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Sistemas Alimentares Inclusivos e Eficientes: efeito do processamento na funcionalidade de farinhas alternativas (propriedades químicas, estruturais e digestibilidade de produto)

Pirassununga
2022

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Muito Obrigado!

I have E.T. on my bicycle

Because giving up won't work

Now I'm riding on my rocketship

And I'm champion of the world

ColdPlay

Santos, Y.J.S. Sistemas Alimentares Inclusivos e Eficientes: efeito do processamento na funcionalidade de farinhas alternativas (propriedades químicas, estruturais e digestibilidade de produto). 2022. 121 f. Dissertação (Mestrado) – Universidade de São Paulo, Faculdade de Zootecnia e Engenharia de Alimentos, Pirassununga, SP, 2022.

RESUMO

Frutas amazônicas estão ganhando destaque e, dentre tais matrizes alimentares, o fruto de pupunha apresenta algumas características interessantes, como o alto teor de compostos fenólicos e o seu potencial antioxidante, características bastante desejadas em matérias primas funcionais. Todavia, dois aspectos ainda são pouco explorados na literatura: a utilização de metodologias alternativas para a quantificação de compostos fenólicos, assim como os efeitos indesejáveis dos compostos fenólicos. Logo, o objetivo deste trabalho foi revisar os trabalhos recentes sobre a utilização de métodos espectroscópicos na determinação de compostos fenólicos em frutos, assim como produzir e caracterizar farinha de pupunha oriunda de seis localidades diferentes quanto as suas características físico-químicas, citotóxicas, inibição da digestibilidade proteica *in vitro*, capacidades antioxidantes pelos métodos de Captura do Radical 2,2-azinobis (3-etilbenzotiazolina-6-ácido sulfônico) (ABTS), Poder de Redução do Íon Ferro (FRAP) e Capacidade de Absorção do Radical Oxigênio (ORAC), teor de compostos fenólicos e análises estruturais a partir de métodos espectroscópicos. Além disso, foi verificada a utilização de equipamento não-destrutivo (MicroNIR) na determinação de compostos bioativos e potenciais antioxidantes pelos métodos de ABTS e FRAP em biscoitos tipos cookies incorporados com proporções de 12,5, 25, 50, 75 e 100% de farinha de pupunha, assim como o produto elaborado foi caracterizado em relação a perda de água, atividade de água, conteúdo de água, volume específico, fator de espalhamento e cor. Os resultados relativos à farinha demonstraram que fatores como a localidade e/ou maturação dos frutos provocaram diferenças significativas entre as amostras, obtendo-se valores médios de 2.11 mgAGE/g, 16,9 mgTE/g, 0,25 mgTE/g e 36,30 mgTE/g para teor de compostos fenólicos e potenciais antioxidantes pelos métodos ABTS, FRAP e ORAC, respectivamente. Os compostos fenólicos da farinha de pupunha apresentaram potencial citotóxico contra células saudáveis (L929) e foi verificado que tal matriz alimentar, quando aplicada em produto lácteo, inibe a ação de enzimas digestivas. Com o uso do equipamento MicroNIR, aliado a técnicas quimiométricas, foi possível elaborar modelos robustos para a previsão dos parâmetros selecionados e produtos com satisfatórios parâmetros de qualidade foram formulados. Concluindo, pode-se verificar que apesar dos benefícios atribuídos aos compostos bioativos, estes também podem exercer efeitos negativos ao metabolismo e que técnicas não invasivas são ótimas ferramentas para a previsão de compostos de interesse minoritários em alimentos.

Palavras-chave: Cookies. Farinha de pupunha. MicroNIR. Predição. Compostos fenólicos.

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ABSTRACT

Amazonian fruits are gaining importance and, among such food matrices, the pupunha fruit presents some interesting characteristics, such as the high content of phenolic compounds and its antioxidant potential, characteristics very desirable for the elaboration of functional raw materials. However, two aspects are still little explored in the literature: the use of alternative methodologies for the quantification of phenolic compounds, as well as the undesirable effects of phenolic compounds. Therefore, the objective of this work was to review recent works about the use of spectroscopic methods in the determination of phenolic compounds in fruits, as well as to produce and characterize pupunha flour from six geographic origins regarding its physicochemical characteristics, cytotoxicity, inhibition of protein digestibility in vitro, antioxidant capabilities by the methods of 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) Radical Capture (ABTS), Iron Ion Reduction Power (FRAP) and Oxygen Radical Absorbance Capacity (ORAC), content of phenolic compounds and structural analysis from spectroscopic methods. Furthermore, the use of non-destructive equipment (MicroNIR) was verified in the determination of bioactive compounds and antioxidant potential by ABTS and FRAP methods in cookies incorporated with proportions of 12.5, 25, 50, 75 and 100% of pupunha flour, as well as the elaborated product was characterized in relation to water loss, water activity, water content, specific volume, spread factor and color. The results for the flour showed that factors such as locality and/or fruit maturity caused significant differences among the samples, obtaining average values of 2.11 mgAGE/g, 16.9 mgTE/g, 0.25 mgTE/g and 36.30 mgTE/g for phenolic compounds content and antioxidant potentials by ABTS, FRAP and ORAC methods, respectively. The phenolic compounds of pupunha flour showed cytotoxic potential against healthy cells (L929) and it was verified that such food matrix, when applied in dairy product, inhibits the action of digestive enzymes. With the use of the MicroNIR equipment, allied to chemometric techniques, it was possible to elaborate robust models for the prediction of the selected parameters and products with satisfactory quality parameters were formulated. In conclusion, it can be verified that despite the benefits attributed to bioactive compounds, these can also exert negative effects on the metabolism and that non-invasive techniques are excellent tools for the prediction of minority compounds of interest in foods.

Keywords: Cookies. Pupunha flour. MicroNIR. Prediction. Phenolic Compounds

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1 INTRODUÇÃO

A região amazônica abriga uma infinidade de espécies de plantas, sendo mais de 2000 espécies de árvores registradas até o momento, porém somente cerca de 10% é utilizada para a produção de óleos essenciais, frutos, fibras e outras aplicações industriais, ou seja, há pouca informação e utilização sobre a vasta maioria das espécies restantes, mesmo entre as mais conhecidas (FERRAZ et al., 2019). Os frutos oriundos dessa região são potencialmente funcionais, com uma variedade de compostos benéficos a saúde, mas que são pouco explorados devido a falta de tecnologias de processamento e falta de conhecimento e difusão a respeito de tais compostos (AVILA-SOSA et al., 2019).

Os compostos funcionais, também chamados de compostos bioativos ou fitoquímicos, são encontrados em pequenas quantidades nos alimentos e sua importância está relacionada à capacidade protetora, na maioria das vezes, em forma de atividade antioxidante (SANTOS et al., 2015). Entre eles, pode-se destacar os polifenóis, carotenóides, fitoesteróis, flavonoides e taninos, sendo que os polifenóis se destacam por ser as principais fontes antioxidantes entre os alimentos de origem vegetal (KARASAWA; MOHAN, 2018).

O efeito protetor dos compostos fenólicos vem sendo demonstrado ao longo dos anos. Em um estudo realizado com a fração fenólica de azeite de oliva, foi constatado um importante efeito relacionado a diminuição da viabilidade de células cancerígenas de mama (MENENDEZ et al., 2007). Compostos fenólicos também apresentaram efeito benéfico contra células cancerígenas dos ovários e foi sugerido seu uso na prevenção e terapia desse tipo de câncer (HE et al., 2015). Já Hamaguchi et al. (2009), concluíram que a administração oral de compostos fenólicos pode prevenir o desenvolvimento de Alzheimer em modelos *in vivo*.

Neste sentido, a presença de elevado teor de compostos fenólicos já foi relatada em algumas polpas de frutos amazônicos como Piquiá (CHISTÉ; MERCADANTE, 2012), Pupunha (ROJAS-GARBANZO et al., 2012), Buriti (RESENDE; FRANCA; OLIVEIRA, 2019a), Bacaba (SANTOS et al., 2015), Cubiu (RODRIGUES; MARIUTTI; MERCADANTE, 2013) e Ubaia-Rubi (SILVA et al., 2007). Na flora amazônica, muitas dessas plantas desconhecidas pelos cientistas e consumidores de grandes centros urbanos, são utilizadas há muito tempo pelos povos indígenas locais para curar, prevenir e tratar doenças degenerativas (SILVA et al., 2007).

Dentre os frutos amazônicos, pode-se destacar o fruto de pupunha, que apesar de ser subutilizado, apresenta grande potencial nutricional e tecnológico (JANICK; PAULL, 2009). O fruto é oriundo da pupunheira (*Bactris gasipaes*), uma palmeira da família *Arecaceae*, com grande cultivo na região da América Tropical, compreendendo os países entre a Costa Rica e o Brasil, sendo encontrado preferencialmente em zonas úmidas (ESPINOSA-PARDO; MARTINEZ; MARTINEZ-CORREA, 2014).

O fruto da pupunha caracteriza-se por apresentar formas e tamanhos distintos. Quando maduros possuem a casca fibrosa de cor amarela, alaranjada ou vermelho intenso e um mesocarpo que varia de amiláceo a oleoso (CARVALHO et al., 2013). O fruto tem um valor nutritivo elevado, rico em lipídios, principalmente ácidos graxos insaturados, predominantemente o ácido oleico (YUYAMA et al., 2003) e, portanto, líquidos a temperatura ambiente e de alto valor nutricional (SANTOS et al., 2017). Além disso, o fruto é rico em carotenóides, fitosteróis, aminoácidos essenciais, retinol, carboidratos, principalmente o amido (CARVALHO et al., 2013; ROJAS-GARBANZO et al., 2011; SANTOS et al., 2017; SANTOS; ALVES; RUÍZ-MÉNDEZ, 2013), revelando-se um fruto que pode ser utilizado para a obtenção de farinha, que pode ser utilizada na produção de diversos tipos de produtos (CARVALHO et al., 2009a).

A utilização da farinha de pupunha é uma alternativa a fim de evitar a saturação do mercado de frutas frescas e também diversificar a oferta do produto. Além disso, o fruto possui elevado teor de amido, dessa forma, quando aquecido a estrutura do granulo pode ser alterada permitindo o desenvolvimento de propriedades tecnológicas de interesse (BEZERRA; SILVA, 2016), aplicação em pães (LIMA; SILVA; FURTADO, 2020), snacks (CARVALHO et al., 2009a), biscoitos/cookie (RIBEIRO et al., 2021), panetones (OLIVERA; MARINHO, 2010) e bolos (KAEFER et al., 2013).

Dentre os produtos destacados, as vendas dos biscoitos no Brasil em 2020 representaram o montante de mais de 4,5 bilhões de reais, equivalendo a 363 mil toneladas de produto, enquanto que o consumo per capita no ano de 2019 foi de cerca de 7kg/hab, com presença em mais de 90% dos lares brasileiros, segundo a Associação Brasileira das Indústrias de Biscoitos, Massas Alimentícias, Pães e Bolos (ABIMAPI) (2020). Somado a esse fato, as exportações desse tipo de produto corresponderam a 115 milhões de dólares, sendo o biscoito o produto que alcançou o maior valor de exportação entre os produtos da cadeia de massas alimentícias, pães e bolos industrializados (ABIMAPI, 2021).

Logo, nota-se que, os biscoitos são amplamente aceitos (MORAES et al., 2010) e representam uma importante fonte de receita para as indústrias. Além disso, o aumento do interesse dos consumidores por alimentos mais saudáveis e práticos, exigiu uma adaptação das formulações existentes no mercado visando o oferecimento de biscoitos enriquecidos/ fortificados com ingredientes funcionais (MONTEIRO; SOUZA; DAMASCENO, 2020).

Todavia, por mais que a produção de farinha a partir do fruto de pupunha represente uma diversificação de seu uso e facilite sua comercialização em outras regiões, principalmente quando aplicada em biscoitos, há lacunas que precisam ser melhor exploradas, principalmente no tocante à quantificação dos compostos fenólicos no produto final.

As metodologias existentes para se estimar a quantidade de compostos fenólicos exploram, na grande maioria das vezes, métodos colorimétricos baseados no ensaio espectrofotométrico de Folin-Ciocalteu (SINGLETON; ORTHOFER; LAMUELA-RAVENTÓS, 1999) onde há, inclusive, padronizações internacionais como a ISO 14502-1 (2005). Todavia, esse ensaio está associado a algumas limitações como: alguns compostos quimicamente semelhantes podem interferir nos resultados, a seleção de padrões para construção de uma curva de calibração pode variar em função da matéria-prima (BASTOLA et al., 2017; PRIOR; WU; SCHAICH, 2005), na etapa de extração dos compostos fenólicos é necessário estabelecer as melhores condições utilizando diferentes agentes de extração e condições de processo, necessita a utilização de diferentes solventes químicos, além de ser uma análise trabalhosa, demorada, e portanto, sujeita a erros (HAMINIUK et al., 2012).

Por outro lado, os métodos espectroscópicos, como o Infravermelho Próximo (NIR), oferecem algumas vantagens que os colocam a frente quando comparados aos métodos tradicionais de análise, como por exemplo a velocidade de realização da análise, pouca ou nenhuma etapa de preparação de amostra e ausência de reagentes (DAS et al., 2016). Seu uso vêm sendo explorado ao longo dos últimos anos para a predição de compostos fenólicos em frutos (AMODIO et al., 2017; AMORIELLO; CICCORITTI; CARBONE, 2019; ARENDSE et al., 2017; BLANCO-DÍAZ et al., 2014; CICCORITTI et al., 2019; OLAREWAJU et al., 2019) e outros parâmetros de qualidade. Outro fato interessante sobre os métodos espectroscópicos é que o desenvolvimento de equipamentos miniaturizados vêm aumentando rapidamente (BEĆ; GRABSKA; HUCK,

2021) e já há estudos que atestam predições mais robustas utilizando equipamentos portáteis em detrimento aos equipamentos de bancada (KANG et al., 2022).

Equipamentos NIR miniaturizados, por mais que apresentem a desvantagem de ter um número reduzido de comprimentos de onda, podem ser considerados ferramentas bastante interessantes e confiáveis para monitorar a qualidade de frutos diretamente no campo ou ser aplicados diretamente ao longo da cadeia de produção facilitando o controle de qualidade de produtos (MALEGORI et al., 2017a).

Contudo, até o presente momento, não há na literatura uma aplicação de equipamento espectroscópico miniaturizado em produtos de panificação para a predição de compostos fenólicos, bem como não há uma caracterização aprofundada da farinha de pupunha, envolvendo aspectos tecnológicos, nutricionais, digestivos e citotóxicos. Diante desse contexto, o presente estudo buscou apresentar uma abordagem inovadora demonstrando o potencial de utilização de NIR portátil para a predição de compostos fenólicos em biscoitos do tipo cookies, assim como uma ampla caracterização da farinha de pupunha.

Dessa forma, essa dissertação está redigida em forma de capítulos, totalizando 3 ao total. O Capítulo 1 teve como objetivo revisar as informações recentes a respeito da aplicação de métodos espectroscópicos, em especial o NIR, para a predição de compostos fenólicos, elucidando suas principais vantagens, limitações e delineando as principais etapas e dificuldades ainda encontradas para a previsão para a quantificação dos compostos fenólicos utilizando esta tecnologia. No Capítulo 2 buscou-se aprofundar os conhecimentos acerca da farinha de pupunha, através de sua ampla caracterização, incluindo análises citotóxicas e de digestibilidade da farinha de pupunha, obtidas de frutos cultivados em seis localidades diferentes da região amazônica, visto que é extremamente importante ter um padrão de qualidade da matéria-prima para o processamento. Por fim, no terceiro capítulo os aspectos tecnológicos de cookies elaborados com as farinhas de pupunha, assim como a utilização do MicroNIR para o desenvolvimento de modelos de predição para o teor de compostos fenólicos capacidade antioxidante dos cookies, puderam ser demonstrados.

Ao final, uma conclusão geral é apresentada em uma seção a parte, visando recapitular e resumir as contribuições dessa pesquisa. Os resultados mais relevantes de cada capítulo foram retomados para que uma correlação direta fosse estabelecida com o objetivo apresentado e, com base nos limites encontrados durante o desenvolvimento deste trabalho, algumas perspectivas de estudos futuros foram propostas.

REFERÊNCIAS

ABIMAPI, ASSOCIAÇÃO BRASILEIRA DAS INDÚSTRIAS DE BISCOITOS, M. A. E P. & B. I. **Biscoitos Industrializados - Nutrição e Indulgência na Cultura Alimentar.** São Paulo. ABIMAPI & ITAL, 2020.

ABIMAPI, ASSOCIAÇÃO BRASILEIRA DAS INDÚSTRIAS DE BISCOITOS, M. A. E P. & B. I. **Estatísticas Gerais**.

AMODIO, M. L. et al. Potential of NIR spectroscopy for predicting internal quality and discriminating among strawberry fruits from different production systems. **Postharvest Biology and Technology**, v. 125, p. 112–121, 2017.

AMORIELLO, T.; CICCORITTI, R.; CARBONE, K. Vibrational spectroscopy as a green technology for predicting nutraceutical properties and antiradical potential of early-to-late apricot genotypes. **Postharvest Biology and Technology**, v. 155, n. June, p. 156–166, 2019.

ARENDSE, E. et al. Development of calibration models for the evaluation of pomegranate aril quality by Fourier-transform near infrared spectroscopy combined with chemometrics. **Biosystems Engineering**, v. 159, p. 22–32, 2017.

AVILA-SOSA, R. et al. Antioxidant Properties of Amazonian Fruits: A Mini Review of in Vivo and in Vitro Studies. **Oxidative Medicine and Cellular Longevity**, v. 2019, 2019.

BASTOLA, K. P. et al. Evaluation of Standards and Interfering Compounds in the Determination of Phenolics by Folin-Ciocalteu Assay Method for Effective Bioprocessing of Biomass. **American Journal of Analytical Chemistry**, v. 08, n. 06, p. 416–431, 2017.

BEĆ, K. B.; GRABSKA, J.; HUCK, C. W. Principles and Applications of Miniaturized Near-Infrared (NIR) Spectrometers. **Chemistry - A European Journal**, v. 27, n. 5, p. 1514–1532, 2021.

BEZERRA, C. V.; SILVA, L. H. M. Pupunha (Bactris gasipaes): General and Consumption Aspects. In: KRISTBERGSSON, K.; OLIVEIRA, J. (Eds.). . **Traditional Foods**. 10. ed. Boston: Integrating Food Science and Engineering Knowledge Into the Food Chain, 2016. p. 399–405.

BLANCO-DÍAZ, M. T. et al. Use of visible and near-infrared spectroscopy for predicting antioxidant compounds in summer squash (Cucurbita pepo ssp pepo). **Food Chemistry**, v. 164, p. 301–308, 2014.

CARVALHO, A. V. et al. Production of third generation snacks by extrusion-cooking of pupunha and cassava flour mixtures. **Brazilian Journal of Food Technology**, v. 12, n. 04, p. 277–284, 2009.

CARVALHO, A. V. et al. Características físicas e químicas de frutos de pupunheira no estado do Pará. **Revista Brasileira de Fruticultura**, v. 35, n. 3, p. 763–768, 2013.

CHISTÉ, R. C.; MERCADANTE, A. Z. Identification and quantification, by HPLC-DAD-MS/MS, of carotenoids and phenolic compounds from the Amazonian fruit Caryocar villosum. **Journal of Agricultural and Food Chemistry**, v. 60, n. 23, p. 5884–5892, 2012.

CICCORITTI, R. et al. FT-NIR spectroscopy and multivariate classification strategies for the postharvest quality of green-fleshed kiwifruit varieties. **Scientia Horticulturae**, v. 257, n. June, p. 108622, 2019.

DAS, A. J. et al. Ultra-portable, wireless smartphone spectrometer for rapid, non-destructive testing of fruit ripeness. **Scientific Reports**, v. 6, n. September, p. 1–8, 2016.

ESPINOSA-PARDO, F. A.; MARTINEZ, J.; MARTINEZ-CORREA, H. A. Extraction of bioactive compounds from peach palm pulp (Bactris gasipaes) using supercritical CO2. **Journal of Supercritical Fluids**, v. 93, p. 2–6, 2014.
FERRAZ, I. D. K. et al. **Guide to Amazonian Fruits, Seeds & Seedlings**. Manaus: Editora INPA, 2019.

HAMAGUCHI, T. et al. Phenolic compounds prevent Alzheimer's pathology through different effects on the amyloid-β aggregation pathway. **American Journal of Pathology**, v. 175, n. 6, p. 2557–2565, 2009.

HAMINIUK, C. W. I. et al. Phenolic compounds in fruits - an overview. **International Journal of Food Science and Technology**, v. 47, n. 10, p. 2023–2044, 2012.
HE, Z. et al. Selecting bioactive phenolic compounds as potential agents to inhibit proliferation and VEGF expression in human ovarian cancer cells. **Oncology Letters**, v. 9, n. 3, p. 1444–1450, 2015.

ISO 14502-1, I.; STANDARD. **Content of Total Polyphenols in Tea - Colorimetric Method Using Folin-Ciocalteu reagent**, 2005.

JANICK, J.; PAULL, R. E. **The Encyclopedia of Fruit & Nuts**. Cambridge, UK: [s.n.]. v. 23

KAEFER, S. et al. Bolo com farinha de pupunha (Bactris gasipaes): análise da composição centesimal e sensorial. **Brazilian Journal of Food Nutrition**, v. 24, n. 3, p. 347–352, 2013.

KANG, R. et al. A comparison of benchtop and micro NIR spectrometers for infant milk formula powder storage time discrimination and particle size prediction using chemometrics and denoising methods. **Journal of Food Engineering**, v. 329, n. January, p. 111087, 2022.

KARASAWA, M. M. G.; MOHAN, C. Fruits as Prospective Reserves of bioactive Compounds: A Review. **Natural Products and Bioprospecting**, v. 8, n. 5, p. 335–346, 2018.

LIMA, D. G.; SILVA, R. F.; FURTADO, M. T. Chemical Composition and

Microbiological Aspects of Enriched Breads with Integral Pulp of Dehydrated Pupunha. **Gestão, Inovação e Tecnologias**, v. 10, n. 1, p. 5352–5366, 2020.

MALEGORI, C. et al. Comparing the analytical performances of Micro-NIR and FT-NIR spectrometers in the evaluation of acerola fruit quality, using PLS and SVM regression algorithms. **Talanta**, v. 165, n. December 2016, p. 112–116, 2017.

MENENDEZ, J. A. et al. Olive oil's bitter principle reverses acquired autoresistance to trastuzumab (Herceptin^{TM}) in HER2-overexpressing breast cancer cells. **BMC Cancer**, v. 7, p. 1–19, 2007.

MONTEIRO, J. D. B.; SOUZA, É. V. DE; DAMASCENO, L. F. Elaboração E Análises Centesimais De Biscoitos Tipo Cookie De Biomassa De Banana Verde (Musa Ssp.). **Avanços em Ciência e Tecnologia de Alimentos - Volume 1**, p. 203–215, 2020.

MORAES, K. S. et al. Avaliação tecnológica de biscoitos tipo cookie com variações nos teores de lipídio e de açúcar. **Ciencia e Tecnologia de Alimentos**, v. 30, n. SUPPL. 1, p. 233–242, 2010.

OLAREWAJU, O. O. et al. Model development for non-destructive determination of rind biochemical properties of 'Marsh' grapefruit using visible to near-infrared spectroscopy and chemometrics. **Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy**, v. 209, p. 62–69, 2019.

OLIVERA, A. M. M. M. DE; MARINHO, H. A. Desenvolvimento de panetone à base de farinha de pupunha (Bactris gasipaes Kunth). **Alim. Nutr. Araraquara**, v. 21, n. 4, p. 595–605, 2010.

PRIOR, R. L.; WU, X.; SCHAICH, K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. **Journal of Agricultural and Food Chemistry**, v. 53, n. 10, p. 4290–4302, 2005.

RESENDE, L. M.; FRANCA, A. S.; OLIVEIRA, L. S. Buriti (Mauritia flexuosa L. f.) fruit by-products flours: Evaluation as source of dietary fibers and natural antioxidants. **Food Chemistry**, v. 270, n. July 2018, p. 53–60, 2019.

RIBEIRO, G. S. et al. Peach palm flour: production, hygroscopic behaviour and application in cookies. **Heliyon**, v. 7, n. 5, 2021.

RODRIGUES, E.; MARIUTTI, L. R. B.; MERCADANTE, A. Z. Carotenoids and phenolic compounds from Solanum sessiliflorum, an unexploited amazonian fruit, and their scavenging capacities against reactive oxygen and nitrogen species. **Journal of Agricultural and Food Chemistry**, v. 61, n. 12, p. 3022–3029, 2013.

ROJAS-GARBANZO, C. et al. Identification and quantification of carotenoids by HPLC-DAD during the process of peach palm (Bactris gasipaes H.B.K.) flour. **Food Research International**, v. 44, n. 7, p. 2377–2384, 2011.

ROJAS-GARBANZO, C. et al. Major physicochemical and antioxidant changes during peachpalm (bactris gasipaes h.b.k.) flour processing. **Fruits**, v. 67, n. 6, p. 415–427,

2012.

SANTOS, M. DOS et al. Amazonian Native Palm Fruits as Sources of Antioxidant Bioactive Compounds. **Antioxidants**, v. 4, n. 3, p. 591–602, 2015.

SANTOS, M. DE F. G. DOS et al. Quality characteristis of fruits and oils of palms native to the brazilian amazon. **Revista Brasileira de Fruticultura**, v. 39, n. 1, 2017.

SANTOS, M. F. G.; ALVES, R. E.; RUÍZ-MÉNDEZ, M. V. Minor components in oils obtained from Amazonian palm fruits. **Grasas y Aceites**, v. 64, n. 5, p. 531–536, 2013.

SILVA, E. M. et al. Antioxidant activities and polyphenolic contents of fifteen selected plant species from the Amazonian region. **Food Chemistry**, v. 101, n. 3, p. 1012–1018, 2007.

SINGLETON, V. L.; ORTHOFER, R.; LAMUELA-RAVENTÓS, R. M. Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin-Ciocalteu Reagent. **Scientia Horticulturae**, v. 299, n. 1974, p. 281–286, 1999.

YUYAMA, L. K. O. et al. Chemical composition of the fruit mesocarp of three peach palm (Bactris gasipaes) populations grown in Central Amazonia, Brazil . **International Journal of Food Sciences and Nutrition**, v. 54, n. 1, p. 49–56, 2003.

Capítulo 1: Application on infrared spectroscopy for the analysis of phenolic compounds in fruits

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ABSTRACT

Recent studies have demonstrated the metabolic benefits of phenolic compounds in human health. However, for the most part, analytical methods for quantification of phenolic compounds are time consuming, laborious, require a high volume of reagents with toxic substances and involve several steps that can result in systematic and instrumental errors. Spectroscopic techniques have been used as an alternative to these methods for the determination of bioactive compounds directly in the food matrix by minimal sample preparation, without using toxic reagents, which is a critical step of traditional methods. Therefore, this overview presents the advantages of these non-destructive methods focusing on infrared spectroscopy, for the quantification of phenolic compounds in fruits. In addition, the main difficulties in applying these spectroscopic techniques are presented, as well as a comparison between the quantification of phenolic compounds by traditional methods and spectroscopic methods. This review concludes that the most widely used are the derivatives combined with the partial least square (PLS) regression method. Furthermore, as much as they have some disadvantages and limitations, infrared spectroscopic techniques can allow for better quality control, resulting in higher productivity in order to create a more sustainable food processing chain, within the principles of green chemistry.

Keywords: bioactive compounds; infrared spectroscopy; non-invasive methods; green chemistry

1 INTRODUCTION

Over the last ten years, the study of bioactive compounds has gained special attention in scientific research due to the health benefits associated to their consumption, such as the elimination of free radicals, protection and regeneration of antioxidant compounds, while inhibiting the formation of pro-oxidants (GARCIA-SALAS et al., 2010; SOARES et al., 2020; XIE et al., 2019; YANG et al., 2018).

The antioxidant effects of phenolic bioactive compounds, from different sources, have been demonstrated to potentially improve the health of people with type 1 and 2 diabetes (BALDISSERA et al., 2017; ORHAN et al., 2013), to have hepatoprotective activities in human hepatic cells (SOBEH et al., 2018) and also to reduce the level of oxidative damage caused by hyperlipidemic diet in mice (CHU et al., 2019). Besides, the intake of phenolic compounds has also been related to some others chronic degenerative diseases such as cancer and Alzheimer's (ALI ASGAR, 2013; ALSUHAIBANI; AL-KURAIEEF, 2019; GUTIÉRREZ-GRIJALVA et al., 2016).

The first polyphenol cataloging database, PhenolExplorer, includes about 500 polyphenols in 400 different product types, ranging from alcoholic beverages to honeys, and most of the registered compounds were found in fruits (NEVEU et al., 2010). The current standard methods for quantification of phenolic compounds in food matrices are laborious, time-consuming, destructive, and use solvents that can generate toxic waste (LUTHRIA, 2006a; RINCÓN et al., 2019). Therefore, fast, straightforward, non-destructive, solvent-free alternative methods for phenolics quantification, in food matrices, using Near-infrared (NIR) and Mid-Infrared (MIR) spectroscopies have been proposed (CICCORITTI et al., 2019; GAUGLITZ, 2004; OLIVERI et al., 2020; RESENDE et al., 2020; SINGH et al., 2020). Table 1 shows the main differences between the infrared and conventional quantification methods.

Taking into consideration that the number of publications related to NIR and MIR non-destructive techniques and phenolic compounds has significantly increased in recent years and that consumers are increasingly aware of the health benefits associated with bioactive compounds and consequently willing to invest for healthier products (BAKER et al., 2022), the objective of this overview is to present the recent (last fifteen years) advances in the quantification of phenolic compounds in food matrices, in particular in fruits, recognized as one of the main sources of phenolics. In addition, pros and cons regarding the use of classical destructive methods as well as the alternative non-

destructive ones, based on infrared (IR) spectroscopies are presented. Due to the importance of the postharvest management, in fact, the need to have a general overview of the technologies available for measuring the quality of fruits and their derivatives is fundamental, since even when harvested under the same conditions, these natural products exhibit very distinct characteristics (LERMA-GARCÍA et al., 2018). Finally, additional perspectives from an analytical point of view and proposals for future studies are presented. The final goal of the present overview is, in fact, to help researchers and industries to develop the most appropriate and reliable analytical strategies for the determination of phenolic compounds in fruits.

2 BRIEF THEORY ABOUT INFRARED METHODS AND SIMPLIFIED PRACTICAL APPROACH TO CHEMOMETRIC

Infrared vibrational spectroscopy uses the electromagnetic radiation between visible and microwave and can be divided into three sub-regions (Stuart, 2005; Tahir et al., 2019): NIR, from 4.000 to 10.000 cm-1; MIR, from 4.000 to 400 cm-1; and Far Infrared (FAR), <400 cm-1. As the operation of the equipment and the theoretical background behind infrared equipment have already been discussed in the literature, we recommend reading the works of Pasquini (2003) and Stuart (2005).

The NIR and MIR spectra are composed of a large amount of data. The evaluation of peaks only, height or area, is not appropriated for quantification of phenolic compounds, therefore, multivariate data analysis must be used to extract hidden information, which is usually referred to as chemometric (LOHUMI et al., 2015). Chemometric methods can be used for exploratory analysis, classification, discriminate and regression methods and specific reviews on these topics can be found at Granato et al. (2018), Lohumi et al. (2015) and Oliveri et al. (2020).

Figure 1 shows the steps involved in non-destructive spectroscopic analyses and the data handling by using chemometric. The first step is the spectroscopic data acquisitions, which can occur either using whole intact fruit, powders or/and crushed fruits. A recent study evaluated the difference between the use of intact and crushed grape fruit in the determination of phenolic compounds, and showed that determination of these compounds in both sample states is possible and comparable, however with high accuracy results for crushed samples (ALEIXANDRE-TUDO; NIEUWOUDT; DU TOIT, 2019).

Table 1 - Difference of the analytical parameters by infrared methods and conventional methods.

Parameters	Infrared methods	Conventional methods
Waste formation	No	The waste comes from the extraction and quantification stages
Toxic waste	No	Waste needs to be disposed of properly so as not to harm the environment
Analysis speed	Fast (minutes)	Slow (hours)
On/in-line monitoring	Yes	No
Non-invasive and non-destructive analysis	Yes	No
Sample preparation	Few or no sample preparation	Samples need to be prepared according to the chosen extraction and quantification method (as detailed in Supplementary Material 1)
Quantitative and qualitative analysis	Yes	Yes
Analysis cost	Low, considering no reagents are required	High, considering that all consumables and reagents need to be constantly purchased
Development of standards and calibration curves for quantification	No, but depends on a first calibration by conventional methods	Colorimetric-based methods need calibration curves/standards for each data collection
Reagent use	No	Yes
Safer chemistry for accident prevention	Yes	No, the stages use many chemical substances that can cause accidents if handled improperly

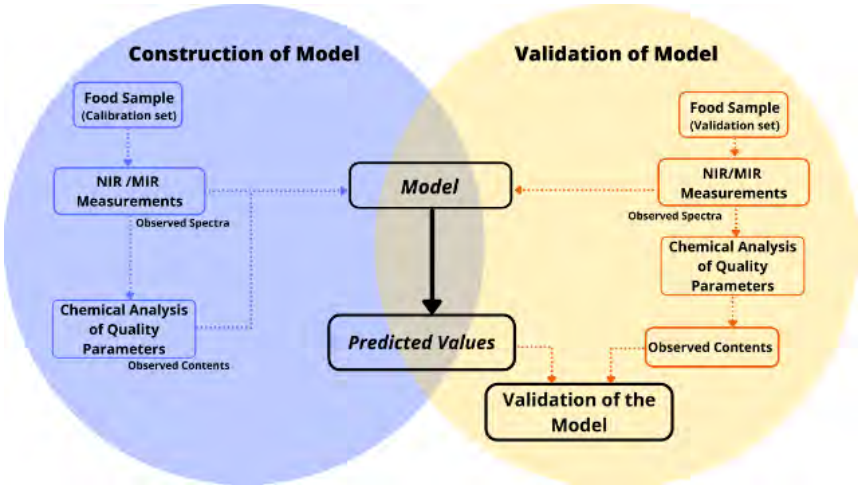
Elaborated by the author

After the data acquisition, multivariate data analysis must be used, for extracting useful information from the fingerprint spectroscopic signals recorded (OLIVERI et al., 2020). The data can be analyzed by supervisioned and non-supervisioned methods. For classification or regression models the samples are divided into two groups: calibration and validation groups. Generally, 75% of the samples are selected for the calibration set and 25% of them are selected for the validation set (LU; RASCO, 2012).

For the calibration and validation procedures, the spectroscopic and chemical data, obtained by standard methods, are processed with multivariate methods and a calibration model is obtained. The model can be evaluated using cross-validation procedure.

For the external validation of the model, spectroscopic and chemical data must be obtained using new samples and the predicted values can be obtained using the spectra data in the calibration model. The predicted and measured values are statically evaluated (CASTRIGNANÒ et al., 2019). An ideal model should have low values of the root mean square errors of calibration (RMSEC), cross-validation (RMSECV) and prediction (RMSEP), and high values of the coefficient of determination in calibration (R^2C), cross-validation (R^2CV) and prediction (R^2P) (MAGWAZA et al., 2016; SU; SUN, 2019; TAHIR et al., 2016). After the development of the validation model, it is only necessary to have the spectra of unknown sample to predict the chemical values, therefore it can be applied as non-destructive analyses.

Figure 1 - Diagram of the steps involved in the quantification of phenolic compounds by non-invasive methods.



Elaborated by the author

3 ANALYSES OF PHENOLIC COMPOUNDS

Quantification of phenolic compounds in food products has been performed with classical destructive methods like chromatographic or spectrophotometric methods using Folin–Ciocalteu reagent and non-destructive methods based on physical methods, like NIR and MIR infrared spectroscopies.

3.1 Conventional Destructive Methods

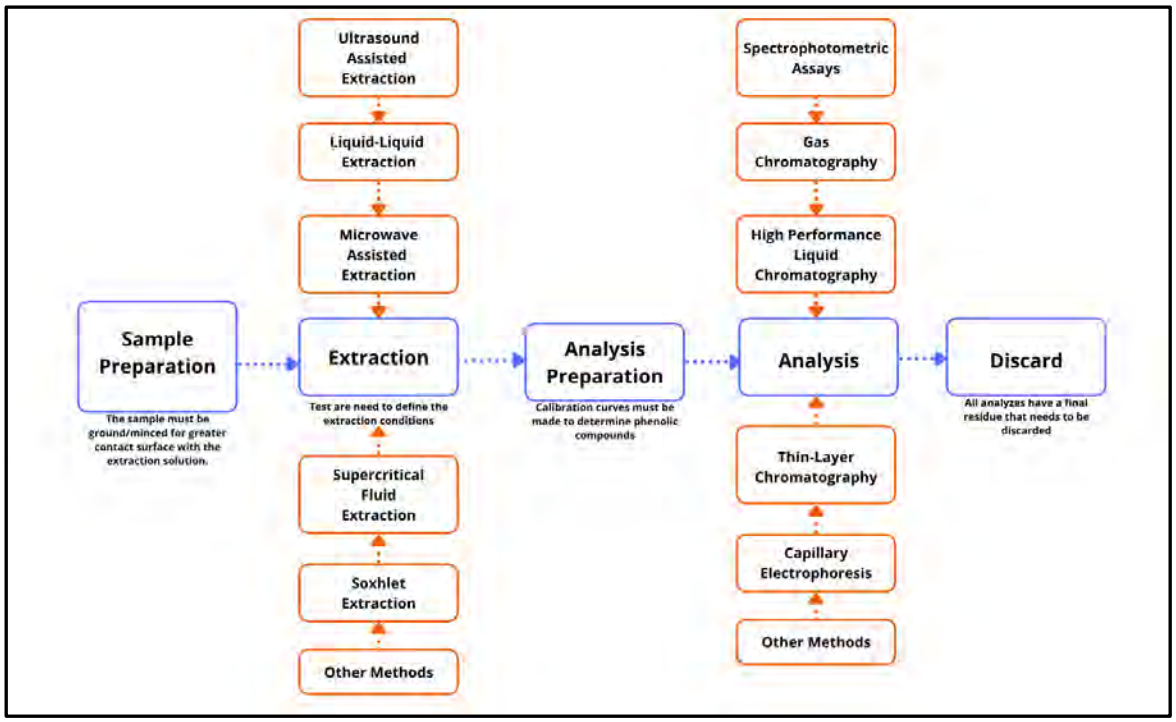
Studies and the respective destructive methods used in the analyses of phenolic compounds in fruits evidenced that the phenolic compounds have been extracted with different procedures and quantified with a chromatographic or spectrophotometric method, using Folin–Ciocalteu reagent (for more details see the Supplementary Material 1). It is possible to underlined the difficulty to reach a consensus on the concentration of the analyzed compound, independent of the fruit matrix analyzed. For example, four different extraction procedures have been used to study Buriti fruits, and the same problem has also been observed in the other fruits like camu-camu, passion fruit, lemon, acerola, blackberry, acai, apple, blueberry, manga and mangaba (Supplementary Material 1). In addition, different analytical methods (chromatographic or spectrophotometric) have been used for the analyses of the same active compound in the same sample/matrix (STRATIL; KLEJDUS; KUBÁŇ, 2006), which makes the results not comparable. Furthermore, these methods are time consuming and require laborious sample preparation, and needs an ecofriendly way to recover or discard the chemical waste at the end of the procedure.

Figure 2 shows the general schematic representation of the traditional methods for determining the concentration of phenolic compounds in food matrices. At first the sample preparation is required, that comprises several steps including freeze-drying, drying, grounding, filtration, and centrifugation (SANTOS-BUELGA et al., 2012). These procedures are necessary to improve sample stability, increase extraction efficiency and eliminate/reduce possible interferences (LUTHRIA, 2006b).

The use of different sample preparation processes, for the different food matrices, is mainly due to their chemical diversity (SANTOS-BUELGA et al., 2012). A reduction in sample particle size is generally recommended, which should be as small as possible to promote better extraction (KHODDAMI; WILKES; ROBERTS, 2013). Mitchell et al. (2017), demonstrated the use of dried and ground cocoa seeds, in which even small particle size variations may have a significant impact on the concentration of

procyanidins recovered. On the other hand, these processes result in errors due to the use of different protocols (MITRA; BRUKH, 2003). Therefore, it is noted that the initial manipulation should be done cautiously, especially if the sample amount is limited. Thus, it is necessary to investigate which treatments can lead to the best results in the databases to ensure the quality and consistency of the analyses.

Figure 2 - Diagram of the steps involved in the quantification of phenolic compounds by traditional methods.



Elaborated by the author; blue boxes represent the general steps in the determination of phenolic compounds by traditional methods; orange boxes exemplify the types of extractions and methods for quantification of phenolic compounds.

After proper sample handling, the extraction of phenolic compounds is carried out. This stage is essential for the recovery and isolation of bioactive compounds and, consequently, several parameters need to be controlled such as the extraction time, temperature, sample-solvent ratio, pressure and enzyme utilization. All these parameters should be constantly optimized to achieve better extraction levels (MEINI et al., 2019; PINELA et al., 2018; SILVA et al., 2015).

Besides this aspect, according to Naczk & Shahidi, (2006) and Stalikas, (2007), the extraction of phenolic compounds also depends on the chemical nature of these compounds; the presence of interfering substances that may interfere with the analysis, such as oils and waxes, sample particle size and storage conditions. Vizzotto & Pereira

(2011), studying extraction optimization processes for phenol determination in blackberry (Rubus sp.), found differences on total phenolic content according to the solvent used.

In addition to the choice of solvent, it is also necessary to choose the most suitable extraction method. Because of their simplicity, efficacy and versatility (STALIKAS, 2007), liquid–liquid and solid-liquid are the most applied for analysis of polyphenolics and phenolics in plants. Other methods such as supercritical solvent, ultrasound and soxhlet extraction are also employed and, in light of these considerations, it is clear that this step is the most time-consuming in the quantification process of phenolic compounds and may lead to significant analytical errors.

After defining the best extraction method, it is necessary to choose the best way to quantify phenolic compounds. The spectrophotometric methods using Folin-Ciocalteu reagent were used in most studies (HAMINIUK et al., 2012, Supplementary Material 1). However, this method requires a calibration curve, most often prepared using gallic acid, which demands even more manipulation time and, depending on the number of samples, even more reagent is discarded. Although it is a simple and inexpensive method, it only leads to a qualitative result, and it is not possible to quantify specific phenolic compounds (KHODDAMI; WILKES; ROBERTS, 2013). In a recent study, three different methods for the determination of phenolic compounds (Folin-Denis Reagent, Folin-Ciocalteu Reagent and Fast Blue BB) were compared under the same extraction condition for three different food matrices (pear orange, passion fruit and palmer mango) and the results showed a huge variation in the results (GUIMARÃES; SALGADO; CARVALHO, 2020). In example, for the pear orange, where the quantification by the Folin-Denis, Folin-Ciocalteu and Fast Blue BB method, were 196 mg of tannic acid/ 100g, 48.83 mg of gallic acid/ 100g and 1.84 mg of gallic acid/ 100g, respectively.

Another way to quantify the phenolic compounds is by using chromatography (ANGELO; JORGE, 2007). The disadvantage of these methods is the number of different existing approaches that could be used (normal phase chromatography, reversed phase chromatography, ion chromatography, etc.) in accordance to the nature of the interactions between the compounds to be analyzed (LAKSHMI, 2015), in other words, an specific chromatography equipment could be used only for determined compounds . It is also necessary to have a highly purified reference standard compound, since these methods depend on it to produce reliable results (WELLS; DANTUS, 1994). These methods also produce chemical wastes that have to be treated before discarding.

3.2 Analysis of Phenolic Compounds using IR Spectroscopies

Probably the main advantage of spectroscopic methods is the possibility to evaluate, measure and predict properties related to several chemical parameters in a single analysis. Therefore, spectroscopic methods can represent a major advance in quality monitoring systems for raw materials and functional/nutraceutical products (COZZOLINO, 2022). Several studies have related the importance of spectroscopic methods for predicting phenolic compounds in fruits. Details about these publications and the evaluation parameters of the regression models built are listed in the Supplementary Material 2.

Many algorithms, including pre-treatments, variable selections, modeling are applied for building a robustness model. Between regression methods used for the calibration model of phenolic compounds quantification, partial least squares regression (PLS) is the most widely used method, with different preprocessing methods strategies. PLS have been used for the calibration model of apricot (AMORIELLO; CICCORITTI; CARBONE, 2019), apples (GIOVANELLI et al., 2014; SCHMUTZLER; HUCK, 2016), strawberries (AMODIO et al., 2017), pomegranate (ARENDSE et al., 2018), grapefruit (OLAREWAJU et al., 2019), green pulp kiwi (CICCORITTI et al., 2019), blueberries (SINELLI et al., 2008), tomato (DING et al., 2016), white and red grapes (XIAO et al., 2018), between others. The use of associated methods with PLS have been also reported in order to increase the accuracy of the model. In example, linear discriminant analysis (LDA), together with the PLS, has been used to classify the fruits according to the storage time, which allowed the selection of strongly correlated variables and, consequently, the identification of more significant regions of the spectra (GIOVANELLI et al., 2014); as well as the radial basis function neural networks (RBF-NN) (DING et al., 2016) which uses linear learning algorithms to complement non-linear algorithms (JIA; ZHAO; DING, 2016), optimizing the accuracy of the models calculated.

Others studies also reported the use of least squares support vector machines (LS-SVM) (DONG et al., 2013; PISSARD et al., 2013; XIAO et al., 2018), back-propagation artificial neural networks (BP-ANN) (Dong et al., 2013), and modified partial least-squares (MPLS) (BLANCO-DÍAZ et al., 2014; NOGALES-BUENO et al., 2014).

The efficiency of phenolic compounds determination in hawthorn fruits, collected in different districts of China, was analyzed by three regression methods (PLS,

BP-ANN and LS-SVM). Dong et al. (2013) demonstrated that the use of BP-ANN, in the classification, and LS-SVM, in the regression, achieves better prediction results. Similarly, Xiao et al. (2018), evaluated the application of the PLS and LS-SVM regression methods for phenolic prediction in two grapefruit varieties and found that the model formulated by LS-SVM obtained better results. Other authors explain that in models using LS-SVM the calibration group samples are structured to a kernel space, where, to switch between the training error and the solution, a smoothing was used for all samples (VALYON; HORVÁTH, 2016). According to Oliveri & Simonetti (2016), artificial neural networks (ANNs) are a versatile qualification and quantification tools with several optimization cycles in order to improve model performance. It is also important underlined, that the use of ANN method was also turned into practical and commercial applications for NIR calibrations, as reported by FOSS Instrument reports (FOSS, 2018).

Nogales-bueno et al. (2014), using MPLS algorithm on NIR data of red grape fruit, proposed yet the use of hyperspectral imaging for the determination of phenolic compounds and demonstrated that this methodology is as effective as the more traditional NIR spectrophotometers. Others studies also highlight the application of hyperspectral imaging in NIR for predicting phenolic compounds with good modeling evaluation parameters (MALEGORI et al., 2017a; MUNERA et al., 2019).

Given these literature results, it can be emphasized that the development of chemometric methods, based on NIR and MIR data, have become valuable tools for food analysis. The technique has been used not only for the analysis of major components, but also for secondary metabolites with low concentrations, such as phenolic compounds. Such perspectives, increasingly aligned with the 4.0 industry concept, show that multivariate calibration has been improved to meet demands and minimize (and in many cases suppress) the negative impacts caused by traditional analytical methods.

4 LIMITATIONS AND DIFFICULTIES OF INFRARED METHODS

Conversely to the application of already optimized method, the development of a reliable predictive model using IR spectroscopy requires a lot of effort, in particular the knowledge of multivariate chemometric methods for analyses of the spectra, to extract useful information about food quality attributes (COZZOLINO et al., 2011; WANG et al., 2015). In addition, multivariate calibration techniques require specific calibration conditions such as sampling, spectrum acquisition and reference data, model validation,

spectral data preprocessing and others (COZZOLINO, 2014). The construction of mathematical and statistical procedures depends on conditions and products, which are analyzed, and could be not generalized. Therefore, the disadvantages of spectroscopic techniques are centered on the need of an initial step of model development, which implies a big effort in term of sample availability, reference analysis and mathematical modeling.

It is worth to notice that results obtained by the combination of spectroscopic measurement and multivariate modelling need to be compared with standard methods with the aim of confirming their reliability. According to these requirements (typical for secondary analytical methods) many actions are taking place to standardize this comparison; in more detail standards for good analytical practices are being written with the aim of make this strategy recognized from a regulatory point of view. As an example it is possible to mention the ISO 21543:2020 - Guidelines for the application of near infrared spectrometry for milk and milk products analysis or the ISO 12099:2017 referred to animal feeding stuffs, cereals and milled cereal products. Nowadays no international standardization is available for polyphenol quantification of fruit quality determination nevertheless the way towards the standardization of spectroscopic techniques is already open.

In this sense, in fact, calibration, the first stage of these spectroscopic analysis, is performed by collecting data of a set of samples with well know analyte concentrations (COZZOLINO, 2012; OZAKI et al., 2006) in the required range. This step also requires different sets of standard analyses. Therefore, it is important to highlight that for developing a good calibration model the quality of the results obtained by the reference methods need to be as high as possible. In more detail, being spectroscopy a secondary analytical method, the reliability of the data used for the training phase will strongly influence the error associated with the predictive model. It is impossible, in fact, to obtain spectroscopic results with a lower analytical error in respect to the error of the reference method because this error is incorporated in the modeling.

For assessing this important issue, recognized strategies validation needs to be performed. In more detail, regarding the quantification of phenolic compounds, the usual reference method is a colorimetric determination; which also quantify other compounds with phenolic groups, without antioxidant activity (PISSARD et al., 2013). Therefore, and consequently, the goodness of calibration determination is the key for developing good models.

Furthermore, each step in the calibration process has to be validated as even reference analyses can incorporate modeling error. Additionally, in order to develop a robust calibration model, a representative quantity of samples is required, making it a labor-intensive process (LI; SUN; CHENG, 2016). Depending on the objective of the study, in order to obtain a robust model with the least possible error, it is necessary to use samples from different varieties and origins, covering a useful variation range of the compound under study.

In addition to the extent of harvest samples required for the construction of a robust model, another difficulty concerns the numerous existing treatments developed to optimize the model prediction in each specific condition (BHATTACHARJEE, 2014; COZZOLINO et al., 2011; WANG et al., 2015), as detailed in the previous section. Therefore, the application of spectroscopic methods in the industry is linked to an appropriate model preparation, which may require much time and specific chemometric knowledge. According to Bhattacharjee (2014), as of software availability is high, choosing a specific data processing package does not mean getting the overall and exclusive solution of the problem.

Another disadvantage is that more sophisticated regression methods require higher number and varieties of samples (JIA; ZHAO; DING, 2016; VALYON; HORVÁTH, 2016), higher level of modeling overfitting (OLIVERI & SIMONETTI, 2016) and higher training time (CHAUCHARD et al., 2004). Therefore, it should be noted that using such regression methods must be done very carefully, since the numerous processes involved increases considerably the risk of overfitting.

In relation to NIR, its applications are more efficient in fruits that have a homogeneous internal structure (LI; SUN; CHENG, 2016). The thickness of the fruit, or its diameter influenced the penetration of the infrared radiation (PISSARD et al., 2018).

Another disadvantage is linked to the fact that each laboratory has its own manual of chemometric techniques, which are elaborated after several analyses and statistics. Above all, the data quality obtained versus yield of the analysis, and the lack of standardization hinders comparing the food analysis institutions (SOBOLEV et al., 2019). This difference can be observed in the data presented in Supplementary Material 2. It is noted there is a lack of important model evaluation data and different pre-treatments applied. However, PLS and pre-processing derivatives were used in most studies, representing a potential modeling to be tested universally for phenolic compounds.

5 CONCLUSION

Although they are considered effective, the most widely used techniques for quantifying phenolic compounds in fruits still need many improvements to be considered sustainable and standardized. This review demonstrates the potential of NIR and MIR infrared technologies, combined with multivariate analysis, as a low-cost, non-destructive or non-invasive method for quantification of phenolic compounds in diverse food. They are rapid methods with minimal or no sample preparation and therefore in consonance with Green Chemistry concept that allows the reduction/elimination of toxic reagents, toxic waste and can be easily automated and used in on line measurements.

However, the combined use of spectroscopic measurement and multivariate modeling still needs to be validated using standard methods. Even though the standardization of spectroscopic techniques is already open, no international standardization is available for polyphenol quantification of fruit quality determination, and is not a simple task as it involves multi-laboratory, multi operators and multiple instruments validation.

Besides this aspect, the concentration and the absolute number of analytes of interest is one of the main issues in the quantification of phenolic compounds in fruit. Independently on the analytical strategy applied, but in particular for secondary techniques like infrared spectroscopy, the development of analytical strategies able to detect and quantify such compounds is a real challenge. To try to overcome this limitation, the availability of a representative and significant number of samples is one of the main tasks that need to be achieved together with the reliability of the reference method used for training the model.

On the other hand, the low-cost portable infrared spectrometers have potential to perform the analyses in the field that can be a useful tool for harvesting the fruit based on phenolic content. Given the actual development of infrared spectroscopy instrumentation it is possible that in near the future, NIR infrared capability could be present in standard smartphone and the consumer may have access to the phenolic non-invasive quantification technology. All this could allow the consumer to choose the food based on chemical composition including phenolic content.

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REFERENCES

AL-QASSABI, J. S. A.; WELI, A. M.; HOSSAIN, M. A. Comparison of total phenols content and antioxidant potential of peel extracts of local and imported lemons samples. **Sustainable Chemistry and Pharmacy**, v. 8, n. December 2017, p. 71–75, 2018.

ALEIXANDRE-TUDO, J. L.; NIEUWOUDT, H.; DU TOIT, W. Towards on-line monitoring of phenolic content in red wine grapes: A feasibility study. **Food Chemistry**, v. 270, n. March 2018, p. 322–331, 2019.

ALI ASGAR, M. Anti-diabetic potential of phenolic compounds: A review. **International Journal of Food Properties**, v. 16, n. 1, p. 91–103, 2013.

ALSUHAIBANI, A. M. A.; AL-KURAIEEF, A. N. Effect of phenolic compounds and fatty acid contents of walnut seeds on streptozotocin-induced diabetes in rats. **Journal of Food Measurement and Characterization**, v. 13, n. 1, p. 499–505, 2019.

AMODIO, M. L. et al. Potential of NIR spectroscopy for predicting internal quality and discriminating among strawberry fruits from different production systems. **Postharvest Biology and Technology**, v. 125, p. 112–121, 2017.

AMORIELLO, T.; CICCORITTI, R.; CARBONE, K. Vibrational spectroscopy as a green technology for predicting nutraceutical properties and antiradical potential of early-to-late apricot genotypes. **Postharvest Biology and Technology**, v. 155, n. June, p. 156–166, 2019.

ANGELO, P. M.; JORGE, N. Compostos fenólicos em alimentos Uma breve revisão. **Revista do Instituto Adolfo Lutz**, v. 66, n. 1, p. 1–9, 2007.

ARENDSE, E. et al. Development of calibration models for the evaluation of pomegranate aril quality by Fourier-transform near infrared spectroscopy combined with chemometrics. **Biosystems Engineering**, v. 159, p. 22–32, 2017.

ARENDSE, E. et al. Evaluation of biochemical markers associated with the development of husk scald and the use of diffuse reflectance NIR spectroscopy to predict husk scald in pomegranate fruit. **Scientia Horticulturae**, v. 232, n. October 2017, p. 240–249, 2018.

BAKER, M. T. et al. Consumer Acceptance toward Functional Foods: A Scoping Review. **International Journal of Environmental Research and Public Health**, v. 19, n. 3, 2022.

BALDISSERA, M. D. et al. Antihyperglycemic, antioxidant activities of tucumã oil (Astrocaryum vulgare) in alloxan-induced diabetic mice, and identification of fatty acid

profile by gas chromatograph: New natural source to treat hyperglycemia. **Chemico-Biological Interactions**, v. 270, p. 51–58, 2017.

BATAGLION, G. A. et al. Simultaneous quantification of phenolic compounds in buriti fruit (Mauritia flexuosa L.f.) by ultra-high performance liquid chromatography coupled to tandem mass spectrometry. **Food Research International**, v. 66, p. 396–400, 2014.

BATAGLION, G. A. et al. Determination of the phenolic composition from Brazilian tropical fruits by UHPLC-MS/MS. **Food Chemistry**, v. 180, p. 280–287, 2015.

BHATTACHARJEE, S. Chemometrics in analytical chemistry. **Journal of AOAC International**, v. 97, n. 1, p. 1–2, 2014.

BLANCO-DÍAZ, M. T. et al. Use of visible and near-infrared spectroscopy for predicting antioxidant compounds in summer squash (Cucurbita pepo ssp pepo). **Food Chemistry**, v. 164, p. 301–308, 2014.

CANTERI, M. H. G. et al. A Comparative Study of Pectin Extracted from Passion Fruit Rind Flours. **Journal of Polymers and the Environment**, v. 18, n. 4, p. 593–599, 2010.

CASTRIGNANÒ, A. et al. Assessing the Feasibility of a Miniaturized Near-Infrared Spectrometer in Determining Quality Attributes of San Marzano Tomato. **Food Analytical Methods**, v. 12, n. 7, p. 1497–1510, 2019.

CHAUCHARD, F. et al. Application of LS-SVM to non-linear phenomena in NIR spectroscopy: Development of a robust and portable sensor for acidity prediction in grapes. **Chemometrics and Intelligent Laboratory Systems**, v. 71, n. 2, p. 141–150, 2004.

CHIRINOS, R. et al. Antioxidant compounds and antioxidant capacity of Peruvian camu camu (Myrciaria dubia (H.B.K.) McVaugh) fruit at different maturity stages. **Food Chemistry**, v. 120, n. 4, p. 1019–1024, 2010.

CHU, L. et al. Chemical composition, antioxidant activities of polysaccharide from Pine needle (Pinus massoniana) and hypolipidemic effect in high-fat diet-induced mice. **International Journal of Biological Macromolecules**, v. 125, p. 445–452, 2019.

CICCORITTI, R. et al. FT-NIR spectroscopy and multivariate classification strategies for the postharvest quality of green-fleshed kiwifruit varieties. **Scientia Horticulturae**, v. 257, n. June, p. 108622, 2019.

COZZOLINO, D. et al. Multivariate data analysis applied to spectroscopy: Potential application to juice and fruit quality. **Food Research International**, v. 44, n. 7, p. 1888–1896, 2011.

COZZOLINO, D. Recent trends on the use of infrared spectroscopy to trace and authenticate natural and agricultural food products. **Applied Spectroscopy Reviews**, v. 47, n. 7, p. 518–530, 2012.

COZZOLINO, D. Sample presentation, sources of error and future perspectives on the application of vibrational spectroscopy in the wine industry. **Journal of the Science of Food and Agriculture**, v. 95, n. 5, p. 861–868, 2014.

COZZOLINO, D. An Overview of the Successful Application of Vibrational Spectroscopy Techniques to Quantify Nutraceuticals in Fruits and Plants. **Foods**, v. 11, n. 3, 2022.

CRUZ, R. G. DA et al. Comparison of the antioxidant property of acerola extracts with synthetic antioxidants using an in vivo method with yeasts. **Food Chemistry**, v. 277, n. June 2018, p. 698–705, 2019.

DING, X. et al. A novel NIR spectroscopic method for rapid analyses of lycopene , total acid , sugar , phenols and antioxidant activity in dehydrated tomato samples. **Vibrational Spectroscopy**, v. 82, p. 1–9, 2016.

DONG, W.; NI, Y.; KOKOT, S. A Near-Infrared Reflectance Spectroscopy Method for Direct Analysis of Several Chemical Components and Properties of Fruit, for Example, Chinese Hawthorn. **Journal of Agricultural and Food Chemistry**, v. 61, p. 540–546, 2013.

DONG, X. et al. The maturity degree, phenolic compounds and antioxidant activity of Eureka lemon [Citrus limon (L.) Burm. f.]: A negative correlation between total phenolic content, antioxidant capacity and soluble solid content. **Scientia Horticulturae**, v. 243, n. August 2018, p. 281–289, 2019.

DUTRA, R. L. T. et al. Bioaccessibility and antioxidant activity of phenolic compounds in frozen pulps of Brazilian exotic fruits exposed to simulated gastrointestinal conditions. **Food Research International**, v. 100, p. 650–657, 2017.

ESCARPA, A.; GONZÁLEZ, M. C. High-performance liquid chromatography with diode-array detection for the determination of phenolic compounds in peel and pulp from different apple varieties. **Journal of Chromatography A**, v. 823, n. 1–2, p. 331–337, 1998.

FERREIRA, D. S.; DE ROSSO, V. V.; MERCADANTE, A. Z. Bioactive compounds of blackberry fruits (Rubus spp.) grown in Brazil | Compostos bioativos presentes em amora-preta (Rubus spp.). **Revista Brasileira de Fruticultura**, v. 32, n. 3, p. 664–674, 2010.

FOSS. Best practice for calibration of NIR instruments with global models. n. May, 2018.

GARCIA-SALAS, P. et al. Phenolic-compound-extraction systems for fruit and vegetable samples. **Molecules**, v. 15, n. 12, p. 8813–8826, 2010.

GAUGLITZ, E. G. **Handbook of spectroscopy**. [s.l: s.n.]. v. 41

GIOVANELLI, G. et al. NIR spectroscopy for the optimization of postharvest apple management. **Postharvest Biology and Technology**, v. 87, p. 13–20, 2014.

GORDON, A. et al. Chemical characterization and evaluation of antioxidant properties of Açaí fruits (Euterpe oleraceae Mart.) during ripening. **Food Chemistry**, v. 133, n. 2, p. 256–263, 2012.

GRANATO, D. et al. Trends in Chemometrics: Food Authentication, Microbiology, and Effects of Processing. **Comprehensive Reviews in Food Science and Food Safety**, v. 17, n. 3, p. 663–677, 2018.

GUIMARÃES, K. C.; SALGADO, D. L.; CARVALHO, E. E. N. Evaluation of different methodologies for the determination of phenolic compounds in tropical fruits. **Brazilian Journal of Food Technology**, v. 23, p. 1–7, 2020.

GUTIÉRREZ-GRIJALVA, E. P. et al. Review: Dietary phenolic compounds, health benefits and bioaccessibility. **Archivos Latinoamericanos de Nutricion**, v. 66, n. 2, p. 87–100, 2016.

HAMINIUK, C. W. I. et al. Phenolic compounds in fruits - an overview. **International Journal of Food Science and Technology**, v. 47, n. 10, p. 2023–2044, 2012.

ISO 12099. **Animal feeding stuffs, cereals and milled cereal products - Guidelines for the application of near infrared spectrometry**. [s.l: s.n.].

ISO 21543. **Milk and milk products — Guidelines for the application of near infrared spectrometry**. [s.l: s.n.].

JIA, W.; ZHAO, D.; DING, L. An optimized RBF neural network algorithm based on partial least squares and genetic algorithm for classification of small sample. **Applied Soft Computing Journal**, v. 48, p. 373–384, 2016.

KHODDAMI, A.; WILKES, M. A.; ROBERTS, T. H. Techniques for analysis of plant phenolic compounds. **Molecules**, v. 18, n. 2, p. 2328–2375, 2013.

LAKSHMI, S. Research and Reviews: Journal of Pharmaceutical Analysis A Review on Chromatography with High Performance Liquid Chromatography (HPLC) and its Functions. **Journal of Pharmaceutical Analysis**, v. 4, n. 1, p. 1–15, 2015.

LERMA-GARCÍA, M. J. et al. Variety Discrimination of Fruits, Edible Plants, and Other Foodstuffs and Beverages by Infrared Spectroscopy. **Comprehensive Analytical Chemistry**, v. 80, p. 127–163, 2018.

LI, J. L.; SUN, D. W.; CHENG, J. H. Recent Advances in Nondestructive Analytical Techniques for Determining the Total Soluble Solids in Fruits: A Review. **Comprehensive Reviews in Food Science and Food Safety**, v. 15, n. 5, p. 897–911, 2016.

LIMA, J. P. et al. The antioxidative potential and volatile constituents of mangaba fruit over the storage period. **Scientia Horticulturae**, v. 194, p. 1–6, 2015.

LIMA, V. L. et al. Total phenolic and carotenoid contents in acerola genotypes

harvested at three ripening stages. **Food Chemistry**, v. 90, n. 4, p. 565–568, 2005.

LOHUMI, S. et al. A review of vibrational spectroscopic techniques for the detection of food authenticity and adulteration. **Trends in Food Science and Technology**, v. 46, n. 1, p. 85–98, 2015.

LÓPEZ-VARGAS, J. H. et al. Chemical, Physico-chemical, Technological, Antibacterial and antioxidant properties of dietary fiber powder obtained from yellow passion fruit (Passiflora edulis var. flavicarpa) co-products. **Food Research International**, v. 51, n. 2, p. 756–763, 2013.

LU, X.; RASCO, B. A. Determination of Antioxidant Content and Antioxidant Activity in Foods using Infrared Spectroscopy and Chemometrics: A Review. **Critical Reviews in Food Science and Nutrition**, v. 52, n. 10, p. 853–875, 2012.

LUTHRIA, D. L. Application of green chemistry principles for extraction of phytolipids and phenolic compounds. **Indian Journal of Chemistry - Section B Organic and Medicinal Chemistry**, v. 45, n. 10, p. 2291–2296, 2006a.

LUTHRIA, D. L. Significance of sample preparation in developing analytical methodologies for accurate estimation of bioactive compounds in functional foods. **Journal of the science of food and agriculture**, v. 86, p. 2266–2272, 2006b.

MAGWAZA, L. S. et al. Development of NIRS models for rapid quantification of protein content in sweetpotato [Ipomoea batatas (L.) LAM.]. **LWT - Food Science and Technology**, v. 72, p. 63–70, 2016.

MALEGORI, C. et al. Comparing the analytical performances of Micro-NIR and FT-NIR spectrometers in the evaluation of acerola fruit quality, using PLS and SVM regression algorithms. **Talanta**, v. 165, n. December 2016, p. 112–116, 2017.

MEINI, M. R. et al. Recovery of phenolic antioxidants from Syrah grape pomace through the optimization of an enzymatic extraction process. **Food Chemistry**, v. 283, p. 257–264, 2019.

MILANEZ, J. T. et al. Pre-harvest studies of buriti (Mauritia flexuosa L.F.), a Brazilian native fruit, for the characterization of ideal harvest point and ripening stages. **Scientia Horticulturae**, v. 202, p. 77–82, 2016.

MILANEZ, J. T. et al. Bioactive compounds and antioxidant activity of buriti fruits, during the postharvest, harvested at different ripening stages. **Scientia Horticulturae**, v. 227, n. September 2017, p. 10–21, 2018.

MITCHELL, A. E.; ROBERTSON, D.; KOH, E. Optimizing the Extraction of Procyanidins Oligomers through Decamer. **Nutrition & Food Science International Journal**, v. 4, n. 3, p. 1–7, 2017.

MITRA, S.; BRUKH, R. **Sample Preparation Techniques in Analytical Chemistry**. United States of America: [s.n.].

MORAES, J. O. et al. Study of rabbiteye blueberry (Vaccinium ashei Reade) in the process of food products. **Ciencia e Tecnologia de Alimentos**, v. 27, n. SUPPL.1, p. 18–22, 2007.

MUNERA, S. et al. Maturity monitoring of intact fruit and arils of pomegranate cv. 'Mollar de Elche' using machine vision and chemometrics. **Postharvest Biology and Technology**, v. 156, n. June, p. 110936, 2019.

NACZK, M.; SHAHIDI, F. Phenolics in cereals, fruits and vegetables: Occurrence, extraction and analysis. **Journal of Pharmaceutical and Biomedical Analysis**, v. 41, n. 5, p. 1523–1542, 2006.

NEVES, L. C. et al. Bioactive compounds and antioxidant activity in pre-harvest camu-camu [Myrciaria dubia (H.B.K.) Mc Vaugh] fruits. **Scientia Horticulturae**, v. 186, p. 223–229, 2015.

NEVEU, V. et al. Phenol-Explorer: an online comprehensive database on polyphenol contents in foods. **Database : the journal of biological databases and curation**, v. 2010, p. 1–9, 2010.

NOGALES-BUENO, J. et al. Determination of technological maturity of grapes and total phenolic compounds of grape skins in red and white cultivars during ripening by near infrared hyperspectral image : A preliminary approach. **Food Chemistry**, v. 152, p. 586–591, 2014.

OLAREWAJU, O. O. et al. Model development for non-destructive determination of rind biochemical properties of 'Marsh' grapefruit using visible to near-infrared spectroscopy and chemometrics. **Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy**, v. 209, p. 62–69, 2019.

OLESZEK, W. et al. Identification of Some Phenolic Compounds in Apples. **Journal of Agricultural and Food Chemistry**, v. 36, n. 3, p. 430–432, 1988.

OLIVEIRA, A. C. et al. Total phenolic content and free radical scavenging activities of methanolic extract powders of tropical fruit residues. **Food Chemistry**, v. 115, n. 2, p. 469–475, 2009.

OLIVERI, P. et al. **Application of Chemometrics in the Food Sciences**. 2. ed. [s.l.] Elsevier Inc., 2020.

OLIVERI, P.; MALEGORI, C.; CASALE, M. Chemometrics: Multivariate Analysis of Chemical Data. **Chemical Analysis of Food, 2nd Edition**, n. June, p. 1–43, 2020.

OLIVERI, P.; SIMONETTI, R. **Chemometrics for Food Authenticity Applications**. [s.l.] Elsevier Ltd, 2016.

ORHAN, N. et al. In vivo and in vitro antidiabetic effect of Cistus laurifolius L. and detection of major phenolic compounds by UPLC-TOF-MS analysis. **Journal of Ethnopharmacology**, v. 146, n. 3, p. 859–865, 2013.

OZAKI, Y.; MCCLURE, W. F.; CHRISTY, A. Near-Infrared Spectroscopy in Food Science and Technology. In: **Near-Infrared Spectroscopy in Food Science and Technology**. [s.l: s.n.]. v. 1p. 405.

PALAFOX-CARLOS, H.; YAHIA, E. M.; GONZÁLEZ-AGUILAR, G. A. Identification and quantification of major phenolic compounds from mango (Mangifera indica, cv. Ataulfo) fruit by HPLC-DAD-MS/MS-ESI and their individual contribution to the antioxidant activity during ripening. **Food Chemistry**, v. 135, n. 1, p. 105–111, 2012.

PASQUINI, C. Near infrared spectroscopy: Fundamentals, practical aspects and analytical applications. **Journal of the Brazilian Chemical Society**, v. 14, n. 2, p. 198–219, 2003.

PINELA, J. et al. Cold extraction of phenolic compounds from watercress by high hydrostatic pressure: Process modelling and optimization. **Separation and Purification Technology**, v. 192, p. 501–512, 2018.

PISSARD, A. et al. Non-destructive measurement of vitamin C , total polyphenol and sugar content in apples using near-infrared spectroscopy. n. May 2012, p. 238–244, 2013.

PISSARD, A. et al. Use of NIR spectroscopy on fresh apples to determine the phenolic compounds and dry matter content in peel and flesh. **Biotechnology, Agronomy and Society and Environment**, v. 22, n. 1, p. 3–12, 2018.

RESENDE, L. M.; FRANCA, A. S.; OLIVEIRA, L. S. Buriti (Mauritia flexuosa L. f.) fruit by-products flours: Evaluation as source of dietary fibers and natural antioxidants. **Food Chemistry**, v. 270, n. December 2017, p. 53–60, 2019.

RESENDE, L. M.; OLIVEIRA, L. S.; FRANCA, A. S. Characterization of jabuticaba (Plinia cauliflora) peel flours and prediction of compounds by FTIR analysis. **Lwt**, v. 133, n. August, p. 110135, 2020.

REYNERTSON, K. A. et al. Quantitative analysis of antiradical phenolic constituents from fourteen edible Myrtaceae fruits. **Food Chemistry**, v. 109, n. 4, p. 883–890, 2008.

RIBEIRO, S. M. R. et al. Antioxidant in mango (Mangifera indica L.) pulp. **Plant Foods for Human Nutrition**, v. 62, n. 1, p. 13–17, 2007.

RIBEIRO, S. M. R. et al. Phenolic compounds and antioxidant capacity of Brazilian mango (Mangifera indica L.) varieties. **Food Chemistry**, v. 110, n. 3, p. 620–626, 2008.

RINCÓN, E. et al. Mechanochemical extraction of antioxidant phenolic compounds from Mediterranean and medicinal Laurus nobilis: A comparative study with other traditional and green novel techniques. **Industrial Crops and Products**, v. 141, n. July, p. 111805, 2019.

RODRÍGUEZ-PULIDO, F. J. et al. Measurement of ripening of raspberries (Rubus idaeus L) by near infrared and colorimetric imaging techniques. **Journal of Food**

Science and Technology, v. 54, n. 9, p. 2797–2803, 2017.

RUFINO, M. S. M. et al. Total phenolic content and antioxidant activity in Acerola, Açaí, Mangaba and Uvaia fruits by DPPH method. **Acta Horticulturae**, v. 841, p. 459–462, 2009.

SANTOS-BUELGA, C. et al. **Extraction and isolation of phenolic compounds**. [s.l: s.n.]. v. 864

SANTOS, G. M. DOS et al. Correlação entre atividade antioxidante e compostos bioativos de polpas comerciais de açaí (Euterpe oleracea Mart). **Archivos Latinoamericanos de Nutricion**, v. 58, n. 2, p. 187–192, 2008.

SCHMUTZLER, M.; HUCK, C. W. Simultaneous detection of total antioxidant capacity and total soluble solids content by Fourier transform near-infrared (FT-NIR) spectroscopy : A quick and sensitive method for on-site analyses of apples. **Food Control**, v. 66, p. 27–37, 2016.

SILVA, P. B. .; DUARTE, C. R. .; BARROZO, M. A. S. Dehydration of acerola (Malpighia emarginata D.C.) residue in a new designed rotary dryer: Effect of process variables on main bioactive compounds. **Food and Bioproducts Processing**, v. 98, p. 62–70, 2016.

SILVA, P. B.; DUARTE, C. R.; BARROZO, M. A. S. A novel system for drying of agro-industrial acerola (Malpighia emarginata D. C.) waste for use as bioactive compound source. **Innovative Food Science and Emerging Technologies**, v. 52, n. November 2018, p. 350–357, 2019.

SILVA, W. P. N. et al. Study of phenol removal by cloud point extraction: A process optimization using experimental design. **Separation and Purification Technology**, v. 152, p. 133–139, 2015.

SINELLI, N. et al. Evaluation of quality and nutraceutical content of blueberries (Vaccinium corymbosum L.) by near and mid-infrared spectroscopy. **Postharvest Biology and Technology**, v. 50, n. 1, p. 31–36, 2008.

SINGH, B. et al. Phenolic composition, antioxidant potential and health benefits of citrus peel. **Food Research International**, v. 132, p. 109114, 2020.

SOARES, J. C. et al. Phenolic profile and potential beneficial effects of underutilized Brazilian native fruits on scavenging of ROS and RNS and anti-inflammatory and antimicrobial properties. **Food and Function**, v. 11, n. 10, p. 8905–8917, 2020.

SOBEH, M. et al. Phenolic compounds from Syzygium jambos (Myrtaceae) exhibit distinct antioxidant and hepatoprotective activities in vivo. **Journal of Functional Foods**, v. 41, n. June 2017, p. 223–231, 2018.

SOBOLEV, A. P. et al. Use of NMR applications to tackle future food fraud issues. **Trends in Food Science & Technology**, v. 91, n. July, p. 347–353, 2019.

SPAGOLLA, L. C. et al. Extração alcoólica de fenólicos e flavonóides totais de mirtilo “Rabbiteye” (Vaccinium ashei) e sua atividade antioxidante. **Revista de Ciencias Farmaceuticas Basica e Aplicada**, v. 30, n. 2, p. 187–191, 2009.

STALIKAS, C. D. Extraction, separation, and detection methods for phenolic acids and flavonoids. **Journal of Separation Science**, v. 30, n. 18, p. 3268–3295, 2007.

STRATIL, P.; KLEJDUS, B.; KUBÁŇ, V. Determination of total content of phenolic compounds and their antioxidant activity in vegetables - Evaluation of spectrophotometric methods. **Journal of Agricultural and Food Chemistry**, v. 54, n. 3, p. 607–616, 2006.

STUART, B. H. **Infrared Spectroscopy: Fundamentals and Applications**. [s.l: s.n.]. v. 8

SU, W. H.; SUN, D. W. Mid-infrared (MIR) Spectroscopy for Quality Analysis of Liquid Foods. **Food Engineering Reviews**, v. 11, n. 3, p. 142–158, 2019.

TAHIR, H. E. et al. Near-Infrared (NIR) Spectroscopy for Rapid Measurement of Antioxidant Properties and Discrimination of Sudanese Honeys from Different Botanical Origin. **Food Analytical Methods**, v. 9, n. 9, p. 2631–2641, 2016.

VALYON, J.; HORVÁTH, G. A robust LS-SVM regression A Robust LS-SVM Regression. **INTERNATIONAL JOURNAL OF COMPUTATIONAL INTELLIGENCE**, v. 3, n. February, p. 148–153, 2016.

VEBERIC, R. et al. Phenolic compounds in some apple (Malus domestica Borkh) cultivars of organic and integrated production. **Journal of the Science of Food and Agriculture**, v. 85, n. 10, p. 1687–1694, 2005.

VIZZOTO, M.; PEREIRA, M. C. Metodologia Científica: Otimização do Processo de Extração de Compostos Fenólicos Antioxidantes de Mirtilo (Vaccinium ashei Reade). **Embrapa Clima Temperado. Boletim de pesquisa e desenvolvimento**, v. 101, p. 19, 2009.

VIZZOTTO, M. et al. Teor de compostos fenólicos e atividade antioxidante em diferentes genótipos de amoreira-preta (Rubus sp.). **Revista Brasileira de Fruticultura**, v. 34, n. 3, p. 853–858, 2012.

VIZZOTTO, M.; PEREIRA, M. C. Amora-preta (Rubus sp.): Otimização do processo de extração para determinação de compostos fenólicos antioxidantes. **Revista Brasileira de Fruticultura**, v. 33, n. 4, p. 1209–1214, 2011.

WANG, H. et al. Fruit quality evaluation using spectroscopy technology: A review. **Sensors (Switzerland)**, v. 15, n. 5, p. 11889–11927, 2015.

WELLS, M.; DANTUS, M. Validation of chromatographic methods. **Analytical Instrumentation Handbook, Third Edition**, n. November, p. 1015–1033, 1994.

XIAO, H. et al. Quality assessment and discrimination of intact white and red grapes

from Vitis vinifera L. at five ripening stages by visible and near-infrared spectroscopy. **Scientia Horticulturae**, v. 233, n. 1, p. 99–107, 2018.

XIE, Y. et al. Immunomodulatory , antioxidant and intestinal morphology-regulating activities of alfalfa polysaccharides in mice. **International Journal of Biological Macromolecules**, v. 133, p. 1107–1114, 2019.

YANG, J. et al. Comparative Effects of Curcumin and Tetrahydrocurcumin on Dextran Sulfate Sodium-induced Colitis and Inflammatory Signaling in Mice. **Journal of Cancer Prevention**, v. 23, n. 1, p. 18–24, 2018.

ZHANG, P.; ZHOU, Z. Postharvest ethephon degreening improves fruit color, flavor quality and increases antioxidant capacity in ‘Eureka’ lemon (Citrus limon (L.) Burm. f.). **Scientia Horticulturae**, v. 248, n. January, p. 70–80, 2019.

SUPPLEMENTARY MATERIAL

Supplementary Material 1 – Current methodologies available on literature in order to quantify total phenolic compounds in different fruit matrix.

Fruit matrix	Raw material condition	Analytical method	Extraction method	Reference
Acerola	Lyophilized pulp powder	Folin–Ciocalteu reagent	Distilled water / agitation - 35 min / 35 ºC Followed filtered vacuum	(CRUZ et al., 2019)
	Fresh pulp crushed and	Folin–Ciocalteu reagent	80% ethanol / 1% HCl conc. / 25 ºC / 20 min followed filtered	(LIMA et al., 2005)
	Dried pulp	Folin–Ciocalteu reagent	Washed with methanol	(SILVA et al., 2019)
	Dried pulp	Folin–Ciocalteu reagent	Ethanol in two ways; 1º - acerola immersed in 93.2 ºGL ethanol/ 2º - Ethanol was sprayed on the residues /1.5h	(SILVA Et al., 2016)
Açai	Lyophilized pulp	High-performance liquid chromatography (HPLC)	500 mg of pulp + 10 mL of acetone-water-formic acid (70%-29%-1%; v/v/v) were sonicated for 10 min / centrifuged for 10 min at 10ºC and 10.000 rpm / residue was extracted once more with 10 mL acetone-water-formic acid / supernatants were combined / extract was vaporized in rotary evaporator (30º) / aqueous supernatant was shaken with 10 mL ethyl acetate / residue was solubilized in 1mL methanol-water-formic acid (50%-49%-1%; v/v/v)	(GORDON et al., 2012)
	Fresh pulp	Ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS)	Benzoic acid-d5 and trans-resveratrol-d4 (IS) were added to 500 g of fruit pulp + 500 mL of methanol in glass blender / liquid extracts were freeze-dried / 1 mg of the dried extracts was dissolved in methanol / filtered in PVDF.	(BATAGLION et al., 2015)
	Fresh pulp	Folin-Denis reagent	Indiscriminate	(SANTOS et al., 2008)
	Fresh pulp and peel	High-performance liquid chromatography (HPLC)	5 and 10 g of peel and pulp were extracted with methanol 1%, 2.6-di-tert-butyl-4-methyphenol (BHT) using ultrasonic bath / sample were extracted with 10 mL for 1h, 10 mL for 30 min and 5 mL for 30 min / three extracted were combined.	(ESCARPA; GONZÁLEZ, 1998)

Apple	Lyophilized pulp and peel	High-performance liquid chromatography (HPLC)	300 g of dried skin and pulp blended with 3x3 L methanol for 5 min / filtered in vacuo to the final volume of 1 L / extract was washed 3x with hexane.	(OLESZEK et al., 1988)
	Frozen pulp and peel	High-performance liquid chromatography (HPLC)	5 and 10 g of peel and pulp were extracted with methanol 1%, 2.6-di-tert-butyl-4-methyphenol (BHT) using ultrasonic bath / sample were extracted with 10 mL for 1h, 10 mL for 30 min and 5 mL for 30 min / three extracted were combined.	(VEBERIC et al., 2005)
Blackberry	Frozen fruit and crushed	Folin–Ciocalteu reagent	5 g of crushed blackberry homogenized with different volumes of methanol and a solvent mixture for 0.2, 4.6 and 24 hours	(VIZZOTTO; PEREIRA, 2011)
	Frozen fruit and crushed	Folin-Ciocalteu reagent	10 g of blackberry with methanol: water (8: 2) in ultrasound for 20 minutes / the sample was filtered through a Buchner funnel and washed with methanol / filtrates were combined and concentrated on a rotary evaporator (<40 °C) up to about 100 mL / solution was centrifuged for 20 min / stored in a freezer (-36 ° C)	(FERREIRA; DE ROSSO; MERCADANTE, 2010)
	Frozen fruit and crushed	Folin-Ciocalteu reagent	5 g of blackberry were crushed with acid ethanol (0.01% HCl) / centrifuged at around 4ºC and 15,000 rpm / supernatant were utilized for analysis	(VIZZOTTO et al., 2012)
Blueberry	Frozen fruit and crushed	Folin-Ciocalteu reagent	5 g of blueberry + different volumes of methanol (10, 20, 30, 40 and 50 mL) / centrifuged for different times (0,2,4,6 and 24 h) at 4 ºC	(VIZZOTO; PEREIRA, 2009)
	Fresh frozen pulp and dried pulp	Folin-Ciocalteu reagent	Solution containing 5 g of treated samples were homogenized for 1 h at 70 ºC in agitator / filtered were transferred for assays tube + 10 mL alcoholic solution	(SPAGOLLA et al., 2009)
	Frozen fruits	Folin-Ciocalteau reagent	Extraction with MetOH and clarification with $Ba(OH)_2$ and $Zn(SO)_4$	(MORAES et al., 2007)
Buriti	Fresh pulp	Folin–Ciocalteu reagent	Indiscriminate	(MILANEZ et al., 2016)
	Lyophilized fruit	Folin–Ciocalteu reagent	Extraction carried out in water and ethanol	(MILANEZ et al., 2018)
	Pulp flour	Modified Folin-Ciocalteu reagent	1g (flour) of Buriti extracted with methanol (50% v/v) and acetone (70% v/v), 40 mL each / mixture centrifuged at 3500 rpm for 15 min / supernatant completed to 100 mL with distilled water	(RESENDE; FRANCA; OLIVEIRA, 2019b)

	Fresh pulp	UHPLC-ESI-MS/MS	Fresh pulp + 500 mL of methanol for 15 min in agitator / centrifuged (5 min; 4000 rpm) / supernatants separated and evaporated to dryness / 1mg of extract dissolved in 1 mL of methanol and filtered.	(BATAGLION et al., 2014)
Camu Camu	Fresh pulp and peel	Folin–Ciocalteu reagent	50g of fruit / 80% methanol; homogenized for 90 s flushed with nitrogen for 3 min; centrifuged at 4000 rpm for 20 min at 4 ºC / supernatant was collected	(CHIRINOS et al., 2010)
	Frozen pulp and peel	Folin-Ciocalteau reagent	Indiscriminate	(NEVES et al., 2015)
	Fresh pulp	High performance liquid chromatography coupled to mass spectrometry (HPLC–	Benzoic acid + trans resveratrol + 500g of fruit pulp / analytes extracted with 500 mL methanol / freeze – dried and 1g was dissolved in methanol / filtered	(BATAGLION et al., 2015)
	Lyophilized fruit	High-performance liquid chromatography (HPLC) and Folin–Ciocalteu reagent	10g of freeze-dried homogenized with 200 mL formic acid + sonicated for 1h / extracts centrifuged – filtered / extracts combined and concentrated under reduced pressure in 40ºC / aliquots (2 – 4 mL) were prepared stored at 20ºC	(REYNERTSON et al., 2008)
Lemon	Pulp and peel powder	Folin–Ciocalteu reagent	2g pulp (1g peel) + 8 mL of methanol + 30 min in ultrasonic at 50ºC / solution centrifuged at 10.000g for 5 min / extraction procedure repeated three times	(ZHANG; ZHOU, 2019)
	Pulp and peel powder	Folin–Ciocalteu reagent	Lemon powder mixed with 15 mL of 80% methanol ultrasonically for 30 min / supernatants were diluted in 50 mL methanol	(DONG et al., 2019)
	Peel powder	Modified Folin-Ciocalteu reagent	Extraction with methanol for 72hr / the extracts suspended in 250 mL of water and partition with hexane, chloroform, ethyl acetate and butanol	(AL-QASSABI; WELI; HOSSAIN, 2018).
Mangaba	Fresh and lyophilized pulp	Folin-Ciocalteu reagent	10 g + 40 mL of methanol: water (50:50 v/v) + 40 mL of acetone: water (70:30 v/v) for 1h / supernatant + 100 mL distilled water.	(RUFINO et al., 2009)
	Fresh pulp	Folin-Ciocalteu reagent	5 g samples + 40 mL of methanol: water (50:50 v/v) for 1 h / centrifuged at 25.400 g for 15 min / supernatant recovered / residue + 40 mL of acetone: water (70:30 v/v) extracted for 1 h and centrifuged / extracts combined and brought to a final volume of 100 mL with distilled water.	(LIMA et al., 2015)

	Fruit and fruit pulp powder	High-performance liquid chromatography (HPLC)	0.5 g + 20 mL of methanol: water (50:50 v/v) / subjected for 30 min in ultrasonic bath at 25 ºC / centrifuged at 6.000 g for 15 min / supernatant recovered / residue + 20 mL of acetone: water (70:30 v/v) / ultrasonic bath for 30 min / extracts combined and brought to a final volume of 50 mL with deionized water	(DUTRA et al., 2017)
Mango	Peel and seed powder	Folin-Ciocalteu reagent	Peel and seed (5 g each) + 20 mL of methanol: water (60:40 v/v) / centrifuged / supernatant was adjusted to 25 mL.	(RIBEIRO et al., 2008)
	Lyophilized pulp	High-performance liquid chromatography (HPLC)	0.5 g of freeze-dried samples + 20 mL of 80% methanol / homogenized and sonicated for 30 min at 30 ºC / centrifuged at 12.000 rpm for 15 min at 5 ºC / filtered.	(PALAFOX-CARLOS; YAHIA; GONZÁLEZ-AGUILAR, 2012)
	Lyophilized pulp	High-performance liquid chromatography (HPLC)	1 g lyophilized material + 10 mL of methanol: water (60:40 v/v) / shaked at 180 rpm for 30 min/ centrifuged at 1.000 g for 10 min / supernatant used immediately.	(RIBEIRO et al., 2007)
Passion fruit	Rind flour	Folin–Ciocalteu reagent	70% ethanol / 24h at -18ºC followed by centrifuge	(CANTERI et al., 2010)
	Fruit albedo powder	High-performance liquid chromatography (HPLC) and Folin–Ciocalteu reagent	Methanol with water followed ultrasonic bath / 2h	(LÓPEZ-VARGAS et al., 2013)
	Solid residual portion (seed and peel)	Folin–Ciocalteu reagent	Hexane and methanol / supernatant - 60 °C rotary evaporator	(OLIVEIRA et al., 2009)

Elaborated by the author

Supplementary Material 2 - Evaluation parameters of the different models arranged in the literature for the quantification of phenolic compounds in fruits.

Wavelength (nm)	Fruit	Calibration				Cross Validations		Prediction			Reference
		Regression Method	Preprocessing Method	RMSEC	R^2C	RMSECV	R^2CV	RMSEP	R^2P	RPD	
1.333-1.064	Pomegranate	PLS	First Derivative + MSC	0.14	76.82	ND	ND	0.12	72.41	1.97	(ARENDSE et al., 2017)
1.333-1.064		PLS	First Derivative + MSC	0.11	86.48	ND	ND	0.11	82.61	2.47	
2.500 – 833	Kiwifruit	PLS	Second Derivative	0.24	0.96	0.56	0.81	0.50	0.94	4.08	(CICCORITTI et al., 2019)
2.380-1.149	Apricot	PLS	Second Derivative	0.44	0.99	1.66	0.96	0.98	0.98	9.65	(AMORIELLO; CICCORITTI; CARBONE, 2019)
2.778-833	Apple	PLS	SNV	41	0.95	54	0.92	ND	ND	ND	(GIOVANELLI et al., 2014)
14.286-2.500	Blueberries	PLS	SNV	ND	ND	0.14	0.93	0.16	0.94	2.21	(SINELLI et al., 2008)
2.778-833		PLS	MSC	ND	ND	0.14	0.96	0.18	0.87	2.05	
2.258-2.357 1.836-1.639	Strawberry	PLS	Smoothing	21.78	0.45	22.35	0.42	19.46	0.45	1.45	(AMODIO et al., 2017)
2.269-1.025	Apple	PLS	Baseline Correction	ND	ND	ND	ND	0.12	0.85	3.0	(SCHMUTZLER; HUCK, 2016)
2.500-400	Grapefruit	PLS	SG (2nd Order)	0.44	0.47	ND	ND	0.37	0.59	1.03	(OLAREWAJU et al., 2019)
1.650-950	Raspberry	PLS	ND	116.3	0.73	121.4	0.73	ND	ND	ND	(RODRÍGUEZ-PULIDO et al., 2017)

1.050-720	Pomegranate	PLS-R PLS-DA	Mean Center + SNV + SG	0.24	0.88	0.24	0.84	0.25	0.86	2.7	(MUNERA et al., 2019)
2.500-800	Chinese Hawthorn	LS-SVM	First Derivative	ND	ND	ND	ND	ND	ND	ND	(DONG et al., 2013)
2.500-900	Grapefruit	LS-SVM	ND	0.193	0.90	ND	ND	0.216	0.87	2.77	(XIAO et al., 2018)
2.500-900		LS-SVM	ND	0.115	0.95	ND	ND	0.150	0.91	3.33	
2.500-400	Apples	LS-SVM	First Derivative + SG	178	ND	ND	ND	140	0.94	5.1	(PISSARD et al., 2013)
2.500-400	Summer squash	MPLS	SNV + DeTrend	0.62	0.79	0.67	0.76	0.73	0.68	1.77	(BLANCO-DÍAZ et al., 2014)
1.650-950	Grapefruit	MPLS	SNV + DeTrend	0.89	1.01	1,07	ND	1.23	ND	ND	(NOGALES-BUENO et al., 2014)
ND	Tomato	RBF-NN	ND	0.04	0.99	ND	ND	0.08	0.95	3.33	(DING et al., 2016)

Elaborated by the author; PLS: partial least square; PLS-DA: partial least square-discriminant analysis; LS-SVM: least squares support vector machines; RBF-NN: radial basis function neural networks; MPLS: modified partial least square; MSC: multiplicative scattering correction; SNV: vector normalization; SG: savitzky-golay; RMSEC: root mean square errors of calibration; RMSECV: root mean square of cross-validation; RMSEP: root mean square of prediction; R^2C: determinant coefficient of calibration; R^2CV: determinant coefficient of cross-validation; R^2P: determinant coefficient of prediction; RPD: residual predictive deviation; ND: not discriminated.

Capítulo 2: Systemic characterization of Pupunha (*Bactris gasipaes*) flour with views of polyphenol content on cytotoxicity and protein in vitro digestion.

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ABSTRACT

Although the Amazonian fruit pupunha *(Bactris gasipaes)* presents interesting properties such as richness in phenolic compounds (PC), it is little consumed. Its application could be expanded by producing a material with a longer shelf life which could be used in different types of products. Therefore, the objective of the present study was to produce pupunha flour (PF) from fruit harvested at different locations and characterized, through various physico-chemical analyses and different spectroscopic techniques, as well as to evaluate its cytotoxic effects and *in vitro* protein digestion. The PF were usefully categorized by principal component analyses and small dependencies on the region of origin were detected. Probably due to its high content of phenolic compounds, PF strongly reduced protein digestion. The cytotoxicity results showed that PF had cytotoxic potential in L929 cells. Nonetheless, considering unique properties of PF applications, there is a need for new forms of flour production to attenuate the results observed.

KEYWORDS: phenolic compounds, Amazonian fruit, NMR, XRD, *in vitro* digestion, inhibition.

1 INTRODUCTION

The Amazon region is home to a large number of fruits that have high nutritional potentials, especially Pupunha (*Bactris gasipaes*), Bacaba (*Oenocarpus bacana*), Buriti (*Mauritia flexuosa*) or Inaja *(Attalea maripa*) (SANTOS et al., 2015). These fruits are rich in essential macronutrients and phytonutrients such as carotenoids, sterols and fatty acids (ARAÚJO et al., 2021; BERTO et al., 2015; VIRGOLIN; SEIXAS; JANZANTTI, 2017). Nevertheless, the use of these fruits has been rarely explored commercially (NERI-NUMA et al., 2018; SILVA; OLIVEIRA, 2018) although this could have a great socioeconomic impact, strengthening local/regional economies.

To reach this objective, developing materials with long shelf-life such as flours offers the possibility to incorporate them into products with greater added value. For example, pupunha flour is suitable to prepare cookies (RECK; MIRANDA, 2016), cakes (MARTÍNEZ-GIRÓN; FIGUEROA-MOLANO; ORDÓÑEZ-SANTOS, 2017), pastas (OLIVEIRA et al., 2006), and snacks (CARVALHO et al., 2009b). In addition, Rojas-Garbanzo et al. (2012) found that the content in phenolic compounds of pupunha was preserved throughout the drying process to produce flour. Similarly, Ribeiro et al. (2021) observed that the carotenoid concentration was maintained after processing cookies using pupunha flour.

Despite those advantages, there are two main limitations to the exploitation of pupunha flour. The variability of characteristics depending on the location where the fruit is harvested is insufficiently documented (YUYAMA et al., 2003). In addition, the presence of polyphenols may represent some health and nutrition disadvantage, depending on their nature and concentration. For example, the tea polyphenol epigallocatechin gallate (EGCG) at 400µM has a clear detrimental effect on HT29 cell viability (D'AGOSTINO et al., 2012), same negative effect found for bitter melon seed in L929 cells (ANDRADE et al., 2022). Furthermore, He, Lv and Yao (2007) and Quesada et al. (1996) found that food phenolic compounds may inhibit enzymes during the digestive process. In the case of proteases, this could lead to reduced protein digestibility.

In this context, a wide dissemination and use of pupunha flour in food systems requires more in-depth research that also considers the regional variability of the raw material. The objective of the present study was first to produce flour from pupunha fruits from different areas of the Amazon region and characterize them thoroughly through various physico-chemical methods (thermal behavior, spectroscopic and diffraction

techniques) so as to generate a fingerprinting database of pupunha. Second, the safety and nutritional aspects were investigated by evaluating the cytotoxic effect of the material produced on L929 cells, as established by ISO 10993-5 (2009), and the impact on protein digestibility when incorporated into a dairy food matrix.

2 MATERIAL AND METHODS

2.1 Material

Pupunha fruits were harvested in the states of Acre and Rondônia (Brazil). After receiving the fruits, they were inspected for any mechanical damage and/or dirt, and then sanitized in sodium hypochlorite solution (100 ppm) for 15 minutes. After this process, they were selected considering uniformity of size and color. For flour production, the fruit was used in its entirety (pulp + peel). Table 1 shows the fruit harvest location.

Table 1 - Harvesting location of the fruit samples used for the manufacture of pupunha flours.

Location of Pupunha Fruit Harvest			
Samples	Latitude	Longitude	City (State)
PF – 01	10º 0’ 25” South	67º 50’ 44” West	CEASA – Rio Branco (Acre)
PF – 02	9º 35’ 33” South	67º 32’ 26” West	Porto Acre (Acre)
PF – 03	9° 56’ 44” South	67º 10’ 35” West	Ramal do Bonal – Rio Branco (Acre)
PF – 04	9º 45’ 15” South	66º 36’ 48” West	Nova Califórnia (Rondônia)
PF – 05	9º 46’ 14” South	66º 21’ 22” West	Extrema (Rondônia)
PF – 06	8º 46’ 3” South	63º 52’ 12” West	Porto Velho (Rondônia)

PF: pupunha flour; elaborated by author.

Pupunha flours (PF) were produced by freeze-drying the fruits (Terroni, LC1500, São Carlos, Brazil) for a period of 48h. The drying time was established based on preliminary tests. At the end of drying, the samples were ground in a knife mill (Marconi, MA340, Piracicaba, Brazil), and a 16 *mesh* sieve was used to standardize their granulometry. After sieved, the flours were stored at -18°C.

2.2 Methods

2.2.1 Proximate composition

The moisture, crude protein, ash, lipid, carbohydrates and crude fiber contents of the flours were determined according to the methodologies defined by AOAC (1980) and AOAC (1985).

2.2.2 Mineral composition

The elemental composition of the flours was determined using the methodology proposed by Nogueira and Souza (2005). The concentrations of sulfur, phosphorus, and boron were analyzed using a colorimetric spectrophotometer (Femto, 600 Soft, Brazil), potassium was analyzed using a flame photometer (Micronal, B462, Brazil) and calcium, iron, magnesium, copper, zinc and manganese were analyzed using an atomic absorption spectrophotometer (Varian, Fast Sequential AA240FS, USA).

2.2.3 Preparation of the phenolic extract

The extraction of free phenolic compounds was performed according to Santos et al. (2015), with adaptations. Initially, 20 mL of water/methanol (50:50; v/v) were added to tubes containing 5 g of flour for the first extraction step. Then, the tubes were homogenized (300 rpm; 1h) in a shaker (Marconi, MA 420, Piracicaba, Brazil) and centrifuged (Eppendorf, 5430R, Germany) at 7830 rpm at 15º C for 10 min. The supernatant was collected and placed in an amber bottle. 20 ml of water/acetone (30:70; v/v) were added to the residue from the previous step and subjected to the same agitation and centrifugation rate. At the end, the methanol and acetone extracts were combined and stored at -18ºC.

2.2.4 Phenolic Content and Antioxidant Potential Analysis

The quantification of total phenolics was determined by the Folin-Ciocalteau method according to Singleton, Orthofer and Lamuela-Raventós (1999). 0.5 mL aliquots of the diluted extract solutions were added to a tube with 2.5 mL of Folin-Ciocalteau reagent. Then, 2.0 mL of sodium carbonate solution (7.5%) was added to each tube, which was homogenized and the solution was kept at rest for another 2 hours. The absorbance reading was performed on a Spectrum One spectrophotometer (Perkin-Elmer, Waltham, MA, USA) at 740 nm and the values were converted into mg of gallic acid/g of flour using a gallic acid standard curve.

The antioxidant potential was measured by the Ferric Reducing Antioxidant Power (FRAP), Radial Capture 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and Oxygen Radical Absorption Capacity (ORAC) methods, all performed in triplicate. The first method, FRAP, was performed following the methodology of Benzie and Strain (1996) with adaptations. Initially, an aliquot (0.1 mL) of extract was removed and then 2.9 mL of the FRAP reagent solution was added. The mixture was incubated in

a thermostated bath (37 °C; 30 min, Marconi, MA 159, Piracicaba, BR). Afterwards, the samples were read at 593 nm in a spectrophotometer (Thermo Scientific, Genesysm 10S UV-Vis, USA) using trolox as a standard.

The analysis by the $ABTS^{\bullet+}$ radical followed the methodology proposed by Larrauri, Rupérez and Saura-Calixto (1997) with modifications. 0.3 mL of extract was added to 3 mL of the $ABTS^{\bullet+}$ radical, homogenized and, after 6 minutes of mixing, the reading was performed in a spectrophotometer at 734 nm (Thermo Scientific, Genesysm 10S UV-Vis, USA). The final result was expressed in TE (Trolox equivalent) in µmol $TE.g^{-1}$.

The antioxidant potential by the ORAC method was performed according to Ou, Hampsch-Woodill and Prior (2001). 25 µL of extract were added to 150 µL of fluorescein and placed under incubation in a microplate for 10 min at 37 ºC in a spectrofluorometer (BMG Labtech, FLUOstar OPTIMA, Germany). Afterwards, 25 µL of AAPPH were added to each well. Fluorescein reduction was measured for 120 min at 528 nm with an excitation of 485 nm. Trolox was used as an external standard and the results were expressed in µmol $TE.g\text{-}^{1}$.

2.2.5 Principal Component Analysis (PCA)

PCA was used as a qualitative method to observe the variation between chemical samples (MANCINI et al., 2020), grouping and/or separating them depending on the similarity of proximate composition data, content of phenolic compounds and antioxidant activity.

2.2.6 Fourier Transform Infrared Spectroscopy

The FTIR measurements were carried out by a Vertex 70 Fourier Bruker spectrometer (Germany) with coupled accessory to measure the Attenuated Total Reflectance (ATR), from 4000 to 400 cm^{-1}, with a spectral resolution of $4cm\text{-}^{1}$ and 32 scans.

2.2.7 X-Ray Diffractometry (XRD)

XRD analyses were conducted on a MiniFlex 300 benchtop diffractometer (Rigaku, Tokyo, Japan). The samples were subjected to Cu-Ka monochromatic UV/ionizing radiation ($\lambda = 0.1542$ nm) under voltage of 30 kV, current of 10 mA, angular range of 3-40º (θ-2θ) and with a step of 0.01º A Relative crystallinity (RC) and relative crystal size (L_{HKL}) referring to the 15.3º, 17.1º, 18.4º, 21.5º and 23.1º crystal planes were

calculated as described by Valencia et al. (2015) and Villas-Boas et al. (2020), respectively, using PAN-analytical™ X'pert high score Plus and PeakFit v. 4.12.

2.2.8 Differential Scanning Calorimetry (DSC)

The thermoanalytical behavior of pupunha flour samples was analyzed in duplicate by DSC, in the range of 0 to 200º C, using a heating ramp of 10º C/min and continuous flow of non-oxidative N_2 atmosphere of 50 mL/min. The parameters of initial transition temperature (Ti), peak transition temperature (Tp) and enthalpy of reaction (Δh) were determined by DSC Q100 equipment using the TAV4 software (TA Instruments, New Castle, PA, USA)

2.2.9 Solid State 13C Nuclear Magnetic Resonance (NMR) Spectroscopy

High-resolution Solid state ^{13}C NMR spectra were acquired using a Bruker® Avance 400 spectrometer (υ = 100.5 MHz for ^{13}C and 400.0 MHz for ^{1}H). The experiments were performed in a 4 mm magic angle sample spinning (MAS) probe at 25.0 ± 0.5 °C. The samples were packed in a 4 mm zirconia rotor and subjected to a spinning frequency of 12000 ± 2 Hz. The spectra were acquired with Cross Polarization pulse sequence with a 90° pulse duration were 2.5 µs and 5.0 µs for ^{1}H and ^{13}C nuclei, respectively, contact time of 1 ms, last delay (d1) of 5 s, n = 1024, acquisition time (t_a) of 40 ms and ^{1}H-^{13}C decoupling frequency of 70 kHz. The chemical shifts were calibrated from the hexamethylbenzene (HMB)methyl peak at 17.3 ppm. Crystallinity values and double helix content were calculated by deconvolution of the spectra from Voigt functions, according to the procedure described by Villas-Boas et al. (2020), using the PeakFit software v. 4.12.

2.2.10 Cytotoxicity of pupunha flour

The phenolic extract of pupunha flour was prepared following the methodology proposed by Rochetti and Fukumasu (2015) with modifications. Initially, 15g of pupunha flour were diluted in 300 mL of water/acetone (70:30; v/v) and water/methanol (50:50; v/v). The mixture was kept at rest for 48 h at room temperature in a dark place. Afterwards, the solution was filtered in a vacuum system and the solvents used for the extraction were removed by distillation under reduced pressure (Fisatom, mod.802, Brazil) for approximately 50 min at 55ºC. At the end of rotaevaporation, the aqueous fraction that contained the phenolic compounds was frozen and lyophilized for 48 h (Terroni, LC1500, São Carlos, Brazil) to obtain the pure phenolic fraction of the pupunha flour.

The cytotoxicity of phenolic extracts from pupunha flour was performed

according to the standard method ISO 10993-5 (2009). Initially, 100 µL/well of commercial cells (L929) were plated, in triplicate, at a concentration of 5×10^3 cells/well in 96 wells, containing DMEM-F12 culture medium (Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12; ThermoFisher, Carlsbad, CA), 10 % fetal bovine serum (InvitrogenTM, Carlsbad, CA) and 1% antibiotics (InvitrogenTM, Carlsbad, CA) and then incubated (37°C / 5% CO_2 / 24 hours). The phenolic extract obtained from pupunha flour was diluted in the culture medium at 0.000128 mg/mL, 0.00064 mg/mL, 0.0032 mg/mL, 0.016 mg/mL, 0.08 mg/mL, 0.4 mg/mL, 2 mg/mL and 10 mg/mL and the cells were incubated again under the described conditions.

After 24 hours, cell viability was analyzed using the MTT colorimetric assay (Thyazolyl Blue Tetrazolium Bromide – Sigma Aldrich). The cytotoxicity index (IC50) was determined using GraphPad Prism 9.2 software.

2.2.11 Pupunha flour incorporation into a yogurt: effects on in vitro protein digestion

The impact of Pupunha flour on protein digestion in a model product (stirred yogurt) was evaluated using the INFOGEST *in vitro* digestion protocol with adaptations (BRODKORB et al., 2019). Yogurts without the addition of flour (control) and with 25% of Pupunha flour incorporated into the formulation were evaluated. Pupunha flour, PF-02, was used and digestions were performed in triplicates.

Briefly, 5 g of yogurt was homogenized with 5 mL of commercial saliva (pooled saliva, LeeBiosolutions, Maryland Heights, USA). After 2 min at 37°C under agitation, the gastric phase of digestion was initiated: 8 mL of simulated gastric fluid (SGF) was added to the mixture and the pH was adjusted to 3.0 with 1 M HCl. 5 µL of $CaCl_2$, 1 mL of pepsin (final concentration 2000U/mL) and distilled water were added to adjust volume to 10 mL. Gastric digestion was performed at 37°C under agitation by 60 min.

The intestinal phase was started by adding 8.5 mL of simulated intestinal fluid (SIF) and the pH was adjusted to 7.0 using 2M NaOH. 2.5 mL of bile solution (final concentration 10mM of bile salts), 40 µL of $CaCl_2$ and 5 mL of pancreatin (final concentration: 100 U/mL based on trypsin activity) were added and the final volume was adjusted to 40 mL using distilled water. The reaction mixture was incubated at 37 °C under agitation for 1 h until the end of digestion.

To monitor the release of free NH_2 groups induced by the enzymatic hydrolysis of protein, samples were collected at 5, 15, 30 and 60 min of both the gastric and intestinal phases of digestion. Free NH_2 groups were measured by the O-phthaldehyde (OPA)

method adapted from Church et al. (1985). 100 mL of reagent was prepared with 2.5 mL of OPA (10 mg/mL in ethanol), 2.5 mL of 20% SDS, 50 µL of β-mercaptoethanol and 95 mL of 20 mM sodium tetraborate. 96-well UV clear plates and a Multiskan™ GO microplate spectrophotometer (Thermo Fisher Scientific, Waltham, USA) were used to measure the absorbance at 340 nm after 10 min of contact with regular agitation between the OPA reagent (100 µL) and diluted samples (50 µL). Each sample was measured in triplicate, and the number of α-amino groups released during hydrolysis was estimated based on the difference in mean absorption at 340 nm between the hydrolyzed and non-hydrolyzed samples, using L-methionine for the calibration curve (0 - 2 mM).

3 RESULTS AND DISCUSSION

3.1 Centesimal Composition

The pupunha flour had a high concentration of carbohydrates (average of 86.35 g/100 g of DM) followed by lipids (average of 8.61 g/ 100 g of DM), proteins (average of 6.34 g/ 100 g of DM) and fibers (average of 2.92 g/100 g of DM) (Table 2). These values are close to those reported by Carvalho et al. (2009), Kaefer et al. (2013) and Rojas-Garbanzo et al., 2012), also for pupunha flour.

The centesimal compositions of the fruits are significantly influenced by the geographic origin. The concentration of carbohydrates, lipids, protein and fiber varied between, 82.4 and 92.0 g/100 g of DM, 4.6 and 11.2 g/100 g of DM, 4.83 and 9.70 g/100 g of DM and 1.7 and 5.4 g/100 g of DM, respectively (Table 2).

Barbosa, Moraes and Chisté (2020) and Yuyama et al. (2003) studied the fruit of Jacaicá (A. *amazonicum*) and Pupunha (*Bactris gasipaes*) from different regions of Brazilian Amazonian states (Pará and Amazonas) and Peru, and also observed significant differences in the centesimal composition of the fruits in function of geographic origin. These results show that the different pupunha growing conditions (rainfall index, type of climate, soil quality, luminosity, and other conditions) affect in the centesimal composition of its fruits, and consequently, the derived food products that use it.

3.2 Mineral Composition

The pupunha flour collected in the states of Acre (PF-01 to PF-03) and Rondônia (PF-03 to PF-06), showed significant differences in the concentration of all macro and microminerals analyzed (Table 2).

Table 2 - Characterization of the chemical composition (elemental composition, phenolic compound content, antioxidant properties) and physical properties, (thermal (DSC) and crystallinity by XRD and NMR techniques) for the six pupunha flours.

Analyze		**Pupunha flour (PF)**						
		PF - 01	**PF - 02**	**PF - 03**	**PF - 04**	**PF - 05**	**PF - 06**	**Average**
Chemical composition	Water content (g/100 g of flour)	$6.41^{b} \pm 0.4$	$5.22^{c} \pm 0.1$	$6.49^{b} \pm 0.4$	$8.01^{a} \pm 0.2$	$4.85^{c} \pm 0.5$	$7.17^{b} \pm 0.3$	**6.36 ± 1.2**
	Protein (g/100 g of DM*)	$6.09^{b} \pm 0.1$	$4.83^{e} \pm 0.01$	$5.70^{c} \pm 0.1$	$6.16^{b} \pm 0.01$	$5.56^{d} \pm 0.01$	$9.70^{a} \pm 0.1$	**6.34 ± 1.7**
	Glycidic fraction (g/100 g of DM)	$11.15^{a} \pm 0.01$	$4.65^{d} \pm 0.6$	$9.10^{b} \pm 0.01$	$8.40^{b} \pm 0.2$	$11.23^{a} \pm 0.2$	$7.15^{c} \pm 0.1$	**8.61 ± 2.5**
	Fiber (g/100 g of DM)	$1.69^{d} \pm 0$	$2.08^{cd} \pm 0$	$2.45^{c} \pm 0.3$	$4.05^{b} \pm 0.2$	$1.93^{cd} \pm 0.1$	$5.36^{a} \pm 0.2$	**2.92 ± 1.5**
	Carbohydrate (g/100 g of DM)	$85.20^{c} \pm 0.6$	$91.99^{a} \pm 0.7$	$87.25^{b} \pm 0.1$	$87.37^{b} \pm 0.3$	$83.91^{d} \pm 0.1$	$82.38^{e} \pm 0.3$	**86.35 ± 3.4**
	Ash (g/100 g of DM)	$2.28^{b} \pm 0.1$	$1.67^{d} \pm 0$	$1.99^{c} \pm 0$	$2.04^{c} \pm 0$	$2.22^{b} \pm 0.1$	$2.58^{a} \pm 0.1$	**2.13 ± 0.3**
Elemental composition	N (g/kg)	$8.7^{b} \pm 0.03$	$7.1^{c} \pm 0.15$	$8.9^{b} \pm 0.67$	$9.3^{b} \pm 0.43$	$9^{b} \pm 0.26$	$13.4^{a} \pm 0.14$	**9.3 ± 0.23**
	P (g/kg)	$0.44^{bc} \pm 0.01$	$0.39^{c} \pm 0.02$	$0.56^{a} \pm 0.06$	$0.41^{c} \pm 0.02$	$0.63^{a} \pm 0.12$	$0.54^{ab} \pm 0.03$	**0.5 ± 0.04**
	K (g/kg)	$8.5^{b} \pm 0.37$	$6^{d} \pm 0.06$	$13^{a} \pm 0.58$	$7.2^{c} \pm 0.06$	$13.3^{a} \pm 0.95$	$8.9^{b} \pm 0.15$	**9.5 ± 0.35**
	Ca (g/kg)	$17.8^{a} \pm 2.16$	$17.7^{a} \pm 4.62$	$2.3^{c} \pm 0.17$	$21.5^{a} \pm 4.98$	$2.3^{c} \pm 0.06$	$8.5^{b} \pm 0.42$	**11.7 ± 2.25**
	Mg	$0.7^{b} \pm 0.09$	$0.4^{cd} \pm 0.03$	$0.5^{c} \pm 0.06$	$0.8^{b} \pm 0.09$	$0.3^{d} \pm 0.1$	$1.2^{a} \pm 0.07$	**0.6 ± 0.03**

	(g/kg)							
	S (g/kg)	0.4^{b} ± 0.01	0.3^{b} ± 0.17	1.9^{a} ± 0.35	0.2^{b} ± 0.04	2.2^{a} ± 0.06	0.4^{b} ± 0.17	**0.9 ± 0.13**
	Cu (mg/kg)	22.9^{ab} ± 2.69	23.6^{ab} ± 5.22	16.4^{ab} ± 6.42	25^{a} ± 3.03	21.9^{ab} ± 6.53	15^{b} ± 2.29	**20.8 ± 1.93**
	Fe (mg/kg)	68.6^{c} ± 4.10	40.2^{d} ± 7.02	312.1^{b} ± 17.04	68.2^{c} ± 6.74	442.6^{a} ± 14.50	58.9^{c} ± 4.60	**165.1 ± 5.43**
	Mn (mg/kg)	13.9^{d} ± 0.87	9.6^{e} ± 0.20	24.9^{a} ± 0.85	19.4^{c} ± 1.66	10^{e} ± 0.28	21.6^{b} ± 0.46	**16.6 ± 0.54**
	Zn (mg/kg)	31.4^{c} ± 0.55	29.4^{c} ± 0.75	44.3^{a} ± 1.23	30.2^{c} ± 1.66	37.9^{b} ± 1.34	38.9^{b} ± 1.54	**35.8 ± 0.44**
Antioxidant properties	Total Phenolics (mgAGE**/g of DM)	1.84^{cb} ± 0.06	2.52^{a} ± 0.03	1.94^{b} ± 0.04	1.96^{b} ± 0.03	1.73^{c} ± 0.15	2.64^{a} ± 0.21	**2.11 ± 0.38**
	FRAP (µMol TE***/ g of DM)	0.23^{c} ± 0.01	0.35^{a} ± 0.02	0.24^{c} ± 0.01	0.19^{d} ± 0.02	0.27^{b} ± 0.01	0.22^{c} ± 0.02	**0.25 ± 0.05**
	ABTS (µMol TE/ g of DM)	13.3^{c} ± 0.5	18.4^{ab} ± 1.3	16.2^{b} ± 1.1	17.1^{b} ± 0.9	16.8^{b} ± 2.4	20.0^{a} ± 3.3	**16.9 ± 2.25**
	ORAC (µMol TE/ g of DM)	35.21^{cb} ± 4.4	38.43^{b} ± 6.4	28.93^{d} ± 5.1	34.65^{cb} ± 2.9	30.18^{cd} ± 2.4	50.38^{a} ± 4.8	**36.30 ± 7.74**
Thermal properties	T_0 (°C)	67.99^{a} ± 1.44	64.21^{a} ± 1.93	66.31^{a} ± 3.88	66.08^{a} ± 4.46	69.70^{a} ± 4.45	69.47^{a} ± 5.64	**67.29 ± 2.14**
	T_p (°C)	113.16^{a} ± 3	109.99^{a} ± 1.70	111.51^{a} ± 3.33	111.11^{a} ± 2.23	111.12^{a} ± 1.87	114.96^{a} ± 1.87	**111.97 ± 1.78**
	T_f (°C)	176.19^{a} ± 1.21	174.02^{a} ± 9.28	176.31^{a} ± 5.60	177.27^{a} ± 4.98	159.77^{a} ± 4.97	168.18^{a} ± 12.04	**171.95 ± 6.81**
	Δ_H (J/g)	123.34^{a} ± 37.54	140.0^{a} ± 31.67	117.69^{a} ± 31.07	145.92^{a} ± 61.01	107.62^{a} ± 40.95	89.89^{a} ± 9.27	**120.74 ± 20.71**

XRD	Relative crystallinity (%)	34.1	36.7	32.9	37.3	35.4	34.3	**35.11 ± 1.3**
	*****L_{HKL} 15.3º	6.0	5.8	5.9	5.7	5.5	5.7	**5.76 ± 0.2**
	17.1º	6.2	5.4	5.5	6.5	6.3	6.3	**6.03 ± 0.5**
	18.4º	5.0	4.7	4.8	6.9	5.9	5.9	**5.53 ± 0.9**
	21.5º	6.4	6.7	6.3	8.1	7.1	6.4	**6.83 ± 0.7**
	23.1º	4.6	4.6	4.6	4.6	4.5	4.5	**4.56 ± 0.06**
NMR	Relative crystallinity (%)	51.1	37.1	48.5	50.5	46.2	44.2	**46.26 ± 5.2**
	Double Helices(%)	61.3	51.5	60.0	61.1	60.9	66.5	**60.21 ± 4.85**

*DM, dry matter; ** GAE, gallic acid equivalent; *** TE, trolox equivalent; and ****L_{HKL}, relative crystal size; Means with equal letters on the same column line do not differ significantly by Duncan's test ($p > 0.05$); elaborated by author.

In the study of elemental concentration of pupunha fruit, Leterme et al. (2005) and Rojas-Garbanzo et al. (2012) reported calcium levels for pupunha flour of 1 g/kg and 0.26 g/kg, respectively, much lower concentrations than the general average found in this study (11.7 g/kg). Furthermore, the levels of micronutrients (iron, copper, manganese and zinc) were also lower than those found in this study. The differences found between the samples – such as pupunha flour samples – for the results obtained in the literature, are probably caused by soil characteristics in each geographic origin, the type of management and, largely, the fertilization used (YUYAMA et al., 2003).

In relation to the daily amounts recommended by the National Research Council (1989), the daily intakes of potassium, manganese and iron are, respectively, of 2000, 2-5 and 10 mg. Therefore, considering that a portion of 250g of food completely prepared with the pupunha flours could already supply 100% of this demand. This result is contrary to that found by Yuyama et al. (2003), also studying pupunha flour, which reported a maximum contribution of 12, 6 and 5% of the recommended daily intake of potassium, iron and manganese, respectively.

3.3 Phenolic Content and Antioxidant Potential Analysis

The pupunha flours showed average concentration of total phenolic compounds of 2.11 mg GAE/g of DM (Table 2). In comparison with the available literature, regardless of the cultivation location, the results found were superior to those found by Danesi et al. (2018) (1.3 mg GAE/g of DM), Rojas-Garbanzo et al. (2012) (0.63 mg GAE/g of DM), Rolando et al. (2019) (0.62 mg GAE/g of DM) and Santos et al. (2015) (0.30 mg GAE/g of DM), all of which also used pupunha flour in their studies. Such discrepancies may result from the different environmental conditions at the harvest locations or from differences in flour processing and even in phenolic compounds extraction methods.

When compared to other flours, the studied samples had an average of phenolic compounds higher and/or comparable to that found in bean (2.88 mgCE/g of DM) and rice (0.90 mgCE/ g of DM) (ARRIBAS et al., 2019), barley (max. 0.68 mg GAE/g of DM) (BONOLI et al., 2004), rye (max. 2.43 mg GAE/g of DM) (MICHALSKA; CEGLIŃSKA; ZIELIŃSKI, 2007), guava (0.84 mg GAE/g) (ALVES; PERRONE, 2015), macaúba pulp (2.62 mg GAE/g) (ANDRADE et al., 2020) and wheat flours (max. 0.37 mg GAE/g) (YU et al., 2004). Thus, the results for pupunha flour show the potential of flour as a source of phenolic compounds for dietary intake.

Considering the average value of the antioxidant activity of the pupunha flour samples, obtained by the FRAP (0.25 µmol TE/g of DM) and ABST (16.9 µmol TE/g of DM) methods (Table 2), it is observed that these were lower and higher, respectively, than those found by Contreras-Calderón et al. (2011) for crushed pupunha pulp (FRAP: 3.98 µmol TE/g; ABTS: 14.1 µmol TE/g). The results obtained by the ORAC method (32.10 µmol TE/g of DM) were similar to those found by Rojas-Garbanzo et al. (2012), also for pupunha flour. These results show that regardless of the phenolic content, the presence of these compounds is not indicative of optimal antioxidant activity, because as previously mentioned, the differences between the techniques, especially in the extraction stage, and the nature of the phenolic compounds, may restrict the antioxidant potential of raw materials.

In addition, like in the results of centesimal composition, the results of phenolic compounds and antioxidant activity of pupunha flour were also significantly affected by the location of the harvested fruits or and even fruit ripeness stage.

3.4 Principal Component Analysis (PCA)

The concentrations of 21 elements (centesimal and elemental composition, phenolic content and antioxidant capacity) of six pupunha flour were analyzed with PCA. Based on the eigenvalues, the first three principal components (PC) explained 88.78% of the total variance. Table 3 shows the loadings of the most significant variables in the first three main components and the variances explained by each component, and Figure 1 shows the score and loads plots for PC1 (39.98%) versus PC2 (34.63%).

Table 3 - Loadings of the variables for the first three principal components.

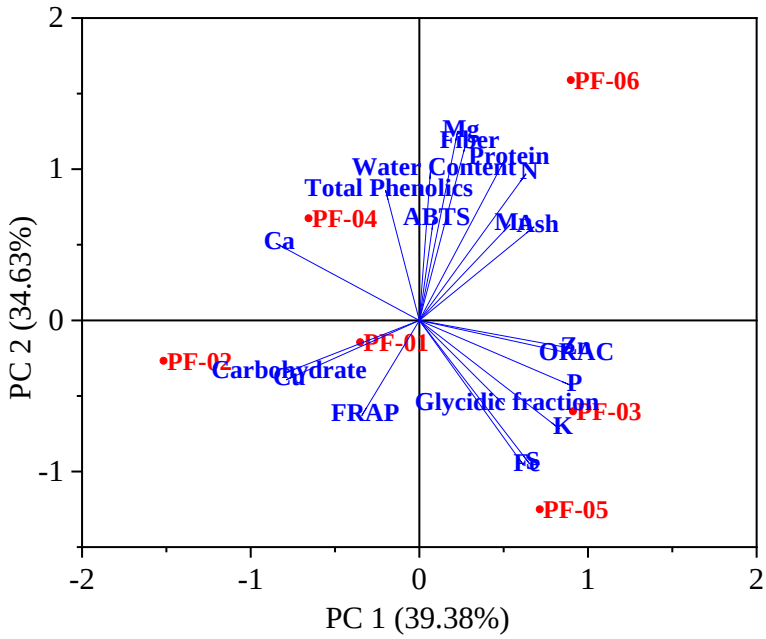
Element	PC1	PC2	PC3
Protein	0,18189	**0,31132**	0,03638
Glycidic fraction	0,17791	-0,16194	**-0,42235**
Nitrogen (N)	0,22493	**0,28161**	0,02527
Phosphorus (P)	**0,32062**	-0,12531	0,08504
Potassium (K)	**0,29574**	-0,20846	-0,01077
Calcium (Ca)	**-0,30245**	0,14812	-0,20758
Magnesium (Mg)	0,08072	**0,36314**	-0,08285
Sulfur (S)	0,23268	**-0,27576**	0,0716
Manganese (Mn)	**0,19148**	0,18386	-0,05208
Zinc (Zn)	**0,32305**	-0,05424	0,14923

Total Phenolics	-0,07122	0,25036	**0,41201**
FRAP	-0,12164	-0,18295	**0,42152**
Percentage of Variance	**39,38%**	**34,63%**	**14,77%**
Cumulative Variance	**39,38%**	**74,01%**	**88,78%**

*Variables marked in bold represent the most significant ones in each principal component; elaborated by author

Considering the most important variables of each PC, Figure 1 shows that samples PF-03, PF-05 and PF-06 contain the largest components of PC1. In addition, the content of phenolic compounds has a higher positive correlation with the antioxidant potential when estimated with the ABTS method than with the others tested methods, as revealed by the shorter distance, and hence more significant relationship (BREDARIOL; CARVALHO; VANIN, 2020), between corresponding data points. This finding confirms that the different methods of measuring antioxidant capacity give quite discordant results.

Figure 1 - Principal component analysis for the parameters of proximate and elemental composition, phenolic concentration and antioxidant activity of pupunha flour harvested in different locations; bi-plot graph of loadings and scores of the aforementioned analyses.



Elaborated by author

Finally, the PCA reveals that the samples were discriminated according to their physicochemical characteristics despite the fact that samples were collected in nearby regions. This fact may be linked, mainly, to three factors: the environmental

characteristics of each harvesting location and also the ripening stage of each fruit and genetic variability.

3.5 Fourier Transform Infrared Spectroscopy

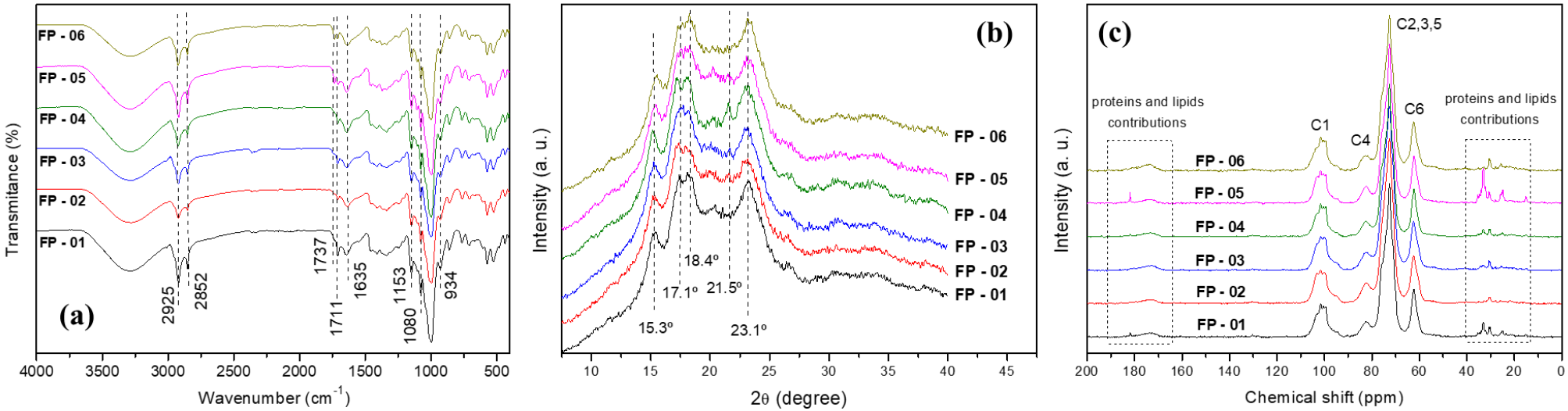
Figure 2a shows the FTIR spectra of the PF samples. These spectra show that the characteristic bands of polysaccharides and lipids are predominant in the spectra. The signals between 2900-2850 cm^{-1} are due to the C-H stretching in large quantities in the saturated fatty acids chain (SANTOS et al., 2020), and the stretching of the C=O bond of the ester carboxyl of around 1730 cm^{-1} (OSIRO et al., 2004; OSIRO; FRANCO; COLNAGO, 2011; PIRES et al., 2019).

The most intense band of the spectra in Figure 2a between 1000 and 1100 cm^{-1} is attributed to the stretching of the C-O bond of saccharides and polysaccharides such as pectin, cellulose and hemicellulose, which are part of the composition of the cell walls of fruits and vegetables. There is a broad base of around 3300 cm^{-1} in these spectra, which correspond to the symmetrical stretching of the O-H bonds of these compounds (LUCARINI et al., 2020; OSIRO; FRANCO; COLNAGO, 2011). Furthermore, the bands of around 1630 cm^{-1} can be visualized due to the asymmetric stretching of the COO- and O-H groups also attributed to the polysaccharides (VALENCIA et al., 2015; YING et al., 2017).

Due to the high concentration of lipids and carbohydrates in the samples, the FT-IR spectrum may not identify the other constituents, such as proteins and phenolic compounds, therefore, it is not possible to attribute characteristic signals to these functional groups.

An analysis of the band intensities between 3000 and 2800 cm^{-1} indicates that the sample PF-01 and PF-05 have a greater amount of lipids and the sample PF-02 the smallest amount of this nutrient, which is in agreement with the analysis of centesimal composition (Table 2). Thus, as the FTIR analysis identified qualitative differences between the PF samples - mainly in the content of polysaccharides and lipids - this tool can be used to quickly investigate and classify the levels of these specific chemical compounds.

Figure 2 – FTIR (a), XRD (b) and solid-state 13C CPMAS (c) data of the six pupunha flour (PF) with the identification of their main vibrational bands; main crystalline planes and chemical shift, respectively



Elaborated by author

3.6 Differential Scanning Calorimetry (DSC)

Table 2 shows the thermodynamic parameters for the pupunha flours. The thermograms revealed only one endothermic peak for all samples, with mean values of T_0, Tp and Δ_H of 67.29 ºC, 111.97 ºC and 120.74 J/g, respectively. In general, the results for the different PF samples did not show significant differences. Studies evaluating the thermal properties of starch from Pupunha (FELISBERTO et al., 2020; NETO et al., 2017; VALENCIA et al., 2015), found lower values than those reported in the present study. This difference can be attributed to a possible protective action of the other constituents of the flour (lipids, carbohydrates, proteins, fibers, etc.) (ANDRADE-MAHECHA; TAPIA-BLÁCIDO; MENEGALLI, 2012; PELISSARI et al., 2012).

The endothermic behavior of the samples can be attributed to the fact that in heterogeneous food systems the thermal curves can come from reactions associated with several components, so the reaction enthalpy is dependent on the compounds that are subject to thermal transitions (HENSHAW et al., 2003). Thus, the enthalpy values found may be largely related to lipids and carbohydrates. On the other hand, Neto et al., (2017) believe that the observed peaks are the result of an order-disorder phase transition of the starch (carbohydrate) under heating during a temperature range (gelatinization) characteristic of the starch source. Also, according to the authors, the amount of amylopectin is one of the factors that determines the enthalpy of the sample, since this structure hinders water accessibility and causes a greater amplitude between the initial and final transition temperatures and, consequently, a slow gelatinization of the more crystalline regions. Another hypothesis associated is that the reaction enthalpy values may be directly related to the non-denaturing of the protein found in the samples during the flour processing. According to Hermansson (1979), when there is protein denaturation during processing, there is little and/or no endothermic peak during DSC analysis. Therefore, as the flour production was carried out by a cold and vacuum process, it can be suggested that the proteins in the samples did not denature.

3.7 X-Ray Diffractometry (XRD)

All samples showed similar diffraction profiles, with the crystalline planes centered at 15.1º, 18.0º and 23.5º (Figure 2b), normally attributed to type C semicrystalline starches (ZHANG et al., 2005). A similar diffraction pattern was found in other studies involving pupunha starch (FELISBERTO et al., 2020; NETO et al., 2017). The type C pattern concentrates the characteristics of types A (normal and waxy cereals)

and B (some tubers and corns with high amylose content), thus the matrices with this type of pattern have distinct characteristics (FELISBERTO et al., 2020), among them, a higher concentration of resistant starch than the other types, which can serve as substrate for the large intestine microbiota (CAI et al., 2014).

The mean relative crystallinity (RC) value of the PF samples was 35.1 ± 1.3%, relatively higher than that achieved by Valencia et al. (2015), while the mean values of relative crystal size (L_{HKL}) were 5.8 ± 0.1, 6.0 ± 0.4, 5.5 ± 0.7, 6.8 ± 0.5 and 4.6 ± 0.04 nm (Table 2), calculated in Supplementary Material (Figure S1). Factors such as the variable composition of the other macronutrients in the flour can considerably affect the RC values (PELISSARI et al., 2012). In the present study, the low variability of RC and L_{HKL} serves as an indication of the low relevance of these parameters for geographic casualties. Thus, the PFs seem to preserve a uniform composition in relation to the crystallinity pattern of the starches, with little dependence on the geographic origin.

3.8 Solid state ^{13}C NMR

Figure 2c shows the solid state ^{13}C CPMAS spectra of the PF samples together with the attribution of the different kinds of carbons in the crystalized starch molecules. The spectra of all samples reveal a profile assigned to the pupunha starch, widely observed for flour samples from other plant species (LOPES-DA-SILVA et al., 2007; TANG; HILLS, 2003). The spectra were similar indicating that the starch crystalline structures show little variation between the samples, in agreement with the XRD. It is also verified that, despite the proximity of spectral profiles between the signals of C1 and C4 carbons, their deconvolution treatment (Figure S2) allowed obtaining slightly different RC values and double helix composition between the samples (Table 2). Generally, these parameters tend to be proportional, since the relative amount of helical structures tends to maintain the spatial regularity of the macromolecular domains and, therefore, the crystalline ordering of the chains. It is important to emphasize that although the interpretation converged due to the close relationship of the CR values by different techniques (XRD and NMR), they do not refer to the same physical origin, XDR observes long-range ordering, while NMR observes short-range ordering (FACCHINATTO et al., 2020, 2021). As both provide complementary information about the general morphology of the samples, it is expected that XRD values are relatively lower as they involve structures other than starch, such as the proteins and lipids, while according to NMR the morphology of the starch can be followed independently of the other components, since

these components do not interfere with the C1 and C4 signals, as indicated in Figure 2c.

According to Table 2, the PF-02 sample showed the highest amorphous characteristic (lowest CR value) calculated by the NMR spectra, while PF-03 showed the lowest CR value calculated by the XRD diffraction patterns, demonstrating that NMR and XRD CR results are different in terms of both order of magnitude and sample ranking.

3.9 In vitro cytotoxicity

The cytotoxicity of PF extracts was evaluated by assessing cell viability at concentrations ranging from 10 – 0.000128 mg/mL. The values were then compared to the control group (100% viable), and the cell viability percentages for the six pupunha flours are shown in Figure 3. The pupunha flour extracts were non-cytotoxic to cells up to a concentration of 0.4 µg/mL. According to ISO 10993-5 (2009) a reduction in cell viability of <70% of the blank indicates cytotoxic potential and, complementing, Andrade et al., (2022) and Burci et al. (2019) demonstrated that extract of interest that induce a 50% reduction in cell viability (IC50) below 1000 µg/mL demonstrate cytotoxic activity. Considering this, the cytotoxic potential of pupunha could be considered not negligible, since a IC50 is reached starting at 0.940 µg/mL, with 67% reduction in cellular viability (data not shown).

In a recent study, it was shown that passion fruit leaf methanolic extract had no toxic effect on L929 cell, but exerted strong metabolic inhibiting effect on cancer cells (HeLA; 10.83 µg/mL) (SISIN; ABDULLAH; SUL'AIN, 2017). Other studies have also found that phenolic extracts from fruits have inhibitory effect on other types of cancer cells, such as HepG2, HeLA, MCS-7, AGS, SW620 (ABUTAHA, 2015; CUSTÓDIO et al., 2011; ELSAYED et al., 2016; NAVARRO et al., 2019).

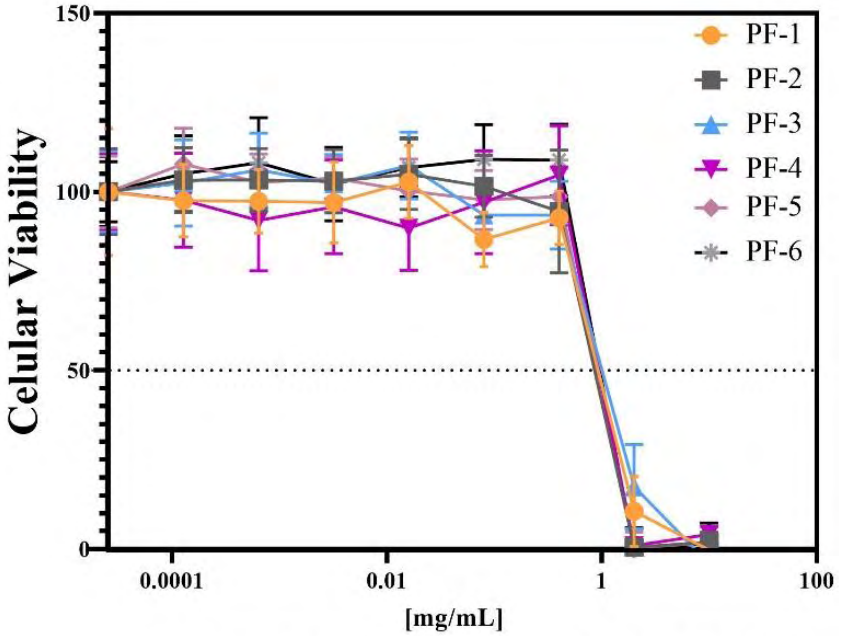
On the other hand, Okonogi et al. (2007) demonstrated that pomegranate peel extract stimulated the metabolic activity of PBMC (human healthy tissue) and Caco-2 (human colon adenoma) cells, therefore, recommending the control of this extract as a supplement, food and /or drug for humans. In another study, proposed by Ampasavate, Okonogi and Anuchapreeda (2010), it was proven that mangosteen peels contained an agent with cytotoxic potential in PBMC cells.

Furthermore, in a study that compared the effect of phenolic extracts from the fruit of *Physalis peruviana*, despite the inactivating effect of cancer cells (HeLA; 60.48 ± 3.28 µg/mL), it was found that the effect against healthy cells (L929) was also quite pronounced (66.62 ± 2.67 µg/mL) (MIER-GIRALDO et al., 2017). Overall, our present

results together with previous reports of the literature on phenolic fruit extracts show that phenolic extracts can exert different effects on different types of cells and, therefore, their use must be cautiously monitored.

Thus, notwithstanding their health-promoting properties, as already reported in the previous sections, phenolic compounds need to be studied in more depth, since their use in food supplements has increased and there is no legislation that regulates the maximum concentration allowed (TRUZZI et al., 2020).

Figure 3 - Results of cytotoxicity assays using the phenolic extract of pupunha flour (FP) samples; values highlighted for each FP batch refer to the results found for the IC50 index of the extracts.



PF-1	PF-2	PF-3	PF-4	PF-5	PF-6
0.8779	0.8587	1.006	0.9610	0.8848	1.052

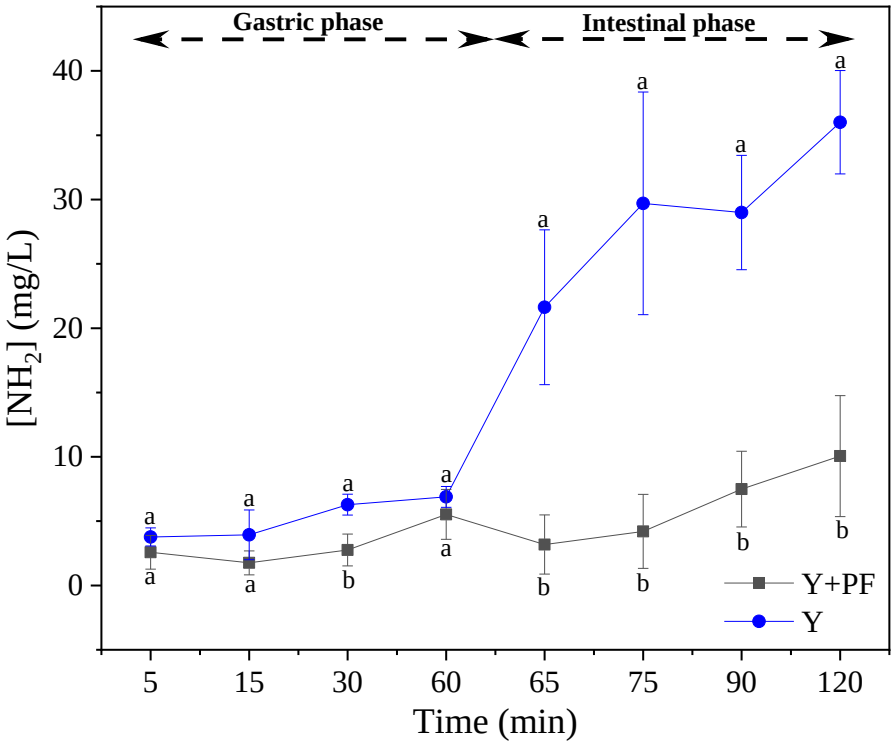
Elaborated by author

3.10 Pupunha flour incorporation into yogurt: effects on *in vitro* protein digestion

Figure 4 presents the *in vitro* protein digestion results, as inferred from the release of free NH2 groups, for yogurt without or with pupunha flour incorporated (25%; w/w). The results obtained with the control yogurt (Y) are typical of what is classically observed during in vitro digestion of foods: a small extent of protein hydrolysis during the gastric phase followed by a more rapid and extensive reaction kinetics during the small intestinal phase (BRODKORB et al., 2019). In the presence of PF into yogurt (Y+P), a clear inhibition of protein digestion was observed, particularly in the intestinal

phase, since the release of NH_2 groups in the reaction medium was significantly lower when compared to the control product (pure yogurt; Y).

Figure 3 - Release of free NH_2 groups concentrations in digestates of yogurt (Y) and yogurt with incorporation of 25% pupunha flour (Y+PF) in the gastric (up to 60 min) and intestinal (65-120min) phases; Y: yogurt; PF: pupunha flour.



Means with equal letters in the same time do not differ significantly by Duncan's test ($p > 0.05$); Elaborated by author

It is unknown whether polyphenols in the PF or some other compound inhibited the digestive enzymatic activity. Clement et al. (2004) and Rojas-Garbanzo et al. (2011) reported the presence of an anti-nutritional factor in PF, but did not identify it. An earlier study (Murillo et al. 1983) reported that the aqueous extract of pupunha flour inhibited the action of proteolytic enzymes and attributed this effect to a low molecular weight component.

The observed inhibition could also be explained, at least partly, by the direct influence of phenolic compounds on digestive proteases. Although this topic is largely discussed in the available literature, the conclusions are still contradictory. Tagliazucchi, Verzelloni and Conte (2005) studied the effect of some beverages and isolated phenolic compounds on pepsin activity during pork digestion, and found that the isolated phenolic compounds favored the digestion process of the evaluated food matrix. The authors also

reported that dealcoholized red wine, green tea and pure phenolics EGCG, resveratrol, catechin and quercetin can increase the concentration of peptides during the digestion of pork.

Lamothe et al. (2014) also demonstrated that green tea polyphenols can inhibit protease activities and lead to a lower rate of proteolysis during the digestion of three distinct dairy products (milk, yogurt, and cheese). Other reports, mostly related to the presence of tannins, have suggested that phenolics act as a protein binding agent that limits digestion (ALFIERI et al., 2019; BARROS; AWIKA; ROONEY, 2012; CAPPAI et al., 2013; DUNN et al., 2015; HE; LV; YAO, 2007; OH; HOFF, 1986; QUESADA et al., 1996). The effect of three phenolic acids (p-Cumalic acid, caffeic acid, gallic acid and chlorogenic acid) on the digestion of β-Conglycinine (7S), an important soy protein, was evaluated, showing there was no effect, either positive or negative, on protein digestion (GAN et al., 2016).

Finally, a recent review concluded that there is no consensus on the influence of tannins on protein digestibility, since there is evidence that such molecules can denature substrates and facilitate the action of proteases, but also can bind to substrates and prevent access to the catalytic site of digestive enzymes (MORZEL; CANON; GUYOT, 2022). Therefore, it appears that the impact of polyphenols on protein digestion is highly dependent on their nature, structure, concentration and it may also differ according to the food matrix used.

Based on the evidence found by other authors, it is suggested that the tannins in PF may act as anti-nutritional factors, although the literature related to the fruit and/or pupunha flour has not identified the presence of these compounds (CARVALHO et al., 2013; ESPINOSA-PARDO; MARTINEZ; MARTINEZ-CORREA, 2014; FELISBERTO et al., 2020; KRONEBERG et al., 1983; MARTÍNEZ-GIRÓN; FIGUEROA-MOLANO; ORDÓÑEZ-SANTOS, 2017; RADICE et al., 2014; RIBEIRO et al., 2021; ROJAS-GARBANZO et al., 2011, 2012).

4 CONCLUSION

Information about unconventional under-utilized fruit, as well as the development of new forms of use with longer and more efficient shelf life, such as flour, have allowed developing products with health appeals. The present study demonstrated by many different physicochemical characterizations on PF, providing data that will contribute to document the literature on this scarcely studied product. Besides for the first

time, this study also demonstrated that the phenolic extract of pupunha flour may pose risks to cellular metabolism, and possibly inhibit gastro-intestinal digestion of protein. In addition, though the flours showed significant differences in relation to centesimal composition, elemental composition and phenolic compound contents, they all demonstrated similar results to the cytotoxicity test, showing that attention must be given to the rampant use of foods with functional appeal to merely one aspect, such as antioxidant capacity.

There is a need for new studies that evaluate the differents ways to obtain flour to verify if such conditions can positively impact the quality parameters, mainly the cytotoxic aspects and the impact on protein digestion, as well as verify its effect on the total phenolic content. As emphasized in other analyses in this study, pupunha flour has physical-chemical properties that can vary depending on the harvest location, thus, it can be applied to a multitude of products.

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REFERENCES

ABUTAHA, N. In vitro antiproliferative activity of partially purified Withania somnifera fruit extract on different cancer cell lines. **Official Journal of the Balkan Union of Oncology (B.U.ON.)**, v. 20, n. 2, p. 625–630, 2015.

ALFIERI, M. et al. Discrimination and prediction of polyphenolic compounds and total antioxidant capacity in sorghum grains. **Journal of Near Infrared Spectroscopy**, v. 27, n. 1, p. 46–53, 2019.

ALVES, G.; PERRONE, D. Breads enriched with guava flour as a tool for studying the incorporation of phenolic compounds in bread melanoidins. **Food Chemistry**, v. 185, p. 65–74, 2015.

AMPASAVATE, C.; OKONOGI, S.; ANUCHAPREEDA, S. Cytotoxicity of extracts from fruit plants against leukemic cell lines. **African Journal of Pharmacy and Pharmacology**, v. 4, n. 1, p. 013–021, 2010.

ANDRADE-MAHECHA, M. M.; TAPIA-BLÁCIDO, D. R.; MENEGALLI, F. C. Physical-chemical, thermal, and functional properties of achira (Canna indica L.) flour

and starch from different geographical origin. **Starch/Staerke**, v. 64, n. 5, p. 348–358, 2012.

ANDRADE, A. C. et al. Prebiotic potential of pulp and kernel cake from Jerivá (Syagrus romanzoffiana) and Macaúba palm fruits (Acrocomia aculeata). **Food Research International**, v. 136, n. March, p. 109595, 2020.

ANDRADE, J. K. S. et al. Bioaccessibility of bioactive compounds after in vitro gastrointestinal digestion and probiotics fermentation of Brazilian fruits residues with antioxidant and antidiabetic potential. **Lwt**, v. 153, n. September 2021, 2022.

AOAC. **Official Methods of Analysis of the Association of Official Analytical Chemists**. 13. ed. Washington, DC: [s.n.]. v. 60

ARAÚJO, F. F. et al. Chemical characterization of Eugenia stipitata: A native fruit from the Amazon rich in nutrients and source of bioactive compounds. **Food Research International**, v. 139, n. November 2020, 2021.

ARRIBAS, C. et al. Bioactive compounds, antioxidant activity, and sensory analysis of rice-based extruded snacks-like fortified with bean and carob fruit flours. **Foods**, v. 8, n. 9, 2019.

BARBOSA, A. P. P.; MORAES, A. F.; CHISTÉ, R. C. Physicochemical characterization and quantification of bioactive compounds of Antrocaryon amazonicum fruits cultivated in Brazilian Amazonia. **CYTA - Journal of Food**, v. 18, n. 1, p. 616–623, 2020.

BARROS, F.; AWIKA, J. M.; ROONEY, L. W. Interaction of tannins and other sorghum phenolic compounds with starch and effects on in vitro starch digestibility. **Journal of Agricultural and Food Chemistry**, v. 60, n. 46, p. 11609–11617, 2012.

BENZIE, I. F. F.; STRAIN, J. J. The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "'Antioxidant Power"': The FRAP Assay Iris. **Analytical Biochemistry**, v. 239, p. 70–79, 1996.

BERTO, A. et al. Bioactive compounds and scavenging capacity of pulp, peel and seed extracts of the Amazonian fruit Quararibea cordata against ROS and RNS. **Food Research International**, v. 77, p. 236–243, 2015.

BONOLI, M. et al. Antioxidant phenols in barley (Hordeum vulgare L.) flour: Comparative spectrophotometric study among extraction methods of free and bound phenolic compounds. **Journal of Agricultural and Food Chemistry**, v. 52, n. 16, p. 5195–5200, 2004.

BREDARIOL, P.; CARVALHO, R. A. DE; VANIN, F. M. The effect of baking conditions on protein digestibility, mineral and oxalate content of wheat breads. **Food Chemistry**, v. 332, n. July, p. 127399, 2020.

BRODKORB, A. et al. INFOGEST static in vitro simulation of gastrointestinal food digestion. **Nature Protocols**, v. 14, n. 4, p. 991–1014, 2019.

BURCI, L. M. et al. Acute and subacute (28 days) toxicity, hemolytic and cytotoxic effect of Artocarpus heterophyllus seed extracts. **Toxicology Reports**, v. 6, n. February 2018, p. 1304–1308, 2019.

CAI, J. et al. Structural and functional properties of C-type starches. **Carbohydrate Polymers**, v. 101, n. 1, p. 289–300, 2014.

CAPPAI, M. G. et al. Pigs use endogenous proline to cope with acorn (Quercus pubescens Willd.) combined diets high in hydrolysable tannins. **Livestock Science**, v. 155, n. 2–3, p. 316–322, 2013.

CARVALHO, A. V. et al. Production of third generation snacks by extrusion-cooking of pupunha and cassava flour mixtures. **Brazilian Journal of Food Technology**, v. 12, n. 04, p. 277–284, 2009.

CARVALHO, A. V. et al. Características físicas e químicas de frutos de pupunheira no estado do Pará. **Revista Brasileira de Fruticultura**, v. 35, n. 3, p. 763–768, 2013.

CHURCH, F. C. et al. An o-phthalaldehyde spectrophotometric assay for proteinases. **Analytical Biochemistry**, v. 146, n. 2, p. 343–348, 1985.

CLEMENT, C. R. et al. Why extensive research and development did not promote use of peach palm fruit in Latin America. **Agroforestry Systems**, v. 61–62, n. 1–3, p. 195–206, 2004.

CONTRERAS-CALDERÓN, J. et al. Antioxidant capacity, phenolic content and vitamin C in pulp, peel and seed from 24 exotic fruits from Colombia. **Food Research International**, v. 44, n. 7, p. 2047–2053, 2011.

CUSTÓDIO, L. et al. Phytochemical Profile, Antioxidant and Cytotoxic Activities of the Carob Tree (Ceratonia siliqua L.) Germ Flour Extracts. **Plant Foods for Human Nutrition**, v. 66, n. 1, p. 78–84, 2011.

D'AGOSTINO, E. M. et al. Interaction of tea polyphenols and food constituents with model gut epithelia: The protective role of the mucus gel layer. **Journal of Agricultural and Food Chemistry**, v. 60, n. 12, p. 3318–3328, 2012.

DANESI, E. D. G. et al. Effects of industrial by-products from orange, peach palm and soybean on the quality traits and antioxidant activity of flours: A response surface approach. **International Food Research Journal**, v. 25, n. 3, p. 1219–1227, 2018.

DUNN, K. L. et al. Interaction of sorghum tannins with wheat proteins and effect on in vitro starch and protein digestibility in a baked product matrix. **Journal of Agricultural and Food Chemistry**, v. 63, n. 4, p. 1234–1241, 2015.

ELSAYED, E. A. et al. In vitro assessment of anticancer properties of moringa peregrina essential seed oil on different cell lines. **Pakistan Journal of Zoology**, v. 48, n. 3, p. 853–859, 2016.

ESPINOSA-PARDO, F. A.; MARTINEZ, J.; MARTINEZ-CORREA, H. A. Extraction of bioactive compounds from peach palm pulp (Bactris gasipaes) using supercritical CO2.

Journal of Supercritical Fluids, v. 93, p. 2–6, 2014.

FACCHINATTO, W. M. et al. Evaluation of chitosan crystallinity: A high-resolution solid-state NMR spectroscopy approach. **Carbohydrate Polymers**, v. 250, n. June, 2020.
FACCHINATTO, W. M. et al. Insight into morphological, physicochemical and spectroscopic properties of β-chitin nanocrystalline structures. **Carbohydrate Polymers**, v. 273, n. March, 2021.

FELISBERTO, M. H. F. et al. Characterization and technological properties of peach palm (Bactris gasipaes var. gasipaes) fruit starch. **Food Research International**, v. 136, n. May, p. 109569, 2020.

GAN, J. et al. Interactions of β-Conglycinin (7S) with different phenolic acids-impact on structural characteristics and proteolytic degradation of proteins. **International Journal of Molecular Sciences**, v. 17, n. 10, 2016.

HE, Q.; LV, Y.; YAO, K. Effects of tea polyphenols on the activities of α-amylase, pepsin, trypsin and lipase. **Food Chemistry**, v. 101, n. 3, p. 1178–1182, 2007.

HENSHAW, F. O. et al. Thermal properties of cowpea flour: A study by differential scanning calorimetry. **Nahrung - Food**, v. 47, n. 3, p. 161–165, 2003.

HERMANSSON, A. M. Methods of studying functional characteristics of vegetable proteins. **Journal of the American Oil Chemists' Society**, v. 56, n. 3, p. 272–279, 1979.
ISO 10993-5, I. O. FOR S. **International Standard International StandardInternational Standard - Tests for in vitro cytotoxicity**, 2009.

JACOBO, F. Q. et al. Caracterización de aceites, tortas y harinas de frutos de ungurahui (Jessenia polycarpa) y aguaje (Mauritia flexuosa L.) de la amazonía peruana. **Revista de la Sociedad Química del Perú**, v. 75, n. 2, p. 243–253, 2009.

KAEFER, S. et al. Bolo com farinha de pupunha (Bactris gasipaes): análise da composição centesimal e sensorial. **Brazilian Journal of Food Nutrition**, v. 24, n. 3, p. 347–352, 2013.

KRONEBERG, M. M. R. et al. Estudio preliminar sobre factores inhibidores de enzimas proteolíticas en la harina de pejibaye (Bactris gasipaes). **Revista de Biología Tropical**, v. 31, n. 2, p. 227–231, 1983.

LAMOTHE, S. et al. Interaction of green tea polyphenols with dairy matrices in a simulated gastrointestinal environment. **Food and Function**, v. 5, n. 10, p. 2621–2631, 2014.

LARRAURI, J. A.; RUPÉREZ, P.; SAURA-CALIXTO, F. Effect of Drying Temperature on the Stability of Polyphenols and Antioxidant Activity of Red Grape Pomace Peels. **Journal of Agricultural and Food Chemistry**, v. 45, n. 4, p. 1390–1393, 1997.

LETERME, P. et al. Chemical composition and nutritive value of peach palm (Bactris gasipaes Kunth) in rats. **Journal of the Science of Food and Agriculture**, v. 85, n. 9, p. 1505–1512, 2005.

LOPES-DA-SILVA, J. A. et al. Rheological and Nuclear Magnetic Resonance (NMR) study of the hydration and heating of undeveloped wheat doughs. **Journal of Agricultural and Food Chemistry**, v. 55, n. 14, p. 5636–5644, 2007.

LUCARINI, M. et al. Grape Seeds : Chromatographic Profile of Fatty Acids and Phenolic Compounds and Qualitative Analysis by FTIR-ATR Spectroscopy. **Foods**, v. 9, n. 10, p. 2–14, 2020.

MANCINI, M. et al. Application of the non-destructive NIR technique for the evaluation of strawberry fruits quality parameters. **Foods**, v. 9, n. 4, 2020.

MARTÍNEZ-GIRÓN, J.; FIGUEROA-MOLANO, A. M.; ORDÓÑEZ-SANTOS, L. E. Effect of the addition of peach palm (Bactris gasipaes) peel flour on the color and sensory properties of cakes. **Food Science and Technology**, v. 37, n. 3, p. 418–424, 2017.

MICHALSKA, A.; CEGLIŃSKA, A.; ZIELIŃSKI, H. Bioactive compounds in rye flours with different extraction rates. **European Food Research and Technology**, v. 225, n. 3–4, p. 545–551, 2007.

MIER-GIRALDO, H. et al. Cytotoxic and Immunomodulatory Potential Activity of Physalis peruviana Fruit Extracts on Cervical Cancer (HeLa) and Fibroblast. **Journal of Evidence-Based Complementary & Alternative Medicine**, v. 22, n. 4, p. 777–787, 2017.

MORZEL, M.; CANON, F.; GUYOT, S. Interactions between Salivary Proteins and Dietary Polyphenols: Potential Consequences on Gastrointestinal Digestive Events. **Journal of Agricultural and Food Chemistry**, 2022.

MURILLO, M. et al. Estudio preliminar sobre factores inhibidores de enzimas proteolíticas en la harina de pejibaye (Bactris gasipaes). **Revista de Biología Tropical**, v. 31, n. 2, p. 227–231, 1983.

NATIONAL RESEARCH COUNCIL. **Recommended Dietary Allowances**. 10th. ed. Washington, DC: The National Academies Press, 1989.

NAVARRO, M. et al. Polyphenolic characterization, antioxidant, and cytotoxic activities of mangifera indica cultivars from Costa Rica. **Foods**, v. 8, n. 9, 2019.

NERI-NUMA, I. A. et al. Small Brazilian wild fruits: Nutrients, bioactive compounds, health-promotion properties and commercial interest. **Food Research International**, v. 103, n. May 2017, p. 345–360, 2018.

NETO, B. A. DE M. et al. Thermal-morphological characterisation of starch from peach-palm (Bactris Gasipaes kunth) fruit (Pejibaye). **International Journal of Food Properties**, v. 20, n. 5, p. 1007–1015, 2017.

NOGUEIRA, A. R. DE A.; SOUZA, G. B. **Manual de Laboratórios: solo, Água, Nutrição Vegetal, Nutrição Animal e Alimentos**São CarlosEmbrapa Pecuária Sudeste, , 2005.

OH, H. IL; HOFF, J. E. Effect of Condensed Grape Tannins on the In Vitro Activity of Digestive Proteases and Activation of Their Zymogens. **Journal of Food Science**, v. 51, n. 3, p. 577–580, 1986.

OKONOGI, S. et al. Comparison of antioxidant capacities and cytotoxicities of certain fruit peels. **Food Chemistry**, v. 103, n. 3, p. 839–846, 2007.

OLIVEIRA, M. K. S. et al. Use of pejibaye flour (Bactris gasipaes Kunth) in the production of food pastas. **International Journal of Food Science and Technology**, v. 41, n. 8, p. 933–937, 2006.

OSIRO, D. et al. A kinetic model for Xylella fastidiosa adhesion, biofilm formation, and virulence. **FEMS Microbiology Letters**, v. 236, n. 2, p. 313–318, 2004.

OSIRO, D.; FRANCO, R.; COLNAGO, A. Spectroscopic Characterization of the Exopolysaccharide of Xanthomonas axonopodis pv. citri in Cu2+ Resistance Mechanism. **Journal of Brazilian Chemical Society**, v. 22, n. 7, p. 1339–1345, 2011.

OU, B.; HAMPSCH-WOODILL, M.; PRIOR, R. L. Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. **Journal of Agricultural and Food Chemistry**, v. 49, n. 10, p. 4619–4626, 2001.

PELISSARI, F. M. et al. Isolation and characterization of the flour and starch of plantain bananas (Musa paradisiaca). **Starch/Staerke**, v. 64, n. 5, p. 382–391, 2012.

PIRES, M. B. et al. Peach palm flour (Bactris gasipae KUNTH): Potential application in the food industry. **Food Science and Technology**, v. 39, n. 3, p. 613–619, 2019.

QUESADA, C. et al. Phenolic inhibitors of α-amylase and trypsin enzymes by extracts from pears, lentils, and cocoa. **Journal of Food Protection**, v. 59, n. 2, p. 185–192, 1996.

RADICE, M. et al. Chemical characterization and antioxidant activity of Amazonian (Ecuador) Caryodendron orinocense Karst. and Bactris gasipaes kunth seed oils. **Journal of Oleo Science**, v. 63, n. 12, p. 1243–1250, 2014.

RECK, I. M.; MIRANDA, N. L. D. E. Chemical Composition and Microbiological Quality of Cookies Formulations With Pupunha Palm Cabbage Flour. **Uningá Review**, v. 27, n. 1, p. 15–18, 2016.

RIBEIRO, G. S. et al. Peach palm flour: production, hygroscopic behaviour and application in cookies. **Heliyon**, v. 7, n. 5, 2021.

ROCHETTI, A. L.; FUKUMASU, H. **Caracterização dos efeitos antitumorais do guaraná sobre modelo murino de células tronco cancerosas**. [s.l.] Universidade de São Paulo - USP, 2015.

ROJAS-GARBANZO, C. et al. Identification and quantification of carotenoids by HPLC-DAD during the process of peach palm (Bactris gasipaes H.B.K.) flour. **Food Research International**, v. 44, n. 7, p. 2377–2384, 2011.

ROJAS-GARBANZO, C. et al. Major physicochemical and antioxidant changes during peachpalm (bactris gasipaes h.b.k.) flour processing. **Fruits**, v. 67, n. 6, p. 415–427, 2012.

ROLANDO, G. L. M. R. et al. Phytochemical characterization of extracts of the mesocarp of Bactris gasipaes and evaluation of its antioxidant power for pharmaceutical dermal formulations. **International Journal of Herbal Medicine**, v. 7, n. 4, p. 56–67, 2019.

SANTOS, M. DOS et al. Amazonian Native Palm Fruits as Sources of Antioxidant Bioactive Compounds. **Antioxidants**, v. 4, n. 3, p. 591–602, 2015.

SANTOS, O. V. DOS et al. Chromatographic profile and bioactive compounds found in the composition of pupunha oil (Bactris gasipaes Kunth): implications for human health TT - Perfil cromatográfico e compostos bioativos presentes na composição do óleo de pupunha (Bactris gasipaes . **Rev. Nutr. (Online)**, v. 33, p. e190146–e190146, 2020.

SILVA RIBEIRO, G. et al. Peach palm flour: production, hygroscopic behaviour and application in cookies. **Heliyon**, v. 7, n. 5, 2021.

SILVA, W. G. DA;; OLIVEIRA, M. N. Edible Popular Fruit in the Amazon Region: Food Importance. In: TODOROV, S. D.; PIERI, F. A. (Eds.). . **Tropical Fruits - From Cultivation to Consumption and Helth Benefits**. 1. ed. New York: [s.n.]. p. 433.

SINGLETON, V. L.; ORTHOFER, R.; LAMUELA-RAVENTÓS, R. M. Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin-Ciocalteu Reagent. **Scientia Horticulturae**, v. 299, n. 1974, p. 281–286, 1999.

SISIN, N. N. T.; ABDULLAH, H.; SUL'AIN, M. D. Antiproliferative, Antioxidative and Compounds Identification from Methanolic Extract of Passiflora Foetida and Its Fractions. **Journal of Analytical & Pharmaceutical Research**, v. 6, n. 1, 2017.

TAGLIAZUCCHI, D.; VERZELLONI, E.; CONTE, A. Effect of some phenolic compounds and beverages on pepsin activity during simulated gastric digestion. **Journal of Agricultural and Food Chemistry**, v. 53, n. 22, p. 8706–8713, 2005.

TANG, H.; HILLS, B. P. Use of 13C MAS NMR to study domain structure and dynamics of polysaccharides in the native starch granules. **Biomacromolecules**, v. 4, n. 5, p. 1269–1276, 2003.

TRUZZI, F. et al. Are supplements safe? Effects of gallic and ferulic acids on in vitro cell models. **Nutrients**, v. 12, n. 6, p. 1–13, 2020.

VALENCIA, G. A. et al. Physicochemical, morphological, and functional properties of flour and starch from peach palm (Bactris gasipaes k.) fruit. **Starch/Staerke**, v. 67, n. 1–2, p. 163–173, 2015.

VILLAS-BOAS, F. et al. Effect of amylolysis on the formation, the molecular, crystalline and thermal characteristics and the digestibility of retrograded starches. **International Journal of Biological Macromolecules**, v. 163, p. 1333–1343, 2020.

VIRGOLIN, L. B.; SEIXAS, F. R. F.; JANZANTTI, N. S. Composition, content of

bioactive compounds, and antioxidant activity of fruit pulps from the Brazilian Amazon biome. **Pesquisa Agropecuaria Brasileira**, v. 52, n. 10, p. 933–941, 2017.

YING, D. Y. et al. Physical properties and FTIR analysis of rice-oat flour and maize-oat flour based extruded food products containing olive pomace. **Food Research International**, v. 100, n. July, p. 665–673, 2017.

YU, L. et al. Comparison of wheat flours grown at different locations for their antioxidant properties. **Food Chemistry**, v. 86, n. 1, p. 11–16, 2004.

YUYAMA, L. K. O. et al. Chemical composition of the fruit mesocarp of three peach palm (Bactris gasipaes) populations grown in Central Amazonia, Brazil . **International Journal of Food Sciences and Nutrition**, v. 54, n. 1, p. 49–56, 2003.

ZHANG, P. et al. Banana starch: Production, physicochemical properties, and digestibility - A review. **Carbohydrate Polymers**, v. 59, n. 4, p. 443–458, 2005.

Capítulo 3: Predição de Compostos fenólicos e Potencial Antioxidante de Cookies produzidos a partir de fruto Amazônico por Calibração Multivariada de dados Espectroscópicos

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RESUMO

A farinha de pupunha (*Bactris gasipaes*) é conhecida por apresentar elevado teor de compostos bioativos, em especial fenólicos em sua composição e, dessa forma, a aplicação dessa matriz alimentar no desenvolvimento de produtos pode representar uma importante estratégia para a veiculação e consumo destes compostos. Por outro lado, a quantificação dos compostos fenólicos nos alimentos ainda é uma técnica complexa e novas ferramentas se mostram necessárias para uma melhor caracterização do produto. Portanto, o objetivo deste trabalho foi avaliar o efeito da utilização de farinha de pupunha na produção de cookies, avaliando os aspectos tecnológicos e químicos do produto, e ainda desenvolver um modelo de predição por espectroscopia no infravermelho próximo (NIR) e quimiometria para quantificação dos compostos fenólicos e capacidades antioxidantes. Cinco formulações de cookies (12.5,25,50,75 e 100%) foram avaliadas em relação ao seu teor de umidade, atividade de água, perda de água, cor, fator de espalhamento, volume específico, fenólicos totais e capacidade antioxidante. A avaliação tecnológica e visual dos cookies demonstrou que o aumento da concentração da farinha de pupunha provocou, em geral, pouca alteração nas diferentes respostas avaliadas. O aumento da concentração de farinha de pupunha provocou aumento do teor de fenólicos (1.30 a 5.55 mgAGE/g) e dos potenciais antioxidantes pelo método ABTS (7.03 a 37.37 mgTE/g) e FRAP (0.09 a 0.72 mgTE/g) dos cookies. Excelentes modelos gerados a partir das análises NIR foram obtidos para o teor de fenólicos (R^2c:0.89; R^2cv:0.86; R^2p:0.80; RPD:2,04) e capacidade antioxidante por FRAP (R^2c:0.95; R^2cv:0.93; R^2p:0.87; RPD:2,73), enquanto que para o método ABTS modelos satisfatórios foram elaborados (R^2c:0.89; R^2cv:0.83; R^2p:0.70; RPD:1.76). Diante do exposto, pode-se concluir que excelentes modelagens foram construídas a partir dos dados de NIR, demonstrando que técnicas espectroscópicas podem prever analitos presentes em baixas concentrações e, portanto, substituir métodos tradicionais. Além disso, a farinha de pupunha pode ser utilizada como matéria prima para a produção de cookies com potencial funcional e a possibilidade de produção de cookies apenas utilizando essa matriz alimentar abre portas para novas formulações de produtos sem glúten.

Palavras-chave: pupunha; produto funcional; NIR; quimiometria.

1 INTRODUÇÃO

A crescente demanda por alimentos funcionais faz com que diversos trabalhos na literatura busquem verificar a presença de nutrientes minoritários e fitonutrientes, como vitaminas, minerais, compostos bioativos e probióticos, em matrizes alimentares pouco exploradas (KHOOZANI; BEKHIT; BIRCH, 2019).

Nesse sentido o fruto de pupunha, bem como a farinha produzida a partir de sua polpa, apresenta uma rica composição de compostos bioativos, em especial de compostos fenólicos e carotenóides (pró vitamina A) (CHISTÉ et al., 2021; FELISBERTO et al., 2020; HEMPEL et al., 2014; PIRES et al., 2019; ROJAS-GARBANZO et al., 2011, 2012). Tais compostos apresentam efeitos citotóxicos contra diversos tipos de células cancerígenas (ABUTAHA, 2015; ELSAYED et al., 2016; SISIN; ABDULLAH; SUL'AIN, 2017), bem como proteger contra doenças crônico-degenerativas, devido ao seu poder antioxidante (GIADA, 2013). Além disso, a farinha de pupunha apresenta alto valor nutricional e energético, com elevado teor de proteínas, lipídios e minerais (RIBEIRO et al., 2021; ROJAS-GARBANZO et al., 2011, 2012) que a fazem ser extremamente atrativas para a formulação de novos produtos.

Atualmente, o consumo do fruto de pupunha é localizado principalmente no Norte do Brasil, onde o fruto é preparado tradicionalmente por meio de cozimento em água salgada (CHISTÉ et al., 2021). Alguns estudos já demostraram a viabilidade da aplicação da farinha de pupunha na preparação de produtos da área de panificação, como biscoitos (RECK; MIRANDA, 2016; SILVA RIBEIRO et al., 2021) e bolos (MARTÍNEZ-GIRÓN; FIGUEROA-MOLANO; ORDÓÑEZ-SANTOS, 2017).

Entretanto, por mais que já se tenha demonstrado o potencial de aplicação da farinha de pupunha, a quantificação de compostos fenólicos nos alimentos por métodos tradicionais, seja em alimentos processados ou *in natura*, é extremamente trabalhosa, necessita de mão de obra especializada e utiliza grande variedade de reagentes (FRIZON et al., 2015).

Tendo isso em vista, a aplicação de metodologias alternativas, como a espectroscopia no Infravermelho Próximo (NIR), para análise de outros parâmetros de qualidades de alimentos vem demostrando ser uma alternativa frente aos métodos tradicionais (ARRUDA DE BRITO et al., 2022; BRASIL; CRUZ-TIRADO; BARBIN, 2022; CAYUELA; WEILAND, 2010; GUIDETTI; BEGHI; BODRIA, 2010; OLIVEIRA et al., 2014). A aplicação de NIR para a previsão de compostos bioativos e potencial

antioxidante em alimentos produzidos com ingredientes ricos em compostos fenólicos vem sendo estudada na literatura, porém até o presente momento os resultados ainda são controversos.

Amodio et al. (2017) avaliaram os parâmetros de qualidade de morangos e, segundo os autores, não foi possível estabelecer predições para o conteúdo fenólico (R^2c: 0.50; R^2cv: 0.44; R^2p:0.42; RPD: 1.45), mesmo testando diferentes pré-processamentos. Resultados semelhantes foram obtidos por Bedini et al. (2013), que não conseguiram realizar predições satisfatórias para compostos fenólicos em cookies comerciais.

Por outro lado, excelentes modelos de predição para fenólicos foram estabelecidos utilizando calibração multivariada e NIR (R^2c: 0.99; R^2p: 0.98; RPD: 3.05) para diferentes tipos de frutas vermelhas (KLJUSURIĆ et al., 2016). Da mesma forma, em um estudo realizado para avaliar a eficiência de equipamento espectroscópico NIR portátil, Saad, Azam e Amer (2022), alcançaram excelentes estatísticas de predição (R^2c:0,91; R^2p:0,90; RPD:3,27) para a previsão de compostos fenólicos em morangos.

Portanto, considerando os aspectos destacados, o objetivo do presente trabalho foi avaliar a aplicação do MicroNIR para quantificar os compostos fenólicos e potencial antioxidante de biscoitos tipo cookie elaborados com diferentes concentrações de farinha de pupunha. Além disso, os produtos foram caracterizados em relação aos aspectos tecnológicos e físico-químicos visando demonstrar a aplicabilidade industrial da farinha elaborada.

2 MATERIAL E MÉTODOS

2.1 Preparação da Farinha

Os frutos de Pupunha utilizados para a elaboração da farinha foram colhidos nos estados do Acre e Rondônia em estágio de maturação ideal pra consumo. Quando recebidos, os frutos foram inspecionados, observando-se danos mecânicos e/ou sujidades e então foram sanitizados em solução de hipoclorito de sódio (100 ppm) por 15 minutos. Após esse período, os frutos em sua forma integral (polpa + casca), foram despolpados e colocados sob congelamento rápido (KlimaEquip, UK05, Brasil) e encaminhados para o processo de liofilização (Terroni, LC1500, Brasil) por 48h. Após secagem, as amostras foram trituradas em moinho do tipo facas (Marconi, MA340, Brasil) com granulometria padronizada em 16 *mesh*, identificadas de acordo com a região de colheita e armazenadas sob temperatura de -18 ºC (Consul, CHB42CBBNA, Brasil).

2.2 Preparação dos Cookies

Cinco formulações de cookies foram desenvolvidas (Tabela 1), segundo metodologia proposta por Pareyt et al. (2008), com adaptações. Inicialmente, margarina e os dois tipos de açúcar foram homogeneizados em batedeira (Kitchen Aid Professional, KPM5 mixer, EUA) durante 3 minutos. Em seguida, foi adicionado água e a mistura foi homogeneizada por mais 2 minutos e somente então a farinha de trigo, de pupunha e o fermento foram adicionados e a massa foi novamente homogeneizada por mais 5 minutos.

A massa crua obtida foi dividida em porções de aproximadamente 12g e de 5 cm de diâmetro. Após pesagem, as amostras foram dispostas em fôrmas e assadas em forno industrial (Pratica Tchnipan, E250, Brasil) sob a temperatura de 165 ºC por 7 minutos. Parte das amostras foram resfriadas a temperatura ambiente (cerca de 30 minutos) para realização de análises de atividade de água, conteúdo de água, dureza, cor e volume específico e outra parte foi armazenada à -18 ºC (Consul, CHB42CBBNA, Brasil) para realização de análises posteriores. Os cookies de farinha de pupunha foram produzidos com farinha de pupunha obtidas de seis diferentes localidades (FPL1, FPL2, FPL3, FPL4, FPL5, FPL6)

Tabela 1 - Formulação dos cookies produzidos com diferentes concentrações de farinha de pupunha.

Ingredientes (g)	**Concentração de farinha de pupunha nos cookies (g/ 100g de farinha total)**				
	12,5	**25**	**50**	**75**	**100**
Farinha de trigo	87,5	75	50	25	0
Farinha de pupunha	12,5	25	50	75	100
Açúcar refinado	36,05	36,05	36,05	36,05	36,05
Açúcar Mascavo	36,05	36,05	36,05	36,05	36,05
Margarina	19,22	19,22	19,22	19,22	19,22
Água	17,18	17,18	17,18	17,18	17,18
Fermento químico	2,09	2,09	2,09	2,09	2,09

Elaborado pelo autor

2.3 Perda de água durante o assamento, conteúdo de água e atividade de água dos cookies após o assamento

A perda de água dos cookies foi determinada a partir da diferença do peso entre a massa dos cookies antes e depois do assamento, sendo expressa em porcentagem (VANIN et al., 2010). Para realização das análises de conteúdo e atividade de água, os cookies já assados foram moídos (Mondial, L1100BI, Brasil). Para a análise de conteúdo de água, utilizou-se a metodologia proposta pela AOAC (2005) com modificações, onde os cookies com diferentes concentrações de farinha de pupunha (0.5 g) foram secos em

estufa (Marconi, FPSTHB2610R-017, China) a 105 ºC por 24h. A atividade de água foi determinada utilizando-se equipamento AquaLab (Meter, Aqualab Series 3 TE, USA) a 25 ºC. Todas as análises foram realizadas em triplicata.

2.4 Análises das Propriedades Físicas dos Cookies

2.4.1 Fator de Espalhamento e Volume Específico

A espessura, diâmetro e o fator de espalhamento dos cookies foram determinados seguindo a metodologia proposta pela AACC 10–50.5 (2000) e o volume específico foi determinado utilizando o equipamento VolScan (Stable Micro Systems, Godalming, Reino Unido). Todas as análises foram realizadas em, pelo menos, triplicata.

2.4.2 Parâmetros de cor

A análise de colorimetria das amostras de cookies foi realizada utilizando-se o equipamento HunterLab (Aeros, Reston, EUA) utilizando o sistema CIELAB, onde os parâmetros de cor L* (luminosidade), a* (-a*:verde; a*:vermelho) e b* (-b*:azul; b*:amarelo) foram avaliados. Os parâmetros de tonalidade (*H*º) e chroma (*C) foram obtidos de acordo com o método proposto por Gonnet (1998). Todas as análises foram realizadas em triplicata.

2.5 Determinação dos Compostos Fenólicos Totais

A extração dos compostos fenólicos totais foi realizada de acordo com a metodologia de Santos et al. (2015) adaptada. Inicialmente amostras trituradas de cookies (Mondial, L1100BI, Brasil) foram pesadas (2,5g), colocadas em tubos, e adicionadas de 10 mL de metanol/água (50:50; v/v). Os tubos foram então homogeneizados por 1 hora a 300 rpm (Marconi, MA420, Brasil) e centrifugados (Eppendorf, 5430R, Alemanha) a 7830 rpm a 10 ºC por 15 minutos. Ao final o sobrenadante foi recolhido e colocado em frasco âmbar.

A segunda etapa da extração consistiu em adicionar acetona/água (70:30; v/v) ao resíduo da fase anterior e realização das mesmas etapas já descritas. Ao final, os extratos metanólicos e acetônicos foram combinados.

Para a quantificação dos compostos fenólicos totais, alíquotas de 0.5 mL dos extratos diluídos foram adicionados a um tubo com 2.5 mL de reagente de Folin-Ciocalteu (1:10; v/v) e 2 mL de solução de carbonato de sódio (7.5%) e então a mistura foi homogeneizada e deixada em repouso por 2 horas sob ausência de luz (SINGLETON; ORTHOFER; LAMUELA-RAVENTÓS, 1999). A leitura de absorbância foi realizada

em um espectrofotômetro (Thermo Scientific, Genesysm 10S UV-Vis, EUA) a 740 nm e os resultados foram convertidos para mgAGE/g utilizando-se uma curva padrão de ácido gálico como referência.

2.6 Determinação dos Potenciais Antioxidantes

Para a determinação do potencial antioxidante dos cookies elaborados dois métodos adaptados foram utilizados: Poder de Redução do Íon Ferro - FRAP (BENZIE; STRAIN, 1996) e Captura do Radical 2,2-azinobis (3-etilbenzotiazolina-6-ácido sulfônico) - ABTS (RE et al., 1999). Ambas as análises foram realizadas utilizando-se o extrato elaborado para determinação de compostos fenólicos e em triplicata para cada formulação de cookie.

Para a realização do método FRAP, uma alíquota (0,1 mL) do extrato diluído foi adicionada de 2.9 mL de solução reagente FRAP. A mistura foi mantida em incubação em banho termostatizado (37 ºC; 30 min, Marconi, MA159, Brasil). Após esse período, realizou-se a leitura das amostras a 593 nm em espectrofotômetro (Thermo Scientific, Genesysm 10S UV-Vis, EUA) e os resultados foram convertidos para mgTE/g utilizando-se uma curva padrão de trolox como referência.

Já para realização do método ABTS, 0.3 mL do extrato diluído foram adicionados a 3 mL do radical $ABTS^{\bullet+}$, homogeneizados por 6 minutos e a leitura foi feita em espectrofotômetro (Thermo Scientific, Genesysm 10S UV-Vis, EUA) a 734 nm. Os resultados foram convertidos para µmolTE/g utilizando-se uma curva padrão de trolox como referência.

2.7 Aquisição dos Espectros de Infravermelho Próximo (NIR) e Processamento de Dados

Os espectros de NIR foram obtidos utilizando um espectrômetro MicroNIR OnSite-W (N1-00208, Viavi Solutions Inc., Milpitas, CA, EUA). A informação espectral foi representada por 125 valores entre 900 e 1670 nm. Cada espectro foi adquirido com 32 scans.

Para construção dos modelos de predição, utilizou-se do algoritmo de regressão dos mínimos quadrados parciais (PLS) - por ser simples, apresentar bom desempenho e de fácil acessibilidade - com validação cruzada leave-one-out (MALEGORI et al., 2017b) e, para a separação dos grupos de predição, utilizou-se do algoritmo Kennard-Stone, onde 75% das amostras foram selecionadas para o grupo de calibração e o restante (25%) foram selecionadas pra o grupo de validação (KENNARD; STONE, 1969)

Ao final, para avaliação da acurácia da modelagem proposta, os parâmetros de raiz quadrada do erro quadrático médio de validação cruzada (RMSECV), raiz quadrada do erro quadrático médio de calibração (RMSEC), raiz quadrada do erro quadrático médio de previsão (RMSEP), coeficiente de correlação linear para a calibração (R^2c), validação cruzada (R^2cv) e predição (R^2p) e desvio residual da predição (RPD) foram analisados (OLIVIERI, 2015)

Todos os dados foram processados utilizando software MatLab, versão R2010a (The MathWorks, Inc) e aplicando algoritmos do PLS Toolbox, versão 8.1.1(Eigenvector Research, Inc).

2.8 Análise de Componentes Principais (PCA)

A PCA foi realizada visto que trata-se de um método qualitativo que consegue mostrar a variação estatística de todas as amostras em uma base química, agrupando-as de acordo com a similaridade espectral (MANCINI et al., 2020). Para tal, foi utilizado o software Unscrambler X 10.4 (CAMO, Oslo Norway) e os dados agrupados foram o teor de compostos fenólicos totais e os potenciais antioxidantes das amostras, bem como os espectros de MicroNIR.

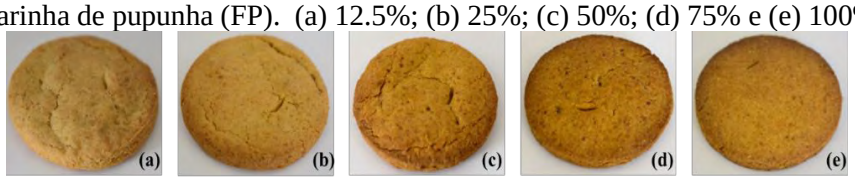
2.9 Análises Estatísticas

Todas as análises de caracterização dos cookies foram realizadas com, no mínimo, triplicata das amostras. Os resultados foram expressos em valor médio ± desvio padrão e avaliados estatisticamente através da diferença entre as médias utilizando o teste de Duncan em intervalo de confiança de 95% utilizando o software SAS (Versão 9.2, SAS, Inc.).

3 RESULTADOS E DISCUSSÃO

Na Figura 1 estão fotos de cookies produzidos com diferentes concentrações de farinha de pupunha (FP), mostrando que o aumento da concentração de FP, de maneira geral, não provocou diferenças visuais significativas nos cookies.

Figura 1 - Imagens fotográficas obtidas dos cookies produzidos com diferentes concentrações de farinha de pupunha (FP). (a) 12.5%; (b) 25%; (c) 50%; (d) 75% e (e) 100%



3.1 Atividade de água, conteúdo de água e perda água durante assamento

A atividade de água é um parâmetro importante relacionado a estabilidade dos alimentos durante armazenamento, uma vez que influencia a taxa de crescimento de microrganismos e reações químicas (ČERVENKA; BROŽKOVÁ; VYTŘASOVÁ, 2006; LIU; CHEN; XU, 2017). Os limites de atividade de água para a deterioração por bactérias, leveduras e bolores são, aproximadamente, de 0.90, 0.85-0.88 e 0.80, respectivamente, e a taxa de reação química diminui drasticamente se a atividade de água for inferior a 0.40-0.30 (INGLETT; CHEN; LIU, 2014). Dessa forma, através dos resultados de atividades de água obtidos, pode-se constatar que independente da concentração de farinha de pupunha utilizada, os cookies apresentaram valor médio de atividade de água de 0,36 ± 0,01, não apresentando diferença significativa em função da concentração de farinha de pupunha utilizada (Tabela 2). Estes resultados, sugerem que os cookies devam apresentar estabilidade microbiológica e química durante armazenamento.

Em relação ao conteúdo de água dos cookies, o aumento da concentração de FP também não provocou variação significativa neste parâmetro, obtendo-se valores médios de 7,11 ± 0,28 (Tabela 2). Este valor é semelhante ao encontrado por Reck & Miranda (2016) e Ribeiro et al., (2021) também para cookies elaborados com farinha de pupunha.

As medições do conteúdo de água em amostras de produtos panificados são, em geral, demoradas e fontes de viés, uma vez que a água pode vaporizar das amostras durante o preparo das amostras (ALCÂNTARA; CARVALHO; VANIN, 2020; VANIN; LUCAS; TRYSTRAM, 2009). Dessa forma, as análises de perda de água de água durante o assamento podem representar um resultado mais viável para entender as diferenças entre as diferentes formulações de produtos avaliadas.

Dessa forma, pode-se verificar que o aumento da concentração de farinha de pupunha provocou redução significativa nos valores de perda de água dos cookies durante o assamento, obtendo-se valores médios entre 11,92 a 10,82% (Tabela 2). Uma das possíveis causas para esse efeito pode estar relacionada a maior concentração de fibras presentes na farinha de pupunha em comparação com a farinha de trigo (MARTÍNEZ-CERVERA et al., 2011), apesar de ambos os valores serem baixos. A absorção de água pelas fibras pode provocar uma maior retenção de umidade nos cookies produzidos com maior concentração de FP. Esse comportamento já foi relatado em biscoitos tipo cookie elaborados a partir da substituição da farinha de trigo por farinha de diferentes tipos de matrizes alimentares, como amaranto (INGLETT; CHEN; LIU, 2015), casca de cacau

(ROJO-POVEDA et al., 2020), jatobá (SOARES et al., 2016) e caroço de abacate (SILVA et al., 2019).

3.2 Propriedades físicas: Fator de Espalhamento, Volume Específico e Parâmetros de Cor

O aumento da concentração de FP provocou, em geral, aumento significativo nos valores de fator de espalhamento dos cookies elaboradas, obtendo-se valores médios entre 6,67 e 7,62 (Tabela 2).

A literatura reporta que o uso de farinhas de alternativas pode provocar a diminuição ou aumento no fator de espalhamento dos cookies. Em geral, o fator de espalhamento é afetado pela competição por água disponível entre ingredientes das farinhas utilizadas (DELCOUR;HOSENEY, 2010). Quando farinhas com elevado teor de fibras são incorporadas na formulação dos cookies observa-se uma redução no fator de espalhamento dos cookies (BECKER et al., 2014; CHAGAS et al., 2021; MUDGIL; BARAK; KHATKAR, 2017; SAHNI; SHERE, 2017). Dessa forma, como a farinha de pupunha não possui elevado teor de fibras (CARVALHO et al., 2013; PIRES et al., 2019), efeito contrário é observado para as amostras de cookies.

Efeito semelhante ao desse estudo, ou seja, aumento nos valores do fator de espalhamento, foi observado em cookies produzidos com maiores concentrações de farinha de subproduto de abacaxi (TOLEDO et al. 2017), farinha de taro (ALFLEN et al. 2016), e farinha de bagaço de uva branca (MILDNER-SZKUDLARZ et al., 2013).

A diminuição da concentração de glúten pode ter provocado uma diminuição na coesão da matriz de proteínas, resultando em uma menor expansão dos cookies, o que por sua vez pode ser observado através dos menores valores de altura dos cookies em função do aumento da concentração de farinha de pupunha (Tabela 2), justificando o aumento no valor de fator de espalhamento observados.

Em relação ao volume específico, concentrações de farinha de pupunha acima de 50 % provocaram redução significativa para este parâmetro (Tabela 2). Mais uma vez, pode-se propor que este comportamento tenha sido influenciado pela redução na formação da rede de glúten e, portanto, aumento da extensibilidade da massa em função do aumento da concentração de farinha de pupunha. Pode-se supor que o volume específico e o fator de espalhamento exercem entre si uma relação inversamente proporcional neste caso.

Tabela 2 - Resultados obtidos para as diferentes análises de caracterização dos cookies produzidos com diferentes concentrações de farinha de pupunha.

Análise	**Concentração da farinha de pupunha (g/ 100g de farinha total)**				
	12,5	**25**	**50**	**75**	**100**
Atividade de Água (Aw)	0,38^{a} ± 0,03	0,37^{a} ± 0,02	0,36^{a} ± 0,01	0,35^{a} ± 0,02	0,35^{a} ± 0,02
Conteúdo de água (%)	6,78^{a} ± 0,35	6,82^{a} ± 0,39	7,25^{a} ± 0,38	7,33^{a} ± 0,52	7,38^{a} ± 0,51
Perda de Água (%)	11,92^{a} ±0,31	11,89^{a} ± 0,38	11,40^{ab} ± 0,30	10,61^{ab} ± 0,31	10,39^{b} ± 0,36
Diâmetro (cm)	52,81^{ab} ± 0,90	53,40^{a} ± 0,96	51,81^{bc} ± 0,71	51,47^{c} ± 0,61	51,21^{c} ± 0,43
Altura (cm)	7,95^{a} ± 0,49	7,70^{ab} ± 0,60	7,34^{bc} ± 0,47	7,15^{cd} ± 0,48	6,79^{d} ± 0,42
Fator de Espalhamento	6,67^{c} ± 0,41	6,98^{bc} ± 0,53	7,10^{bc} ± 0,44	7,26^{ab} ± 0,47	7,62^{a} ± 0,48
Volume Específico (cm³/g)	1,59^{a} ± 0,04	1,59^{a} ± 0,07	1,54^{ab} ± 0,04	1,45^{bc} ± 0,03	1,40^{c} ± 0,05
L	62,35^{a} ± 2,62	62,21^{a} ± 1,37	60,12^{ab} ± 2,47	58,08^{b} ± 2,08	54,23^{c} ± 4,53
a*	11,84^{c} ± 1,07	13,93^{b} ± 0,65	16,98^{a} ± 1,22	17,78^{a} ± 0,66	17,79^{a} ± 1,28
b*	34,76^{c} ± 1,45	38,28^{b} ± 0,70	41,78^{a} ± 2,12	42,85^{a} ± 1,40	42,62^{a} ± 2,06
C*	36,18^{c} ± 0,59	40,91^{b} ± 0,76	44,95^{a} ± 1,86	46,41^{a} ± 1,19	46,21^{a} ± 1,67
H°	70,92^{a} ± 1,49	70,07^{ab} ± 0,67	68,35^{bc} ± 1,56	67,43^{c} ± 1,28	67,30^{c} ± 2,18

* Médias seguidas por letras iguais na mesma linha não diferem significativamente entre si pelo teste de Duncan ($p > 0,05$); elaborado pelo autor.

3.3 Parâmetros de cor

A cor é uma característica descritiva bastante relevante para a aceitação do consumidor (BOLEK, 2022). Pode-se verificar o aumento da concentração de farinha de pupunha nos cookies provocou variação significativa nos parâmetros L, a* e b*, C* e H^o (Tabela 2), e consequentemente, na coloração do cookie (Figura 1). Esse comportamento também foi relatado para cookies produzidos a partir de farinha de alcachofra-girassol (*Helianthus tuberosus L.*) (LEE et al., 2016), tamarindo (Tamarindus indica) (BOLEK, 2022) e cabaça (*Lagenaria siceraria*) (MUHAMMAD et al., 2022). Esse fato pode ser atribuído ao escurecimento enzimático e reação de Maillard durante o processo de assamento. A farinha de pupunha apresenta um elevado teor de polifenóis (ROJAS-GARBANZO et al., 2012; SANTOS et al., 2015), que são substratos para as polifenoloxidases, que na presença de oxigênio provocam a formação de pigmentos escuros resultantes da polimerização da quinina. Associado a isso, por conter mais açúcares que a farinha de trigo, a farinha de pupunha pode favorecer as reações de escurecimento não enzimático (Maillard) (MILDNER-SZKUDLARZ et al., 2013).

3.4 Compostos Fenólicos Totais e Potencial Antioxidante

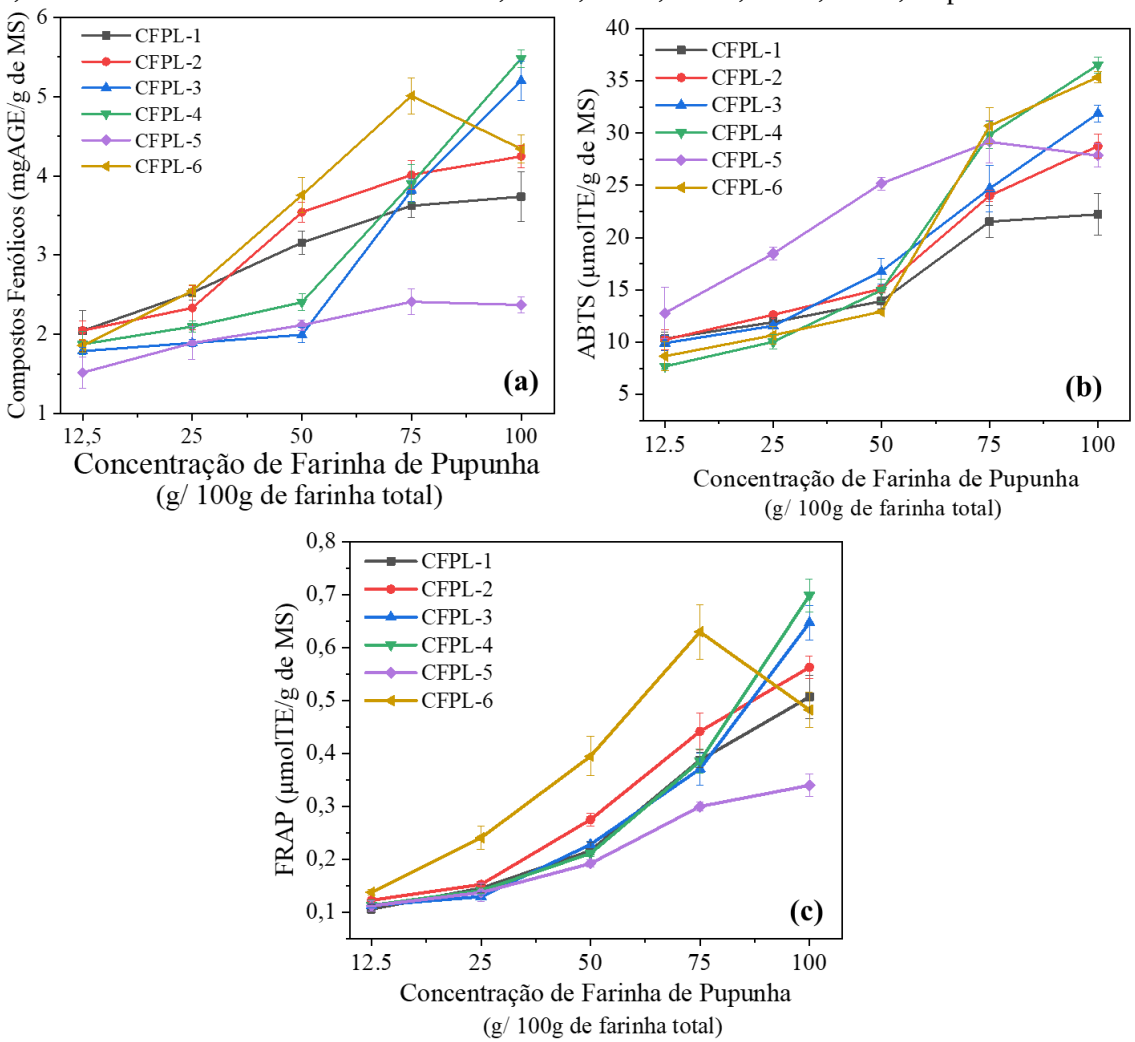
Os resultados de compostos fenólicos totais e potencial antioxidante (Figura 2) foram analisados considerando as diferentes concentrações de farinhas de pupunha utilizadas para a produção dos cookies, e também o local de colheita dos frutos utilizadas para a produção das farinhas, visto que tais diferenças foram consideradas na elaboração da modelagem para a predição de tais parâmetros (seções 3,5 e 3,6).

De modo geral, observa-se que o aumento da concentração da farinha de pupunha provou aumento significativo da concentração de compostos fenólicos totais nos cookies produzidos (Figura 2a). De modo geral, os cookies elaborados apresentaram teores de compostos fenólicos superiores a cookies produzidos com 15% de farinha de bergamota (LAGANA et al., 2022), 70% de farinha de casca de goiaba (BERTAGNOLLI et al., 2014), 100% de farinha de alfarroba (BABIKER et al., 2020) e 9% de farinha de epicarpo de maracujá (NING et al., 2021). Estes resultados evidenciam o elevado potencial da farinha de pupunha como ingrediente rico em compostos fenólicos.

Além disso, observa-se que tal comportamento ocorre de maneira acentuada para os cookies produzidos com as farinhas de pupunha das localidades (CFP) 3 e 4 (CFP 3 e CFP4), e de maneira mais gradual nos cookies produzidos com CFP-1, CFP-2 e CFP-5. Tais diferenças podem ter ocorrido devido as diferentes regiões de colheita, visto que a

localização de colheita e as condições de cultivo podem interferir diretamente na composição bioativa de frutos (USLU; ÖZCAN, 2020), e, consequentemente, no produto elaborado.

Figura 2 - (a) Concentração de compostos fenólicos totais, (b) potencial antioxidante pelo método ABTS e (c) FRAP para os cookies produzidos com diferentes concentrações de farinha pupunha obtidas de diferentes localidades (CFPL); as amostras CFPL-1, CFPL-2, CFP-3, CFPL-4, CFPL-5, CFPL-6 são oriundas das farinhas FPL1, FPL2, FPL3, FPL4, FPL5, FPL6, respectivamente.



Elaborado pelo autor

Os resultados do potencial antioxidante pelos métodos ABTS e FRAP (Figura 2b e 2c, respectivamente) demonstraram que o teor de compostos fenólicos está diretamente relacionado com o potencial antioxidante e, consequentemente, contribui para uma maior atividade antioxidante à medida que a concentração de farinha de pupunha aumenta nas formulações de cookies.

A amostra CFPL-6 não teve sua capacidade antioxidante diminuída na formulação de 100% pelo método ABTS, mas pelo método FRAP, sim. Já a amostra

CFPL-5 demonstrou um decréscimo em seu potencial antioxidante pelo método ABTS, mas não pelo método FRAP. Tal diferença, novamente, pode ser explicada pelas características de cada farinha utilizada.

3.5 Métodos de Referência, Análise de Componentes Principais (PCA) e Interpretação do Espectro de NIR das amostras

Os intervalos dos valores de referência utilizados como variáveis preditoras nas modelagens feitas para o teor de compostos fenólicos totais, capacidade antioxidante pelos métodos ABTS e FRAP são mostrados na Tabela 3. Os resultados foram divididos de acordo com os grupos de calibração (67 amostras) e validação (23 amostras) e apresentam a faixa de valores das variáveis utilizadas, valor médio e desvio padrão para cada grupo.

Tabela 3 - Intervalo de dados dos métodos de referência utilizados para a construção das modelagens para as amostras de cookie de farinha de pupunha com diferentes concentrações de farinha de pupunha.

Análise de Referência	***Calibração (67 amostras)**			***Validação (23 amostras)**		
	Intervalo	**Média**	**Desvio Padrão**	**Intervalo**	**Média**	**Desvio Padrão**
Compostos fenólicos (mgAGE/g de resíduo seco)	5,55-1,68	3,09	1,19	4,16-1,30	2,66	0,87
ABTS (µmol TE/g de resíduo seco)	37,37-7,03	19,96	9,23	30,76-7,64	17,01	7,26
FRAP (µmol TE/g de resíduo seco)	0,73-0,09	0,31	0,19	0,47-0,10	0,23	0,11

*90 amostras utilizadas para a construção das modelagens ao total (calibração + validação); elaborado pelo autor

A Figura 3a apresenta o *scoreplot* (amostras) utilizando os componentes principais 1 (PC-1) e 2 (PC-2), e também mostra os *loadings plot* (análises de referência), no que diz respeito aos dados físico-químicos utilizados para a predição. A PCA é uma ferramenta matemática que tem como objetivo representar a variação existente dentro de um conjunto de dados, em outras palavras, visa encontrar “respostas” que serão usadas para caracterizar as amostras (GRANATO et al., 2018b).

Observa-se uma boa separação entre as amostras nos quadrantes de acordo com a concentração de farinha de pupunha utilizada na formulação dos cookies, onde as

variâncias para PC-1 e PC-2 explicam 98.5%, dos dados. Além disso, é possível notar que a partir da região de maior influência (PC-1), quanto maior concentração de PF nos cookies, maior os efeitos que a concentração de compostos fenólicos e os potenciais antioxidantes (ABTS e FRAP) exercem sobre as amostras, localizando-as para o lado positivo da PC-1.

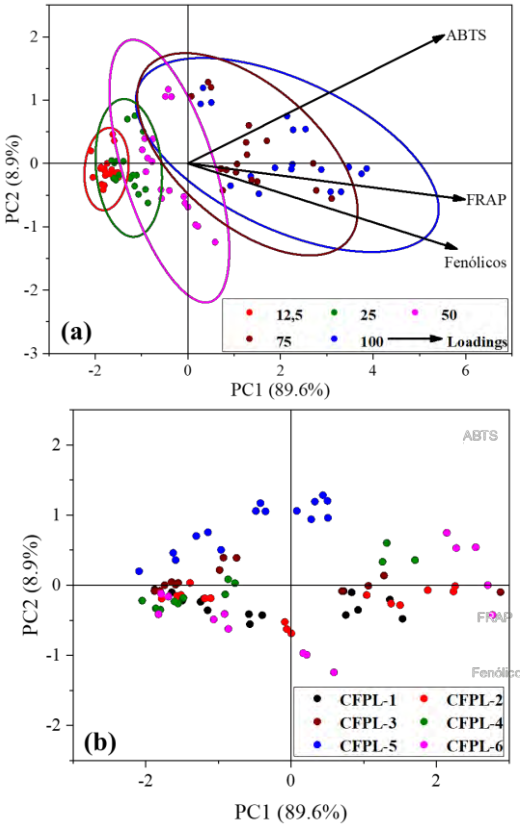
Por outro lado, ao se analisar o efeito do local de colheita dos frutos usados para a produção das farinhas utilizadas na formulação dos cookies pode-se observar que este efeito não foi significativo a ponto de classificar as amostras (Figura 3b). Tal fato indica que as regiões de colheita apresentaram características de teor de compostos fenólicos e potenciais antioxidantes semelhantes, confirmando que a variabilidade dos cookies sofre maior influência pela proporção de farinha. Bizzani et al. (2017) também não observou agrupamentos oriundos de parâmetros extrínsecos em amostras de laranjas e destaca que isso ocorre devido a características semelhantes entre as amostras obtidas sob diferentes condições, o que por sua vez indica uma boa variação na distribuição da amostragem.

O espectro NIR médio de cada formulação de cookies após tratamento com a segunda derivada e suavização de Savitzky-Golay (janela de 15 pontos e ordem polinomial 2) revela as principais bandas de absorção nas regiões de 1131, 1200,1349-1386, 1316, 1397, 1450 e 1588 nm (Figura 4a). A utilização do tratamento da segunda derivada diminui os efeitos da dispersão da luz, resultando na eliminação de linha de base entre os espectros e aumentando a resolução dos picos e, consequentemente, facilitando a análise.(KADDOUR; MOREL; CUQ, 2008).

A região centrada em 1200 nm é decorrente à componentes de lipídios e carboidratos (HERNÁNDEZ-HERNÁNDEZ et al., 2022; WIMONSIRI et al., 2017). Já a banda centrada em 1316 e em 1588 nm são atribuídas as vibrações C-H (estiramento C-H e deformação C-H) e ao estado de ligação de hidrogênio intermolecular que podem estar associadas a hidratação do amido assim como à alteração na estrutura de proteínas em função de mudanças em sua estrutura secundária (KADDOUR; MOREL; CUQ, 2008).

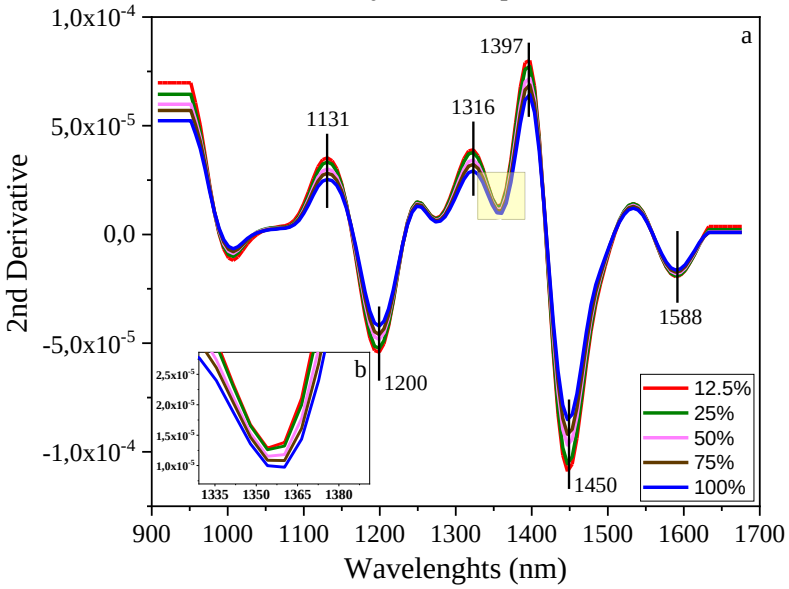
A absorção nas bandas centradas em 1450 nm são devido às combinações O-H simétricas e antissimétricas e também aos modos de alongamento antissimétricos e de deformação das moléculas de água (LUCAS et al., 2008; WIMONSIRI et al., 2017).

Figura 3 - (a) Score e loading plot dos componentes principais 1 (PC-1) e 2 (PC-2) para as análises de teor de compostos fenólicos e potenciais antioxidantes pelos métodos ABTS e FRAP feitas nas amostras de cookies elaborados com diferentes concentrações de farinha de pupunha; (b) Score plot dos componentes principais (PC-1 e PC-2) obtidos através das análises de teor de compostos fenólicos e potenciais antioxidantes com base na localização de colheita dos frutos utilizados para a fabricação da farinha.



Elaborado pelo autor

Figura 4 - (a) médias dos espectros tratados pelo processamento de segunda derivada e Savitzky-Golay (janela de 15 pontos e ordem polinomial 2) para cada concentração de farinha de pupunha nos cookies; (b) aumento da escala na região de 1330-1386 possivelmente relacionada a concentração de compostos fenólicos.



Elaborado pelo autor

Apesar de bandas relacionadas a componentes de menor concentração, como os compostos fenólicos, serem difíceis de ser detectadas por análise visual de espectros (HERNÁNDEZ-HERNÁNDEZ et al., 2022), a curta região espectral entre 1349 e 1386 nm está associada a presença de compostos fenólicos (HASHIMOTO et al., 2018; MACAVILCA; CONDEZO-HOYOS, 2020) e, a partir de uma expansão da escala (Figura 4b), é possível verificar que as intensidades de absorbância nesses comprimentos aumenta à medida que a concentração de compostos fenólicos também aumenta.

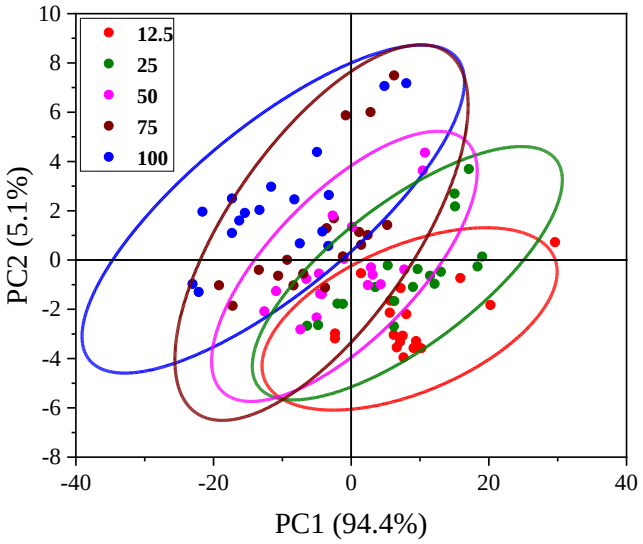
Portanto, considerando as colocações a respeito dos resultados químicos e analisando a Figura 4, observa-se que a espectroscopia NIR apresenta potencial para discriminar e, provavelmente, quantificar de modo não invasivo o teor de compostos fenólicos e potenciais antioxidantes nas amostras de cookies.

3.6 Desenvolvimento dos Modelos de Calibração e Validação

3.6.1 Análise dos Componentes Principais dos Espectros de NIR

Assim como ocorreu para a PCA utilizando apenas as análises de referência, quando o espectro bruto dos cookies foi analisado nota-se uma diferenciação entre as amostras produzidas com diferentes concentrações de farinha de pupunha, mesmo que não tão clara quanto no caso anterior (Figura 5). Apenas dois componentes principais foram necessários para descrever mais de 95% da variabilidade total dos dados para as diferentes formulações de cookies.

Figura 5 - Análise de componentes principais (PCA) de 93 espectros brutos de cookies produzidos com diferentes concentrações de farinha de pupunha (12.5, 25, 50, 75 ou 100 g/ 100g de farinha total).



Elaborado pelo autor

A formação de grupos em função da concentração de farinha de pupunha nos cookies pode ser observada na PCA. Também é possível observar que a medida que a concentração de farinha de pupunha aumenta, há uma maior dispersão entre dados do mesmo grupo, o que pode ser explicado pelo fato da PCA ser uma técnica não supervisionada que relaciona as variâncias com índices e agrupamentos de dados (LIMA et al., 2020).

3.6.2 Modelos de Calibração e Validação Cruzada PLS por NIR

A principal vantagem do algoritmo PLS para previsão de dados espectroscópicos é que, durante a análise do espectro completo, as variáveis são correlacionadas e não há exigência de que sigam uma distribuição normal (LIMA et al., 2022) e por isso é utilizado na grande maioria das vezes para predição com dados de NIR.

Diferentes modelos de regressão foram desenvolvidos para o conjunto espectral e as estatísticas de regressão das modelagens elaboradas podem ser observadas na Tabela 4. Vale ressaltar que diferentes pré-processamentos foram avaliados (dados não mostrados), mas apenas aqueles que apresentaram os melhores resultados foram apresentados. Os pré-processamentos tem como objetivo remover e/ou reduzir ruídos nos espectros que não são relacionados à absorção química da luz e, consequentemente, aos compostos de interesse (ALBANELL; MIÑARRO; CARRASCO, 2012), podendo ser usados de forma singular ou em combinações (ALFIERI et al., 2019). Além disso, cada conjunto de dados de referência foi processado individualmente.

O algoritmo PLS utiliza informação das duas matrizes de dados (espectros vs referência) e as relacionam de forma a dar origem as variáveis latentes (VL), que representam a quantidade de variáveis significativas capazes de prever com maior facilidade, e menor erro associado, a composição química desejada a partir da modelagem proposta (LIMA et al., 2020).

As VL são selecionadas a partir do erro mínimo do erro quadrático médio da validação cruzada (RMSECV) e também a partir pelo teste de *Student vs Laverage* e T^2 *Hotelling*, que também auxiliam na identificação de valores anômalos (LIMA et al., 2020; RIBEIRO et al., 2009). De modo geral, quanto menor o número de VL selecionadas, mais simples será o modelo, não sendo aconselhável selecionar mais que 8 a fim de evitar super ajuste *(overfitting*) (CARBAS et al., 2020).

Tabela 4 - Parâmetros de avaliação das modelagens elaboradas para os grupos de calibração e validação cruzada para a determinação de compostos fenólicos e potenciais antioxidantes (ABTS e FRAP) em amostras de cookies com diferentes concentrações de farinha de pupunha por NIR

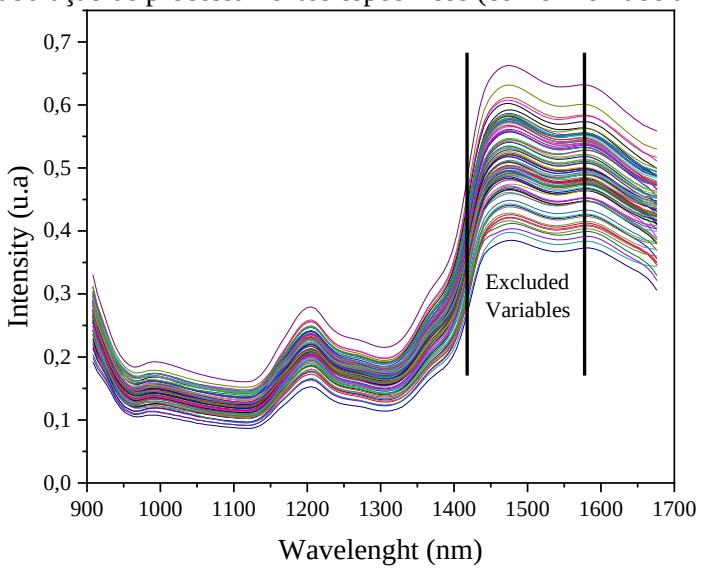
Análise Química	**Estatística de Regressão da Modelagem - Calibração**						
	Pré – Processamento	**Condição do Espectro**	**VL**	**R^2c**	**R^2cv**	**RMSEC**	**RMSECV**
Compostos Fenólicos	*Detrend + Mean Center*	Inteiro	5	0.87	0.84	0.42	0.47
	Mean Center	Inteiro	6	0.89	0.84	0.39	0.46
	*OSC**	Inteiro	3	0.80	0.77	0.52	0.57
	Smoothing (ordem polinomial 2 e janela de 15 pontos)	***Inteiro***	***6***	***0.89***	***0.86***	***0.38***	***0.44***
ABTS	*Detrend*	Inteiro	6	0.83	0.78	3.72	4.30
	Smoothing (ordem polinomal 2 e janela de 15 pontos) + Detrend	Inteiro	4	0.71	0.64	4.90	5.47
	Smoothing (ordem polinomal 2 e janela de 15 pontos)	Inteiro	7	0.82	0.76	3.85	4.52
	Detrend + OSC	Inteiro	3	0.85	0.80	3.50	4.06
	Detrend + OSC	***Corte na região de 1416 a 1577 nm***	***3***	***0.89***	***0.83***	***3.06***	***3.72***
	OSC	Corte na região de 1416 a 1577 nm	6	0.82	0.77	3.79	4.36
	Detrend + Smoothing (ordem polinomal 2 e janela de 15 pontos)	Corte na região de 1416 a 1577 nm	8	0.87	0.81	3.24	3.95
FRAP	*Mean Center*	Inteiro	6	0.93	0.90	0.05	0.06
	Mean Center + Detrend	Inteiro	6	0.94	0.92	0.05	0.05
	OSC	Inteiro	3	0.88	0.85	0.07	0.07
	****SNV + Smoothing (ordem polinomial 2 e janela de 7 pontos)***	***Inteiro***	***6***	***0.95***	***0.93***	***0.04***	***0.05***

	**OSC + Smoothing (ordem polinomial 2 e janela de 7 pontos)*	Inteiro	3	0.87	0.85	0.07	0.07
	Mean Center	Corte na região de 1416 a 1577 nm	6	0.93	0.90	0.05	0.06
	Smoothing (ordem polinomial 2 e janela de 7 pontos)	Corte na região de 1416 a 1577 nm	5	0.92	0.90	0.05	0.06

*OSC: correção de sinal ortogonal; SNV: variação normal padrão; pré-processamentos marcados em negrito e itálico são indicativos de melhores modelos ajustados; elaborado pelo autor.

Entre os diferentes pré-processamentos avaliados, alguns foram aplicados no espectro inteiro e outros no espectro com comprimentos de ondas excluídos. A seleção de comprimentos de ondas é feita pois em algumas regiões podem haver muitos dados irrelevantes, ruidosos e não confiáveis e sua remoção normalmente melhora as previsões e reduz a complexidade do modelo (ANDERSEN; BRO, 2010; CHENG; SUN, 2017). O espectro integral e a localização da região excluída estão na Figura 6.

Figura 6 - Espectros originais das 93 amostras de cookies elaborados com diferentes concentrações de farinha de pupunha e identificação da zona excluída (1416-1577 nm) durante a elaboração de processamentos específicos (conforme Tabela 4).



Elaborado pelo autor

O desempenho e a robustez do conjunto de calibração foram avaliados usando os coeficientes de determinação de calibração (R^2c) e validação cruzada (R^2cv), erro quadrático médio de calibração (RMSEC) e validação cruzada (RMSECV). De modo

geral, um modelo robusto deve apresentar altos coeficientes de determinação e baixos valores de erros quadráticos, bem como uma pequena diferença entre eles (MAGWAZA et al., 2016; PUTTIPIPATKAJORN; PUTTIPIPATKAJORN, 2020; TAHIR et al., 2016; ZHU et al., 2017). De modo mais específico, um R^2 entre 0.50 e 0.65 indica uma predição ruim, onde apenas uma discriminação entre concentrações altas e baixas será observada, entre 0.66 e 0.81 indica previsões quantitativas aproximadas, enquanto R^2 maior que 0.82 e menor que 0.90 indica uma boa previsão e, acima disto, são consideradas previsões excelentes (KAROUI et al., 2006).

Os resultados observados para os quatro modelos desenvolvidos para predição de polifenóis totais tiveram coeficientes de determinação de calibração (R^2c) e validação cruzada (R^2cv) entre 0.80-0.89 e 0.77-0.87, respectivamente, com bons erros quadráticos de calibração (0.38-0.52 mgAGE/g) e validação cruzada (0.42-0.57 mgAGE/g) (Tabela 4). Embora a dificuldade de prever o conteúdo fenólico em diferentes tipos de cookies comerciais por NIR já tenha sido discutida, principalmente devido à baixa concentração deste componente nesse tipo de matriz alimentar (BEDINI et al., 2013), os valores de R^2 acima de 0.82 representam modelos classificados como bons e aceitáveis (CHENG; SUN, 2017). Em um estudo recente, Alfieri et al., (2019), determinaram o teor de fenólicos em grãos de sorgo por NIR e encontraram valores de coeficientes de determinação semelhantes ao desse estudo (R^2c: máx. 0.88; R^2cv: máx. 0.76). Por outro lado, a previsão de polifenóis em chás instantâneos comerciais não teve tanto sucesso utilizando o algoritmo PLS (R^2c: máx de 0.73; RMSEC: 6.17) (BAI et al., 2022). Dentre as modelagens elaboradas para o teor de fenólicos, as estatísticas de regressão para o modelo *Smoothing* foram consideradas as melhores, esse pré-processamento consegue otimizar a relação sinal/ruído e, consequentemente, remover os ruídos aleatórios de dados espectrais (CEN; HE, 2007).

Apesar da faixa de concentração dos potenciais antioxidantes ser baixa, principalmente no caso do método FRAP (0,73-0,09 mgTE/g), e não serem compostos químicos específicos e sim uma propriedade inerente aos compostos presentes, os modelos previstos para as capacidades antioxidantes também se mostraram bastante satisfatórios. Os melhores coeficientes de determinação de calibração (R^2c) para os métodos ABTS e FRAP foram de 0.89 e 0.95, respectivamente, associados a RMSEC e RMSECV baixos, além disso, a previsão feita para o potencial antioxidante pelo método FRAP se mostrou melhor do que aquele gerado para o método ABTS. Carbas et al. (2020), realizaram predições de potenciais antioxidantes em feijão pelo mesmo algoritmo

e conseguiram estatísticas semelhantes para os métodos ABTS (R^2c: máx. 0.97; R^2cv: máx 0.90) e FRAP (R^2c: máx. 0.97; R^2cv: 0.86). Tahir et al. (2016) discriminaram méis de diferentes regiões botânicas sudanesas através de espectroscopia NIR e também alcançaram modelos satisfatórios para predição de capacidade antioxidante pelo método FRAP (R^2c: máx. 0.96; RMSECV: máx. 10.70; VL: máx. 14).

Em relação a etapa de validação, as estatísticas de regressão – coeficientes de determinação de predição (R^2p), erro quadrático médio de predição (RMSEP) e desvio de predição residual (RPD) – foram avaliadas. A Figura 7 ilustra os gráficos de dados mensurados *versus* preditos dos melhores modelos para detectar os parâmetros químicos desejados, bem como as estatísticas de regressão da etapa de validação e, assim como ocorre na etapa de calibração, o desempenho da predição é definida por baixos erros de RMSEP e alto R^2p (LIU et al., 2011).

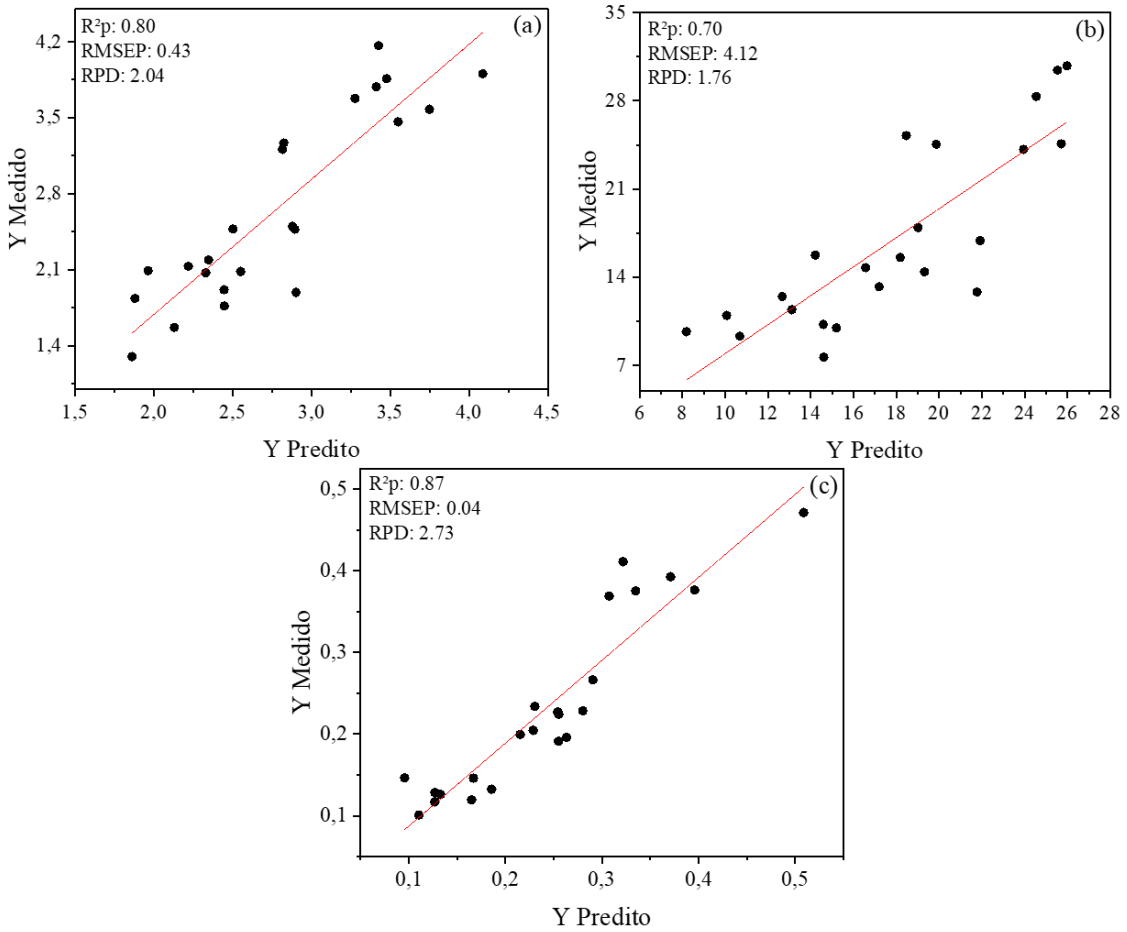
Já em relação ao RPD, esta é uma medida utilizada para indicar quão satisfatórios foram os desempenhos da etapa de calibração (FERRER-GALLEGO et al., 2011) e a classificação das modelagens segundo essa estatística são excelentes (RPD>2), razoáveis (1.4<RDP<2) e não confiáveis (RPD<1.4) (CHANG et al., 2001).

Dessa forma, de acordo com a avaliação dos modelos, é possível realizar excelentes predições utilizando o método FRAP e compostos fenólicos totais, sendo essas a primeira e a segunda melhores predições elaboradas, respectivamente, mas apenas modelos razoáveis utilizando o método ABTS.

Em um estudo recente, desenvolvido para prever diferentes parâmetros de qualidade em grãos de cacau, a previsão do teor de compostos fenólicos foi considerada apenas razoável, com valor de R^2c, R^2cv e RPD de 0.87, 0.66 e 1.69, respectivamente (HERNÁNDEZ-HERNÁNDEZ et al., 2022). Um outro estudo, idealizado pelos mesmo autores, relata que modelos de calibração para atividade antioxidante podem ser usados para discriminar amostras, dada tal função atribuída na literatura aos fenóis (HERNÁNDEZ-HERNÁNDEZ et al., 2021) e, portanto, é possível elaborar boas previsões, mesmo não sendo compostos químicos dos alimentos.

Diferente do encontrado nesse estudo, Carbas et al., (2020), conseguiram melhores predições para o método ABTS do que para o FRAP e Tahir et al. (2016) conseguiram predições similares ao elaborado nesse estudo para compostos fenólicos e capacidade antioxidante por FRAP em méis sudaneses. Já Kljusurić et al. (2016) alcançaram excelentes predições de compostos fenólicos em frutas vermelhas usando NIR (R^2c: 0.99; R^2p: 0.98; RPD: 3.05).

Figura 7 - Regressão linear dos dados preditos versus dados medidos para o conjunto de validação para (a) compostos fenólicos, (b) potencial antioxidante pelo método ABTS e (c) potencial antioxidante pelo método FRAP em cookies elaborados com diferentes concentrações de farinha de pupunha.



Elaborado pelo autor

Logo, os parâmetros de avaliação indicam a possibilidade de determinação de compostos bioativos totais e indicadores de qualidade antioxidante em amostras de cookies elaborados com diferentes concentrações de farinha de pupunha. Tal constatação é de extrema importância, considerando que até o presente momento foi encontrado apenas um estudo que avaliou a predição de compostos fenólicos em biscoitos comerciais, sendo que os resultados não foram satisfatórios (BEDINI et al., 2013), e demais estudos sobre a aplicação de técnicas espectroscópicas e calibração multivariada em cookies foram focados na previsão de diferentes componentes químicos majoritários, como umidade, proteína, lipídios, glúten, xantinas e carboidratos (BEDINI et al., 2013; CAYUELA-SÁNCHEZ et al., 2020; LANCELOT et al., 2020; RADOŠ et al., 2021; WIMONSIRI et al., 2017).

4 CONCLUSÃO

Através dos resultados apresentados pode-se concluir que a farinha de pupunha apresenta um grande potencial de aplicabilidade industrial, uma vez que os cookies produzidos apresentaram propriedades tecnológicas bastante interessantes, além da possibilidade de formulação de um produto feito integralmente de farinha de pupunha. Tal formulação representa um grande passo na elaboração de produtos voltados para os consumidores que possuem a doença celíaca, por ser um produto sem glúten, e que pode ser caracterizado também como um produto funcional, resultando em um alto valor agregado.

Somado a esse fato, a utilização de metodologias alternativas, como o MicroNIR, pode aprimorar e agilizar sistemas de controle de qualidade em linhas de produção de alimentos, permitindo que a análise seja feita fora de um ambiente controlado, diretamente nas amostras e sem uso de reagentes.

REFERÊNCIAS

AACC 10–50.5, A. A. OF C. C. **Approved methods of the American association of cereal chemists**. [s.l.] Amer Assn of Cereal Chemists, 2000. v. 1

ABUTAHA, N. In vitro antiproliferative activity of partially purified Withania somnifera fruit extract on different cancer cell lines. **Journal of B.U.ON.**, v. 20, n. 2, p. 625–630, 2015.

ALBANELL, E.; MIÑARRO, B.; CARRASCO, N. Detection of low-level gluten content in flour and batter by near infrared reflectance spectroscopy (NIRS). **Journal of Cereal Science**, v. 56, n. 2, p. 490–495, 2012.

ALCÂNTARA, R. G. DE; CARVALHO, R. A. DE; VANIN, F. M. Evaluation of wheat flour substitution type (corn, green banana and rice flour) and concentration on local dough properties during bread baking. **Food Chemistry**, v. 326, n. April, p. 126972, 2020.

ALFIERI, M. et al. Discrimination and prediction of polyphenolic compounds and total antioxidant capacity in sorghum grains. **Journal of Near Infrared Spectroscopy**, v. 27, n. 1, p. 46–53, 2019.

ALFLEN, T. A. et al. Partial substitution of wheat flour with taro (Colocasia esculenta) flour on cookie quality. **Revista Ciencias Exatas e Naturais**, v. 18, n. 2, p. 202–212, 2016.

AMODIO, M. L. et al. Potential of NIR spectroscopy for predicting internal quality and discriminating among strawberry fruits from different production systems. **Postharvest Biology and Technology**, v. 125, p. 112–121, 2017.

ANDERSEN, C. M.; BRO, R. Variable selection in regression-a tutorial. **Journal of Chemometrics**, v. 24, n. 11–12, p. 728–737, 2010.

AOAC, A. OF O. A. C. **Official Methods of AnalysisOfficial Methods of Analysis**, 2005.

ARRUDA DE BRITO, A. et al. Non-destructive determination of color, titratable acidity, and dry matter in intact tomatoes using a portable Vis-NIR spectrometer. **Journal of Food Composition and Analysis**, v. 107, n. October 2021, 2022.

BABIKER, E. E. et al. Physico-chemical and bioactive properties, fatty acids, phenolic compounds, mineral contents, and sensory properties of cookies enriched with carob flour. **Journal of Food Processing and Preservation**, n. June, p. 1–10, 2020.

BAI, X. et al. Near-infrared spectroscopy and machine learning-based technique to predict quality-related parameters in instant tea. **Scientific Reports**, v. 12, n. 1, p. 1–8, 2022.

BECKER, F. S. et al. Incorporation of Buriti Endocarp Flour in Gluten-free Whole Cookies as Potential Source of Dietary Fiber. **Plant Foods for Human Nutrition**, v. 69, n. 4, p. 344–350, 2014.

BEDINI, A. et al. Rapid and Simultaneous Analysis of Xanthines and Polyphenols as Bitter Taste Markers in Bakery Products by FT-NIR Spectroscopy. **Food Analytical Methods**, v. 6, n. 1, p. 17–27, 2013.

BENZIE, I. F. F.; STRAIN, J. J. The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "'Antioxidant Power"': The FRAP Assay Iris. **Analytical Biochemistry**, v. 239, p. 70–79, 1996.

BERTAGNOLLI, S. M. M. et al. Bioactive compounds and acceptance of cookies made with Guava peel flour. **Food Science and Technology**, v. 34, n. 2, p. 303–308, 2014.

BIZZANI, M. et al. Non-invasive spectroscopic methods to estimate orange firmness, peel thickness, and total pectin content. **Microchemical Journal**, v. 133, p. 168–174, 2017.

BOLEK, S. Valorization of microwave roasted Tamarindus indica seed in functional biscuit production and effects on rheological and textural properties of biscuit dough. **Journal of Food Science and Technology**, v. 59, n. 5, p. 2080–2086, 2022.

BRASIL, Y. L.; CRUZ-TIRADO, J. P.; BARBIN, D. F. Fast online estimation of quail eggs freshness using portable NIR spectrometer and machine learning. **Food Control**, v. 131, n. May 2021, p. 108418, 2022.

CARBAS, B. et al. Prediction of Phytochemical Composition, In Vitro Antioxidant Activity and Individual Phenolic Compounds of Common Beans Using MIR and NIR Spectroscopy. **Food and Bioprocess Technology**, v. 13, n. 6, p. 962–977, 2020.

CARVALHO, A. V. et al. Características físicas e químicas de frutos de pupunheira no

estado do Pará. **Revista Brasileira de Fruticultura**, v. 35, n. 3, p. 763–768, 2013.

CAYUELA-SÁNCHEZ, J. A. et al. Compositional method for measuring the nutritional label components of industrial pastries and biscuits based on Vis/NIR spectroscopy. **Journal of Food Composition and Analysis**, v. 92, n. January, p. 103572, 2020.

CAYUELA, J. A.; WEILAND, C. Intact orange quality prediction with two portable NIR spectrometers. **Postharvest Biology and Technology**, v. 58, n. 2, p. 113–120, 2010.

CEN, H.; HE, Y. Theory and application of near infrared reflectance spectroscopy in determination of food quality. **Trends in Food Science and Technology**, v. 18, n. 2, p. 72–83, 2007.

ČERVENKA, L.; BROŽKOVÁ, I.; VYTŘASOVÁ, J. Effects of the principal ingredients of biscuits upon water activity. **Journal of Food and Nutrition Research**, v. 45, n. 1, p. 39–43, 2006.

CHAGAS, E. G. L. et al. Enrichment of antioxidants compounds in cookies produced with camu-camu (Myrciaria dubia) coproducts powders. **Lwt**, v. 137, n. October 2020, 2021.

CHANG, C.-W. et al. Near-Infrared Reflectance Spectroscopy-Principal Components Regression Analyses of Soil Properties. **Soil Science Society of America Journal**, v. 65, n. 2, p. 480–490, 2001.

CHENG, J. H.; SUN, D. W. Partial Least Squares Regression (PLSR) Applied to NIR and HSI Spectral Data Modeling to Predict Chemical Properties of Fish Muscle. **Food Engineering Reviews**, v. 9, n. 1, p. 36–49, 2017.

CHIREMBA, C.; TAYLOR, J. R. N.; DUODU, K. G. Phenolic content, antioxidant activity, and consumer acceptability of sorghum cookies. **Cereal Chemistry**, v. 86, n. 5, p. 590–594, 2009.

CHISTÉ, R. C. et al. Carotenoid and phenolic compound profiles of cooked pulps of orange and yellow peach palm fruits (Bactris gasipaes) from the Brazilian Amazonia. **Journal of Food Composition and Analysis**, v. 99, n. December 2020, p. 103873, 2021.

DELCOUR, J. A.; HOSENEY, R. C. Principles of Cereal Science and Technology. AACC International Press. 2010.

ELSAYED, E. A. et al. In vitro assessment of anticancer properties of moringa peregrina essential seed oil on different cell lines. **Pakistan Journal of Zoology**, v. 48, n. 3, p. 853–859, 2016.

FELISBERTO, M. H. F. et al. Characterization and technological properties of peach palm (Bactris gasipaes var. gasipaes) fruit starch. **Food Research International**, v. 136, n. May, p. 109569, 2020.

FERRER-GALLEGO, R. et al. Determination of phenolic compounds of grape skins during ripening by NIR spectroscopy. **LWT - Food Science and Technology**, v. 44, n. 4, p. 847–853, 2011.

FRIZON, C. N. T. et al. Determination of total phenolic compounds in yerba mate (Ilex paraguariensis) combining near infrared spectroscopy (NIR) and multivariate analysis. **Lwt**, v. 60, n. 2, p. 795–801, 2015.

GIADA, M. DE L. R. Food Phenolic Compounds: Main Classes, Sources and Their Antioxidant Power. In: **Oxidative Stress and Chronic Degenerative Diseases - A Role for Antioxidants**. [s.l: s.n.]. p. 87–112.

GONNET, J. F. Colour effects of co-pigmentation of anthocyanins revisited - 1. A colorimetric definition using the CIELAB scale. **Food Chemistry**, v. 63, n. 3, p. 409–415, 1998.

GRANATO, D. et al. Use of principal component analysis (PCA) and hierarchical cluster analysis (HCA) for multivariate association between bioactive compounds and functional properties in foods: A critical perspective. **Trends in Food Science and Technology**, v. 72, n. December 2017, p. 83–90, 2018.

GUIDETTI, R.; BEGHI, R.; BODRIA, L. Evaluation of grape quality parameters by a simple vis/nir system. **American Society of Agricultural and Biological Engineers**, v. 53, n. 2, p. 477–484, 2010.

HASHIMOTO, J. C. et al. Quality Control of Commercial Cocoa Beans (Theobroma cacao L.) by Near-infrared Spectroscopy. **Food Analytical Methods**, v. 11, n. 5, p. 1510–1517, 2018.

HEMPEL, J. et al. Lipid-dissolved γ-carotene, β-carotene, and lycopene in globular chromoplasts of peach palm (Bactris gasipaes Kunth) fruits. **Planta**, v. 240, n. 5, p. 1037–1050, 2014.

HERNÁNDEZ-HERNÁNDEZ, C. et al. Viability of near infrared spectroscopy for a rapid analysis of the bioactive compounds in intact cocoa bean husk. **Food Control**, v. 120, n. July 2020, 2021.

HERNÁNDEZ-HERNÁNDEZ, C. et al. Rapid screening of unground cocoa beans based on their content of bioactive compounds by NIR spectroscopy. **Food Control**, v. 131, n. February 2021, 2022.

INGLETT, G. E.; CHEN, D.; LIU, S. Physical properties of sugar cookies containing chia-oat composites. **Journal of the Science of Food and Agriculture**, v. 94, n. 15, p. 3226–3233, 2014.

INGLETT, G. E.; CHEN, D.; LIU, S. X. Physical properties of gluten-free sugar cookies made from amaranth-oat composites. **LWT - Food Science and Technology**, v. 63, n. 1, p. 214–220, 2015.

JAN, U. et al. Characterization of cookies made from wheat flour blended with

buckwheat flour and effect on antioxidant properties. **Journal of Food Science and Technology**, v. 52, n. 10, p. 6334–6344, 2015.

KADDOUR, A. A.; MOREL, M. H.; CUQ, B. Description of batter mixing using near infrared spectroscopy. **Journal of Cereal Science**, v. 48, n. 3, p. 698–708, 2008.

KAROUI, R. et al. Mid-infrared spectrometry: A tool for the determination of chemical parameters in Emmental cheeses produced during winter. **Diary Science & Technology**, v. 86, n. 1, p. 83–97, 2006.

KENNARD, R. W.; STONE, L. A. Computer Aided Design of Experiments. **Technometrics**, v. 11, n. 1, p. 137–148, 1969.

KHOOZANI, A. A.; BEKHIT, A. E. D. A.; BIRCH, J. Effects of different drying conditions on the starch content, thermal properties and some of the physicochemical parameters of whole green banana flour. **International Journal of Biological Macromolecules**, v. 130, p. 938–946, 2019.

KLJUSURIĆ, J. G. et al. Near-infrared spectroscopic analysis of total phenolic content and antioxidant activity of berry fruits. **Food Technology and Biotechnology**, v. 54, n. 2, p. 236–242, 2016.

LAGANA, V. et al. Formulation of Biscuits Fortified with a Flour Obtained from Bergamot By-Products (Citrus bergamia, Risso). **Foods**, v. 11, 2022.

LANCELOT, E. et al. Prediction of water content in biscuits using near infrared hyperspectral imaging spectroscopy and chemometrics. **Journal of Near Infrared Spectroscopy**, v. 28, n. 3, p. 140–147, 2020.

LEE, Y. J. et al. Characterizing Texture, Color and Sensory Attributes of Cookies Made with Jerusalem Artichoke (Helianthus tuberosus L.) Flour Using a Mixture Design and Browning Reaction Kinetics. **International Journal of Food Engineering**, v. 12, n. 2, p. 107–126, 2016.

LIMA, A. B. S. DE et al. Fast quantitative detection of black pepper and cumin adulterations by near-infrared spectroscopy and multivariate modeling. **Food Control**, v. 107, n. August 2019, p. 106802, 2020.

LIMA, A. B. S. DE et al. Spectroscopy NIR and MIR toward predicting simultaneous phenolic contents and antioxidant in red propolis by multivariate analysis. **Food Chemistry**, v. 367, n. August 2021, 2022.

LIU, F. et al. Detection of Organic Acids and pH of Fruit Vinegars Using Near-Infrared Spectroscopy and Multivariate Calibration. **Food and Bioprocess Technology**, v. 4, n. 8, p. 1331–1340, 2011.

LIU, S. X.; CHEN, D.; XU, J. Evaluation of Gluten-Free Amaranth and Navy Bean Flour Blends on Quality of Sugar Cookies. **Journal of Food Research**, v. 6, n. 6, p. 63, 2017.

LUCAS, A. et al. Prediction of dry matter, fat, pH, vitamins, minerals, carotenoids, total antioxidant capacity, and color in fresh and freeze-dried cheeses by visible-near-infrared reflectance spectroscopy. **Journal of Agricultural and Food Chemistry**, v. 56, n. 16, p. 6801–6808, 2008.

MACAVILCA, E. A.; CONDEZO-HOYOS, L. Assessment of total antioxidant capacity of altiplano colored quinoa (Chenopodium quinoa willd) by visible and near-infrared diffuse reflectance spectroscopy and chemometrics. **Lwt**, v. 134, n. May, p. 110182, 2020.

MAGWAZA, L. S. et al. Development of NIRS models for rapid quantification of protein content in sweetpotato [Ipomoea batatas (L.) LAM.]. **LWT - Food Science and Technology**, v. 72, p. 63–70, 2016.

MALEGORI, C. et al. Comparing the analytical performances of Micro-NIR and FT-NIR spectrometers in the evaluation of acerola fruit quality, using PLS and SVM regression algorithms. **Talanta**, v. 165, n. December 2016, p. 112–116, 2017.

MANCINI, M. et al. Application of the non-destructive NIR technique for the evaluation of strawberry fruits quality parameters. **Foods**, v. 9, n. 4, 2020.

MARTÍNEZ-CERVERA, S. et al. Cocoa fibre and its application as a fat replacer in chocolate muffins. **LWT - Food Science and Technology**, v. 44, n. 3, p. 729–736, 2011.

MARTÍNEZ-GIRÓN, J.; FIGUEROA-MOLANO, A. M.; ORDÓÑEZ-SANTOS, L. E. Effect of the addition of peach palm (Bactris gasipaes) peel flour on the color and sensory properties of cakes. **Food Science and Technology**, v. 37, n. 3, p. 418–424, 2017.

MILDNER-SZKUDLARZ, S. et al. White grape pomace as a source of dietary fibre and polyphenols and its effect on physical and nutraceutical characteristics of wheat biscuits. **Journal of the Science of Food and Agriculture**, v. 93, n. 2, p. 389–395, 2013.

MUDGIL, D.; BARAK, S.; KHATKAR, B. S. Cookie texture, spread ratio and sensory acceptability of cookies as a function of soluble dietary fiber, baking time and different water levels. **LWT - Food Science and Technology**, v. 80, p. 537–542, 2017.

MUHAMMAD, S. et al. Exploring the potential of bottle gourd (Lagenaria siceraria) flour as a fat mimetic in biscuits with improved physicochemical and nutritional characteristics and anti-diabetic properties. **Italian Journal of Food Science**, v. 34, n. 2, p. 50–66, 2022.

NING, X. et al. Cookies fortified with purple passion fruit epicarp flour: Impact on physical properties, nutrition, in vitro starch digestibility, and antioxidant activity. **Cereal Chemistry**, v. 98, n. 2, p. 328–336, 2021.

OLIVEIRA, G. A. et al. Comparison of NIRS approach for prediction of internal quality traits in three fruit species. **Food Chemistry**, v. 143, p. 223–230, 2014.

OLIVIERI, A. C. Practical guidelines for reporting results in single- and multi-component analytical calibration: A tutorial. **Analytica Chimica Acta**, v. 868, p. 10–22, 2015.

PAREYT, B. et al. The role of gluten in a sugar-snap cookie system: A model approach based on gluten-starch blends. **Journal of Cereal Science**, v. 48, n. 3, p. 863–869, 2008.

PIRES, M. B. et al. Peach palm flour (Bactris gasipae KUNTH): Potential application in the food industry. **Food Science and Technology**, v. 39, n. 3, p. 613–619, 2019.

PUTTIPIPATKAJORN, A.; PUTTIPIPATKAJORN, A. Development of calibration models for rapid determination of moisture content in rubber sheets using portable near-infrared spectrometers. **Journal of Innovative Optical Health Sciences**, v. 13, n. 2, p. 1–12, 2020.

RADOŠ, K. et al. The quality and shelf life of biscuits with cryo-ground proso millet and buckwheat by-products. **Journal of Food Processing and Preservation**, n. March, p. 1–12, 2021.

RE, R. et al. ANTIOXIDANT ACTIVITY APPLYING AN IMPROVED ABTS RADICAL CATION DECOLORIZATION ASSAY. **Free Radical Biology & Medicine**, v. 26, p. 1231–1237, 1999.

RECK, I. M.; MIRANDA, N. L. D. E. Chemical Composition and Microbiological Quality of Cookies Formulations With Pupunha Palm Cabbage Flour. **Uningá Review**, v. 27, n. 1, p. 15–18, 2016.

RIBEIRO, G. S. et al. Peach palm flour: production, hygroscopic behaviour and application in cookies. **Heliyon**, v. 7, n. 5, 2021.

RIBEIRO, J. S. et al. Prediction of sensory properties of Brazilian Arabica roasted coffees by headspace solid phase microextraction-gas chromatography and partial least squares. **Analytica Chimica Acta**, v. 634, n. 2, p. 172–179, 2009.

ROJAS-GARBANZO, C. et al. Identification and quantification of carotenoids by HPLC-DAD during the process of peach palm (Bactris gasipaes H.B.K.) flour. **Food Research International**, v. 44, n. 7, p. 2377–2384, 2011.

ROJAS-GARBANZO, C. et al. Major physicochemical and antioxidant changes during peachpalm (bactris gasipaes h.b.k.) flour processing. **Fruits**, v. 67, n. 6, p. 415–427, 2012.

ROJO-POVEDA, O. et al. Physical properties and consumer evaluation of cocoa bean shell-functionalized biscuits adapted for diabetic consumers by the replacement of sucrose with tagatose. **Foods**, v. 9, n. 6, 2020.

SAAD, A. G.; AZAM, M. M.; AMER, B. M. A. Quality Analysis Prediction and Discriminating Strawberry Maturity with a Hand-held Vis–NIR Spectrometer. **Food**

Analytical Methods, v. 15, n. 3, p. 689–699, 2022.

SAHNI, P.; SHERE, D. M. Physico-chemical and sensory characteristics of carrot pomace powder incorporated fibre rich cookies. **Asian Journal of Dairy and Food Research**, v. 36, n. 04, p. 327–331, 2017.

SANTOS, M. DOS et al. Amazonian Native Palm Fruits as Sources of Antioxidant Bioactive Compounds. **Antioxidants**, v. 4, n. 3, p. 591–602, 2015.

SILVA, I. G. DA et al. Elaboration and sensory analysis of cookies made from avocado lump flour. **Brazilian Journal of Food Technology**, v. 22, p. 1–10, 2019.

SILVA RIBEIRO, G. et al. Peach palm flour: production, hygroscopic behaviour and application in cookies. **Heliyon**, v. 7, n. 5, 2021.

SINGLETON, V. L.; ORTHOFER, R.; LAMUELA-RAVENTÓS, R. M. Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin-Ciocalteu Reagent. **Scientia Horticulturae**, v. 299, n. 1974, p. 281–286, 1999.

SISIN, N. N. T.; ABDULLAH, H.; SUL'AIN, M. D. Antiproliferative, Antioxidative and Compounds Identification from Methanolic Extract of Passiflora Foetida and Its Fractions. **Journal of Analytical & Pharmaceutical Research**, v. 6, n. 1, 2017.

SOARES, J. M. et al. Jatobá flour added to cookies: chemical composition and sensorial acceptance by children. **Revista Brasileira de Pesquisa em Saúde**, v. 18, n. 3, p. 74–82, 2016.

TAHIR, H. E. et al. Near-Infrared (NIR) Spectroscopy for Rapid Measurement of Antioxidant Properties and Discrimination of Sudanese Honeys from Different Botanical Origin. **Food Analytical Methods**, v. 9, n. 9, p. 2631–2641, 2016.

TOLEDO, N. M. V. et al. Influence of pineapple, apple and melon by-products on cookies: physicochemical and sensory aspects. **International Journal of Food Science and Technology**, v. 52, n. 5, p. 1185–1192, 2017.

USLU, N.; ÖZCAN, M. M. The effect of irrigation and harvest time on bioactive properties of olive fruits issued from some olive varieties grown in Mediterranean region. **European Food Research and Technology**, v. 246, n. 12, p. 2587–2599, 2020.
VANIN, F. M. et al. Simulating the formation of bread crust in a DMTA rheometer. **Journal of Cereal Science**, v. 51, n. 3, p. 277–283, 2010.

VANIN, F. M.; LUCAS, T.; TRYSTRAM, G. Crust formation and its role during bread baking. **Trends in Food Science and Technology**, v. 20, n. 8, p. 333–343, 2009.

WIMONSIRI, L. et al. Rapid analysis of chemical composition in intact and milled rice cookies using near infrared spectroscopy. **Journal of Near Infrared Spectroscopy**, v. 25, n. 5, p. 330–337, 2017.

ZHU, H. et al. Hyperspectral Imaging for Predicting the Internal Quality of Kiwifruits Based on Variable Selection Algorithms and Chemometric Models. **Scientific Reports**, v. 7, n. 1, p. 1–13, 2017.

2 CONCLUSÃO GERAL

Pela primeira vez um estudo abrangente, incorporando desde aspectos tecnológicos, nutricionais e em nível celular, foi realizado utilizando a farinha de pupunha oriundas de diferentes localidades da Amazônia, onde foi descoberto que, apesar dos parâmetros físico-químicos serem bastante diferentes, por exemplo o teor de fenólicos, lipídios e carboidratos, variarem em função do local de colheita e/ou nível de maturação da fruta, os aspectos tecnológicos, como a estrutura e conformação do amido, não variaram substancialmente. Ainda em relação a farinha, foi demonstrado que os compostos fenólicos presente em sua composição podem apresentar potencial citotóxico e que a matriz alimentar, nas condições testadas, pode inibir a absorção de proteínas no metabolismo e, portanto, há de se tomar cuidado com o uso desenfreado de alimentos com apelos antioxidantes.

A espectroscopia NIR, aliada a calibração multivariada, foi capaz de classificar e prever metabólitos secundários presente em pequenas frações em biscoitos tipo cookie elaborados com diferentes concentrações de farinha de pupunha, assim como também os efeitos antioxidantes que estes causam, demonstrando serem ferramentas extremamente versáteis.

3 PERSPECTIVAS FUTURAS

Trabalhos anteriores relatam que o processo de cozimento do fruto de pupunha consegue eliminar a presença de um inibidor, ainda não identificado, das enzimas digestivas (principalmente a tripsina) (BEZERRA; SILVA, 2016; ROJAS-GARBANZO et al., 2011). O presente estudo colocou novamente esta questão em evidencia, uma vez que a farinha de pupunha foi produzida por liofilização, e seu uso em um alimento modelo, iogurte, demonstrou que há prejuízos na absorção proteica durante a digestão. Logo, considerando todos os aspectos tecnológicos e nutricionais que a farinha de pupunha oferece, é interessante verificar se haverá diferenças entre os processos de fabricação (fruto in natura *versus* fruto cozido) e obtenção da farinha (processo de secagem em estufa *versus* liofilização), e ainda sua aplicação na elaboração de cookies contendo essa matriz alimentar em concentrações elevadas, acima de 50%.

Outra perspectiva interessante a ser abordada relaciona-se a elaboração de modelagens preditivas, feitas utilizando um equipamento espectroscópico portátil, como o MicroNIR, na previsão de aspectos nutricionais e em nível celular. A maioria dos espectrômetros utilizados em aplicações industriais ou laboratoriais são “grandes” e requerem um computador para capturar dados, o que faz com que muitos dados espectroscópicos sejam limitados a ambientes controlados (DAS et al., 2016) e uma das grandes vantagens dos instrumentos portáteis é a capacidade de se conectarem via *bluetooth* a um smartphone, uma tecnologia que permite que o equipamento realize leituras e obtenha espectros ao ar livre, ou seja, abrindo portas para realização da análise diretamente no campo ou em locais onde o NIR de bancada não consegue chegar (LAN et al., 2021).

Logo, avaliar a aplicabilidade da metodologia NIR baseada em métodos lineares e não lineares utilizando smartphones para avaliar a interferências dos compostos fenólicos na citotoxicidade e digestibilidade da proteína nos produtos elaborados torna-se extremamente interessante do ponto de vista nutricional e comercial, visto que é crescente o anseio da indústria/academia por métodos mais rápidos, mais baratos e seguros para a determinação de propriedades dos alimentos.

REFERÊNCIAS

BEZERRA, C. V.; SILVA, L. H. M. Pupunha (Bactris gasipaes): General and Consumption Aspects. In: KRISTBERGSSON, K.; OLIVEIRA, J. (Eds.). **Traditional Foods**. 10. ed. Boston: Integrating Food Science and Engineering Knowledge Into the Food Chain, 2016. p. 399–405.

DAS, A. J. et al. Ultra-portable, wireless smartphone spectrometer for rapid, non-destructive testing of fruit ripeness. **Scientific Reports**, v. 6, n. September, p. 1–8, 2016.

LAN, Z. et al. Rapid evaluation on pharmacodynamics of Curcumae Rhizoma based on Micro-NIR and benchtop-NIR. **Journal of Pharmaceutical and Biomedical Analysis**, v. 200, 2021.

ROJAS-GARBANZO, C. et al. Identification and quantification of carotenoids by HPLC-DAD during the process of peach palm (Bactris gasipaes H.B.K.) flour. **Food Research International**, v. 44, n. 7, p. 2377–2384, 2011.

ANEXOS

Anexo A - Carta de submissão do artigo “Application on infrared spectroscopy for the analysis of phenolic compounds in fruits”

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Application on Infrared Spectroscopy for the Analysis of Phenolic Compounds in Fruits

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Anexo B – Carta de submissão do artigo “Systemic characterization of Pupunha (Bactris gasipaes) flour with views of polyphenol content on cytotoxicity and protein in vitro digestion”

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