic Forest in Brazilian territory

Legend: ~ Brazil states. Datum WGS-84

Native small producers from these regions have juçara culture as a relevant economical activity, as each part of the plant has different applications, thus the risk of extinction is also considered an important issue for the local population (Conab 2016). In a sustainable approach, the whole tree can be used in different ways (Fig. 2): palm leafs and body provide material for construction and handicrafts, and root and oil can be used in folk medicine for the treatment of respiratory diseases such as flu and pneumonia (Macía et al. 2011). The fruit of juçara is considered a valuable part of the tree, being commonly commercialized in pulp form, which consists of fragments of epicarp (peel) and mesocarp (pulp) obtained after softening and depulping through technological processes. Fibers originated from the mesocarp can be used in the manufacture of bakery products, and the hard seed, adherent to the endocarp, can be used as filter in water treatment, as fertilizer or for the production of seedlings that can be used for continuous growth and reforestation of the areas where there has been extinction (Pereira et al. 2017).

Juçara fruit can come of native forest (extractivism) or cultivated forests. No data on fruit production has been collected so far by the Brazilian Institute of Geography and Statistics; consequently, there is no information on the amount of fruit produced yearly. However, the Ministry of the Environment of Brazil ranked the main production areas in the country: Santa Catarina, São Paulo, Rio Grande do Sul, Rio de Janeiro and Espírito Santo (Conab 2016). According to Conab - National Supply Company (2016), fruit production can achieve 193 tons per year, being the state of Santa Catarina, in the south of Brazil, the largest producer, concentrating 84% of juçara production (Conab 2016). Juçara pulp commercialization has also increased considerably, driven by the açaí market that negotiates about 40 thousand tons of pulp per year. The minimum price of juçara and açaí fruit is US $ 0.78 and US $ 0.42 per kg, respectively, with the pulp price being about five times higher than that of the fruit *in natura* (Conab 2019). Nowadays juçara pulp is no longer just a product that

aims to increase the income of small local farmers, it has become a valuable raw material. A greater income can be obtained from the industrialization and consumption of juçara fruit rather than from wood production, which reinforces the need for increasing its utilization. The production of juçara fruit may be estimated as 2.5 bunches per tree/year which corresponds to approximately 8 kilograms of fruits per palm tree. In an already established seven-year-old plantation the total fruit yield was 2,500 kg per hectare per year, reaching a total income of US $ 25,886.47, more profitable than that was obtained from wood market (US$ 3,997.78) (Maier et al. 2019).

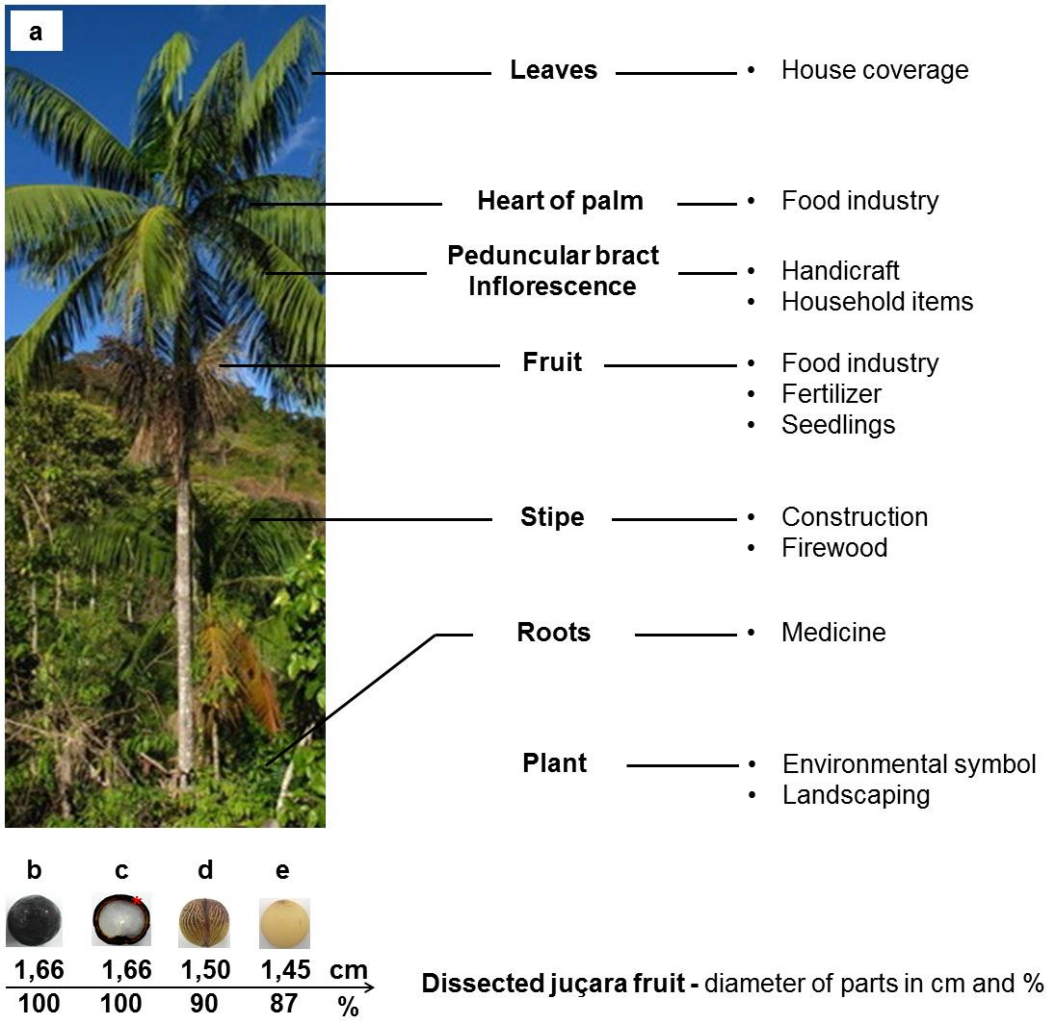

Fig. 2 Juçara palm tree and main applications (a), fruit *in natura* (b), fruit in cross section, evidencing the dark pulp* and white seed (c), fiber seed (d), fiber-free seed - endosperm (e)

For almost a century, juçara palm has been commercially valued only for the extraction of its palm heart, which consists in cutting the whole palm. In the 1970s, Brazil became the world's major producer, consumer and exporter of juçara palm heart. In 2001, the Brazilian market consumed 100,000 tons / year of juçara palm heart, equivalent to an area of 130,000 hectares, and juçara tree alone accounted for almost 97% of the Brazilian palm heart marketed, which almost dragged it into extinction. In 1995, Brazil continued to be the main producer and consumer of palm heart, exporting 30% of the production (Corpei 2001). However, in 2008 juçara was classified as an endangered species in accordance with Normative Instruction n. 06, from September 23, 2008, which defines the "Official List of the Brazilian Flora Threatened to Extinction" (Brazil 2008), due to deforestation and illegal extraction of palm heart. In 2016, Brazilian Law nº 6,209 (Brazil 2016) instituted the Policy of Incentive to the Cultivation of Vegetable Species, which included the obtainment of Palmito and Açaí. This new policy had the purpose to stimulate a sustainable management of native species; the installation of agroindustries for processing and packaging of derived products; and the acquisition of necessary machinery and equipment. All those points together intended to reduce the consumption of palm heart obtained from predatory extraction, which represented approximately 80% of the palm heart consumed in the country. In an attempt to encourage a more sustainable management, the surveillance of juçara palm heart illegal trade has been intensified, and at the same time, the exploitation of fruits considerably increased as the pulp has become a more commercially interesting product.

Studies on the biochemical composition of juçara indicated that it can be considered a 'superfruit' due to its content of bioactive compounds, such as anthocyanins, which can suggest its utilization in formulations of products with functional allegation (Felzenszwalb et al. 2013; Ribeiro et al. 2019a; Ribeiro et al. 2019b). According to analysis on the research platforms, Scopus and Sciencedirect, over the last 20 years, research concerning juçara fruit

has been mainly conducted by areas such as i) Agricultural and Biological Sciences (55%), ii) Environmental Science (13%), iii) Biochemistry, Genetics and Molecular Biology (9%), iv) Medicine (8%) and v) Chemistry (5%). Other areas are responsible for 10% of the publications.

This review aims to present juçara's botanical aspects, traditional culture, innovative products, biochemical composition and observations about production chain and current market. The focus on its potential applications has the objective of increasing its visibility contributing to its preservation.

2. Botanical and morphological description

The family Arecaceae (Palmae) is represented by approximately 3,500 species, with about 240 genera, and has tropical palm trees species naturally found in the Americas as representative members (Navia et al. 2007; Vedel-Sørensen et al. 2013).

Palm heart can be harvested from several palm species, however most products available in stores come from the juçara palm (*Euterpe edulis*), peach palm (*Bactris gasipaes*) and açaí palm (*Euterpe oleracea* and *Euterpe precatoria*). While *E. edulis* is native to the Brazilian Atlantic Forest, the other species are from the Amazon (Moon 2017).

E. edulis is a palm tree with approximately 15 meters of height, and 15 cm of stipe diameter. The apex has a group of pinnate leaves, with about 2.0 to 2.5 m length that stand out easily from the plant (Soares et al. 2014) (Fig. 2a). At the base of the stipe, there is a visible cone of roots colored from brown to red.

An important feature of juçara is that it is a single stem palm, therefore it does not produce tillers. This characteristic results in the death of the plant during the extraction of the palm heart. Another important characteristic is that *E. edulis* takes 8 to 12 years to produce high-quality palm heart, whereas *B. gasipaes* palm can be harvested just 18 months after

planted. Over the time, adult trees produce less seeds and dispersal and germination are reduced, leading to population decline and possible extinction (Moon 2017). Moreover, its germination is slow and uneven, as occurs with the majority of palm species, being influenced by intrinsic factors and related to the environment (Tiberio et al. 2012). For this reason, the use of fruits from juçara palm tree has emerged as a form of sustainable exploitation.

Juçara fruit is non-climacteric, small and rounded, with a color that varies from green to black-violet during the ripening process. The average fruit weight is approximately 1.7 g with a diameter of 1.5 cm. It has a very thin fibrous-fleshy mesocarp, with hard endosperm that constitutes 87.5% of the diameter of the fruit and up to 90% of its weight (Fig 2b-e) (Bicudo et al. 2014). Each palm produces on average three inflorescences that generate bunches with 3 to 5 kg of fruits, and each kg of fruit contains approximately 750 units. Harvest period goes from April to November, with differences among Brazilian states (Conab 2016).

3. Chemical composition

Physico-chemical and nutritional characteristics

In Brazil, palm heart is defined as the edible part of palm from the Palmae family, found mainly in tropical and subtropical climates. It is the white, soft and fibrous tissue found in the palm's stem. Palm heart from juçara tree (*Euterpe edulis*) is considered the favorite for both international and Brazilian consumption (Corpei 2001; Fantini and Guries 2007). However, currently extraction in an extractive manner is not allowed due to the risk of extinction. The chemical composition may vary according to season and soil. According to Table 1 juçara fruit presents richer composition when compared to palm heart, mainly regarding phenolic compounds and vitamin C. In this case, the fruit can be considered a better source of bioactive compounds with antioxidant activity. Nowadays, besides the sustainable

concern that is more widespread, people are more interested in a healthy diet, which can lead to an increase in the consumption of foods with a functional appeal. In this way, juçara fruit has more nutritional advantages than palm heart, consequently increasing its commercial value.

According to other chemical characteristics, the pH of juçara fruit and pulp ranges from 4.8 to 5.6 (da Silva et al. 2014; Inada et al. 2015; Moreira et al. 2017) and the content of soluble solids from 3.0% to 4.9% (Inada et al, 2015; Moreira et al. 2017). Other berries, such as blueberry and strawberry, generally contain about 15% soluble solids (Nile and Park 2014).

Juçara is considered a source of energy due to its higher lipid content in comparison with other fruits varying from 18.45% to 50.18% (w/w) in dry weight basis (dwb) (da Silva et al. 2014; Inada et al. 2015; Moreira et al., 2017). In other tropical fruits, such as araça, uvaia (da Silva et al. 2014), jaboticaba (Inada et al. 2015) and mango (Moreira et al., 2017), the total lipid content is much lower, varying from 0.19% to 2.2% (w/w), while in apple, papaya, banana, guava, kiwi, blackberry, red raspberry, strawberry, blueberry and cherry the lipid content varies from (0.1% to 0.42% w/w) (de Souza et al. 2014). Açaí pulp is the most similar to juçara pulp in relation to total lipid content (33.49% to 48.0% w/w) (Ferreira et al. 2016; Gordon et al. 2012). A study, Borges et al. (2011) identified 16 fatty acids in juçara fruit, with a predominance of monounsaturated fatty acids (45.53% to 56.82%), being oleic acid (44.63% to 55.61%) the major component. In that study, polyunsaturated fatty acids represented 18.79% to 26.03% of the total lipid content, and the main constituents were linoleic (18.19% to 25.36%) and linolenic (0.60 to 1.46%) acids (Borges et al. 2011; Schulz et al. 2015). The predominance of unsaturated fatty acids, such as monounsaturated fatty acids, is associated with the reduction of cardiovascular disease (Cheng et al. 2016; Ooi et al. 2015).

Table 1 Nutritional characteristics of juçara fruit and commercial palm heart

Characteristic	Juçara fruit	Palm heart[a]	Reference
Total acidity g/100g	0.19-1.67	0.5-1.10	Berbari et al. (2008); Moreira et al. (2017); Paim et al. (2016); Vieira et al. (2013)
Moisture content g/100g	56.36-92.0	87.68-92.50	Berbari et al. (2008); Campos et al. (2019); Corpei (2001); da Silva et al. (2013); Hiane et al. (2011); Moreira et al. (2017); Vieira et al. (2013)
Carbohydrate g/100g	2.03-6.75	2.6-4.60	Berbari et al. (2008); Corpei (2001); da Silva et al. (2013); Moreira et al. (2017); Paim et al. (2016); Salvi and Katewa (2014)
Lipids g/100g	3.17-4.98	0.44-0.50	Berbari et al. (2008); Borges et al. (2011); Corpei (2001); da Silva et al. (2013); Hiane et al. (2011); Moreira et al. (2017); Salvi and Katewa (2014); Vieira et al. (2013)
Protein g/100g	0.25-0.70	1.2-2.50	Berbari et al. (2008); Borges et al. (2011); Corpei (2001); da Silva et al. (2013); Hiane et al. (2011); Moreira et al. (2017); Salvi and Katewa (2014); Vieira et al. (2013)
Ashes g/100g	0.34-0.41	0.8- 1.20	Berbari et al. (2008); Corpei (2001); da Silva et al. (2013); Hiane et al. (2011); Moreira et al. (2017); Salvi and Katewa (2014); Vieira et al. (2013)
Fiber g/100 g	3.41-5.24	0.7-9.01	Corpei (2001); Garcia et al. (2019); Hiane et al. (2011); Inada et al. (2015); Salvi and Katewa (2014); Vieira et al. (2013)
Total phenolic mg/100g	302.10-968.95	202-241	Argentato et al. (2017); Hiane et al. (2011); Moreira et al. (2017); Paim et al. (2016); Pereira et al. (2019); Rufino et al. (2010); Shimizu et al. (2011)
Vitamin C mg/100g	36.67-186	5.52-23.10	Campos et al. (2019); Corpei (2001); Cravo et al. (2017); Hiane et al. (2011); Rufino et al. (2010)
Iron mg/100g	1.2-6.91	1.5-1.58	Corpei (2001); da Silva et al. (2013); Guergoletto et al. (2017); Pupin et al. (2018); Salvi and Katewa (2014)

Phosphorus mg/100g	11.2-140	109-380	Corpei (2001); da Silva et al. (2013); Guergoletto et al. (2017); Salvi and Katewa (2014)
Calcium mg/100g	35.4-110	81-490	Corpei (2001); da Silva et al. (2013); Guergoletto et al. (2017); Salvi and Katewa (2014)
Calories kcal/100g	66-83	11.64-33.00	Berbari et al. (2008); Corpei (2001); Garcia et al. (2019); Hiane et al. (2011); Inada et al. (2015)

[a]Analyzed Palm hearts: Australian royal palm (*Archontophoenix alexandrae* and *A. cunninghamiana*), Açaí (*Euterpe oleracea*), Pupunha (*Bactris gasipaes*), Wild date palm (*Phoenix sylvestris*), Guariroba (*Syagrus oleracea*) and Juçara (*Euterpe edulis*).

Saturated fatty acids represented 24.32% to 28.89% of total lipid content, mainly palmitic acid (20.25% to 25.00%) (Borges et al. 2011). Other tropical fruits such as avocado, pineapple, banana, papaya, passion, watermelon and melon present lower concentrations of oleic and linoleic acids (0.02% to 36.7% and 0.25% to 19.6% respectively) (Morais et al. 2017).

The fatty acids content in juçara pulp is essential for the digestion, absorption and transport of lipid soluble vitamins and phytochemicals, such as carotenoids (Pinard et al. 2014). Carotenoid content in juçara pulp and fruit was also determined. Values ranged from 414 μg 100 g^{-1} to 737.5 μg 100 g^{-1} of fresh matter (da Silva et al. 2014; Inada et al. 2015), which were greater than in other tropical fruits like açaí (Ribeiro et al. 2010), grumixama, araçá (da Silva et al. 2014), jaboticaba, blackberry, papaya, watermelon and guava (Inada et al. 2015). Carotenoids, which are isoprenoid secondary metabolites, present in the juçara pulp and fruit, can avoid vitamin A (retinol $C_{20}H_{30}O$) deficiency in mammals, due to their action as precursors of this compound (Álvarez et al. 2015). The major component was *all-trans*-lutein (2.9 mg 100 g^{-1}) and *all-trans*-β-carotene, *all-trans*-α-carotene and 9-cis-β-carotene (0.38 mg 100 g^{-1} to 2.7 mg 100 g^{-1}) were the main precursors of vitamin A (da Silva et al. 2014).

Unlike most fruits, juçara fruit and pulp have high protein content (6 to 7.6% w/w) (da Silva et al. 2014; Guergoletto et al. 2016; Moreira et al., 2017). Carbohydrates are the most abundant macronutrients in plants, due to their cell wall composition. Fresh fruits vary greatly in their carbohydrate content. Juçara has low carbohydrate content (de Melo et al. 2016) representing a total dry matter content in the range of 28.3% to 42.5% (w / w) (da Silva et al. 2014; Guergoletto et al. 2016; Inada et al. 2015; Moreira et al., 2017). Those values were lower than in other fruits like grape, banana, melon, pineapple, papaya, jabuticaba, mango, araça, grumixama and uvaia (up to 90% w / w of total dry matter) (Hui, 2006; Vicente et al., 2009; da Silva et al. 2014). Regarding the sugar profile, it is possible to find in juçara fruit

mainly sucrose, fructose and glucose (Guergoletto et al. 2016). A study (Inada et al. 2015) found values of fructose and glucose of 0.5% and 0.3%, respectively, in fresh weight basis of juçara pulp, content lower than in other fruits (4.00% to 12.64%) (Shanmugavelan et al. 2013).

Juçara fruit is considered rich in minerals. According to previous studies, the mineral content varies from 3.47 to 8.8% (w / w) (da Silva et al. 2014; Moreira et al., 2017). The mineral profile showed the presence of 17 chemical elements (phosphorus, sulfur, potassium, calcium, magnesium sodium, cobalt, aluminum iron, manganese, zinc, copper, nickel, selenium, cadmium, boron and molybdenum) (Inada et al. 2015; Schulz et al. 2015).

According to published data, a 200 g portion of juçara pulp has higher mineral levels than the Recommended Daily Intake (RDI), and it is significant for adults and children aged 7 to 10 years, mainly for magnesium, copper, zinc, iron and manganese, besides increasing the zinc intake of vegetarians, since the lack of meat in their diet leads to low levels of this mineral in the human body (da Silva et al. 2013).

Juçara fruit can also contribute to the daily intake of vitamins A, C and E (da Silva et al. 2014; Inada et al. 2015). A portion of 100 g of juçara contains 186 mg of ascorbic acid, two times higher than what is found in açaí fruits (84 mg 100 g^{-1}) (Rufino et al., 2010), and almost six times higher than in banana, mango and papaya (14-33 mg 100 g^{-1}) (Siriamornpun and Kaewseejan 2017). The consumption of juçara fruits also contributes to the dietary intake of vitamin E, as 200 g of fruits represent 4.5% of the RDI for adults.

Dietary fiber is one of the main components of plant foods and its importance in nutrition and health is widely recognized. Juçara fruit is a good source of dietary fiber (de Melo et al. 2016), a content of 27.1 to 28.3% (w / w) on dry weight was previously reported (da Silva et al. 2014; Inada et al. 2015). According to data (Inada et al. 2015), a 100 g portion of juçara pulp could provide approximately 17% of the recommended daily intake for fiber.

Similar value of dietary fiber was reported for açaí fruits (*E. oleracea*), 20 to 30.9% (w / w) in dry weight (Sangronis and Sanabria 2011). Many studies have addressed the role of dietary fiber in preventing cardiovascular diseases, cancer, obesity, diabetes and intestinal disorders (Buttriss and Stokes 2008).

Bioactive compounds: polyphenols

Polyphenols are a highly heterogeneous group with approximately 10,000 compounds (Ooi et al. 2015), which, according to their chemical characteristics, are responsible for physiological roles in plants and consumers (Boncler et al. 2017). Based on their structure they can be classified mainly as: flavonoids, phenolic acids, lignans and stilbenes (Zhou et al. 2016) and an important group of flavonoids are anthocyanins. In juçara, the two main anthocyanins are cyanidin-3-O-rutinoside and cyanidin-3-O-glucoside (Guergoletto et al. 2016; Inada et al. 2015), being the anthocyanins cyanidin-3,5-diglucoside, cyanidin-3-sambubioside, cyanidin-3-rhamnoside, pelargonidin-3-glucoside, pelargonidin-3-rutinoside, peonidin-3-glucoside, peonidin-3-rutinoside, and malvidin-3-glucoside also identified (Argentato et al. 2017; Bicudo et al. 2014; Brito et al. 2007; Garcia-Mendoza et al. 2017; Guergoletto et al. 2016; Inada et al. 2015).

Although sensory characteristics of juçara fruit (*E. edulis*) are similar to those of açaí fruits (*E. oleracea* and *E. precatoria)*, the nutritional properties seem to be more relevant, which is interesting for the development of potentially functional products (Siqueira et al. 2018). Juçara fruits have two to three times more anthocyanins than açaí (*E. oleracea* and *E. precatoria*) fruits (Siqueira et al. 2018; Teixeira et al. 2015), ranging from 634.26 to 2,929 mg cyanidin-3-glucoside equivalents 100 g^{-1} fruits (Table 2), and those molecules have been associated with a protective effect against oxidative damage. As flavonoids with multiple

hydroxyl groups in their structure, anthocyanins have a high antioxidant capacity, able to eliminate free radicals (Brewer 2011).

Table 2 Bioactive compound content and antioxidant scavenging capacity of juçara fruit and non-traditional Brazilian tropical fruits also considered *superfruits*

Fruits	Total anthocyanins[a]	Total phenolics[b]	Antioxidant capacity[c]	Reference
Juçara	634.26-2,929	415.1-9,778.20	2,400.14-6,450	Argentato et al. (2017) Cunha Júnior et al. (2016); Pereira et al. (2019); Peron et al. (2017); Moreira et al. (2017); Shanmugavelan et al. (2013)
Açaí	372.8-1,100	1,452-3,437	1,196-2,693.1	Alcázar-Alay et al. (2017); Ferreira et al. (2016); Garzón et al. (2017);
Acerola	18.9-215	914.2-1,738.9	1,605-9,660	Maciel et al. (2010); Mariano-Nasser et al. (2017); Mezadri et al. (2008); Rufino et al. (2010); Vasavilbazo-Saucedo et al. (2018)
Jabuticaba	58.1-315	460.9-2,420	1,200-6,000	Inada et al. (2015); Lima et al. (2011); Wu et al. (2012); Wu et al. (2013)
Jambolão	79-210.9	354.9-1,639.7	2,383-5,500	Brandão et al. (2011); Faria et al. (2011); Haroon et al. (2015); Singh et al. (2016)

[a] mg cyanidin-3-glucoside equivalents 100 g^{-1} in dry weight basis; [b] mg gallic acid equivalents 100 g^{-1} in dry weight basis; [c] TEAC – Trolox Equivalent Antioxidant Capacity and ORAC – Oxygen Radical Absorbance Capacity (μmol of Trolox equivalents 100 g^{-1} in dry weight basis)

The maximum value, also stands out when compared with other 3 non-traditional Brazilian tropical fruits with recognized importance but also little used, like acerola

(*Malpighia emarginata*), jabuticaba (*Myrciaria cauliflora*) and jambolão (*Syzygium cumini*), presenting a higher content of anthocyanins and total phenolic compounds (Table 2). This demonstrates the healthy and technological potential of the juçara fruit to be commercialized as a *superfruit*.

Furthermore, juçara fruit and pulp are excellent sources of polyphenols, with total content varying from 415.1 to 9,778.20 mg equivalents of gallic acid 100 g^{-1} in dry weight basis (Table 2), being more concentrated than in açaí (98.9 to 3,437 mg 100 g^{-1}) (Garzón et al. 2017; Gordon et al. 2012), red raspberry, blueberry, cherry (305.38 to 850 mg 100 g^{-1}) (de Souza et al. 2014), jabuticaba (815 mg 100 g^{-1}) (Inada et al. 2015) and banana, mango, papaya (19 to 327 mg 100 g^{-1}) (Siriamornpun and Kaewseejan 2017).

In relation to the phenolic profile, 32 compounds have already been identified in juçara (Table 3), 17 phenolic acids (protocatechuic, *p*-coumaric, vanillic, gallic, caffeic, ferulic, syringic, sinapic, ellagic, chlorogenic, benzoic, *p*-hydroxybenzoic, 3,4-dihydroxybenzoic, 3,4-dihydroxyphenylacetic, *p*-hydroxyphenylacetic, *trans*-cinnamic and *m*-coumaric acids), 13 flavonoids (apigenin, kaempferol, aromadendrin, catechin, epicatechin, quercetin, taxifolin, myricetin, isoquercetin, rutin, hispidulin, luteolin deoxyhexosyl-hexoside and dihydrokaempferol-hexoside), 1 stilbene (resveratrol) and 1 phenol aldehyde (vanillin) (Argentato et al. 2017; Bicudo et al. 2014; Borges et al. 2011; Guergoletto et al. 2016; Inada et al. 2015; Schulz et al. 2015; Schulz et al. 2017; Siqueira et al. 2018).

Table 3 Content of bioactive compounds found in juçara fruit and their health beneficial action

Bioactive Compound	Content	Isolated Compounds in juçara fruit	Identified health benefits	Reference
Anthocyanins				
Cyanidin-3-rutinoside	966-1565[a]	Argentato et al. (2017); Bicudo et al. (2014); Brito et al. (2007); Garcia-Mendoza et al. (2017); Guergoletto et al. (2016); Inada et al. (2015)	- antioxidant, anti-inflammatory, antitumor, antiartherosclerotics, antimicrobial and anticarcinogenic activities; - induction of apoptosis; - neuroprotective effects; - reduction of risk of coronary heart disease; - reduction of cellular oxidative damage; - protective effect against oxidative stress; - prevention of cardiovascular diseases; - promotion of obesity and diabetes control benefits: ↓insulin resistance, ↑insulin secretion, ↓plasma glucose; - improvement of visual and brain functions.	Cassidy (2018); Del Pozo-Insfran et al. (2004); Khoo et al.(2017); Pereira et al. (2017); Ramos et al. (2014); Smeriglio et al. (2016); Yousuf et al. (2016)
Cyanidin-3-glucoside	322-1358[a]	Argentato et al. (2017); Bicudo et al. (2014); Brito et al. (2007); Garcia-Mendoza et al. (2017); Guergoletto et al. (2016); Inada et al. (2015)		
Malvidin-3-glucoside	33.2[a]	Guergoletto et al. (2016)		
Peonidin-3-rutinoside	0.47-16.2[a]	Bicudo et al. (2014); Garcia-Mendoza et al. (2017); Guergoletto et al. (2016)		

Pelargonidin-3-glucoside	8-15.6[a]	Brito et al. (2007); Guergoletto et al. (2016)		
Cyanidin-3-sambubioside	13[a]	Brito et al. (2007)		
Cyanidin-3-rhamnoside	7[a]	Brito et al. (2007)		
Pelargonidin-3-rutinoside	5[a]	Bicudo et al. (2014); Brito et al. (2007)		
Peonidin-3-glucoside	0.28-1.59[a]	Bicudo et al. (2014); Garcia-Mendoza et al. (2017)		
Cyanidin 3, 5- diglucoside	nq	Bicudo et al. (2014)		
Phenolic acids				
Ferulic acid	2.59-34[a] 1.48-1.48[b]	Bicudo et al. (2014); Borges et al. (2011); Inada et al. (2015); Schulz et al. (2017); Siqueira et al. (2018)	- involvement in various physiological activities, such as nutrient uptake, enzyme activity, protein synthesis; - high antioxidant capacity; - radical scavenging capacity; - protection against injuries induced by reactive oxygen species; - ability to interact with proteins; - inhibition of pathogens and virus; - anti-deposition of triglycerides; - anti-inflammatory and anti-allergic effects through processes involving reactive oxygen species; - anti-carcinogenic and anti-mutagenic effects; - inhibition of the formation of DNA single strand breaks; - vasodilatory activities due to their antioxidant property;	Basli et al. (2017); Gong et al. (2019); Ozcan et al. (2014); Pernin et al. (2019); Vithana et al. (2019); Zhang et al. (2019)
Protocatechuic acid	2.43-22.51[a] 6.29-33.32[b]	Bicudo et al. (2014); Borges et al. (2011); Schulz et al. (2015); Schulz et al. (2017); Siqueira et al. (2018)		
Vanillic acid	8.51-16.51[a]	Bicudo et al. (2014); Schulz et al. (2017); Siqueira et al. (2018)		
p-hydroxybenzoic acid	3.4-14.75[a]	Bicudo et al. (2014); Inada et al. (2015)		
Ellagic acid	0.07-7.15[a]	Schulz et al. (2017)		

p-coumaric acid	0.19-6.96[a] 0.38-1.26[b]	Bicudo et al. (2014); Borges et al. (2011); Guergoletto et al. (2016); Inada et al. (2015); Schulz et al. (2015); Schulz et al. (2017); Siqueira et al. (2018)	- reduction of the incidence of non-communicable diseases: cardiovascular diseases, diabetes, cancer and stroke; - modulation of the gut microbiota.	
Syringic acid	2.96-6.83[a]	Bicudo et al. (2014); Schulz et al. (2017); Siqueira et al. (2018)		
3,4-dihydroxyphenylacetic acid	5.9[a]	Inada et al. (2015)		
Sinapic acid	2.38-5.29[a]	Bicudo et al. (2014); Schulz et al. (2017)		
3,4-dihydroxybenzoic acid	1.1-4.5[a]	Inada et al. (2015)		
Gallic acid	0.21-4.4 7.97-52.25[b]	Bicudo et al. (2014); Borges et al. (2011); Inada et al. (2015); Schulz et al. (2015); Schulz et al. (2017); Siqueira et al. (2018)		
p-hydroxyphenyl acetic acid	2.6[a]	Inada et al. (2015)		
Chlorogenic acid	0.21-1.33[a]	Bicudo et al. (2014); Schulz et al. (2017); Siqueira et al. (2018)		

m-coumaric acid	0.4[a]	Inada et al. (2015)		
Caffeic acid	0.11-0.39[a]	Bicudo et al. (2014); Schulz et al. (2017); Siqueira et al. (2018)		
trans-cinnamic acid	0.03[a]	Inada et al. (2015)		
Benzoic acid	nq	Siqueira et al. (2018)		
Flavonoids				
Rutin	0.17-9.4[a]	Guergoletto et al. (2016); Schulz et al. (2015); Schulz et al. (2017); Siqueira et al. (2018)	- radical-scavenging capacity; - ability to interact with proteins; - inhibition of pathogens; - anti-deposition of triglycerides; - anti-inflammatory and anti-allergic effects through processes involving reactive oxygen species; - anti-carcinogenic and anti-mutagenic; - reduction of the incidence of non-communicable diseases: cardiovascular diseases, diabetes, cancer and stroke; - antiobesity and cardioprotective effects; - blocking EGFR tyrosine kinase activity; - Inhibition of human CYP_1A_1 activities and formation of diolepoxide $_2(DE_2)$ and B[a]P activation.	Ballard et al. (2019); Basli et al. (2017); Ozcan et al. (2014); Perez-Vizcaino and Fraga (2018); Pernin et al. (2019)
Aromadendrin	2.04-7.64[a]	Schulz et al. (2015); Schulz et al. (2017)		
Taxifolin	1.09-6.50[a]	Schulz et al. (2015); Schulz et al. (2017)		
Quercetin	0.82-4.8[a] 17.59-36.34[b]	Borges et al. (2011); Guergoletto et al. (2016); Schulz et al. (2015); Schulz et al. (2017); Siqueira et al. (2018)		
Isoquercetin	0.36-1.39[a]	Schulz et al. (2017)		
Kaempferol	0.16-0.88[a]	Schulz et al. (2015); Schulz et al. (2017)		
Catechin	2.34-0.66[a]	Borges et al. (2011);		

	0.74-16.24[b]	Schulz et al. (2017)		
Hispidulin	0.15-0.29[a]	Schulz et al. (2015)		
Epicatechin	0.03-0.17[a] 6.83- 30.56[b]	Borges et al. (2011); Schulz et al. (2017)		
Myricetin	0.03-0.09[a]	Schulz et al. (2015); Schulz et al. (2017)		
Apigenin	0.02-0.03[a] 25.4[b]	Argentato et al. (2017); Schulz et al. (2017)		
Dihydrokaempferol-hexoside	66.4[b]	Argentato et al. (2017)		
Luteolin deoxyhexosyl-hexoside	37.6[b]	Argentato et al. (2017)		
Stilbene				
Resveratrol	0.01-0.06[a]	Schulz et al. (2015); Schulz et al. (2017)	- antioxidant properties; - neuroprotective actions; - anti-aging and antimicrobial properties - cardiovascular protective, antiplatelet, anti-inflammatory, blood glucose-lowering and anticancer activities, exhibiting a complex mode of action; - effects on genes as well as the heart, breast, prostate, uterus, and immune system; - sustainability of healthy nerves and important brain functions including cognitive processes; - Inhibition of cell proliferation and down regulation of telomerase activity.	Anekonda (2006); Basli et al. (2017); Kuršvietienė et al. (2016); Manach et al. (2004); Ozcan et al. (2014); Vestergaard and Ingmer (2019)
Phenol aldehyde				

Vanillin	1.50-16.51[a]	Bicudo et al. (2014); Schulz et al. (2017)	- antioxidant properties; - antimicrobial activity; - Inhibition of protein oxidation and lipid peroxidation.	Bezerra et al. (2017); Cava-Roda et al. (2012); Kamat et al. (2000); Li et al. (2019)

[a]mg/100g dry matter; [b] mg/ 100g fresh matter; nq: not quantified.

Usually the antioxidant capacity of juçara is superior to other fruits such as açaí (0.03 to 64.5 μmol Trolox g^{-1}) (Gordon et al. 2012), jabuticaba (670 μmol Trolox g^{-1}) (Inada et al. 2011), mango (75.5 μmol Trolox g^{-1}) (Moreira et al., 2017), banana and papaya (1.47 to 5.37 DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging IC_{50} (mg mL^{-1})) (Siriamornpun and Kaewseejan 2017).

The main healthy effect of juçara fruit is related to antioxidant scavenging capacity, which also proved to be effective on *in vivo* studies. The consumption and utilization of juçara, under specific conditions and doses were able to: 1) decrease the lipid peroxidation of healthy humans (Schulz et al. 2015); 2) restore the fecal content of *Bifidobacterium* spp, improving intestinal barrier integrity in the offspring of rats exposed to juçara polyphenols in the intrauterine and lactation periods (Morais et al. 2015); 3) preserve lean mass and decrease blood glucose and triacylglycerol in newborn rats (Argentato et al. 2017); 4) have a protective effect being considered anticancer, antimutagenic, antimicrobial, anti-inflammatory, antineurodegenerative (Nile and Park 2014); 5) protect against the UVB-induced oxidative damage (Nile and Park 2014). Other health benefits of bioactive compounds from juçara fruit are given in Table 3.

4. Applications of juçara palm fruits

All components of juçara palm can be used in different fields as shown in Fig. 2. However, the fruit and the palm heart are the most used for industrial purposes. In order to add value to juçara fruit, new uses are proposed such as food ingredient and supplement, as shown in the diagram of applications for the use of juçara palm fruits carried out after a bibliographic review (Fig. 3) (Moreira et al. 2017; Pereira et al. 2017).

Furthermore, anthocyanins, extracted from juçara fruit, are an alternative in the food industry, as potential substitutes for synthetic food colorants (Lima et al. 2019) which are

nowadays of public concern because of the adverse effect of synthetic dyes on human health, particularly affecting neurological functions and behavioral patterns (Sigurdson et al. 2017).

Anthocyanins extracted from juçara fruit can also be used by the cosmetic industry, as a natural dye in the manufacture of cosmetics, such as lipsticks, creams and lotions (Nile and Park 2014).

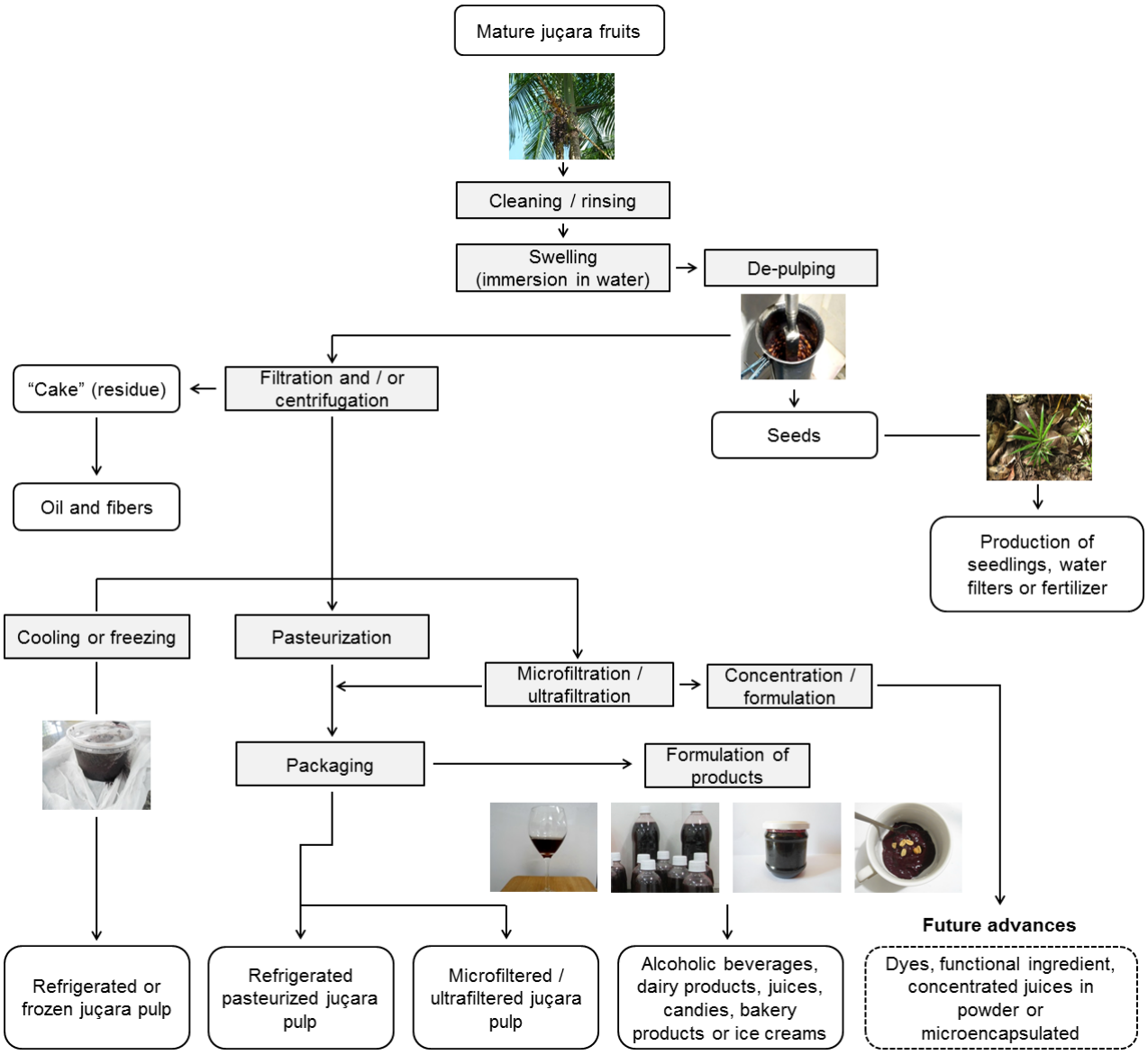

Fig. 3 Flow diagram of proposing different methods of juçara fruit processing

The oil extracted from the juçara fruits is rich in polyunsaturated fatty acids, being linoleic, palmitic and oleic acids the main components. It can be used in the cosmetic

(Felzenszwalb et al. 2013) and pharmaceutical industries, in the manufacture of creams and capsules.

Whichever form of use, due to its physicochemical and nutritional composition (Schulz et al. 2016), juçara fruit and pulp are perishable at room temperature, requiring conservation technologies after harvest. Pasteurization methods followed by freezing are the most widely used, however other technologies such as high pressure treatment (Moreira et al. 2017) and spray drying have also been studied to preserve the bioactive compounds and nutritional value increasing the product's shelf life, improving storage and transportation stability and reducing costs of cold chain procedures (Guergoletto et al. 2017; Paim et al. 2016). Those methods combined have also been used to provide alternative products to the market, such as pasteurized and high pressure fruit juices, probiotic fermented and spray dried juices (Guergoletto et al. 2017; Lima et al. 2019; Moreira et al. 2017; Paim et al. 2016), the latter being an alternative to lactose intolerant or allergic people with dairy restrictions.

5. Market potential

Although pulp commercialization is more profitable than fruit, it implies greater financial investment for the implementation of a production facility (Conab 2019), and its cost will vary with the processing technology.

Maier et al. (2019) found that the cost of implementing a project to produce juçara fruit and cutting your trunk for wood production can reach US$ 2,716.62 per hectare, having as products, the juçara fruit for commercial sale and the timber for community use. According to the authors, throughout 30 years invested on scientific projects, the estimated total amount of labor costs would be US$10,296.54 (69.1 %) and inputs cost would be US$ 4,611.74 (30.9 %), being the total income from the juçara fruit US$ 25,886.47. Projects like this can help the permanence of small producers in the field, reducing migration to the city.

The data available on the production of juçara showed that in 2012 the national production of juçara palm fruits was approximately 193 tons (Conab 2016). In 2013, the production of açaí fruits was much greater than juçara, 202,216 tons. Data showed that açaí pulp was mainly commercialized to USA, Japan and South Korea. Juçara also has potential to be part of this trading market. The value of açaí and juçara pulp exported reached US$ 17 million in 2012, corresponding to approximately 6 thousand tons of pulp (Conab 2016).

Although the market is growing, the commercialization of juçara pulp is still small compared to açaí pulp. This is due to a low production due to planting difficulties requiring extractive production, the concentrated production in a specific period of the year and non-standardization of pulp production. In this context, studies that focus on the use and production of juçara pulp will encourage the region development, as its commercialization on industrial scale will generate employment and income for the local population, besides contributing to the appreciation of the value of this species, favoring its preservation.

6. Conclusion and future prospects

Encouraging juçara fruit management rather than palm heart commercialization is a project with strong social approach, great environmental and economically promising footprint.

Data on juçara composition demonstrates that it assumes great importance in human nutrition due to its rich chemical content, which includes fatty acids, proteins, minerals, vitamins and dietary fibers. In addition, this fruit presents a great variety of phenolic compounds with bioactive potential, such as anthocyanins, with proved health benefits. This suggests that the use of juçara in the development of food products or even in cosmetics may represent a viable alternative for the use and valorization of these fruits. In addition, the high content of minerals and antioxidants found in juçara and the combination of other key success

factors for a food product, such as the origin of the plant (Atlantic Forest Biome), high biological activity (it is rich in bioactive molecules) and the environmental benefit (reduction of the slaughter of the palms of juçara in function of the valorization of their fruits) represents an excellent opportunity for the use of the pulp of fruits of juçara, with great potential of impact throughout the entire production chain of the food and cosmetic industry.

Despite the small number of industries that process this raw material, a small market is starting to appear, and there is an increasing interest for healthier products and sustainable production. It is of common sense that the variety of ecosystems should be preserved as well as the species individually. Traditional technological procedures are used in order to increase the variety of products that can be obtained from juçara fruit, such as heat treatment (i.e. pasteurization), dehydration and technological alternatives with less deleterious effect that intend to preserve the bioactive composition such as drying methods (i.e. spray drying). All those methods have as main purpose to increase the conservation and stability of juçara fruit or pulp, and increase juçara commercialization. Those technological approaches may need a substantial financial investment and/or face difficulties to be implemented in a large production scale. However, there is a need to value the juçara fruit culture in order to stimulate the commercialization of derivates from the fruit.

Juçara fruit has a short harvesting period, it is considered a highly perishable product, and its functional properties need to be preserved until consumption. Whereas that most workers responsible for the fruit harvesting and first processing are in the middle of the atlantic forest biome in Brazil, it is necessary to invest on standard methods making it easier to producers for a more sustainable culture procedure to increase juçara fruit conservation, reducing the palm tree extraction. In addition, marketing approaches that brings attention to the consumption of juçara, would increase consumers interest on juçara palm fruits.

The combined efforts of producers, research institutions and innovative industries will provide technological alternatives for the use of the fruits of juçara, making it possible to remove this tree from the list of endangered species.

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CAPÍTULO 2

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Spray drying of juçara pulp aiming to obtain a “pure” powdered pulp without using carrier agents

Spray drying of juçara pulp aiming to obtain a "pure" powdered pulp without using carrier agents

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Abstract

The aim of this work was to investigate the possibility of obtaining a juçara pulp powder by spray drying with no carrier agent addition. The pulp was filtered and then dried in a lab scale spray dryer. The process yield was relatively high (66%) and a highly anthocyanin-rich (7,079 ± 83 mg/100g cyanidin-3-glucoside) powder was obtained. Total phenolic content (14,305 ± 551 mg/100g) and antioxidant capacity (4,155 ± 92 μmol of Trolox/g) were satisfactorily preserved after drying, showing similar or better results than those reported using carrier agents, as well as solubility, bulk density and morphology. Finally, pulp powder was stable for 103 days, at different storage conditions, with respect to color, anthocyanin content and antioxidant capacity, showing potential to be used as a natural colorant or antioxidant in food products.

Keywords: *Euterpe edulis*, spray drying, anthocyanin, antioxidant capacity, storage stability.

1. Introduction

Juçara (*Euterpe edulis* Martius) is a palm tree native to the Brazilian Atlantic Forest, which has as main exploitation product the palm heart. However, its unsustainable culture has placed this specie on the list of threatened endangered plants [1]. Therefore, the economic valorization of juçara fruits can be a more sustainable alternative for its management and environment preservation [2, 3], since this tree is a key species for the Atlantic Forest biome.

Juçara fruit is a black-violet color berry due to the high anthocyanin content (634.26-2,929 mg/100g), with cyanidin-3-rutinoside and cyanidin-3-glucoside as main compounds [4-6]. Anthocyanin pigments can be used as natural food colorants, being an alternative to synthetic food dyes. Lately, the reduction on the utilization of synthetic dyes has attracted the consumers concerned about safety and the adverse effects on human health, particularly on neurological functions, allergies and behavioral effects [7, 8]. Besides the use as natural dyes, anthocyanins are associated with potential health benefits, such as anticancer, antimutagenic, antimicrobial, anti-inflammatory, antineurodegenerative effects and protection against the UVB-induced oxidative damage, both *in vitro* and *in vivo* [7, 9]. In this way, juçara pulp has a promising functional application in the food industry.

Juçara fruit and pulp are highly perishable at room temperature, due to their high pH and high moisture content, which favors the occurrence of chemical and enzymatic reactions, leading to product deterioration. Spray drying is a well established technology used to dry fruit pulps, resulting in a longer shelf life and wider industrial application. The process results in powders with good quality and low water activity, which are easier to transport and store [6, 10]. However, sugars and organic acids present in fruit pulps have low glass transition temperatures and high hygroscopicity, resulting in higher stickiness, low yield and flow-related problems. To prevent these problems, the addition of carrier agents, such as

carbohydrates and proteins, to the feed solution before processing is often recommended [11-13].

Spray drying of juçara pulp using carrier agents was reported by Lacerda et al. [2], Carvalho et al. [6], Bicudo et al. [10], Guergoletto et al. [12], Paim et al. [14], Santana et al. [15] and Bernardes et al. [16]. These authors used different types of carrier agents such as gelatin, gum Arabic, maltodextrin, inulin, oligofructose, starch, whey protein and soy protein to microencapsulate juçara pulp by spray drying, in order to act as a protective barrier, avoiding chemical and physical reactions and the deleterious effects of external factors, and aiming at improving the physical characteristics of the final product. However, the addition of these agents may promote color change as most of them are of white color, as well as "dilution" of bioactive compounds content and antioxidant capacity of juçara pulp. Therefore, the production of juçara pulp powder without the addition of carrier agents is a promising alternative for the development of a natural additive to be used as a functional ingredient, richer in bioactive compounds when compared to those produced with carrier agents. To the best of our knowledge, there is no report on the spray drying of juçara pulp without the addition of carrier agents.

Therefore, the aim of this work was to obtain a juçara pulp powder with no carrier agent addition and to evaluate its physicochemical characteristics, as well as its stability under different storage conditions. Results were compared to those already reported for juçara pulp spray dried with carrier/encapsulating agents.

2. Material and methods

2.1. Material

Frozen juçara pulp was purchased from a rural producer, located at Rio Pomba (Minas Gerais, Brazil). Juçara fruit was harvested in Rio Pomba between the coordinates 21° 09' 19.2" and 21° 09' 09.3" S and 43° 09' 12.5" and 43° 08' 58.8" W, in November 2016. The pulp was stored at -18°C.

2.2. Methods

2.2.1. Sample preparation

Juçara pulp was thawed, homogenized for 3 minutes with an industrial blender (Walita LiqStar, RI1784) and filtered using a 1.8 mm pore diameter sieve. This procedure reduced the solids content from 9.7% to 6.5%, in order to facilitate the spray drying process, preventing clogging of the spray nozzle. Whole and filtered pulp were characterized for moisture content, soluble solids, acidity, proteins, lipids and ash [17], total carbohydrates [17], total phenolic compounds [18, 19], total monomeric anthocyanins [20], instrumental color and antioxidant capacity by two methods: $ABTS^{\bullet+}$ (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulphonate) scavenging capacity [21] and ORAC (Oxygen Radical Absorbance Capacity) [22].

2.2.2. Histological section

In natura juçara fruit was cut in cross section and its pericarp was observed and photographed in a light microscope, through the preparation of fresh histological slide. No dyes were used due to the natural coloration of the tissues.

2.2.3. Spray drying

The process was performed in a laboratory scale spray dryer LabPlant^{TM} SD-06 (Huddersfield, England), with a 2.0 mm diameter nozzle and main spray chamber of 500 × ϕ 215 mm. The filtered pulp was fed into the main chamber through a peristaltic pump. Compressor air pressure was 0.25 MPa, the feed flow rate was 9 g/min, drying air speed was 4.3 m/s, inlet and outlet air temperature were 160 ± 2°C and 86 ± 2°C. Spray drying process yield was calculated as the relationship between the total solids obtained after drying and the total solids in the initial feed solution. The process was made in quadruplicate, using 1 L for each replicate.

2.2.4. Spectrophotometric analysis

For both filtered and powdered juçara, total phenolic content was determined using Folin-Ciocalteu reagent according to the method described by Singleton and Rossi [18] and modified by Georgé et al. [19]. Results were expressed as mg gallic acid equivalent (GAE) per 100 g of sample.

Anthocyanin content was determined using the spectrophotometric pH differential method as described by Giusti and Wrolstad [20]. Results were expressed as mg cyanidin-3-glucoside per 100 g of sample.

The antioxidant capacity was determined using ORAC [22] and $ABTS^{\bullet+}$ radical cation scavenging activity [21]. For both methods, results were expressed as μmol of Trolox equivalents per g of sample. All analysis were performed in triplicate.

2.2.5. High Performance Liquid Chromatography - HPLC Analysis

Extracts from filtered and juçara pulp powder were obtained according to the methodology described by Gouvêa et al. [23] and Pérez-Jiménez et al. [24] for analysis of anthocyanins and flavonoids and phenolic acids, respectively. The extracts were analyzed by

high performance liquid chromatography (HPLC) using Waters® Alliance model 2695 and e2695 chromatograph (Milford, USA). The results obtained were compared to the spectral of compounds control curve (cyanidin-3-glucoside, cyanidin-3-rutinoside, 3, 4-dihydroxybenzoic acid, 4-hydroxybenzoic acid, catechin, vanillic acid, epicatechin, ferulic acid, rutin and luteolin).

2.2.6. Juçara pulp powder characterization

2.2.6.1. Water activity and moisture content

Water activity (a_w) of juçara pulp powder was measured directly at 25°C using an AquaLab® analyzer (Model S3TE B, Washington, USA). Moisture content was determined using a vacuum oven until constant weight, according to AOAC method nº. 925.10 [17].

2.2.6.2. Water solubility

Water solubility of juçara pulp powder was determined as described by Cano-Chauca et al. [25]. Briefly, powder (1g) was dissolved in water (100 mL), centrifuged at 2000 rpm for 15 minutes and the amount of soluble solids in the supernatant was determined gravimetrically.

2.2.6.3. Bulk density

For determination of bulk density, 2 g of powder were transferred to a 50 mL graduated cylinder [10]. The bulk density was calculated by dividing the powder mass by the volume occupied in the cylinder. The measurements were carried out at 25 ± 2°C.

2.2.6.4. Hygroscopicity

Hygroscopicity was determined by storing powdered juçara pulp (500 mg) in a desiccator with saturated NaCl (34.0 M, 75.0% relative humidity) solution at 25 ± 2°C for 7

days. After this period, powder was weighed and hygroscopicity was expressed as percentage [26].

2.2.6.5. Particle size distribution

Particle size distribution was measured using a laser light diffraction instrument, MICROTRAC S3500 (Microtrac Inc., Montgomery Ville, USA) based on light scattering technique. Analysis was conducted in duplicate and in three cycles, using isopropyl alcohol as the carrier fluid (refractive index of 1.37). Result was expressed as the volumetric mean diameter ($D_{4,3}$). Particles size dispersion was calculated as the coefficient of variation of the particle size distribution analysis [2].

2.2.6.6. Morphology

Powder morphology was observed in a scanning electron microscope TM3000 (Hitachi, Japan), operated at 15 kV under low vacuum. Samples were previously left in desiccator with anhydrous calcium chloride for 20 days to remove surface moisture.

2.2.6.7. Instrumental color

Instrumental color of juçara pulp powder was determined using Minolta CR-400 colorimeter (Konica Minolta Meter, Osaka, Japan), with illuminant D65 and view angle of 2º. The CIELab color space was used to determine the color components: L^* [black (0) to white (100)], a^* [greenness (−) to redness (+)] and b^* [blueness (−) to yellowness (+)]. The cylindrical coordinates C^* (Chroma) and ^oh (hue angle) were also determined. For the stability test, the total color difference (ΔE^*) was calculated according to Eq. (1).

$$\Delta E^* = \sqrt{(L^*_{t_0} - L^*_{t_1})^2 + (a^*_{t_0} - a^*_{t_1})^2 + (b^*_{t_0} - b^*_{t_1})^2} \quad (1)$$

Where L^*, a^* and b^* are the color coordinates at initial time (t_0) and after i days of storage (t_i).

2.2.7. Storage stability at 25º C and 7ºC

In order to investigate the storage stability of dried juçara pulp, samples were equally and individually distributed in 10 g bags (5.0 × 7.0 cm) composed by metallized polyester and polyethylene. Half bags were sealed with oxygen and the other half was vacuum sealed, in order to evaluate the effect of oxygen on powder stability. The influence of temperature was analyzed by storing the bags at 25°C and 7°C. Samples were analyzed each 7 days during the first 28 days, then each 15 days until complete 103 days of storage, with respect to moisture content, total anthocyanins, antioxidant capacity and color.

2.2.8. Statistical analysis

Results were obtained in triplicate and statistically treated by analysis of variance, using the software SISVAR 5.6. Mean analysis was performed using Tukey procedure at $p \leq 0.05$.

3. Results and discussion

3.1. Juçara pulp characterization

Table 1 shows the chemical characterization of whole and filtered juçara pulp. As expected, the filtered juçara pulp presented lower total solid content and higher soluble solids content, proteins, lipids and ash (in a dry basis). This increase is possibly due to the fact that part of the pulp (total solids) is retained in the sieve during filtration, reducing the carbohydrate content and increasing other components concentration per portion.

Phenolic compounds, anthocyanins and antioxidant capacity of triturated-filtered juçara pulp also increased (Table 1), which shows that trituration and filtration procedure reduced the pulp particle size, releasing a higher amount of bioactive compounds. These higher values are also related to the reduction in total solid content, since results are expressed in a dry basis.

Table 1. Chemical characterization of full and filtered juçara pulp (*Euterpe edulis* Martius).

Analyzed item**	Full pulp	Filtered pulp
Moisture content (%wet basis)	90.35±0.08b	93.47±0.01a
Soluble solids (ºBrix %wet basis)	4.63±0.15b	5.73±0.06a
Acidity (% citric acid wet basis)	0.31±0.01a	0.31±0.00a
Proteins (%dry base)	8.62±8.62b	11.01±11.01a
Lipids (%dry base)	17.30±0.13b	27.26±0.19a
Ash (%dry base)	4.31±0.33b	9.19±0.17a
Total carbohydrates (%dry base)	69.76±0.17a	52.54±0.94b
Total phenolic compounds (mg/100g dry base)	9,071.87±85.90b	15,807.22±40.77a
Total anthocyanins (mg cyanidin-3-glucoside /100g dry base)	2,928.64±28.58b	6,240.11±30.43a
ABTS antioxidant capacity (µmol of Trolox/g dry base)	504.97±6.83b	790.08±15.47a
ORAC antioxidant capacity (µmol of Trolox/g dry base)	2,400.10±107.78b	3,750.19±253.75a
L*	12.54±0.04a	11.56±0.13b
a*	6.68±0.01b	7.80±0.12a
b*	-13.08±0.01b	-9.82±0.11a
C	14.69±0.02a	12.62±0.11b
ºh	297.12±1.07b	308.51±0.68a

**Mean of three replicates. Values expressed as mean and standard deviation. Means followed by the same letter do not differ statistically at 5% probability by the Tukey test.

3.2. Process yield

The drying process yield was 66%, which could be considered a good result, considering the values commonly observed for laboratorial scale spray dryers. According to Bhandari et al. [27], in general, spray drying with 50% recovery of the powder is considered to be successful. Lacerda et al. [2] obtained lower process yield (21.5% to 53.1%) in the

microencapsulation of juçara pulp by spray drying, using starch sodium octenyl succinate (OSA starch), inulin and/or maltodextrin as carrier agents.

Process yield similar to the present study was reported by Santana et al. [15] in the microencapsulation by spray drying of juçara pulp with gum Arabic, modified starch, whey protein and/or soy protein. According to the authors, the addition of carrier agents of high molecular weight was necessary to avoid large deposition on the main chamber and cyclone walls, due to the feed viscosity.

In comparison to other tropical fruits, juçara pulp presented low amount of carbohydrates (52.54±0.94%, dry basis) and organic acids (0.31% citric acid, wet basis), which facilitates the spray drying process. Thus, even without the addition of carrier agents, it was possible to obtain a good process yield. In addition, pulp with significant content of total solids (6.5%) was used to feed the dryer, and the powder generated in both drying chamber and dryer was collected, which also explain the high calculated process yield.

3.3. Bioactive compounds and antioxidant capacity

Table 2 shows the characterization of the processed juçara pulp used to feed the dryer (filtered pulp), the pulp powder obtained after spray drying and the bioactive compounds retention. As the focus of this work was to obtain a powdered product, bioactive compounds and antioxidant capacity were calculated in a dry basis.

Table 2. Bioactive compounds and antioxidant capacity of filtered and powdered juçara pulp, and retention of bioactive compounds

Analyzed item*	Filtered pulp	Powdered pulp	Retention (%)
Total phenolic compounds (mg/100g dry base)	15,814.84±40.77a	14,305.08±550.58b	90.45±3.32
Total anthocyanins (mg/100g cyanidin-3- glucoside dry base)	6,240.11±30.43b	7,079.19±82.67a	113.45±1.9
ABTS antioxidant capacity (μmol of Trolox/g dry base)	790.08±15.47[a]	858.59±44.28a	108.63±3.5
ORAC antioxidant capacity (μmol of Trolox/g dry base)	3,750.19±253.75a	4,155.39±92.50a	111.26±10.20

* Mean of three replicates. Values expressed as mean and standard deviation. Means followed by the same letter do not differ statistically at 5% probability by the Tukey test.

HPLC analysis allowed observing that comparatively the retentions of anthocyanins and total phenolic compounds determined by different methods followed the same trend, but with different contents (Table 3). Spectrophotometric methods such as Folin-Ciocalteu method can overestimate the total phenolic content, since other compounds than phenols can be accounted, such as thiols, vitamins, amino acids, proteins, nucleotide bases, unsaturated fatty acids, carbohydrates, organic acids, inorganic ions, metal complexes, aldehydes, and ketones. Thus, Folin-Ciocalteu method can be considered a measurement of total antioxidant capacity, since phenolic compounds are the most abundant antioxidants in many fruits [30].

Carrier agents used to increase the glass transition temperature are also used to protect the bioactive compounds of fruits from oxidation [15]. In the present work, even without the addition of carrier agents, no losses of bioactive compounds were observed (7,079.19 mg cyanidin-3-glucoside / 100g juçara powder). Although we have not used carrier agents, juçara pulp components themselves, such as proteins and carbohydrates, may have a protective effect against degradation of these bioactive compounds.

Lacerda et al. [2] and Paim et al. [14] found lower anthocyanin levels when analyzing microencapsulated juçara pulp (330-2,420 mg/100g). These differences in relation to the present study can be explained by the low concentration of juçara pulp in the

microencapsulated products due to its dilution with the addition of carrier agent. In addition, low levels of anthocyanins in microencapsulated juçara pulps were reported by Lacerda et al. [2] with low yields in the encapsulation process (21.5-61.1%).

Table 3. Anthocyanins, phenolic acids and non-anthocyanin flavonoids of filtered and powdered pulp, and retention of bioactive compounds by HPLC.

Analyzed compounds * (mg/100g dry base)	Filtered pulp	Powdered pulp	Retention (%)
Cyanidin-3-glucoside	1.145.79±29.89[a]	1.244.47±38.54a	108.61±0.53
Cyanidin-3-rutinoside	2.031.93±135.47a	2.334.04±72.42a	115.00±4.10
Total anthocyanins	**3,177.72±165,35a**	**3,578.51±110,96a**	**112.67±2,37**
3, 4-dihydroxybenzoic acid	0.21±0.00a	0.08±0.01b	35.54±0.24
4-Hydroxybenzoic acid	0.46±0.00a	0.21±0.00b	46.42±0.00
Catechin	0.46±0.00a	0.18±0.01b	39.79±3.13
Vanillic acid	1.07±0.00a	0.49±0.01b	45.95±0,67
Epicatechin	1.91±0.11[a]	0.80±0.01b	41.70±1.98
Ferulic Acid	0.31±0.00a	0.13±0.01b	43.11±4.69
Rutin	1.53±0.00a	0.72±0.0b	46.76±0.47
Luteolin	1.15±0.11[a]	0.26±0.01b	23.15±3.43
Total phenolic compounds**	**3,184.82±165.11a**	**3,581.39±110.93a**	**112.51±2.35**

* Mean of two replicates. Values expressed as mean and standard deviation. Means followed by the same letter do not differ statistically at 5% probability by the Tukey test. ** Sum of the phenolic compounds found in the juçara (cyanidin-3-glucoside, cyanidin-3-rutinoside, protocatechuic acid, 4-hydroxybenzoic acid, catechin, vanillic acid, epicatechin, ferulic acid, rutin and luteolin).

Anthocyanins were fully retained and showed a slight increase after drying (Table 2 and 3). These increase on anthocyanin content may have occurred because the anthocyanic compounds are present, in most part, in the cell vacuoles (Figure 1) and, during drying process, cellular disruption caused by the high shear and heat may have promoted a greater release of these compounds.

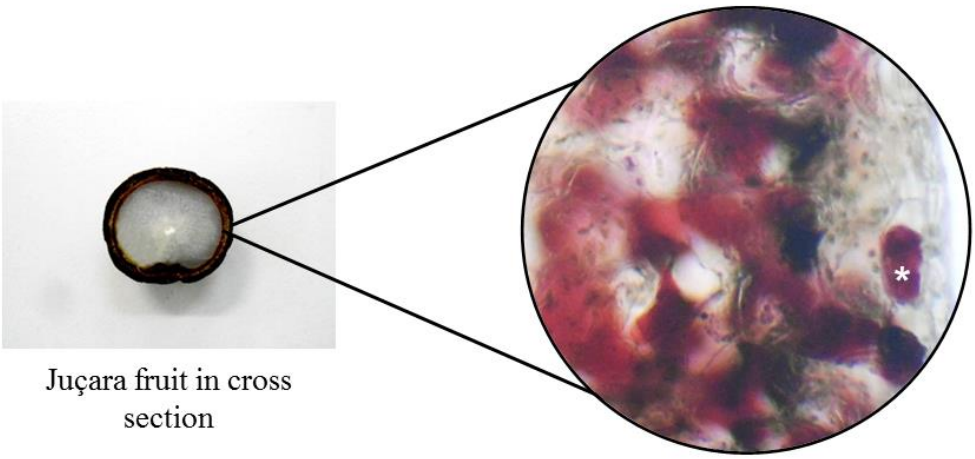

Figure 1. Juçara fruit cross section, evidencing (*) cell with vacuole full with phenolic compounds. Histological section visualized on an optical microscope with a magnification of 400× (natural coloring of tissues).

Under similar drying conditions and quantification by spectrophotometry test, lower anthocyanin retention was reported for açai pulp microencapsulated with maltodextrin (77-86%) [26], jabuticaba extract microencapsulated with maltodextrin, gum arabic and modified starch (79%-100%) [31] and juçara pulp microencapsulated with maltodextrin, gum arabic and / or gelatin (64%-88% %) [10]. Lacerda et al. [2] quantified by HPLC anthocyanins from juçara pulp microencapsulated with modified starch, inulin and maltodextrin and also found lower retention values than the present study (6%-67%).

For antioxidant capacity, no significant increase ($p > 0.05$) was observed for the ABTS and ORAC methods, while total phenolic compounds had a retention of 90% by the Folin-Ciocalteu method. The loss of 10% of the phenolic compounds may be related to the degradation or copigmentation of the compounds, which alters its wavelength, reducing its detection in spectrophotometric analyses. In addition, a reduction of phenolic acids and non-anthocyanin flavonoids was observed by HPLC, after drying (Table 3). However, antioxidant

capacity presented an increase (Table 2), probably due to the compounds copigmentation. Similar results of increased antioxidant capacity was observed by Reque et al. [32] when analyzing blueberry fruit. In this case, the increase of the antioxidant capacity was attributed to the formation of compounds with high antioxidant capacity, also originated from the copigmentation phenomenon.

3.4. Physical properties

The development of new ingredients to be used in the food industry needs to attend chemical and physical parameters such as a_w, instrumental color, particle size, hygroscopicity, solubility and bulk density. The a_w and moisture content are related to the product shelf life, as it is directly related to microbial growth and enzymatic and non-enzymatic reactions. The juçara pulp powder presented low a_w and moisture content values (0.29 and 1.54%, repectively), and hygroscopicity of 11.62% (Table 4), being considered adequate for this type of product. The a_w near 0.3 and moisture content lower than 5% inhibits microbial growth and oxidative reactions, and the product can be stored for long-term [2, 13], which suggests that the juçara pulp powder would be stable. These values are in the same range observed by other authors for microencapsulated juçara pulp powders [2, 10].

Solubility is considered the most reliable criteria to evaluate the powder behavior in an aqueous solution. This parameter refers to the ability of powders to form solution or suspension in water [10]. In general, a high solubility is desired for powdered products. According to Table 4, the observed solubility (72.93%) indicated that the juçara pulp powder was relatively highly soluble in water, being appropriate to be used as colorant or raw material for the development of new products.

Table 4. Physical properties of spray dried juçara pulp powder.

Properties*	Values
Water activity (a_w)	0.29±0.00 a 25,1°C
Moisture content (%wet basis)	1.54±0.16
Solubility (%)	72.93±0.28
Bulk density (g/cm^3)	0.42±0.01
Hygroscopicity (%)	11.62±0.30
L*	14.76±0.91
a*	8.00±0.27
b*	-4.04±1.89
C	9.06±1.01
^{o}h	346.90±0.82

*Mean of three replicates. Values expressed as mean and standard deviation.

According to Tontul and Topuz [13], bulk density is dependent on the size, shape, and surface properties of powder particles, and powders with a smooth and uniform surface have greater bulk density. The bulk density of the juçara pulp powder (0.42 g/cm^3) was higher than that found by Bicudo et al. [10], who analyzed juçara pulp powder (0.20-0.37 g/cm^3) microencapsulated with maltodextrin, gum arabic and / or gelatin. Knowledge of food density is fundamental for material properties studies and for industrial processes in order to adapt the most suitable conditions for storage, processing, packaging and distribution [33]. High bulk density is desirable to reduce transportation costs and packaging. In addition, lower apparent densities result in a larger package volume and the higher amount of occluded air inside the powders can lead to a higher possibility of pigment oxidative degradation, resulting in lower storage stability [10, 34].

The juçara pulp powder was visually purple with low *L** values (14.76), low positive *a** values (8.00) and $^o h$ of 346.90, which corresponds to a predominantly dark purple powder (Table 4). Bicudo et al. [10] and Lacerda et al. [2] found higher values of *L** and *a** varying from 10.8 to 32.48 and 23.9 to 54.5, respectively, when a microencapsulated juçara pulp powder was analyzed. This difference may be related to the addition of carbohydrates (maltodextrin, gum arabic and starch) as encapsulating agents, which interfere in the powder

color, making it lighter and with the red tonality more evident, due to the predominantly white color of the added carbohydrates.

The physical properties (density, compressibility and fluidity) of a food powder are highly dependent on particle size and size distribution [35]. Figure 2 shows the particle size distribution for the juçara pulp powder. The powder showed a unimodal distribution, i.e. a distribution with a predominant peak. The particles exhibited a large range of sizes (2.3 μm to 1,674 μm), with volumetric mean diameter ($D_{4,3}$) of 124.5 μm. A broad size range is common in powders produced by spray drying. Smaller values were found for microencapsulated juçara pulps (6.23 to 40.5 μm) analyzed by Bicudo et al. [10], Lacerda et al. [2] and Paim et al. [14]. The larger size observed in the present study may be related to the larger diameter of the atomizer nozzle used (2 mm) for the juçara pulp drying. In addition, the absence of wall material may have allowed the aglomeration due to the pulp sugars.

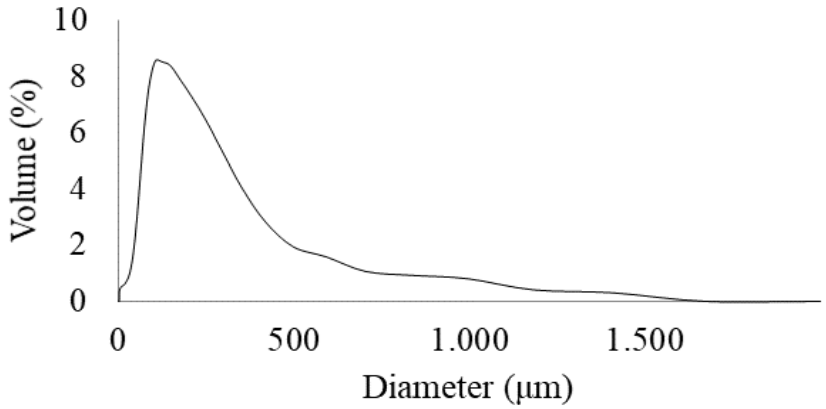

Figure 2. Particle size distribution of spray dried juçara pulp powder.

Particle size is one of the most important physical parameters, as it can influence the powder flow, blending of different components, segregation and compaction of a mixture [33]. In addition, this property significantly influences the powder processing, handling and

shelf life, which may interfere in the taste, color and texture of the product, being these characteristics important for consumer's consideration [13, 36].

According to Schubert et al. [37], very fine powders may have low wettability, making them difficult to use. A smaller particle size increases the particle surface area causing higher affinity to water [33], which leads to the formation of a viscous layer in the liquid surface, representing an obstacle to the capillarity flow throughout the intergranular pores. Thus, depending on the application of these fine powders, a process of agglomeration can be applied, aiming to increase particle size and improving the instantaneity of powders. The juçara pulp powder produced in the present study dispenses agglomeration process due to its larger particle size. In the work carried out by Lacerda et al. [2], the conditions that led to bigger microparticles were preferred in relation to the smaller ones, as they presented smaller dispersity of the particle size, which is as important as the average diameter.

Narrower particle size dispersity represents more homogeneous physicochemical properties, such as water solubility and hygroscopicity. In the present study, particle size dispersity was 8.6% (Figure 2), similar to what was found by Lacerda et al. [2] (4.6%-30.2%).

3.5. Scanning electron microscopy

Morphological characterization of the juçara pulp powder (Figure 3) showed most of the particles with spherical shape resulting from the fast water evaporation. When the drying temperature is sufficiently high, the water evaporates very quickly and the particle surface becomes dry and hard, which is essential for the protection of internal anthocyanins.

Due to the formation of loose bonding bridges some juçara pulp powder particles were agglomerated. However, this agglomeration still allowed a high bulk density of the juçara pulp powder produced in the present study. Agglomerated products have low bulk density which influences the powder properties, such as flowability and instant characteristics [10].

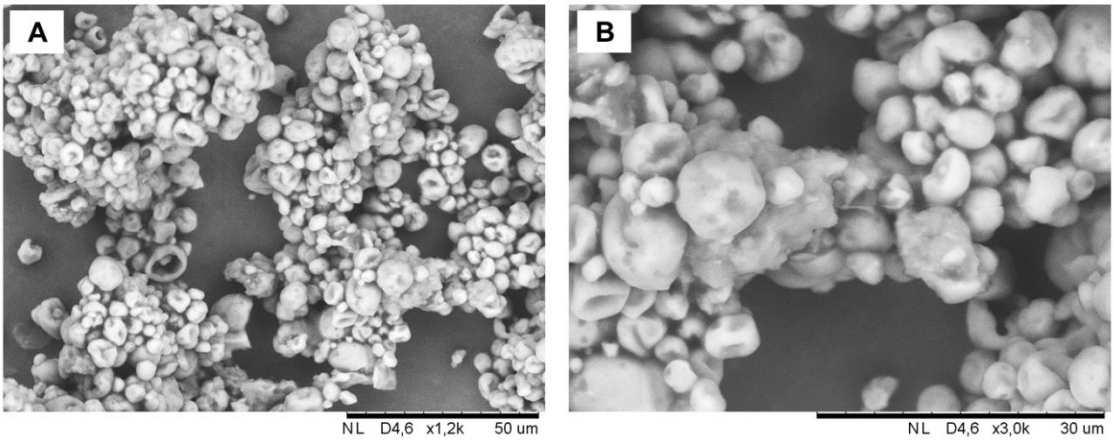

Figure 3. Scanning electron microscopy images of juçara powder at different magnifications: (A) x 1200 and (B) x 3000.

3.6. Storage stability

Stability of color (*ΔE** and h^o), anthocyanins, antioxidant capacity ($ABTS^{\bullet+}$ and ORAC) and moisture content of juçara pulp powder stored at 25°C and 7°C, both in the presence and absence of oxygen, for 103 days, is shown in Figure 4. As a general trend, juçara powder color became slightly lighter ($L^*_{t_0} = 14.76$ and $L^*_{t_0} = 15.98$) and less red ($a^*_{t_0} = 8.00$ and $a^*_{t_{103}} = 7.70$) during storage, which led to small changes in *ΔE**, corresponding to the total color difference between the *L*a*b** parameters in the initial and final times. Based on the *ΔE** values, the powder stored at 7°C in the absence of oxygen showed lower *ΔE** (0.76) ($p < 0.05$), which indicates greater stability. The powder stored at 25ºC in the absence of oxygen showed the highest variation after 103 days of storage ($p < 0.05$). No significant difference ($p > 0.05$) was observed between powders stored at 25°C and 7°C in the presence of oxygen. The *Hue* angle (°*h*) of the powders stored at 7°C tended to a more purple color (337°), significantly differing ($p < 0.05$) from the powders stored at 25°C (average 324°). The powder stored at 25ºC in the presence of oxygen differed ($p < 0.05$) from the powder stored in the absence of oxygen after 103 days of storage, showing a lower value for ^oh, which indicates

a less purple coloration (323º). According to Obón et al. [38], color differences become eye evident for ΔE^*> 5.0. All powders stored in the four conditions showed ΔE^* <3.0, indicating that the instrumental color differences of juçara powder after 103 days storage at 25°C or 7°C would not be differentiated without the use of a colorimeter. Bernardes et al. [16] observed greater ΔE^* variation (4.49) when analyzing microcapsulated juçara powder with different carrier agents and incorporated in a gelatin model system, again with no distinction to human eyes.

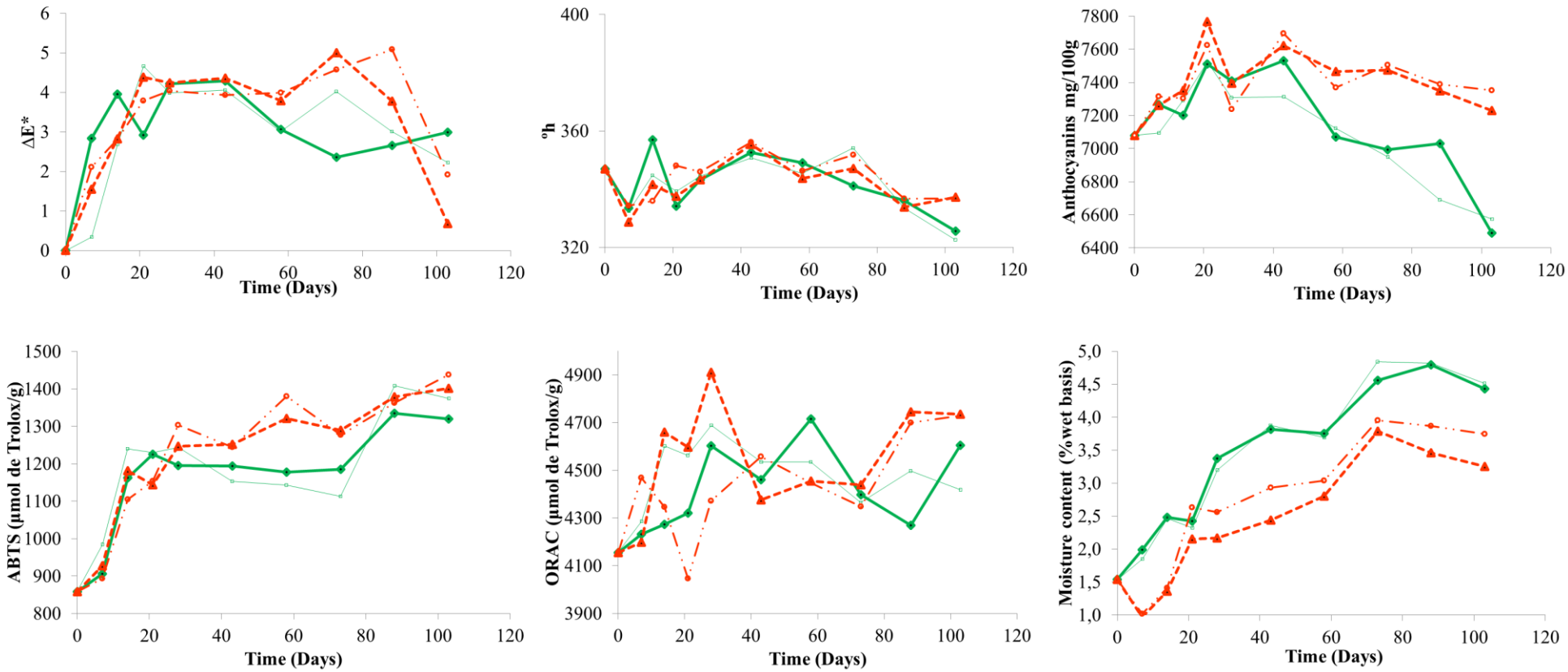

Figure 4. Stability of color (ΔE^* and h^o), anthocyanin content and antioxidant capacity (ORAC and ABTS$^{\bullet+}$ assays) of juçara pulp powder stored at 25ºC (full line) and 7ºC (dashed line), vacuum sealed (closed symbols) and in contact with oxygen (open symbols) for 103 days.

The anthocyanin content remained unchanged after 103 days of storage at 25°C and 7°C, even under exposure to oxygen, and no significant difference ($p > 0.05$) was observed after 103 days of storage. Similar result was observed by Lacerda et al. [2] when analyzing microencapsulated juçara pulp stored for 38 days at 50°C, even under light exposure. This stability could be explained by copigmentation interactions between anthocyanins and other phenolic compounds [39], which prevented its transformation to less stable forms. This interaction involves the carbinol pseudo-base form of anthocyanins, leading to color loss upon complexation, possibly explaining the apparent divergence between anthocyanins stability and color change in juçara powders [2]. Cortez et al. [40] recommended the use of anthocyanins in the form of powder to increase the food stability during product shelf life. Due to that, the juçara powder obtained in the present study has a promising application as food colorant or ingredient, especially in dry or instant powdered products.

After 103 days of storage, the antioxidant capacity of the juçara powder stored in the four conditions increased 61% on average for the ABTS method and 11% on average for the ORAC method, except for the powder stored at 25°C in the presence of oxygen, which remained stable for the ORAC method. Storage at 25°C in the presence and absence of oxygen resulted in a lower antioxidant capacity ($p < 0.05$) by the ORAC and ABTS methods, respectively, when compared to the other storage conditions. No significant difference ($p > 0.05$) was observed after 103 days of storage, for the evaluated conditions. Brownmiller et al. [39] also observed little alteration on the antioxidant capacity by the ORAC method during the storage of blueberries that were canned in syrup, canned in water, pureed, and juiced (clarified and nonclarified), indicating that the formation of polymers compensated the loss of antioxidant capacity. Since the anthocyanin content was not altered during storage, the increase in antioxidant capacity may be due to the copigmentation reactions of anthocyanins

with other phenolic compounds, forming compounds that may have a higher antioxidant capacity.

Moisture content increased and tended to stability for all storage conditions, however the moisture increase did not lead to significant losses of bioactive compounds, in contrast to the observed by Paim et al. [14], which related the loss of bioactive compounds from microencapsulated juçara pulp to higher water absorption over time. Storage at 7ºC in the absence of oxygen resulted in a lower moisture content after 103 days of storage ($p < 0.05$), followed by storage at 7ºC in the presence of oxygen. Storage at 25ºC did not differ among them ($p > 0.05$) and showed the highest moisture content after 103 days of storage ($p < 0.05$).

4. Conclusions

Spray drying was efficient for producing juçara powder, allowing the retention of 100% of anthocyanin content and antioxidant capacity. The absence of carrier agents did not interfere in the product quality, generating a powder with high anthocyanin concentration (7,079.19±82.67 mg/100g cyanidin-3-glucoside dry basis), not diluted by the use of carrier agents. Bioactive compounds content and antioxidant capacity value were stable in all the storage conditions evaluated during 103 days. The obtained juçara powder is an alternative to add value to this fruit chain. As a suggestion of application, the powder could be used as natural additive or colorant in food products such as beverages, powdered juices, ice creams, dairy and bakery products, as well as in the reconstitution of the juçara juice itself.

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CAPÍTULO 3

Artigo submetido para a revista *Food Chemistry* - ISSN 0308-8146

In vitro gastrointestinal digestion influence on phenolic compounds profile of fresh and powdered juçara pulp

***In vitro* gastrointestinal digestion influence on phenolic compounds profile of fresh and powdered juçara pulp**

Influence of digestion on phenolic compounds profile of powdered juçara pulp

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Abstract

Bioactive compounds bioaccessibility of juçara fruit and its products, and their effects on intestinal microbiota still need to be explored. The bioacessibility of phenolic compounds and the antioxidant capacity of juçara pulp and juçara powder after gastrointestinal digestion was evaluated. The behavior of colonic microbiota (*Bifidobacterium, Lactobacillus, Clostridium* and *Escherichia coli*) after 24 h colonic fermentation was also evaluated. After digestion, it was determined that 26.4% of the total phenolic content of juçara pulp (666.5 mg/100g) and 21.0% of juçara powder (787.21 mg/100g) reached the colon, respectively. In juçara pulp, an increase of 158.7% (ABTS) and 76.8% (ORAC) on the antioxidant capacity (1,306.4 and 4,242.9 μmol of Trolox/g respectively) was observed. In juçara powder an increase of 22.7% (ABTS) and 6.2% (ORAC) on the antioxidant capacity (1,048.9 and 4,411.0 μmol of Trolox/g, respectively) was observed. Microbial fermentation of juçara pulp resulted in an increase on short-chain fatty acids production (738.2% in juçara pulp; 774.0% powdered juçara) and *Bifidobacterium* cell count of 1 Log cycle. A smaller increase was observed in the *E. coli* population of the microbiota exposed to juçara pulp and powder, and a decrease in ammonia production of 100.8% and 127.0% respectively for pulp and powder, when compared to a negative control (reactor without the addition of fresh or powdered juçara pulp). Powdered juçara presented high content of phenolic compounds and the potential to modulate the intestinal microbiota, being an alternative to act as a food ingredient.

Keywords: *Euterpe edulis*, bioacessibility, colonic fermentation, microbial modulation, ammonia ion, short-chain fatty acids.

1. Introduction

Juçara (*Euterpe edulis*) is a native palm of the Brazilian Atlantic Forest with a black-violet color berry due to a high anthocyanins content (425.76 to 1,353 mg/100g dry base), presenting cyanidin-3-rutinoside and cyanidin-3-glucoside as main compounds (Carvalho et al., 2016; Guergoletto et al., 2016; Schulz et al., 2015). Anthocyanin pigments can be used as a natural food colorant being an alternative to synthetic food dyes. Lately the reduction on the utilization of synthetic dyes has attracted public attention regarding safety and adverse effects on human health, particularly on neurological functions, allergies and behavioral effects (Khoo et al., 2017; Sipahli et al., 2017). Besides the use as natural dyes, anthocyanins are associated with potential health protective effect, such as anticancer, antimutagenic, antimicrobial, anti-inflammatory, antineurodegenerative and protection against the UVB-induced oxidative damage, both *in vitro* and *in vivo* (Khoo et al., 2017; Silván et al., 2016). Thereby, juçara pulp has a promising functional application in the food industry.

However, juçara pulp is highly perishable at room temperature, due to a high pH (4.77) and moisture content (90.35%), which favors the occurrence of chemical and enzymatic reactions, leading to product deterioration. Spray drying is a well established technology used to dry fruit pulps, resulting on a longer shelf life and wider industrial application (Tontul and Topuz, 2017). The technological process results in powders with higher bioactive compounds concentration, better stability and lower water activity, which are easier to transport and store (Pereira et al., 2019).

The *in vivo* effect of phenolic compounds depends on several factors, such as the organism ability to absorb them and changes on chemical molecule structure along the gastrointestinal tract. In this case, a high concentration of phenolic compounds on the product is not a guarantee to a high bioavailability. It is estimated that 90-95% of the polyphenols resists to digestion and accumulate in the lumen of the large intestine, where the colonic

microbiota, through fermentation process, produce low molecular weight metabolites that may be the real responsible for the health effects (Guergoletto et al., 2016; Tuohy et al., 2012). Thus, the non-digestible food ingredients, fermented by the intestinal microbiota, has an *in loco* influence affecting the composition and / or activity of the gastrointestinal microbiota, conferring health benefits to the host (Gibson et al., 2004; Maccaferri et al., 2012).

Currently, it is estimated that 500-1,000 different microbial species inhabit the gastrointestinal tract, reaching the highest concentrations in the colon (up to 10^{12} cells per gram of feces). However, only a few bacterial species (e.g. *Escherichia coli*, *Bifidobacterium* sp., *Lactobacillus* sp., *Bacteroides* sp., *Eubacterium* sp.) have influence in the metabolism of phenolics (Cardona et al., 2013), being these generally selected to microbial modulation studies. *Bifidobacterium* sp. and *Lactobacillus* sp. have health-promoting properties, such as protection of the host against pathogens by competitive exclusion and provision of nutrients through the breakdown of non-digestible dietary carbohydrates (Gibson and Roberfroid, 1995; O'Callaghan and Sinderen, 2016), whilst others have been associated with deleterious effects, such as inflammatory bowel diseases and irritable bowel syndrome (Rastall et al., 2005) such as certain members of the genera *Clostridium* (Guergoletto et al., 2016) and *Escherichia coli*.

This study aimed to investigate the phenolic compounds bioaccessibility of a juçara pulp powder submitted to *in vitro* digestion and colonic fermentation simulation and to determine the changes promoted by the phenolics compounds in the human microbiota.

2. Material and methods

2.1 Material

Juçara pulp was purchased from farmer located in the city of Rio Pomba (Minas Gerais State, Brazil). The fruits were harvested between the coordinates 21° 09' 19.2" and 21° 09' 09.3" S and 43° 09' 12.5" and 43° 08' 58.8" W, on November 2016.

2.2 Methods

2.2.1 Production of juçara powder

Juçara powder was produced by spray drying, as described by Pereira et al., 2019. The process was performed in a laboratory scale spray dryer LabPlant^{TM} SD-06 (Huddersfield, England), with a 2.0 mm diameter nozzle and main spray chamber of 500 x 215 mm. Pulp (4 kg) at 20°C was fed into the main chamber through a peristaltic pump, with air pressure compressor of 0.25 MPa. The feed flow rate used was 9 g/min, inlet and outlet air temperature were 160 ± 2°C and 86 ± 2°C, respectively. In order to maintain homogeneity, the pulp was kept under magnetic agitation throughout the process.

2.2.2 *In vitro* simulated gastrointestinal digestion

The *in vitro* simulated gastrointestinal digestion was performed using the method described by Gião et al. (2012), for juçara pulp and juçara powder. The procedures for *in vitro* gastrointestinal digestion are described in Figure 1.

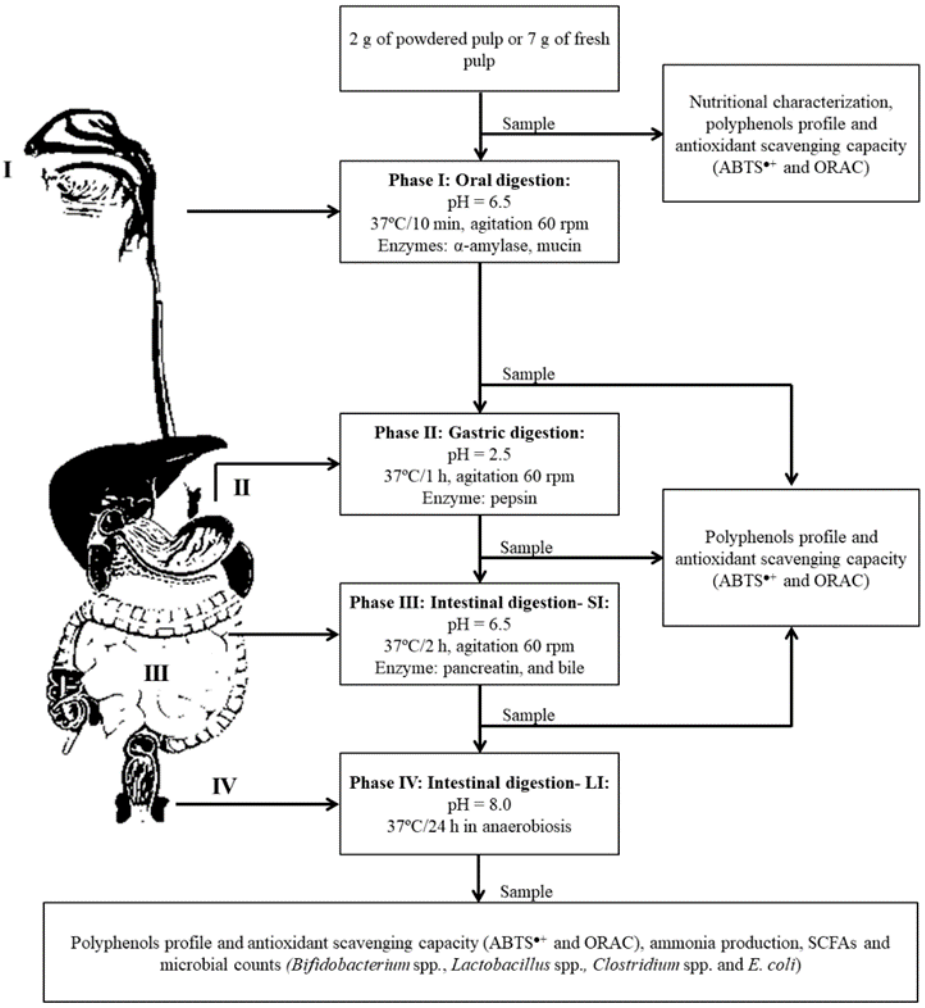

Figure 1 - Graphical representation of *in vitro* digestion performed for fresh and powdered juçara pulp (ABTS - 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulphonate), ORAC - Oxygen Radical Absorbance Capacity, SI - Small Intestine, LI - Large Intestine and SCFAs - Short-chain fatty acids).

Fresh and powdered juçara pulp were exposed to a simulated digestion procedure. As the powdered pulp is considered to be more concentrated, a smaller amount (2g) was exposed to digestion compared to the fresh pulp (7g). Each sample was first homogenized in a saliva solution with α-amylase (0.029g/100 mL) and mucin (0.01g/100 mL) (Sigma®, USA). After 10 minutes the content was mixtured into a gastric solution (pH 2.5) with pepsin (2g/50mL) (Sigma®, USA), and left under agitation at 60 rpm for 1 hour at 37°C. After 1 hour bile

solution (2g/ 50 mL) and pancreatin lipase, 0.25g/ 25mL and 0.125g /25 mL, respectively (Sigma®, USA) were added. Then, the volume had the pH adjusted to 6.5 with NaOH 1M and stayed under agitation 60 rpm for 2 hours at 37°C (Gião et al., 2012). On each phase (oral, gastric and enteric) a sample of 5 mL was collected for chemical characterization. After intestinal digestion in the small intestine (phase III, Figure 1), 5 mL of the digested samples were placed in sterile Schott bottles (reactors) containing 5 mL of human feces diluted 1:10 in phosphate buffer (pH 6.5) and 45 mL of basal nutrient broth (Guergoletto et al., 2016) (phase IV, Figure 1). Immediately after inoculation, aliquots of the reactors were separated for analysis in time 0 hour (T0h).

2.2.3 Microbiological analysis

The reactors (T0h) were conditioned in microaerophilic jar using microaerophilic atmosphere generator (Microaerobac®, Brazil) and incubated at 37°C for 24 h (T24h). Samples from time 0h and 24h were homogenized and serially diluted in peptone water 0.1% and *pour plated* in culture medium MRS (de Man, Rogosa, and Sharpe, TM Media®, India) acidified with Glacial Acetic acid to pH 5.4 for *Lactobacillus* spp. determination. For *Clostridium* spp. count, the RCA (Reinforced Clostridial Agar, BD Difco™, Europe) was utilized. RCA supplemented with selective and differential compounds according to Muñoa and Pares (1988) was applied for *Bifidobacterium* spp. enumeration. All culture media were incubated at 37°C under anaerobic conditions for 5 days. Selective count of *Escherichia coli* was performed using Petrifilm® EC count plates (3M Company, St. Paul, MN, USA), incubated at 45°C / 2 days on aerobic conditions. Fecal samples were collected from a single individual, female, 28 years old, in good health who had not ingested antibiotics or probiotics for at least 6 months before the study (Aas et al., 2003). Samples were collected, on site, on the day of the experiment, and placed on sterile fecal collection containers (Guergoletto et al.,

2016; Tauxe et al., 2015). The simulated digestion was performed 3 times in order to observe reproducibility.

A reactor containing only the feces with the basal nutrient broth was used as control. Thus, the control does not have the presence of fresh pulp and juçara powder.

2.2.4 Ion ammonia determination

For the measurement of ammonia production, samples from T0h and T24h were evaluated using commercial kit HANNA® Checker®HC model HI715 according to the manufacturer's manual.

2.2.5 Short-chain fatty acids determination

Short-chain fatty acids production (SCFAs) was quantified by high resolution gas chromatography on an Agilent 7890 equipment, using a FFAP (Free Fatty Acid Phase) fused silica capillary column (25m x 0.2mm x 0.30μm) with temperature programming from 40 to 230°C, injector temperature and detector of 250 and 280°C, respectively (Adorno et al., 2014). The injector was operated in split-flow mode at 1:50 ratio and 1 microliter injection. For quantification, calibration curves were established using area and concentration ratio for crotonic, acetic, propionic, butyric, isobutyric, valeric, isovaleric, hexanoic, heptanoic and octanoic acids. Crotonic acid was used as an internal standard.

2.2.6 Phenolic content assays

Phenolic compounds characterization was evaluated in fresh and powdered pulp and in each sample of the simulated digestion (oral, gastric, enteric, colonic T0h and colonic T24h samples).

2.2.6.1 Phenolic compounds profile

Extracts from fresh and powdered juçara pulp were obtained as described by Gouvêa et al. (2012) and Pérez-Jimenéz et al. (2008) for anthocyanins and total phenolic compounds analysis, respectively. The extracts were analyzed by high performance liquid

chromatography (HPLC) using a Waters® Alliance model 2695 and e2695 chromatograph (Milford, USA) (Gouvêa et al., 2012; Pérez-Jimenéz et al., 2008). The results obtained were compared with control compounds curve spectrum (cyanidin-3-glucoside, cyanidin-3-rutinoside, protocatechuic acid, 4-hydroxybenzoic acid, catechin, vanillic acid, epicatechin, ferulic acid, rutin and luteolin).

2.2.7 Antioxidant assays

Antioxidant capacity was determined in fresh and powdered pulp and in each sample of the simulated digestion (oral, gastric, enteric, colonic T0h and colonic T24h samples). The antioxidant activity was determined using ORAC (Zuleta et al., 2009) and ABTS radical cation scavenging activity (Re et al., 1999). For both methods, results were expressed as μmol of Trolox equivalents per g of sample. All analysis were performed in triplicate.

To remove the influence of water on the results, bioactive compounds and antioxidant capacity were calculated in a dry basis.

2.2.8 Statistical analysis

Results were obtained in triplicate and statistically treated by analysis of variance, using the software SISVAR 5.6. Mean analysis was performed using Tukey procedure at $p \leq 0.05$.

3. Results and discussion

3.1 Fresh and powdered juçara pulp bioactive characterization

The yield of the drying process was 66%, which can be considered efficient. In general, spray drying with at least 50% recovery of the powder is a successful process (Lacerda et al., 2016; Lee et al., 2018). Chemical characteristics of fresh and powdered juçara pulp (*Euterpe edulis* Martius) are shown in Table 1. Fresh juçara pulp presented high antioxidant capacity, anthocyanins and phenolic compounds content. Juçara powder presented

a higher antioxidant capacity, anthocyanins and phenolic compounds content), indicating that the drying process concentrated the bioactive compounds from the pulp. The increase of phenolic compounds could be explained by their presence, in most part, in the cell vacuoles (Pereira et al., 2019). During drying process, a cellular disruption may have promoted a greater release. According to Perdana et al. (2013) high pressure and rapid dehydration of spray drying are responsible for the alteration on cell membrane characteristic causing an irreversible collapse. It is possible to suggest that these structural alterations were responsible for the antioxidant capacity increase.

Table 1. Chemical characterization of fresh and powdered pulp from *Euterpe edulis* Martius fruit.

Analyzed item[1]	Fresh pulp	Powdered pulp
Moisture content (%)[2]	90.35±0.08[a]	1.54±0.16[b]
ABTS antioxidant capacity (μmol of Trolox/g) [3]	504.97±6.83[b]	858.59±44.28[a]
ORAC antioxidant capacity (μmol of Trolox/g) [3]	2,400.10±107.78[b]	4,155.39±92.50[a]
Cyanidin-3-glucoside (mg/100g) [3]	901.86±13.11[b]	1,244.47±38.54[a]
Cyanidin-3-rutinoside (mg/100g) [3]	1,489.59±21.68[b]	2,334.04±72.42[a]
Total anthocyanins by HPLC	**2,391.46±34,79[b]**	**3,578.51±110.96[a]**
3, 4-dihydroxybenzoic acid (mg/100g) [3]	27.65±0.01[b]	35.95±0.07[a]
4-Hydroxybenzoic acid (mg/100g) [3]	18.2±0.28[b]	30.5±0.71[a]
Catechin (mg/100g) [3]	0.67±0.00[b]	1.8±0.00[a]
Vanillic acid (mg/100g) [3]	30.02±0.03[a]	28.38±0.03[b]
Epicatechin (mg/100g) [3]	8.25±0.35[a]	8.00±0.00[a]
Ferulic acid (mg/100g) [3]	21.38±0.53[a]	20.25±0.35[b]
Rutin (mg/100g) [3]	8.14±0.20[a]	1.5±0.00[b]
Luteolin (mg/100g) [3]	7.1±0.28[b]	39.5±0.71[a]
Total phenolic compounds by HPLC[4]	**2,522.86±34.73[b]**	**3,744.39±111.41[a]**

[1]Mean of three replicates. Values expressed as mean and standard deviation. Means followed by the same letter do not differ statistically at 5% probability by the Tukey test. [2]Result expressed on wet basis. [3]Result expressed on dry basis. [4]Sum of the phenolic compounds identified and quantified in juçara.

3.2 Changes in bioactive composition during digestion and fermentation by colonic bacteria

Table 2 shows the changes in bioactive composition during digestion and fermentation by colonic bacteria. Anthocyanins concentration was higher in gastric digestion (phase II)

when compared to intestinal digestion in the small intestine (phase III) (Table 2). These changes in concentration may be due to lower stability of anthocyanins at high pH found on small intestine (Guergoletto et al., 2016; McDougall, et al., 2007). The acid pH on gastric phase (pH 2.0) promotes a predominance of anthocyanins in the *flavylium cation* form, which shows intensely reddish color, although on a higher pH found in the small intestine, the balance is displaced for chalcones formation. Guergoletto et al. (2016) when analyzing lyophilized juçara pulp also observed a high content of phenolic compounds in the gastric phase when compared to the intestinal phase.

Phenolic compounds content was reduced throughout *in vitro* gastrointestinal digestion, 26.4% and 21.0% of phenolic compounds content from fresh and powder pulp, respectively, remained at the end of digestion in the small intestine (phase III phase, Table 2), being an important target for gut microbiota and *in loco* colon antioxidant activity. Greater retention was observed by Guergoletto et al. (2016), when 46% of total phenolic contents remained after juçara digestion, being an important source of antioxidant activity that could reach the colon. When compared to total phenolic compounds, there was greater reduction in anthocyanin content in both juçara pulp and powder (Table 2).

Possibly this reduction in the content of phenolic compounds is due to metabolization with consequent changes in phenolic compounds during digestion with the release of lower molecular weight compounds and possibly higher antioxidant capacity. Like this, changes in the phenolic compounds and anthocyanins content were possibly responsible for the alteration on the antioxidant capacity, resulting in an increase on antioxidant capacity in the large intestine after colonic fermentation. In juçara pulp an increase of 158.70% (ABTS) and 76.78% (ORAC) on the antioxidant capacity and in juçara powder an increase of 22.7% (ABTS) and 6.15% (ORAC) on the antioxidant capacity after 24 h fermentation (Table 2). The highest increase in antioxidant capacity observed for fresh pulp may be related to the

greater release of phenolic compounds through digestion phases, since fresh pulp did not undergo previous structural alterations in the drying process.

Table 2. Content of total phenolic compounds, total anthocyanins and antioxidant capacity (ABTS and ORAC) using spectrophotometric methods and HPLC, of fresh pulp and powdered juçara submitted to the *in vitro* human digestion simulation

Analyzes[1] (mg/100g dry base)	I phase: Oral digestion	II phase: Gastric digestion	III phase: Intestinal digestion - SI	IV phase: Intestinal digestion - LI
Fresh pulp				
ABTS µmol of Trolox/g	414.08±4.49	786.75±3.18	1,322.98±6.08	1,306.38±0.09
ORAC µmol of Trolox/g	2,643.37±132.29	7,275.83±70.88	6,966.54±8.67	4,242.85±0.99
Cyanidin-3-glucoside	687.73±11.72	551.37±8.57	176.39±38.38	N.D
Cyanidin-3-rutinoside	1,437.60±5.86	1,042.15±0.00	440.96±47.97	87.62±0.00
3, 4-dihydroxybenzoic acid	26.68±0.00	15.15±0.86	20.35±9.6	100.77±6.20
4-Hydroxybenzoic acid	17.77±0.53	6.79±0.17	7.33±3.45	28.92±1.24
Catechin	N.D	4.67±2.31	N.D	N.D
Vanillic acid	29.41±0.23	24.96±0.17	8.55±4.03	23.62±11.21
Epicatechin	N.D	11.45±5.40	N.D	N.D
Ferulic acid	21.71±0.12	8.79±0.09	9.77±0.19	N.D
Rutin	8.08±3.81	6.36±2.99	1,95±2,03	N.D
Luteolin	7.08±0.29	7.816±3.68	1.17±1.22	13.12±6.23
Total phenolic compounds by HPLC[2]	**2,236.06±3.22**	**1,679.50±0.60**	**666.47±66.21**	**254.05±9.94**
Powdered juçara				
ABTS µmol of Trolox/g	717.70±6.49	1,998.50±15,40	1,125.64±1.73	1,048.90±0.08b
ORAC µmol of Trolox/g	3,361.67±235.58	19,331.71±30.85	16,301.78±36.87	4,411,04±0.17
Cyanidin-3-glucoside	991.54±1.87	648.27±1.87	223.54±4.45	57.08±0.00
Cyanidin-3-rutinoside	1,884.06±0.00	1,233.16±3.73	522.63±4.45	342.46±0.00
3, 4-dihydroxybenzoic acid	29.77±1.02	8.27±0.04	7.04±9.23	115.15±1.01
4-Hydroxybenzoic acid	25.83±0.81	9.28±0.02	7.72±3.64	29.25±0.61
Catechin	N.D	N.D	N.D	N.D
Vanillic acid	22.91±0.29	19.46±0.00	13.96±6.58	37.89±17.86
Epicatechin	N.D	10.38±4.89	N.D	N.D
Ferulic acid	21.70±0.62	8.29±0.019	10.86±0.09	5.35±2.52
Rutin	1.29±0.61	1.39±0.65	1.18±0.56	N.D
Luteolin	37.63±0.37	6.83±3.22	0.28±0.27	4.28±2.02
Total phenolic compounds by HPLC[2]	**3,014.73±2.75**	**1,945.30±9.40**	**787.21±19.25**	**591.46±18.77**

[1]Mean of three replicates. Values expressed as mean and standard deviation. Result expressed on dry basis. [2]Sum of the phenolic compounds identified and quantified in juçara using HPLC method. SI - small intestine, LI - large intestine. N.D - not detected

According to Guergoletto et al. (2016) compounds which are not degraded in human digestion can influence the growth of beneficial bacteria. In this case, the antioxidant capacity

can be related to the digestion effect that possibly altered the composition and / or structure of the phenolic compounds due to reactions in the gastrointestinal environment, and / or the metabolism of the colonic microbiota. Attri et al. (2017), when analyzing fruit juice submitted to the *in vitro* digestion method (until the small intestine phase), also observed a significant increase ($p < 0.05$) in the phenolic compounds content and in the antioxidant capacity. Haas et al. (2019) observed an increase in the polyphenols content and antioxidant capacity and a significant reduction ($p < 0.05$) in anthocyanin content when analyzed grape juice using *in vitro* gastrointestinal simulation until the intestinal phase. In contrast to the present study, Schulz et al. (2017) observed loss of antioxidant capacity (55-78%) after *in vitro* gastrointestinal digestion of juçara fruits. These authors attributed the reduction of antioxidant capacity to the decrease in the content of phenolic compounds and / or transformation into different structural forms with other chemical properties. These alterations could be due to the pH changes during *in vitro* digestion and interference of antioxidant phenolics with other constituents of the sample matrix.

It is worth mentioning that the reduction in the content of phenolic compounds especially in the colonic fermentation phase (phase IV), may be related to a series of chemical transformations performed by the intestinal microbiota fermentation. As consequence of their low bioacessibility, most of phenolic compounds reach the large intestine where, mediated by the action of local microbiota, a series of related metabolites are produced and accumulated, such as SCFAs and smaller phenolic compounds (Mosele et al., 2015). The content of phenolic compounds can indicate that gut microbiota manages to use phenolic compounds and dietary fibers as energy source, producing health beneficial metabolites.

The antioxidant capacity can be influenced by many factors, including the quantification method, free radical generator and the radical used in the measurement. Thus, according to Zulueta et al. (2009) the use of different analytical methods to determine the

antioxidant capacity is a key factor to help in the interpretation of the result. ABTS and ORAC are methods that detect the antioxidant capacity of compounds of hydrophilic and lipophilic nature (Prior et al., 2005), being widely used for fruits and vegetables. Despite this similarity, ABTS is a method based on electron transfer and ORAC is a method based on hydrogen atom transfer (Apak et al., 2007; Zulueta et al., 2009). The differences in the antioxidant capacities found with the ABTS and ORAC methods (Table 2) were due to the different nature of the two assays. Juçara presented higher antioxidant capacity when measured with ORAC than ABTS method (504.97 and 858.59 μmol of Trolox/g of pulp and powder respectively), which might be because of the presence of OH groups in the structure of anthocyanins. Besides that, the ORAC method is the only one so far that combines the total inhibition time and the percentage of the free radical damage by the antioxidant into a single quantity, ensuring that, by the end of the process, all the antioxidants compounds in the sample have reacted with the radicals generated, not underestimating the result as in the ABTS method (Zulueta et al., 2009).

The phenolic profile in both juçara pulp and powder and during the digestion process is shown in Table 2. It was possible to find anthocyanins, phenolic acids and flavonoids. The anthocyanins detected in juçara pulp were cyanidin-3-rutinoside and cyanidin-3-glucoside, respectively. As for phenolic acids, different concentrations were obtained for fresh pulp and juçara powder. Fresh pulp presented a higher concentration of phenolic acids, such as vanillic acid, protocatechuic acid, ferulic acid and 4-hydroxybenzoic acid. In the powdered pulp was possible to find flavonoid, luteolin and phenolic acids such as, protocatechuic acid, 4-hydroxybenzoic acid and vanillic acid as main compounds. This difference may be related to the structural changes occurred in the powdered pulp due to its obtainment processing. At oral and gastric phases, small amounts of anthocyanins were modified. Significant increase of the flavonoid epicatechin was observed in the gastric phase possibly due to a more favorable

stabilization pH (2.0), where the *flavilium cation* structure predominates. After the gastric phase, anthocyanins had a significant decrease when compared to the original sample and the flavonoids catechin and epicatechin were degraded completely.

After colonic fermentation cyanidin-3-glucoside, ferulic acid and rutin were not detected on fresh pulp and for the powdered pulp only rutin was not detected. In contrast, protocatechuic acid, 4-hydroxybenzoic acid, vanillic acid and luteolin slightly increased after colonic fermentation in both pulps, which may explain the increase in antioxidant capacity (Table 2). Catechin and epicatechin, were not detected in phase III digestion, in this case showing no possible influence on microbial growth in the large intestine (phase IV), all other components detected in the original sample were accessible in the large intestine, being available to be metabolized by the colonic microbiota (Table 2).

Regular flavanol-rich food consumption is likely to result in a general increase in the antioxidant capacity in the large intestine (Tzounis et al., 2008). The increase of flavonoids may indicate the metabolism of polyphenols by the colon bacteria, leading to the production of other smaller phenolic compounds which could be considered as metabolites with health benefits. Tzounis et al. (2008) also observed variations on the flavonoids content due to specific metabolic transformations, such as the bacterial conversion of catechin to epicatechin. These same authors observed that flavanols can be metabolized by the human colonic bacteria even in the presence of more favorable carbon sources, such as sucrose; but when flavanols are in lower concentrations protein and amino acids can also be used (Tuohy et al., 2012) increasing ammonia production (Table 3). Thus, phenolic compounds that escape from human digestion can be used as substrates for beneficial bacteria growth (Guergoletto et al., 2016), inhibiting pathogenic bacteria colonization.

3.3 Changes in bacterial populations after *in vitro* colonic fermentation

The simulation of the microbial fermentation process that occurs in the large intestine aims to determine the influence of the undigested dietary compounds in the modulation of the intestinal microbiota. Table 3 shows the results of microbial counts before and after 24h colonic fermentation of fresh and powder juçara pulp after being submitted to an *in vitro* oral, gastric and enteric digestion.

Table 3. Microbial population counts before and after colonic fermentation of fresh and powder juçara pulp

Fresh pulp	Microbial count (CFU Log^{10})[1]			
	Bifidobacterium	*Lactobacillus*	*Clostridium*	*E. coli*
Neg. Control (T0h)	4.49±0.02[b]	4.18±0.26[b]	6.76±0.09[a]	6.64±0.13[b]
Neg. Control (T24h)	4.69±0.12[a]	7.24±0.23[a]	7.53±0.30[a]	7.94±0.02[a]
Juçara (T0h)	4.52±0.11[b]	4.10±0.21[b]	6.38±0.74[b]	6.43±0.12[b]
Juçara (T24h)	5.85±0.06[a]	5.17±0.12[a]	7.19±0.23[a]	6.99±0.01[a]
Powdered pulp	*Bifidobacterium*	*Lactobacillus*	*Clostridium*	*E. coli*
Neg. Control (T0h)	4.93± 0.00[a]	4.32± 0.03[b]	6.78± 0.03[a]	6.86± 0.05[b]
Neg. Control (T24h)	4.47± 0.10[b]	6.08± 0.02[a]	6.71± 0.02[a]	7.67± 0.03[a]
Juçara (T0h)	4.67± 0.20[a]	4.26± 0.05[b]	6.55± 0.06[b]	6.57±0.04[b]
Juçara (T24h)	5.09± 0.37[a]	5.28± 0.17[a]	6.71± 0.04[a]	6.85± 0.05[a]

[1]Mean of three digestion processes. Values expressed as mean and standard deviation. Means followed by the same letter do not differ statistically at 5% probability by the Tukey test. CFU: Colony Formation Unit. Neg. Control - negative control (reactor without the addition of fresh or powdered juçara pulp).

The use of fecal inoculants is more significant for *in vitro* methods compared to the use of pure cultures as it ensures that a representative variety of bacterial species are exposed to the sample material. In this case, the use of feces probably provides an accurate representation of the events in the distal colon (Gibson et al., 2004). The presence of the tested bacterial groups before and after fermentation (Table 3) indicates that the anaerobic

microbiota was well preserved in our system, even with the manipulation promoted, and could ferment the available substrates.

Fresh and powdered juçara pulp increased *Bifidobacterium* counts and reduced the increase in *Escherichia coli* counts in approximately 1 Log cycle, when compared to negative control after fermentation for 24h (Table 3). These results indicate that juçara pulp has the potential to influence on bacteria growth. Guergoletto et al. (2016) also observed significant changes ($P < 0.001$) in bifidobacteria numbers in response to juçara after 24 h of fermentation. No positive effect of juçara pulp was observed for other microbial groups, such as *Lactobacillus* and *Clostridium*. The lower growth of *Lactobacillus* group microorganisms may be related to the antibacterial effect of polyphenols (Duda-Chodak 2012; Gwiazdowska et al., 2015).

The reduction of the increase of *E. coli* count cell, compared to the negative control, can indicate the preference of beneficial bacteria in fermenting juçara pulp, reducing *E. coli* by competition. The antimicrobial effect on certain bacterial groups during juçara fermentation, can also be related to the presence of phenolic compounds in the pulp. Rodríguez-Pérez et al. (2016) observed that phenolic compounds extracted from cranberry (*Vaccinium macrocarpon*) could inhibit the growth of *E. coli* ($p < 0.05$). These results suggest that phenolic compounds such as anthocyanins and their metabolites may induce a positive modulation on the intestinal bacterial population.

The concentration of phenolic compounds after the intestinal phase, was greater than that was reported by Guergoletto et al. (2016) by HPLC (2.79%). The similarity between the results of the pulp and powder may be related to the amount of anthocyanins that are accessible in the large intestine (Table 2). This result suggests that the juçara pulp processing did not reduce its bioaccessibility, thus small amounts of powdered pulp can be digested,

obtaining bioactive benefits similar to those of the juçara pulp digestion. In this case, powder form facilitates the use, since small doses are sufficient to promote a health benefit.

3.4 Ammonia and short-chain fatty acid production

In order to evaluate the metabolism of fibers from the pulps digested by the human fecal microbiota, samples collected at 0 h and 24 h were analyzed for the production of ammonia and SCFAs (Table 4).

Table 4. Concentrations of major short-chain fatty acid before (T0h) and after (T24h) fermentation of fresh and powdered juçara

Fresh pulp[1]	Ammonia (mg/L) content		Short-chain fatty acid (mmol/L) content			
	Ammonia	Ammonia variation (%)	Acetic acid	Acetic acid Variation (%)	Propionic acid	Propionic acid variation (%)
Neg. Control (T0h)	17.04±0.07^{b}	88.92	1.37±0.34^{b}	213.80	ND	-
Neg. Control (T24h)	32.28±0.13^{a}		4.30±0.19^{a}		NQ	
Juçara (T0h)	44.90±8.92^{a}	-11.92	0.74±0.20^{b}	738.20	NQ	-
Juçara (T24h)	39.55±2.93^{a}		6.17±0.25^{a}		0.09±0.01	
Powdered pulp[1]	Ammonia	Ammonia variation (%)	Acetic	Acetic Variation (%)	Propionic	Propionic variation (%)
Neg. Control (T0h)	27.66±0.53^{b}	88.40	1.32±0.24^{b}	370.54	ND	-
Neg. Control (T24h)	52.11±1.07^{a}		6.20±0.69^{a}		0.19±0.04	
Juçara (T0h)	177.47±11.00^{a}	-38.56	0.99±0.13^{b}	774.03	ND/NQ	-
Juçara (T24h)	109.03±2.50^{b}		8.67±0.84^{a}		0.94±0.08	

[1]Mean of three digestion processes. Values expressed as mean and standard deviation. Means followed by the same letter do not differ statistically at 5% probability by the Tukey test. ND: not detected, NQ: not quantified. Neg. Control - negative control (reactor without the addition of fresh or powdered juçara pulp).

Supplementation with fresh and powdered juçara pulp reduced the production of ammonia in 11.92% and 38.56%, respectively. The negative control (reactor without the addition of fresh and powdered juçara pulp) increased on average 88% the ammonia

concentration, which corroborates with the results found for bacterial growth in reactors containing juçara (Table 3). This suggests that the deamination and decarboxylation of dietary and/or endogenous protein by microorganisms in the gastrointestinal tract were altered by supplementation with juçara pulp. Similar result was observed by Chen et al. (2017) and Chen et al. (2018) when analyzing the effect of dietary supplementation with α-ketoglutarate on intestinal microbiota and ammonia levels after fermentation. These authors also observed increase on *Bifidobacterium* and reduction of *Escherichia coli* cell count and ammonia content, as well as Hansen et al. (2007) that by adding 15% of inulin in diet reduced the ammonia concentration on 33%.

According to Piva et al. (2002) energy is the limiting factor for microbial growth in the large intestine. When no alternative nutrient source was added, there was an increase on ammonia content in the negative control, related to the process of the deamination and the decarboxylation of medium proteins by colonic bacteria. When there is a high protein content available in the intestine, deamination reactions produce more ammonia (Apajalahti and Vienola, 2016), which explains the initial ammonia values.

It is due to this fact that there may be increased proteolysis and the consequent release of toxic substances such as ammonia and amines (Apajalahti and Vienola, 2016). Ammonia is a toxic catabolite that interferes with the metabolism and integrity of intestinal mucosal cells (Apajalahti and Vienola, 2016; Topping and Visek, 1977), thus, its reduction is beneficial to the individual health. In this sense the pulp powder brought more benefits than fresh juçara pulp, since the reduction of the ammonia production was even greater (38.56%) when compared to the fresh pulp (11.92%) (Table 3). This result may be related to the easier access of microorganisms to the nutrients of the powdered pulp due to the removal of water from the food matrix.

The intestine of humans and animals is an anaerobic habitat with high density of bacteria and fermentation acids (Russell and Diez-Gonzalez, 1997). The content of bound and free phenolic compounds that reaches the large intestine, are metabolized by beneficial bacteria as a source of energy, in this way supplementation with juçara may lead to lower production of ammonia and amines and a reduction of pathogenic bacteria populations, due to the competition for sites of adhesion and modification of the intestinal conditions. A higher concentration of beneficial bacteria increases acid production, such SCFA, leading to an intestinal pH reduction, which is important to inhibit potentially pathogenic bacteria, such as *E. coli*. In addition, the metabolism of phenolic compounds originates a series of low molecular weight metabolites, which may be the real responsible for the beneficial effects on the host health (Guergoletto et al., 2016; Tuohy et al., 2012).

Fermentation of juçara resulted in a higher production of SCFAs compared to the negative control. After 24 h incubation, the fermentation of juçara increased the production of acetic acid on 738.20% for juçara pulp and 774.03% for powdered juçara, against an increase of 213.80% in juçara pulp control and 370.54% in juçara powder control. Propionic acid was only detected on fresh pulp after fermentation (0.09 mmol propionic acid /L), and on pulp powder its concentration (0.94 mmol propionic acid /L) after the fermentation was almost five times higher than the control (0.19 mmol propionic acid /L). In the present study, no butyric acid was detected in the analyzed samples (Table 4).

Guergoletto et al. (2016) observed that fermentation of juçara pulp resulted in a higher ($P < 0.05$) production of acetate and propionate, and lower amounts of butyrate, compared to the negative control after 24 h of fermentation. Gullon et al. (2015) also observed a significant increase ($P < 0.05$) in the acetic, propionic and butyric acids after *in vitro* 24 h fermentation of the pomegranate peel flour by colonic microbiota.

According to Inada et al. (2015), dietary fibre make up more than 64% of carbohydrates in juçara fruits and its fractions. Dietary carbohydrates, especially resistant starches and dietary fiber, are substrates for fermentation which results on the production of SCFAs, primarily acetate, propionate, and finally butyrate. These SCFAs may reduce the risk of developing gastrointestinal disorders, cancer, and cardiovascular disease (Wong et al., 2006; Gill et al., 2018). They may also regulate immune responses and the composition of the intestinal microbiota (Gill et al., 2018). Therefore, an increase in SCFAs production, as observed in the present study, may result in a protective effect to the host.

4. Conclusions

This study provides relevant information on the behavior of phenolic compounds throughout the simulation of digestion and colonic fermentation. A reduction on phenolic compounds content and an increase on antioxidant capacity were observed. Changes in the phenolic compounds content were possibly responsible for the alteration on the antioxidant capacity, resulting in an increase on antioxidant capacity in the large intestine after colonic fermentation. The increase in the growth of *Bifidobacterium* and short-chain fatty acids production, and the reduction in the increase of *Escherichia coli* count and ammonia concentration, shows a beneficial modulation tendency. Powdered and fresh juçara obtained similar results, both in bioactive behavior and in microbial modulation. Thus, obtained juçara powder is an alternative to add value to this fruit chain, in addition to characterize it as a potentially functional food.

Conflict of Interest

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

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CAPÍTULO 4

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Formulation of a mixed juice: suitability of whole apple juice as a vehicle for applying juçara pulp powder

Formulation of a mixed juice: suitability of whole apple juice as a vehicle for applying juçara pulp powder

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Abstract

Juçara pulp powder was added to a commercial apple juice, to evaluate the suitability of whole apple juice as a vehicle for the application of powdered juçara pulp. The bioaccessibility of phenolic compounds and the colonic fermentation of the juice were evaluated. Colonic microbial fermentation of apple juice with juçara resulted in an increase on short-chain fatty acids production (1.69 mmol/L) and *Bifidobacterium* cell count (0.67 cycles Log), and a reduction on the ammonia production (28.22%) was observed. After digestion, 31.31% of anthocyanins (5.06 mg cyanidin-3-glucoside and 2.95 mg cyanidin-3-rutinoside /200g of apple juice with juçara) reached the colon and an increase of 52.94% on the antioxidant capacity (21.62 and 1,786.64 μmol of Trolox/mL ABTS and ORAC, respectively) was observed after 24h fermentation representing a potential *in loco* antioxidant activity. Apple juice supplemented with juçara powder showed good acceptance on a sensory study. Most consumers (58.75%) reported that they would buy apple juice added with juçara powder if it was for sale, as it was tasteful and presented health benefits. Thus, apple juice is a viable matrix for the addition of juçara powder, allowing the maintenance of the bioacessibility of phenolic compounds and antioxidant capacity. Moreover, juçara powder showed potential to be used as a natural dye and potentially functional ingredient.

Keywords: *Euterpe edulis*, apple juice, bioacessibility, anthocyanins, global acceptability.

1. Introduction

Juçara (*Euterpe edulis*) is a native palm from the Brazilian Atlantic Forest, its fruit is a black-violet color berry rich in anthocyanins (425.76 to 1,353 mg/100g dry base), with cyanidin-3-rutinoside and cyanidin-3-glucoside as main compounds (Guergoletto, Costabile, Flores, Garcia & Gibson, 2016; Schulz et al., 2015). Anthocyanin based pigments can be used as a natural food colorant alternatively to synthetic food dyes. Besides the technological approach, anthocyanins have been associated to some health protective effects (Khoo, Azlan, Tang & Lim, 2017).

In a previous work, our group developed a juçara pulp powder rich in phenolic compounds with high antioxidant capacity, in order to obtain a food ingredient with health benefits (Pereira, Beres, Gomes, Tonon & Cabral, 2019). Fruit powder represents an alternative to enrich the functionality of new beverage mainly on the development of juice blends (Gironés-Vilaplana et al., 2012).

Apple juice represents a proper base to develop blends because of its natural sweet taste. However, apple juice can be considered not attractive due to its color, and in addition, it has a low concentration of bioactive compounds. Approximately, 20% to 25% of the Brazilian apple harvest is destined for the juice production, a rising market due to a higher concern for healthy foods (Brazilian Apple Yearbook, 2019). Pleasant taste and functional proprieties are crucial for the development of foods and beverages with a functional appeal.

This work aimed to evaluate adequacy of apple juice as a vehicle for application of juçara pulp powder, as well as to investigate the sensory acceptance and purchase intention. The bioaccessibility of the formulated beverage was determined by *in vitro* digestion, followed by colonic fermentation. In addition, a possible microbiota modulation was also investigated.

2. Material and methods

2.1 *Material*

Juçara fruit (*E. edulis*) was harvested in Rio Pomba (Minas Gerais State, Brazil), between the coordinates 21° 09' 19.2" and 21° 09' 09.3" S and 43° 09' 12.5" and 43° 08' 58.8" W, on November 2016. The pulp was purchased from a rural producer and stored at -18°C. Commercial integral apple juice (Casal Piccoli®) was purchased in a local market of Rio de Janeiro.

2.2 *Methods*

2.2.1 Spray drying

The process was performed in a laboratory scale spray dryer LabPlant™ SD-06 (Huddersfield, England), with a 2.0 mm diameter nozzle and main spray chamber of 500 x 215 mm. Pulp (15 kg) with 6.53% solids (at 20°C), previously filtered using a 1.8 mm pore diameter sieve, was fed into the main chamber through a peristaltic pump, with air pressure compressor of 0.25 MPa. The feed flow rate used was 9 g/min, inlet and outlet air temperature were 160 ± 2°C and 86 ± 2°C, respectively (Pereira et al., 2019). In order to maintain homogeneity, the pulp was kept under magnetic agitation throughout the process.

2.2.2 Beverage formulation

Three formulations of mixed juice were evaluated, using different concentrations of apple juice and juçara powder (Table 1). The formulations were based on the final anthocyanin content in the juice produced. The addition of 4% of juçara pulp powder is equivalent to a final juice with the same anthocyanin content found on *in natura* juçara pulp.

Table 1 Formulations of apple and juçara mixed juices.

Formulation	Whole apple juice (%)	Juçara pulp powder (%)
1	99.5	0.5
2	98	2
3	96	4

Apple juice and juçara powder were mixed an industrial blender (LB-25MB, Skymsen®, Brazil) for 1 min at room temperature. Based on the previous experience of the study group, the beverages were pasteurized at 92° C for 30 s in a scraped surface heat exchanger (FT25D, Armfield®, UK) and hot packed (83° C) in glass bottles inside aseptic chamber (Micro Thermics®, USA). Then it was cooled and stored at room temperature (Figure 1).

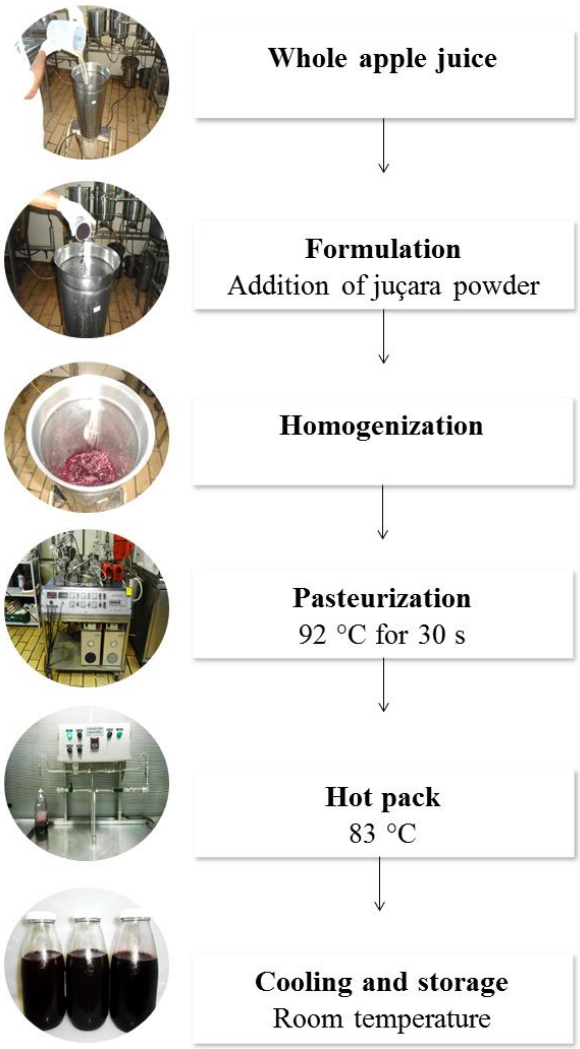

Fig. 1. Flowchart of production of the mix juice from apple and juçara.

2.2.3 Sensory analysis

Consumer evaluation was conducted with individuals (males and females) who indicated that liked juice. The tests were carried out at Embrapa Food Technology, Sensory and Instrumental Evaluation Laboratory, Rio de Janeiro - RJ, Brazil. The mixed juices (Table 1) were evaluated for appearance, consistency and global acceptance, using a structured hedonic scale of nine points, varying from: 1 – "I extremely disliked it" to 9 – "I extremely liked it" (Meilgaard, Civille & Carr, 1999). The Intention to Purchase, açaí and juçara fruit juice frequency of consumption, and the most consumed type of juice, were also evaluated. An amount of 30 mL of each sample was offered in plastic cups (50 mL) at refrigeration temperature (7 ° C), each sample was coded with a 3 digit number, and they were offered all at once in a balanced order. Panelists were instructed to try from left to right, cleaning the palate between samples with water and a salty cookie.

2.2.4 Physical chemical characterization

The best sensory evaluated mixed juice was characterized for moisture content, soluble solids, acidity, pH, proteins, lipids, ash and total carbohydrates (AOAC, 2010). Instrumental color was determined using Color Quest XE colorimeter (HunterLab, UK).

2.2.5 Bioaccessibility analysis

The *in vitro* digestion simulating gastrointestinal process was performed using the method described by Gião et al. (2012). The simulating gastrointestinal process started directly in the gastric phase, owing to the short time that a liquid product remains in the mouth. The sample with best sensory performance (7 g) was added to a gastric solution (pH 2.5) with pepsin (Sigma®, USA), and left under agitation for 1 hour at 37°C. It had the pH adjusted to 6.5 with NaOH 1M and stayed under agitation for 2 hours at 37°C. On each phase (gastric and enteric) a sample of 5 mL was collected for

chemical characterization. After small intestinal digestion, 5 mL of each triplicate was added into sterile Schott bottles (reactors) containing 5 mL of human feces diluted 1:10 in phosphate buffer and 45 mL of basal nutrient broth (Guergoletto et al., 2016), which was also used as the negative control. Immediately after preparation, aliquots of the reactors were separated for analysis in time 0 hour (T0h). The reactors (T0h) were conditioned under microaerophilic conditions (atmosphere generator Microaerobac®, Brazil) and placed in incubator at 37°C for 24 h (T24h).

2.2.6 Microbiological analysis

Aliquots from T0h and T24h were homogenized and serially diluted in peptone water 0,1% and *pour plated* in culture medium MRS (deMan, Rogosa, and Sharpe, TM Media®, India) acidified with Glacial Acetic acid to pH 5.4 for *Lactobacillus* determination. For *Clostridium* count, the RCA (Reinforced Clostridial Agar, BD Difco™, Europe) was utilized. RCA supplemented with selective and differential compounds according to Muñoa and Pares (1988) was applied for *Bifidobacterium* enumeration. All culture media were incubated at 37°C under anaerobic conditions for 5 days. *E. coli* count was performed using Petrifilm® EC (3M Company, St. Paul, MN, USA), incubated at 45°C / 2 days on aerobic conditions. A reactor containing only feces and basal nutrient broth was used as control. Fecal samples were collected from a single individual, female, 28 years old, in good health who had not ingested antibiotics or probiotics for at least 6 months before the study (Aas, Gessert & Bakken, 2003). Samples were collected, on site, on the day of the experiment and placed on sterile feces vial (Guergoletto et al., 2016). The simulated digestion was performed three times in order to observe reproducibility.

2.2.7 High Performance Liquid Chromatography (HPLC)

Analysis were performed for the original sample and for each gastrointestinal fractions. For phenolic compounds and total anthocyanins analysis it was used the methodology described by Pérez-Jiménez et al. (2008) and Gouvêa, Santiago, Pacheco, Godoy and Cabral (2012), respectively. The extract was analyzed by high performance liquid chromatography (HPLC) using Waters® Alliance model 2695 and e2695 chromatograph (Milford, USA). The results obtained were compared with standards spectral curve (cyanidin-3-glucoside, cyanidin-3-rutinoside, 3, 4-dihydroxybenzoic acid, 4-hydroxybenzoic acid, catechin, vanillic acid, epicatechin, ferulic acid, rutin and luteolin).

2.2.8 Spectrophotometric analysis

The antioxidant activity was determined using ABTS (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulphonate)) radical cation scavenging activity (Re et al., 1999) and ORAC (Oxygen Radical Absorbance Capacity) (Zuleta, Esteve & Frígola, 2009), for both methods, results were expressed as μmol of Trolox equivalents per g of sample.

Analysis was conducted for the juice and for each phase of the simulated digestion (gastric, enteric, colonic T0h and colonic T24h).

2.2.9 Digestion metabolite production

After fecal microbiota fermentation, the production of ion ammonia and short-chain fatty acids (SCFAs) were analyzed. Samples from T0h and T24h were evaluated using commercial kit HANNA® Checker®HC model HI715 for ammonia content, according to the manufacturer's manual.

SCFAs were quantified on T0h and T24h by high resolution gas chromatography on Agilent 7890 equipment using a column FFAP (Free Fatty Acid Phase) fused silica capillary (25m x 0.2mm x 0.30μm) with temperature programming from 40 to 230°C,

injector temperature and detector of 250 and 280°C, respectively (Adorno et al., 2014). The injector was operated in split-flow mode at 1:50 ratio and 1µL volume injection. In order to quantify, calibration curves were used to relate peak area and concentration ratio for crotonic acid and acetic, propionic, butyric, isobutyric, valeric, isovaleric, hexanoic, heptanoic and octanoic acids. Crotonic acid was used as an internal standard.

2.2.10 Statistical analysis

Results were obtained in triplicate and statistically treated by analysis of variance, using the software SISVAR 5.6. Mean analysis was performed using Tukey test at $p \leq 0.05$.

3. Results and discussion

3.1 *Sensory evaluation*

Apple and mixed juices are shown in Figure 2.

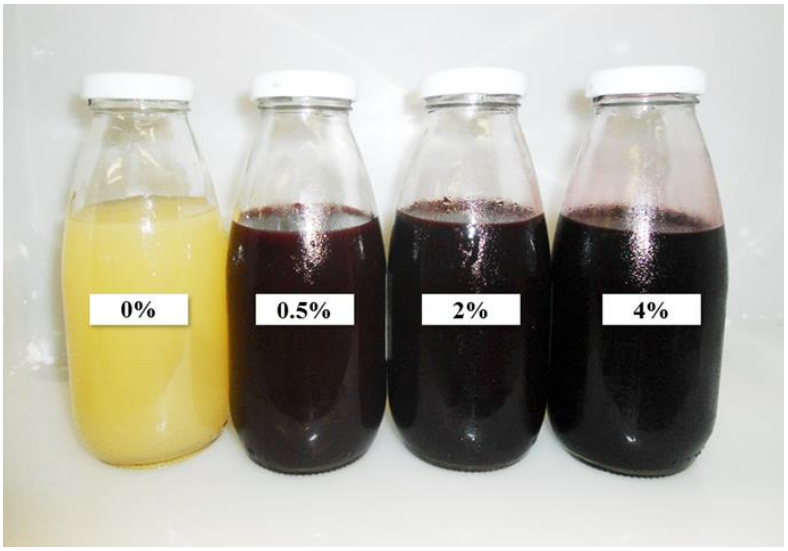

Fig. 2. Visual characteristic of apple juice (0% juçara powder) and of mixed juices (0.5%, 2% and 4% juçara pulp powder).

The sensory panel consisted on 80 non-trained consumers, over 18 years old and predominantly female (55%). An often fruit juice consumption was pointed out by

66.25% of panelists, being 31.25% frequently consuming açai juice. However, 80.00% of tasters had never consumed juçara juice (Figure 3a). The study showed that whole juice is most consumed (76.25%), followed by concentrated juice (45.00%), fruit nectar (42.50%), liquid refreshment (22.50%) and powder refreshment (17.50%) (Figure 3b). As for the intention to buy, 58.75% would buy the formulated juices by being tasty and healthy (Figure 3c).

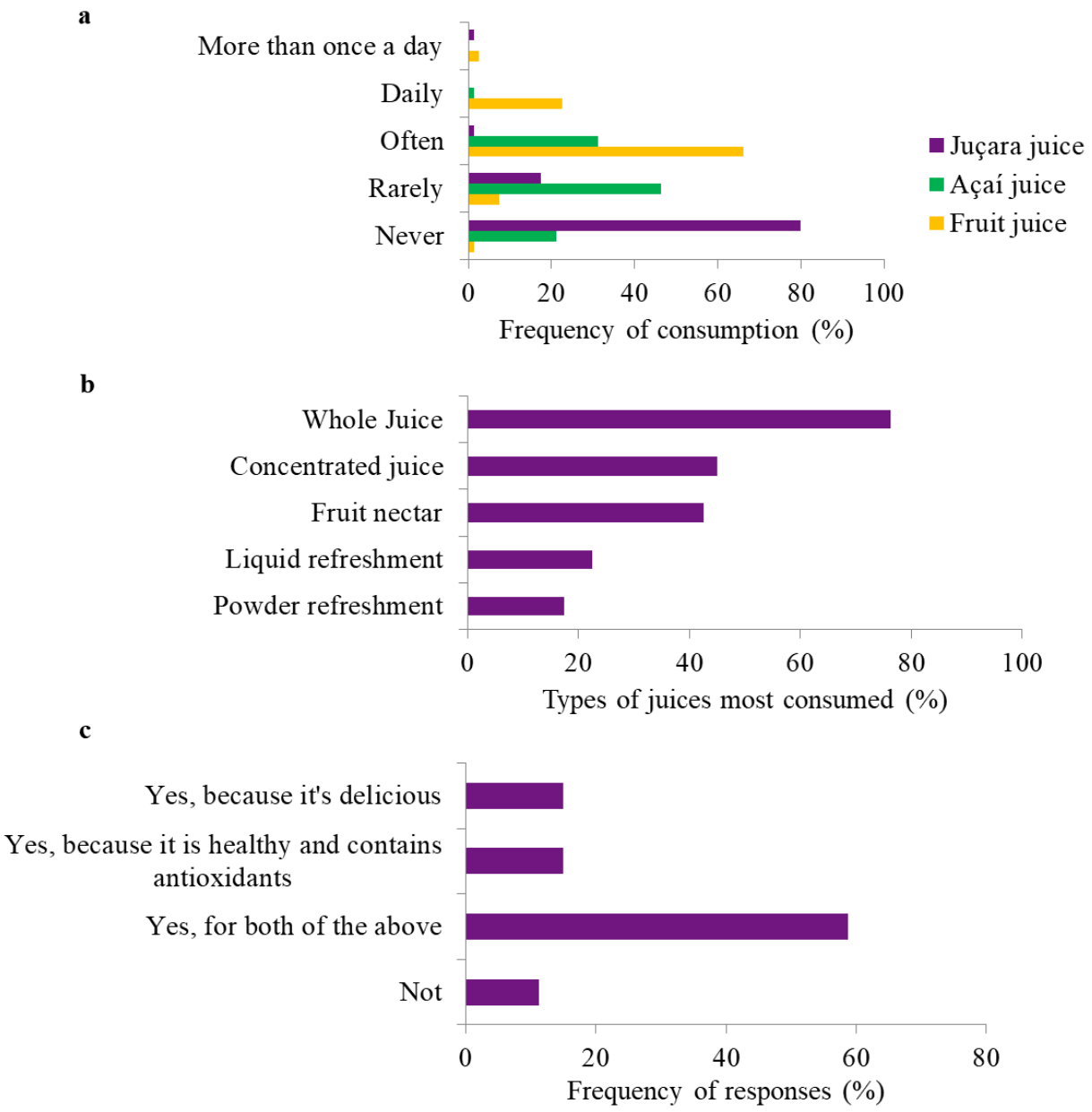

Fig. 3. Frequency of consumption of fruit juices, açai juice and juçara juice (a), types of juices most consumed (b) and reasons to purchase the apple and juçara juices (c).

The results (Table 2) indicate that mixed juices with 0.5% and 2% of juçara pulp were the most accepted by the panelists, and there were no significant differences between them ($p > 0.05$). The juice with 4% of juçara pulp was the least accepted ($p < 0.05$), with global acceptability score of 5.79.

Table 2 Attributes score according to sensory evaluation.

Formulation[1]	Appearance	Texture	Global acceptability
1	7.41[ab]	6.93[ab]	6.99[a]
2	7.78[a]	7.44[a]	7.21[a]
3	7.18[b]	6.74[b]	5.79[b]

[1]Formulation 1, 2 and 3: 0.5%, 2% and 4% of juçara pulp powder. Means followed by the same letter do not differ statistically at 5% probability by the Tukey test.

Although no significant difference was observed between the formulation 1 and 2, only the juice with 0.5% of juçara pulp was further characterized as it represented the most economical product. Considering that a higher concentration of apple juice is interesting due to the market availability and the low availability of juçara pulp in the market makes industrial processing difficult.

3.2 *Physical and chemical characterization*

The results were expressed for a portion of 200 mL of juice, as recommended by Brazil (2003). Addition of juçara powder provided the whole apple juice 52.99 mg cyanidin-3-glucoside / 200mL (Table 3) which represents higher value when compared to other sources of anthocyanins such as commercial grape juices (1.17 a 66.80 mg/L) (Malacrida & Motta, 2005). These results indicate the possibility of using apple juice supplemented with juçara pulp powder as a source of anthocyanins. The increase on anthocyanins content contributed to a color change from yellow to purple (Figure 2). Protein (0.25 g), lipid (0.96 g) and ash (0.36 g) content also increased, without alteration on the caloric value, which is important for those who are on a restricted diet (Table 3).

No major difference was observed in soluble solids content, acidity and pH, which may be interesting for the utilization of juçara powder as a food colorant or supplement.

Table 3 Physical and chemical characterization of apple juice and apple juice added with juçara pulp powder.

Analyzed item [1]	Apple juice[2]	Apple juice with 0.5% juçara powder
Moisture content (%)	-	87.63±0.02
Soluble solids (ºBrix)	11.87±0.06[a]	12.00±0.10[a]
Total acidity (%)	3.79±0.01[b]	3.84±0.02[a]
pH	3.48±0.03[a]	3.52±0.04[a]
Proteins (g)	-	0.25±0.01
Lipids (g)	-	0.96±0.25
Ash (g)	-	0.36±0.03
Fibers (g)	-	not detected
Carbohydrates (g)	25[3a]	23.2±0.00[b]
Total anthocyanins (mg cyanidin-3-glucoside /200mL)	not detected	52.99±1.11
L*	40.39±0.07[a]	27.55±0.01[b]
a*	-1.32±0.01[b]	2.98±0.01[a]
b*	5.05±0.03[a]	-0.16±0.02[b]
C	5.22±0.03[a]	2.92±0.02[b]
ºh	104.69±0.15[b]	356.84±0.35[a]
Energy value (kcal)	105[3a]	102.32±0.00[b]

[1] Mean of three replicates, results are expressed for a portion of 200 mL. Values represent mean and standard deviation. [2] It does not contain significant amounts of proteins, lipids and fibers according to the manufacturer. – unrealized analysis. [3] According to the manufacturer.

3.3 *Bioactive composition during digestion and colonic fermentation*

Knowledge about the changes in bioactive compounds during digestion and their bioaccessibility is relevant to determine the functional proprieties of food and ingredients, in order to verify their true benefits to human health. Analysis of the phenolic compounds revealed that cyanidin-3-rutinoside and chlorogenic acid were the main compounds, and 4-hydroxybenzoic acid, catechin, epicatechin and rutin were also identified (Table 4). In the present study, addition of 0.5% juçara powder provided the mixed juice a phenolic compounds content of 87.34 mg / 200 mL (sum of the phenolic compounds found before digestion) and anthocyanins of 13.85 mg / 200 mL (sum of anthocyanins found before digestion) by HPLC (Table 4). Due to this, the addition of

juçara pulp powder in beverages may be an alternative for the enrichment of juices with antioxidant compounds. Content of phenolic compounds and anthocyanins was reduced throughout *in vitro* gastrointestinal digestion. At the end of digestion in the small intestine, 31.31% of total anthocyanins (1.47 mg cyanidin-3- glucoside and 2.95 mg cyanidin-3-rutinoside /200g of apple juice with juçara) were available for the colonic bacteria. There were no anthocyanins detected neither phenolic compounds (chlorogenic acid, hydroxybenzoic acid, catechin, epicatechin and rutin) after digestion in the large intestine.

Table 4 Contents of bioactive compounds detected by HPLC and antioxidant capacity (ABTS and ORAC) using spectrophotometric methods, of apple juice with 0.5% juçara powder submitted to an *in vitro* human digestion simulation.

Sample[1]	Chlorogenic acid (mg/200mL)	4-Hydroxybenzoic acid (mg/200mL)	Catechin (mg/200mL)	Epicatechin (mg/200mL)	Rutin (mg/200mL)	Anthocyanins (mg cyanidin-3- glucoside /200mL)	Anthocyanins (mg cyanidin-3-rutinoside /200mL)	ABTS (µmol of Trolox/g)	ORAC (µmol of Trolox/g)
Juice before digestion	63.05±0.00^{b}	0.15±0.00^{a}	4.84±0.00^{a}	5.19±0.00^{a}	0.26±0.00^{a}	5.06±0.08^{a}	8.79± 0.61^{a}	11.37±1,11^{c}	1,543.78±395.23^{a}
II phase: gastric digestion	27.08±0.00^{c}	ND	0.79±0.00^{d}	0.73±0.00^{d}	ND	2.19±0.00^{c}	3.95±0.00^{c}	8.78±0.57^{d}	193.87 ±58.46^{c}
III phase: intestinal digestion - SI	22.78±0.00^{d}	ND	0.88±0.00^{c}	0.76±0.00^{c}	ND	1.47±0.00^{d}	2.95±0.00^{d}	13.99±0.50^{b}	392.07±41.78^{c}
IV phase: intestinal digestion - LI (T24h)	ND	ND	ND	ND	ND	ND	ND	21.62± 0.85^{a}	1,786.64±74.89^{a}

[1]Mean of three digestion processes. Values expressed as mean and standard deviation. Means followed by the same letter do not differ statistically at 5% probability by the Tukey test. SI - small intestine, LI - large intestine.

The concentration of anthocyanins was higher after gastric digestion (2.19 mg cyanidin-3-glucoside and 3.95 mg cyanidin-3-rutinoside /200mL apple juice with juçara) when compared to small intestine digestion (1.47 mg cyanidin-3-glucoside and 2.95 mg cyanidin-3-rutinoside /200mL apple juice with juçara) (Table 4). These changes in concentration may be due to the lower stability of anthocyanins at high pH found on small intestine (Guergoletto et al., 2016). The acid pH on gastric phase (pH 2.0) promotes a predominance of anthocyanins in the flavylium cation form that shows intensely reddish coloration, although on a higher pH found in the small intestine, the balance is displaced for chalcones formation.

Antioxidant capacity increased 90.15% (ABTS) after the digestion of apple juice with juçara powder (Table 4), mainly after colonic fermentation, possibly due to changes in the phenolic compounds content, due to changes arising from the digestion process or colonic fermentation, which may have led to the formation of compounds with higher antioxidant capacity.

According to Guergoletto et al. (2016) compounds which are not degraded in human digestion can influence the growth of beneficial bacteria, which suggests a structural modification of anthocyanins, caused by reactions in the gastrointestinal environment or due to the metabolism of the colonic microbiota. The influence in the antioxidant capacity is related to transformation into different structural forms with other chemical properties, caused by pH changes during *in vitro* digestion and phenolics compounds interaction with sample matrix.

3.4 *Changes in bacterial populations after fermentation in vitro culture*

The use of fecal inoculants is more significant for *in vitro* methods compared to the use of pure cultures as it ensures that a representative variety of bacterial species is exposed to the test material (Gibson, Probert, Loo, Rastall & Roberfroid, 2004). The increasing of the

tested bacterial groups showed that the anaerobic microbiota was well maintained in the system and could ferment the substrates (Table 5).

Table 5 Microbial population before and after the colonic fermentation of the apple juice with 0.5% juçara powder.

Apple juice with 0.5% juçara powder	Microbial count (CFU Log^{10})[1]			
	Bifidobacterium	*Lactobacillus*	*Clostridium*	*E. coli*
Neg. control (T0h)	5.11± 0.20[a]	4.22 ± 0.31[b]	6.41± 0.15[b]	6.20± 0.24[a]
Neg. control (T24h)	5.20±0.00[a]	7.31± 0.22[a]	7.63 ± 0.52[a]	5.55± 0.08[b]
Juice (T0h)	5.27± 0.06[b]	4.52± 0.10[b]	6.36±0.08[b]	6.21±0.12[a]
Juice (T24h)	5.87± 0.06[a]	6.36± 0.12[a]	8.29± 0.14[a]	6.22± 0.16[a]

[1]Mean of three digestion processes. Values expressed as mean and standard deviation. Means followed by the same letter do not differ statistically at 5% probability by the Tukey test. CFU: Cell Formation Unit. Neg. control - negative control (reactor without the addition juice).

Apple juice with juçara increased *Bifidobacterium* counts (0.67 logarithm cycle), when compared to negative control after 24h fermentation, result also observed by Guergoletto et al. (2016). However, no positive effects on the growth of other microorganisms were observed (Table 5). When compared to the negative control, there was a higher increase in the growth of *Clostridium* and *E. coli* and a smaller increase in the growth of *Lactobacillus* for apple juice with 0.5% juçara, which may indicate that the proportions used, or the formulated juice did not bring beneficial effect in relation to such microbial groups.

3.5 *Ammonia and short-chain fatty acid production*

Ammonia is a toxic catabolite that interferes with the metabolism and integrity of intestinal mucosal cells (Apajalahti & Vienola, 2016). Supplementation of apple juice with juçara pulp powder reduced the production of ammonia (-18.84%), while the negative control increased 9.38% (Table 6). This suggests that the deamination and decarboxylation of dietary

and / or endogenous protein by microorganisms in the gastrointestinal tract are altered by supplementation with mixed juice.

Fibers and phenolic compounds can reach the large intestine where, mediated by the action of local microbiota, a series of metabolites are accumulated, such as SCFAs and smaller phenolic compounds (Mosele, Macià & Motilva, 2015).

Table 6 Concentrations of major short-chain fatty acid at 0 h and 24 h of fermentation of juice and negative control after the digestion of the apple juice with 0.5% juçara powder.

Apple juice with 0.5% juçara powder	Ammonia (mg/L) and short-chain fatty acid production (mmol/L)[1]		
	Ammonia	Ammonia variation (%)	Acetic acid
Neg. control (T0h)	25.68±0.13[b]	9.38	NQ
Neg. control (T24h)	28.08±0.07[a]		0.96±0.03
Juice (T0h)	44.93±1,16[a]	-18.84	NQ
Juice (T24h)	36.47±2.97[b]		1.69±0.10

[1]Mean of three digestion processes. Values expressed as mean and standard deviation. Means followed by the same letter do not differ statistically at 5% probability by the Tukey test. NQ: not quantified. Neg. control - negative control (reactor without the addition juice).

The increase of ammonia in the negative control can be explained by the increase of the deamination and the decarboxylation of the excess proteins by colonic bacteria (Apajalahti & Vienola, 2016).

Some phenolic compounds can reach the large intestine, being metabolized by bacteria as a source of energy, supplementation with apple juice with juçara may lead to lower production of ammonia and amines. In addition, the metabolism of phenolic compounds originates a series of low molecular weight metabolites, which may be the real responsible for the beneficial effects on health related to the consumption of phenolic compounds (Guergoletto et al., 2016).

Fermentation of apple juice with juçara resulted in a production higher of acetic acid compared to the negative control. After 24 h of incubation, the fermentation of the apple juice with juçara produced the largest amount of acetic acid (1.69 mmol/L) when compared to negative control which had a production of 0.96 mmol/L (Table 6), that is, 0.73 mmol / L was produced more with the addition of apple juice with juçara. In the present study no propionic acid and butyric acid were detected in the analyzed sample.

Guergoletto et al. (2016) and Gullon, Pintado, Fernández-López, Pérez-Álvarez and Viuda-Martos (2015) observed that 24h fermentation of juçara pulp and pomegranate peel flour, respectively, resulted in a greater ($P < 0.05$) production of acetate and propionate, however the first observed lower amounts of butyrate, while the second also had an increase in this SCFAs. Suggesting the influence of the microbial fermentation on the concentration of these metabolites.

Dietary carbohydrates, especially resistant starches and dietary fiber, are substrates for fermentation leading to the production of SCFAs, primarily acetate, propionate and butyrate. These SCFAs may reduce the risk of developing gastrointestinal disorders, cancer, and cardiovascular disease. They may also regulate immune responses and the composition of the intestinal microbiota (Gill, van Zelm, Muir & Gibson, 2018). Therefore, a greater increase in SCFAs production may result in a protective effect.

4. Conclusions

The developed mixed beverage with apple juice and juçara powder represented an alternative product with technological, nutritional and functional benefits, including significant amount of antioxidant compounds. The product was well accepted by consumers, showing a market potential. Throughout the simulation of digestion and colonic fermentation a reduction on anthocyanins and an increase in antioxidant capacity were observed. The

increase in the population of *Bifidobacterium* and in the short chain fatty acids concentration showed a beneficial microbiota modulation trend. Whole apple juice proved to be a good base for application of powdered juçara pulp and juçara powder can be suitable to be used as a natural dye and to add functional value to apple juice.

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4 CONCLUSÃO

O fruto da juçara tem potencial para ser utilizado como polpa ou ingrediente na preparação de muitos alimentos, devido ao seu rico conteúdo de compostos essenciais, que inclui ácidos graxos, proteínas, minerais, vitaminas e fibras dietéticas. Além disso, este fruto apresenta uma grande variedade de compostos fenólicos com potencial bioativo, como as antocianinas.

Apesar das dificuldades para a utilização da polpa de juçara, devido a sua perecibilidade, as pesquisas estão continuamente expandindo. A desidratação da polpa presenta-se como uma solução para o aumento da vida comercial e promove uma maior flexibilização dos potenciais mercadológicos desse produto.

A secagem por pulverização da polpa de juçara é uma alternativa para o processamento desta fruta, uma vez que o pó obtido é mais fácil de manusear, embalar e transportar do que a própria polpa. O processo de secagem por atomização utilizado neste estudo foi eficiente para a proteção das antocianinas e capacidade antioxidante da polpa de juçara. Além disso, a secagem por pulverização pode ser utilizada como uma técnica de preservação de compostos bioativos em que a alta retenção de antocianinas foi alcançada. A não utilização de agentes encapsulantes não interferiu na qualidade do produto, gerando um pó com maior concentração de antocianinas, além da redução de variáveis e custos de processo, por não utilizar agente carreador.

Os pós de juçara armazenados sob refrigeração (7 ºC), sem a presença de oxigênio, foram mais estáveis que os armazenados à temperatura ambiente (25 ºC), no entanto, o conteúdo de compostos bioativos e a capacidade antioxidante foram estáveis em todas as condições de armazenamento. Neste caso, o pó armazenado a 25 ºC na presença ou ausência de oxigênio pode reduzir os custos causados pela cadeia do frio. A polpa de juçara em pó produzida nas condições apresentadas é um produto de valor agregado, com potencial para ser aplicado como matéria-prima ou aditivo natural, em alimentos, como bebidas, suco em pó, sorvetes comestíveis, produtos lácteos e de padaria, bem como na reconstituição do próprio suco. A bioacessibilidade dos compostos fenólicos da polpa de juçara fornece informações relevantes sobre o comportamento das antocianinas e compostos fenólicos totais durante a simulação da digestão e fermentação colônica. Foi observada uma redução no teor de compostos fenólicos totais e aumento da capacidade antioxidante no intestino grosso após a fermentação colônica. O aumento no crescimento da produção de *Bifidobacterium* e de ácidos graxos de cadeia curta e a redução do aumento da contagem de *Escherichia coli* e da concentração de amônia mostram uma tendência benéfica à modulação. O suco de maçã adicionado com juçara em pó e sem outros ingredientes foi um produto apreciado pelos consumidores. Ao longo da simulação da digestão e fermentação colônica, foram observadas uma redução nas antocianinas e um aumento na capacidade antioxidante. O aumento no crescimento de *Bifidobacterium* mostrou uma tendência de modulação benéfica. Assim, a juçara em pó pode ser uma alternativa para agregar valor a produtos alimentícios, além de se caracterizar como um ingrediente potencialmente funcional.

Uma maior conscientização sobre o conteúdo nutricional e os benefícios de consumo da juçara para a saúde e o meio ambiente podem contribuir para consolidação de um mercado consumidor brasileiro e internacional. Os esforços combinados de produtores, instituições de pesquisa e indústrias inovadoras para divulgar as possibilidades de aplicação da polpa de juçara provavelmente aumentarão a comercialização dos frutos de juçara e seus derivados, o que possivelmente permitirá remover esta planta da lista de espécies ameaçadas de extinção.

Estudos futuros sobre a estabilidade dos produtos adicionados de juçara em pó e estudos em humanos são necessários para avaliar a aplicação industrial e potencial funcional do produto desenvolvido.

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