UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ENGENHARIA DE ALIMENTOS

KAREN LINELLE DE OLIVEIRA SANTOS

APLICAÇÃO DE MÉTODOS CONVENCIONAIS DE EXTRAÇÃO E HIDRÓLISE ENZIMÁTICA PARA RECUPERAÇÃO DE COMPOSTOS ANTIOXIDANTES DE BRÁCTEAS DE BANANEIRA

APPLICATION OF CONVENTIONAL METHODS OF EXTRACTION AND ENZYMATIC HYDROLYSIS FOR THE RECOVERY OF ANTIOXIDANT COMPOUNDS FROM BANANA BRACTS

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Tese apresentada à Faculdade de Engenharia de Alimentos da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do título de Doutora em Ciência de Alimentos.

Thesis presented to the School of Food Engineering of the University of Campinas in partial fulfillment of the requirements for the degree of doctor in Food Science.

Orientador: Prof. Dr. Ruann Janser Soares de Castro

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"Que eu jamais me esqueça que Deus me ama infinitamente,
que um pequeno grão de alegria e esperança dentro de cada um
é capaz de mudar e transformar qualquer coisa, pois
a vida é construída nos sonhos e concretizada no amor"
(Chico Xavier)

RESUMO

A banana, é uma das pseudofruta mais populares e cultivadas principalmente em regiões tropicais e subtropicais como o Brasil, um dos cinco maiores produtores mundiais. Devido à grande demanda, a bananicultura gera altos volumes de resíduos provenientes de seus frutos e árvore; as brácteas da bananeira, em especial, são descartadas durante o cultivo da banana por ser considerado resíduo. Estudos têm demonstrado que as brácteas podem ser exploradas como uma fonte relevante de compostos bioativos. Tradicionalmente, a extração de compostos bioativos é realizada utilizando solventes orgânicos, que apresentam alta eficiência de extração, mas possuem algumas desvantagens como volatilidade, toxicidade e inflamabilidade. Diversas tecnologias de extração têm sido avaliadas para recuperação de compostos bioativos de matrizes alimentares para reduzir os impactos da utilização de solventes. Desta forma, o objetivo deste projeto foi avaliar os efeitos da extração convencional com a utilização de solventes e a hidrólise enzimática visando a obtenção de extratos de brácteas de bananeira de três variedades (Maçã, Nanica e Prata) com as melhores propriedades antioxidantes. Neste estudo também foram investigados os efeitos de variáveis em cada processo de extração, na extração convencional foram avaliados o solvente mais adequado (água, metanol e acetona) e a influência das variáveis de extração (temperatura, agitação e relação sólido-solvente); na hidrólise enzimática, foi determinada a melhor enzima (pectinase, protease e celulase) utilizada isoladamente ou em combinação para a recuperação de compostos com propriedades antioxidantes. Para a avaliação das propriedades antioxidantes foram utilizados os métodos ABTS, DPPH, FRAP, assim como a determinação de compostos bioativos incluindo compostos fenólicos totais, teor de flavonoides totais e taninos condensados de brácteas de bananeira. Adicionalmente, os extratos com as melhores propriedades antioxidantes de cada processo de extração avaliados foram submetidos à identificação de compostos fenólicos por cromatografia líquida de alta eficiência (HPLC). A combinação de solventes escolhida para a extração de compostos bioativos para as variedades Maçã e Prata foi água e acetona, em proporções iguais e, para a variedade Nanica, a mistura de água (1/6), metanol (2/3) e acetona (1/6) resultando, de forma geral, em aumentos acima de 8 vezes na atividade antioxidante avaliados pelos métodos ABTS, DPPH e FRAP quando comparados com os extratos obtidos com solventes isolados. Os parâmetros da extração definidos para as variedades de brácteas de bananeira estudadas foram: temperatura de 37,5ºC, agitação de 100 rpm e uma relação sólido-solvente de 0,50% m/v. Em relação à hidrólise enzimática, os resultados mostraram que as propriedades

antioxidantes e o teor de compostos fenólicos totais apresentaram aumentos significativos após o uso de enzimas e/ou combinações. A mistura ternária de pectinase, protease e celulase resultaram em aumentos acima de 65% nas propriedades antioxidantes em todas as variedades avaliadas. Os compostos bioativos identificados por HPLC em extratos incluíram rutina, ácidos cumárico, ferúlico, sináptico e vanílico. Desta forma, a obtenção de tais compostos a partir de uma matriz de baixo custo com a utilização de procedimentos simples e eficientes, contribuirão com o uso sustentável de um subproduto proveniente da bananicultura.

Palavras-chave: coração de bananeira; extração assistida por enzimas; resíduos agroindustriais; bioatividades, antioxidantes naturais.

ABSTRACT

Banana is one of the most popular and cultivated pseudofruits, mainly in tropical and subtropical regions such as Brazil, one of the five largest producers in the world. Due to the large amount, banana industry generates high volumes of agro-industrial waste from its fruits and tree; banana bracts, in particular, are discarded during banana cultivation as they are considered waste. Studies have shown that bracts can be explored as a relevant source of bioactive compounds. Traditionally, the extraction of bioactive compounds is performed using organic solvents, which have high extraction efficiency, but have some disadvantages such as volatility, toxicity and flammability. Several extraction technologies have been evaluated for the recovery of bioactive compounds from food matrices to reduce the impacts of solvent use. Thus, the objective of this project was to evaluate the effects of conventional extraction with the use of solvents and enzymatic hydrolysis in order to obtain extracts from banana bracts of three varieties (Maçã, Nanica and Prata) with the best antioxidant properties. In this study, the effects of variables in each extraction process were also investigated; the conventional extraction, the most suitable solvent (water, methanol and acetone) and the influence of extraction variables (temperature, agitation and solid-solvent ratio) were evaluated; in enzymatic hydrolysis, the best enzyme (pectinase, protease and cellulase) used singly or in combinations for the recovery of compounds with antioxidant properties was determined. For the evaluation of antioxidant properties, ABTS, DPPH, FRAP methods were used, as well as the determination of bioactive compounds including total phenolic compounds, total flavonoid content and condensed tannins of banana bracts. Additionally, the extracts with the best antioxidant properties from each extraction process evaluated were submitted to the identification of phenolic compounds by high performance liquid chromatography (HPLC). The combination of solvents chosen for the extraction of bioactive compounds for the Maçã and Prata varieties was water and acetone, in equal proportions and, for the Nanica variety, the mixture of water (1/6), methanol (2/3) and acetone (1/6) resulting, in general, in increases up to 8-fold in the antioxidant activity evaluated by the ABTS, DPPH and FRAP methods when compared with the extracts obtained with isolated solvents. The extraction parameters defined for the banana bract varieties studied were: temperature of 37.5ºC, agitation of 100 rpm and solid-solvent ratio of 0.50% m/v. Regarding enzymatic hydrolysis, the results showed that the antioxidant properties and the total phenolic compounds showed significant increases after the use of enzymes and/or combinations. The ternary mixture of pectinase, protease and cellulase

resulted in increases up to 65% in antioxidant properties in all varieties evaluated. Bioactive compounds identified by HPLC in extracts included rutin, coumaric, ferulic, synaptic and vanillic acids. Thus, obtaining such compounds from a low-cost matrix using simple and efficient procedures will ensure that they contribute to the sustainable use of a by-product from banana industry.

Keywords: banana flower; enzyme assisted extraction; agroindustrial waste; bioactivities, natural antioxidants.

SUMÁRIO

1. Introdução geral

A banana (*Musa* spp.), originária da região do Sudeste Asiático, é mundialmente cultivada e considerada uma cultura de expressiva importância social, econômica e nutricional (Vu et al., 2019). Estima-se que anualmente são produzidos em todo o mundo cerca de 119 milhões de toneladas de bananas (FAO, 2020). Devido à alta produção, elevados volumes de resíduos provenientes de seus frutos e árvore, incluindo pseudocaule, folhas, inflorescências e cascas também são gerados. No Brasil, o sistema produtivo é caracterizado pela junção de grandes cultivos comerciais associados à produção em pequenas/médias propriedades, com o uso intensivo de mão de obra familiar (Baptistella et al., 2019). Assim, a utilização de resíduos oriundos da bananicultura configura uma oportunidade de agregar valor nesta cadeia, representando um ganho social e econômico para os produtores. Diante deste cenário, as questões ambientais têm alavancado o interesse por processos sustentáveis e os resíduos agroindustriais tornaram-se uma valiosa matriz, pois são reconhecidamente uma das principais fontes de compostos bioativos (Lau et al., 2020). A bráctea, estrutura pertencente à inflorescência da bananeira, tem sido explorada principalmente devido à sua rica composição nutricional e perfil de compostos bioativos. Incentivado por este potencial, a revisão da literatura apresentada no Capítulo I inclui a realização de uma análise bibliométrica com o objetivo de expor os dados mais recentes, identificar as lacunas de conhecimento e abordar as tendências para esta área de pesquisa.

Os compostos bioativos em plantas estão disponíveis em baixas concentrações, assim, a escolha de um método de extração adequado é um dos principais desafios para a recuperação de biomoléculas, sendo este um aspecto definitivo na determinação do teor de compostos que será transferido para o extrato (Shahidi et al., 2019). Desse modo, para a recuperação de compostos fenólicos insolúveis, diversos métodos podem ser empregados com o objetivo de extrair tais componentes das matrizes vegetais (Acosta-Estrada et al., 2014). Embora a extração convencional seja amplamente abordada na literatura, estudos sobre a extração de compostos bioativos de brácteas de bananeira com o uso de diferentes solventes ainda são escassos; tal abordagem foi discutida no Capítulo II.

Associado à importância de investigações a respeito da extração, esta tecnologia também é governada por outros parâmetros tais como a matriz, tamanho de partícula da amostra, a razão sólido-solvente, tipos de solventes orgânicos, temperatura, pressão e tempo (Alara et al., 2021). Desse modo é crucial selecionar os fatores que terão maior impacto na matriz e selecionar as melhores condições para a maximização da extração de compostos

bioativos e suas propriedades biológicas (Hassan et al., 2021). A influência de diferentes fatores de extração assim como a determinação da melhor condição foi abordada no Capítulo III.

Ainda que os métodos convencionais de extração com uso de solventes estejam em operação, diversas estratégias são investigadas para o incentivo de técnicas sustentáveis como alternativa à extração com solventes (De la Peña-Armada et al., 2020). Neste sentido, a hidrólise enzimática tem demonstrado grande potencial de aplicação por ser uma tecnologia de baixo impacto ambiental e alta recuperação, devido à especificidade e regiosseletividade das enzimas (Liu et al., 2016). O estudo da hidrólise das brácteas de bananeira visando a obtenção de extratos com o uso de diferentes enzimas de forma isolada ou combinada foi relatado no Capítulo IV. Esta abordagem pode oferecer uma oportunidade para a exploração de resíduos provenientes da bananicultura por meio de um processo ambientalmente seguro e eficiente.

CAPÍTULO I: Banana inflorescence as a promising resource of bioactive compounds for health promotion: an updated review and bibliometric analysis

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Abstract

Background: Banana is one of the most consumed fruits around the world and its inflorescence is a low-cost agricultural by-product. However, besides the potential as a bioactive compounds source, banana inflorescence is considered an agricultural waste and largely under-exploited.

Scope and approach: Encouraged by the bioactive potential of banana inflorescence, this review aims to analyze scientific literature available for this material and to approach new research trends that point toward future applications. Bibliometric studies and scientific literature review were used to gain a one-stop overview, identify knowledge gaps, and propose trend clusters for investigation.

Key findings and conclusions: Banana inflorescence have been explored mainly due to its phenolic compounds, antidiabetic and antioxidant properties. Although this matrix can be further explored in the field of Food Science and Technology, the published studies have shown high potential for food applications due to its rich nutritional composition, physicochemical properties and profile of bioactive compounds.

Keywords: Banana flower; agricultural waste, functional properties, bioactivities, natural antioxidants, bibliometrics.

1. Introduction

Food processing and agroindustrial activities generate several tons of wastes and by-products worldwide, which can represent a potential problem due to its prejudicial environmental impacts when not properly treated. This material is considered one of the cheapest and most abundant sources of biomass but remains largely underexploited, despite several studies have demonstrated that it could be an outstanding source of a wide range of bioactive compounds (Gullón, Gullón, Romaní, Rocchetti, & Lorenzo, 2020; Kumar, Srivastav, & Sharanagat, 2021; Qin & Xi, 2021).

Banana (*Musa acuminata*) is considered one of the most consumed pseudofruit worldwide due to its highly nutritional value and it has a relevant economic interest. In 2019, 116 million tons of banana were produced in more than 150 countries and the emphasis goes to India, China, Indonesia, Brazil and Ecuador that are considered the major producers of the world (FAO, 2019). The largest producer, India, for instance, generates several tons (220 tonnes) per hectare of underused by-products and wastes (Shah, Reddy, Banerjee, Ravindra Babu, & Kothari, 2005).

Concerning its agro-industrial production chain, banana industry generates large amount of agricultural waste with high value-added, such as some banana plant structures (pseudostem, leaves, inflorescences and peels) that have been reported to have medicinal properties (Mathew & Negi, 2017). Banana inflorescence, also known as flower or blossom, is typically treated as an agricultural waste and most of it is disposed during the banana harvesting, left on the plantations as a soil cover or discarded into rivers or on roadsides, generating serious environmental problems (Chai et al., 2018; Schmidt, Prestes, Kubota, Scapin, & Mazutti, 2015).

Contradictory to this, the banana inflorescence is widely used by communities from different regions for culinary purposes, folk medicine and mainly for the numerous bioactivities that include: antioxidant, anti-diabetic and cardiovascular-protective activities (Arun, Thomas, Reshmitha, Akhil, & Nisha, 2017; Ramu et al., 2017a; Sheng et al., 2014b; Sitthiya, Devkota, Sadiq, & Anal, 2018).

These substantial advances increasing the number of scientific papers including excellent review articles, the oldest of them discussed extensively the utilization of banana by-products in various food and non-food applications in association to the sustainability (Padam, Tin, Chye, & Abdullah, 2014).

From another perspective, one of the research groups provided an overview of nutritional attributes, bioactive components and potential health-promoting properties of the

banana inflorescence (Lau et al., 2020). In a more recent publication, the authors summarized benefits of banana peel and banana blossom via the nutritional and chemical properties for utilization in the food industry (Kraithong & Issara, 2021). Despite the amount of information available, some fields of knowledge remain insufficiently explored or unknown as well as the lack of commercial exploitation for production and consumption purposes which encourages the search for additional information. Nevertheless, this paper helps a researcher understand the trends and potential applications, based on available scientific data using bibliometric analysis. This tool allows to quantify and measure academic output using the data available in the scientific database with easy visualization of the indicators presented as clusters and networks (Almeida, Castro, Travália, & Forte, 2021).

Aiming to fill this literature gap, this work aims to provide a comprehensive analysis based on a combination of bibliometric and review methods to give the current state about banana inflorescence research as well as future prospects.

2. Banana inflorescence

Banana inflorescence is a complex spike consisting of a stout peduncle located at the end of the stalk or peduncle on which the flowers are arranged in clusters (Robinson & Saúco, 2010). It is a long oval shaped structure also known as “banana flower”, “blossom” or “heart” (Figure 1). Most of bananas have red, purple or violet bracts; this color variation is correlated with anthocyanins composition (Pazmiño-Durán, Giusti, Wrolstad, & Glória, 2001). The internal structure namely bracts are located under each of which are male and female flowers arranged in rows. It is a perishable structure due to its high moisture content (7.36 – 90.73%) consequently, its storage at high temperatures causes rotting and chilling temperature causes the inflorescence browning. The inflorescence is removed from plantain to prevent growth and maturation fruits interference (Jha, Meghwal, Prabhakar, & Singh, 2021; Lau et al., 2020). Thereby, it is considered an agricultural waste of banana crop and is mostly discarded in abundance in culture fields and/or rivers and soil, causing serious environmental damage. Studies concerning the proximate composition showed that inflorescence is a great source of nutrients, existing data differs among its numerous varieties, generally contains crude fibre (0.08 – 70.07%), carbohydrate (10.18 – 95.61%), protein (1.43 – 19.60%) and lipid (0.39 - 5.79%) (Lau et al., 2020). The proteins present in this structure have an interesting amino acid profile, containing glutamic acid, aspartic acid, leucine, alanine, proline, arginine, cysteine and serine in greater amounts and lysine in lesser abundance (Ramu et al., 2017a). Regarding the lipids, banana inflorescence has a great source of unsaturated fatty acids such as oleic, linoleic

and α-linolenic acids that representing more than 60% of the total fatty acids. The consumption of these fatty acids leads to a reduced risk of cardiovascular diseases (Sheng et al., 2010). The composition of minerals is quite varied, with potassium, at the highest concentration, followed by calcium, magnesium and iron (Basumatary & Nath, 2018). Dietary fibre, one of the main constituents of banana inflorescence has been known for its benefits regarding preventive role on chronic diseases including type II diabetes, cardiovascular diseases and some types of cancer (Macagnan, da Silva, & Hecktheuer, 2016).

Figure 1. The structure of banana inflorescence and their internal parts.

Furthermore, several studies have been reported that banana inflorescence could be explored as an interesting source of bioactive compounds, including antioxidant properties often associated with the prevention of chronic and degenerative diseases (Basumatary and Nath, 2018; Bhaskar et al., 2012; Ramu et al., 2017; Sheng et al., 2011), antidiabetic activities (Bhaskar et al., 2011; Prakasan and Saraswathy, 2013; Ramu et al., 2014; Sheng et al., 2014), cytotoxic and apoptosis-inducing (Arun et al., 2018; Revadigar et al., 2017; Timsina and Nadumane, 2014), antimicrobial (Jawla et al., 2012; Padam et al., 2012; Tin et al., 2015), anti-inflammatory (Divya et al., 2016; Gautam and Jachak, 2009; Nisha and Mini, 2013) and cardiovascular-protective activities (Arun et al., 2017).

3. Literature Research methodology and data statistics

A mixed method was used for the bibliometric analysis followed by a review of the literature. The literature dataset was collected from Web of Science (WOS) (scientific database) during the month of August 2021. The terms “banana inflorescence” OR “banana bract” OR “banana flower” OR “banana heart” were used in the “topic item” that includes the title, abstract, keywords, and keywords plus of articles indexed in the Web of Science Core Collection (Figure 2).

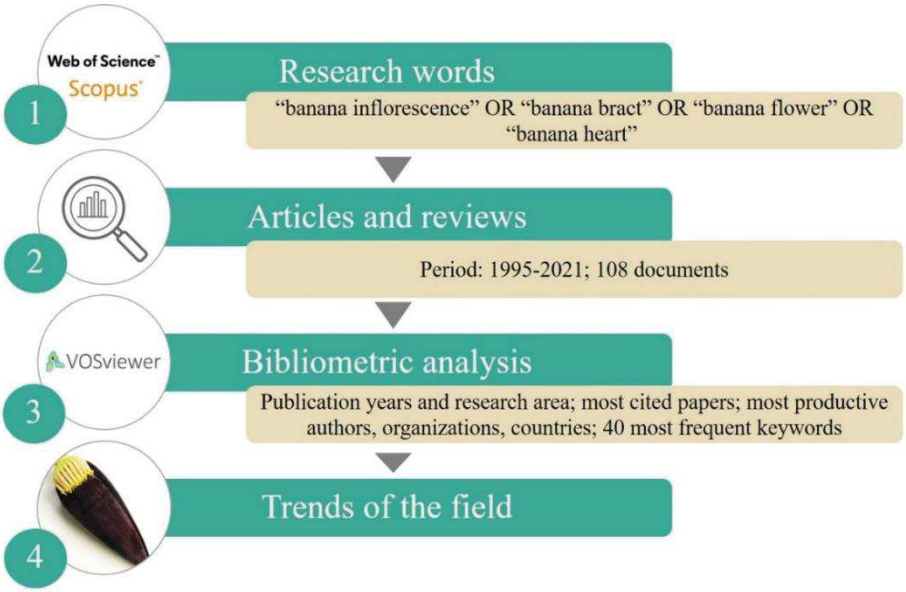

Figure 2. Steps carried out for the construction of the bibliometric analysis of banana inflorescence.

The search was conducted without year filter in order to ensure that all the studies reported in the database were obtained, but one filter of document types was applied to be selected only research and review articles. Using these parameters, 108 studies comprising 102 research articles (94.44%) and 6 review articles (5.56%) were retrieved. From the data, we extracted the number of publications, WOS categories, top 10 most cited papers, top 10 most productive authors, organizations, and countries, and 40 most frequent keywords. Additionally, the data of most productive countries were processed using the VOSviewer software (Java version 1.8.0_261) (van Eck & Waltman, 2010; van Eck & Waltman, 2014) in order to gather information concerning international collaborations among countries and the 40 most frequent keywords were processed to evaluate the trends on banana inflorescence.

4. Bibliometric analysis

4.1 Study of publications and most cited papers

Studies concerning banana inflorescence begun to be published from 1995. Figure 3 shows the evolution on the number of publications and the categories that these studies have been grouped according to WOS. In general, Figure 3 shows that the number of studies about banana inflorescence has been increasing over the years with some oscillations in specific periods. From 1995 to 2009 were published 12 articles with a mean of 1 work by year in the fields of Agronomy, Virology, and Plant Sciences. These studies were concentrated in these fields because researches were mainly about the identification and control of banana bract

mosaic potyvirus (BBMV); an aphid-transmitted virus that was responsible to yield losses of up to 40%, being a high concern to the banana culture (Rodoni, Ahlawat, Varma, Dale, & Harding, 1997).

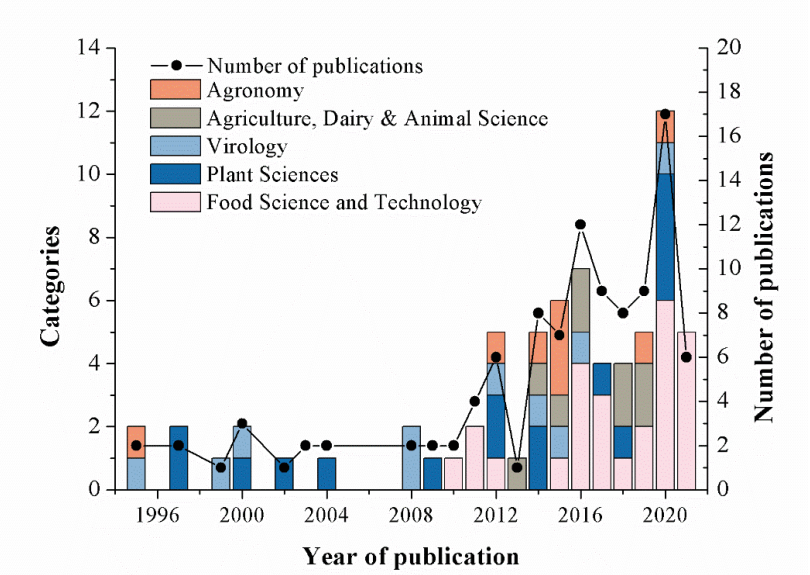

Figure 3. Evolution of the number of publications related to banana inflorescence over the years in main research categories published in Web of Science and Scopus.

In 2011, the first study on Food Science and Technology field was published in *Journal of Food Biochemistry* (Bhaskar, Shobha, Sambaiah, & Salimath, 2011a). The authors studied the ingestion effects of banana inflorescence (*Musa* sp. var. Elakki bale) and stem on intestinal and renal enzymes activities of streptozotocin-induced diabetic rats. They reported that the incorporation of 5% inflorescences on the basal diet and stem on diet of diabetic rats decreased the urine volume, urine sugar and fasting blood sugar, through the decrease of sucrase, maltase, and lactase activities. This study was very important to the field, since it demonstrated the main beneficial effects of banana inflorescence and stem when applied as a feed/food ingredient. This raw material can be incorporated in the diet of diabetic individuals to make them more tolerant to hyperglycemia, as suggested by the authors. From 2012 to 2016, there was a remarkable increase on the number of studies about banana inflorescence. In this period, 34 articles were published, being grouped in four different categories, pointing the versatility of this raw material that is studied by researchers from different areas. The highest number of publications (17) over the years was achieved in 2020, demonstrating the growing interest concerning banana inflorescence and its potential uses. In addition, the Food Science and Technology field has attracted great interest among researches with 26 studies until now. These papers approached the use as ingredient in foods, as well their composition and biological

properties evaluation, which will be discussed in this review. The most 10 cited papers concerning banana inflorescence until now are shown in Table 1.

The oldest articles are the most cited due to their length of index time as observed for the three most cited papers (published between 2000 and 2008) (Jiménez-Castro, Buller, Sganzerla, & Forster-Carneiro, 2020). The first most cited paper entitled 'Antihyperglycaemic activity of *Musa sapientum* flowers: Effect on lipid peroxidation in alloxan diabetic rats' was published in *Phytotherapy Research* journal (Impact factor; IF = 5.87) with 147 citations (Pari & Umamaheswari, 2000). Alloxan is a toxic glucose analogue, which destroys insulin-producing cells in the pancreas when administered to rodents. This causes an insulin-dependent diabetes mellitus (called "alloxan diabetes") in these animals, with characteristics similar to type 1 diabetes in humans. This study has been widely cited once it provides support to other researches, such as the antihyperglycemic and antioxidant potential of banana flowers chloroform extract. In addition, the authors reported that extract consumption for 30 days by diabetic rats resulted in a significant reduction in blood glucose and glycosylated hemoglobin, in an increase in total hemoglobin, as well as in the reduction on the body weight and decrease on free radical formation in the tissues.

The second, the third and the fourth most cited articles received, respectively, 107, 69, and 33 citations (Ha et al., 2008; Sharman, Thomas, & Dietzgen, 2000; Thomas, Geering, Gambley, Kessling, & White, 1997). These studies were published in Plant Sciences and Virology fields and were mainly focused on development of primers and techniques as RT-PCR (Real Time Polymerase Chain Reaction) to the detection, identification, and genomic characterization of potyvirus, the virus which attack banana inflorescence as already mentioned previously. In this sense, these studies were very important to this area since they contributed to better control of potyvirus, as well to avoid significant losses of bananas.

The fifth and the ninth most cited papers received 32 and 27 citations, respectively, and were published by the same research group in 2011 and 2012 (Bhaskar, Mahadevamma, Chilkunda, & Salimath, 2012; Bhaskar, Shobha, Sambaiah, & Salimath, 2011b). The ninth most cited paper was used as base to the fifth most cited, and in both works, banana inflorescence from *elakki* bale variety was studied.

Table 1. The most 10 cited papers concerning banana inflorescence until August 2021 evaluated by VOSviewer.

Rank	Title	Journal	NC	IF	References
1st	Antihyperglycaemic activity of *Musa sapientum* flowers: Effect on lipid peroxidation in alloxan diabetic rats	Phytotherapy Research	147	5.87	Pari & Umamaheswari (2000)
2nd	Design and application of two novel degenerate primer pairs for the detection and complete genomic characterization of potyviruses	Archives of Virology	107	2.57	Ha et al. (2008)
3rd	Development of a multiplex immunocapture PCR with colourimetric detection for viruses of banana	Journal of Virological Methods	69	2.01	Sharman, Thomas & Dietzgen (2000)
4th	Purification, properties, and diagnosis of banana bract mosaic potyvirus and its distinction from abaca mosaic potyvirus	Phytopathology	33	4.02	Thomas et al. (1997)
5th	Banana (*Musa sp. var. elakki bale*) flower and pseudostem: dietary fiber and associated antioxidant capacity	Journal of Agricultural and Food Chemistry	32	5.27	Bhaskar et al. (2012)
6th	Dietary sources and their effects on animal production and environmental sustainability	Animal Nutrition	32	6.38	Wanapat et al. (2015)
7th	Isolation and characterization of an α-glucosidase inhibitor from *Musa spp.* (Baxijiao) flowers	Molecules	29	4.41	Sheng et al. (2014)
8th	Characterization and expression of the coat protein-coding region of banana bract mosaic potyvirus, development of diagnostic assays and detection of the virus in banana plants from five countries in southeast Asia	Archives of Virology	28	2.57	Rodoni, Dale & Harding (1999)
9th	Beneficial effects of banana (*Musa sp. var. elakki bale*) flower and pseudostem on hyperglycemia and advanced glycation end-products (AGEs) in streptozotocin-induced diabetic rats	Journal of Physiology and Biochemistry	27	4.15	Bhaskar et al. (2011)
10th	Banana bract mosaic-virus - characterization using potyvirus specific degenerate PCR primers	Archives of Virology	26	2.57	Bateson & Dale (1995)

IF: Impact factor; NC: number of citations.

In 2011, the authors studied the effect of the ingestion of banana inflorescence on hyperglycemia and advanced glycation end-products (AGEs) of streptozotocin-induced diabetic rats, while in 2012, they characterized the dietary fiber content and phytochemical profile of inflorescence. Both works also studied the antioxidant potential of powdered banana inflorescence. In summary, the authors observed high dietary fiber content (65%) and polyphenols: epicatechin (12.53 μg mg^{-1}), gallic acid (10.86 μg mg^{-1}), syringic acid (10.26 μg

mg^{-1}), and ferulic acid (5.73 μg mg^{-1}) were the major compounds observed and were associated with the high antioxidant potential against DPPH radicals, ferric reducing power, and superoxide radical scavenging of samples. Additionally, it was observed that diabetic symptoms including hyperglycemia, polyuria, polyphagia, polydipsia, urine sugar, and body weight were attenuated from ingestion of the incorporation of 5% inflorescences on the basal diet, besides inhibit the formation of AGEs (hyperglycemia and advanced glycation end-products). The sixth most cited paper (NC 32) is a review article that focuses on the dietary sources and their influence on animal production related to environmental issues (Wanapat, Cherdthong, Phesatcha, & Kang, 2015). The seventh most cited paper was 'Isolation and characterization of an α-glucosidase inhibitor from *Musa* spp. (Baxijiao) flowers' in *Molecules* journal (IF = 4.41) (Sheng et al., 2014a). In this study, the authors isolated and identified by one-dimensional NMR technique the following compounds: vanillic acid, ferulic acid, β-sitosterol, daucosterol, and 9-(4′-hydroxyphenyl)-2-methoxyphenalen-1-one. They observed that these compounds showed strong α-glucosidase inhibitory effects (IC_{50} values ranging from 3.86 to 2004.58 mg L^{-1}). The enzyme α-glucosidase participates in the completion of carbohydrate digestion, releasing glucose for intestinal absorption; its inhibition reduces the increase of blood glucose level after a meal (post prandial glycemic) (Dra et al., 2019)In addition, they observed that the β-sitosterol, daucosterol, and 9-(4′-hydroxyphenyl)-2-methoxyphenalen-1-one were the most effective compounds with an inhibition effect better than acarbose, a standard inhibitor. The eighth (Rodoni, Dale, & Harding, 1999) and the tenth (Bateson & Dale, 1995) most cited papers were also published in Plant Sciences and Virology fields and focused on the identification and characterization of banana bract mosaic potyvirus (BBMV) in banana inflorescence. These studies are oldest, reinforcing the concern with the potyvirus at that time, as already mentioned previously. It can be observed that the most cited papers concerning banana inflorescence cover studies with BBMV, demonstrating the importance on control of this virus for banana culture in specific periods (from 1995 to 2008). However, the Food Science and Technology has been gaining prominence in studies on banana inflorescence due to their bioactivities, as reported in Figure 3. This area exceeded the number of publications in virology and plant sciences fields.

4.2 Authors, organizations, countries, and keywords analysis

Table 2 shows the most productive researchers, organizations, and countries responsible for the high number of published papers and citations concerning banana inflorescence. Dr. Metha Wanapat (Wanapat, M) and Dr. Sungchhang Kang (Kang, S) are the most productive authors occupying the first and second ranking, respectively, due to its high

number of published papers. Both authors published together 8 papers, focusing on effects promoted by the incorporation of banana inflorescence in ruminant diets. Their most current studies showed that inflorescence improved the digestibility of nutrients and milk production, fermentation efficiency and buffering capacity of rumen, and a remarkable reduction of rumen methane (CH_4) production (Kang & Wanapat, 2018; Kang, Wanapat, Cherdthong, & Phesatcha, 2016; Kang, Wanapat, & Viennasay, 2016; Kang, Wanapat, & Cherdthorng, 2014; Wanapat, Ampapon, Phesatcha, & Kang, 2019). Dr. Ramasamy Selvarajan (Selvarajam, R) and Dr. Velusamy Balasubramanian (Balasubramanian, V) are the third and the sixth most influent authors, respectively, having published 5 studies together. Their studies aimed to characterize the genome and molecular diversity of BBMV to develop tests of detection and alternatives to control it and improve the productivity of bananas in India (Balasubramanian & Selvarajan, 2012, 2014; R. Selvarajan, Balasubramanian, Jeyabaskaran, Pandey, & Mustaffa, 2009; Ramasamy Selvarajan, Balasubramanian, et al., 2020; Ramasamy Selvarajan, Kanichelvam, Balasubramanian, & Sethurama Subramanian, 2020). It is important to highlight that both authors are from National Research Centre for Banana (India), the third most influent organization on ranking, a research institute focused on increasing the production and productivity of banana and plantations through strategic research. MSc. Yesmin Ara Begum (Begum, Y.A.) and Dr. Sankar Chandra Deka (Deka, S.C.) have been significantly contributing with their studies concerning banana inflorescence to the Food Science and Technology field. In addition, both researchers are from Tezpur University, the second most influent organization, and their researches may have contributed for this position in ranking. Begum and Deka studied the extraction of pectin from banana inflorescence to be used as wall material for anthocyanins nanoparticles (Begum & Deka, 2017a), and the microencapsulation to preserve their bioactivities (Begum & Deka, 2017b). Also, they studied extraction strategies to obtain dietary fiber (DF) from banana inflorescence and the influence on their structural, thermal, and physicochemical properties (Begum & Deka, 2019b) and the incorporation of anthocyanins in DF as a matrix to preserve their bioactivities (Begum, Baishya, Das, Chakraborty, & Deka, 2020). The latest publications evaluated the inflorescence in functional breads (Begum, Chakraborty, & Deka, 2020). These studies demonstrated the several and potential uses of banana inflorescence and their bioactive compounds in foods.

Table 2. The most influent authors, organizations (the affiliations of the top 10 most influent authors) and countries concerning publications of banana inflorescence until August 2021 evaluated by VOSviewer.

Ranking	Identification	ND	NC
	Authors		
1st	Wanapat, P.	9	83
2nd	Kang, S.	8	80
3rd	Selvarajan, R.	8	62
4th	Deka, S. C.	8	30
5th	Begum, Y. A.	6	30
6th	Balasubramanian, V.	5	27
7th	Thomas, J.	4	124
8th	Bhaskar, J. J.	4	80
9th	Salimath, P. V.	4	80
10th	Phesatcha, K.	4	42
	Organizations		
1st	Khon Kaen University	9	83
2nd	Tezpur University	9	44
3rd	National Research Centre for Banana	8	51
4th	National Institute of Education – Singapore	6	90
5th	Central Food Technological Research Institute – Mysore	5	74
6th	Chinese Academy of Tropical Agricultural Sciences	5	42
7th	Federal University of Paraná	4	5
8th	Queensland University of Technology	4	161
9th	Pooja Bhagavat Memorial Mahajana Post Graduate Centre - Mysore	3	43
10th	Sri Jayachamarajendra College of Engineering	3	43
	Countries		
1st	India	42	539
2nd	Thailand	14	101
3rd	China	10	93
4th	Brazil	9	45
5th	USA	8	60
6th	Malaysia	8	36
7th	Philippines	8	28
8th	Australia	7	277
9th	Cambodia	7	65
10th	Colombia	3	21

ND: number of documents; NC: number of citations

Dr. John E. Thomas (Thomas, JE) from Queensland University of Technology is the seventh most influent author, having published 4 studies between 1997 and 2004, being cited 124 times (Gambley, Thomas, Magnaye, & Herradura, 2004; Sharman et al., 2000; Thomas et al., 1997). Their studies also were focused on the development of PCR methods for detection of BBMV in bananas and were important to give support to other researchers that study the potyvirus. Moreover, noteworthy that two of its four published articles were the third and fourth most cited papers (Table 1).

Dr. Jamuna J. Bhaskar (Bhaskar J.) and Dr. Salimath Paravans V. (Salimath, P. V) are the eighth and the ninth most influent authors, respectively, and published together the fifth and the ninth most cited papers (Table 1). The main focus of their studies was to characterize the dietary fiber content and demonstrate the antidiabetic and antioxidant potential of banana inflorescence (Bhaskar, Mahadevamma, et al., 2012; Bhaskar, Mahadevamma, Vishwanatha, & Salimath, 2010; Bhaskar, Salimath, & Nandini, 2011).

Dr. Kampanat Phesatcha (Phesatcha, K.) is the tenth most influent researcher and collaborated with the researches concerning the use of banana inflorescence in ruminant diets performed by the Dr. Wanapat and Dr. Kang (first and second most influent authors).

The most influent organizations (Table 2) are the affiliations of the top 10 most influent authors. Their studies contributed for maintenance of these institutions on ranking due to the high number of publications.

For the countries, in addition to listed ranking in Table 2, an international researches collaboration between the 10 most productive countries was evaluated by co-authorships analysis using VOSviewer (Figure 3). The node (circle) size represents the number of publications, and edge thicknesses and color indicate the cluster to which the item belongs (Almeida, Travália, Gonçalves, Forte, & Soares, 2021).

India, Thailand, and China published 42, 14, and 10 papers, respectively. The greatest number of publications in these countries can be due to the high world production of bananas in Asia. According to FAOSTAT (www.fao.org/faostat/), Asia was responsible for 54.1% of world production of bananas with a production of approximately 49.3 million of tons. Between them, India was the first most influent country because gathers the majority of most cited authors and organizations. In addition, as can be seen in Figure 4, the India possess a collaboration network with Australia, the second country with the highest number of citations (277) on ranking.

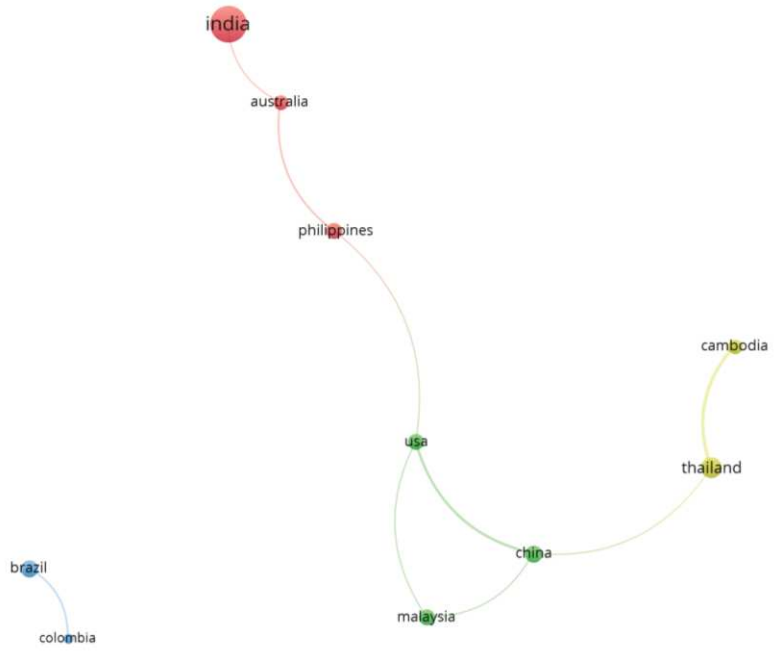

Figure 4. Collaborative networks among the 10 countries that most published scientific articles related to banana inflorescence.

Thailand and China possess collaborations with other countries (Cambodia, Malaysia, and USA), which may have favored their remarkable positions on ranking. In summary, all 10 countries analyzed possess international collaborations between them, which contribute to the increase of researches and maintenance of those countries as the most productive on studies with banana inflorescence. For a better understanding of the current interest of researchers, a keyword analysis was used as a strategy to obtain an overview of the trends related to banana inflorescence. There are two fields on keywords in Web of Science (WOS) database, known as author keywords (AKs, terms choosed by authors) and keywords plus (KP, generated upon a special algorithm from cited titles, they cannot be changed). This study analyzes only the author keywords. The article titles are the most visible and the reader's first contact and author´s keywords acting as a filter in file searches, both play an essential role in bibliometrics (Rodríguez-Rojas, Ospina, Rodríguez-Vélez, & Arana-Florez, 2019). Figure 5 shows the author keywords map, where the node (circle) size represents the number of times that the keywords were used, the edge thicknesses indicate the cluster to which the item belongs, and the color represents the average publication year of the works in which the word was used. Between the studies analyzed, 320 different AKs were found. However, here, we used the most frequent 40 AKs, those that were used at least twice on the papers.

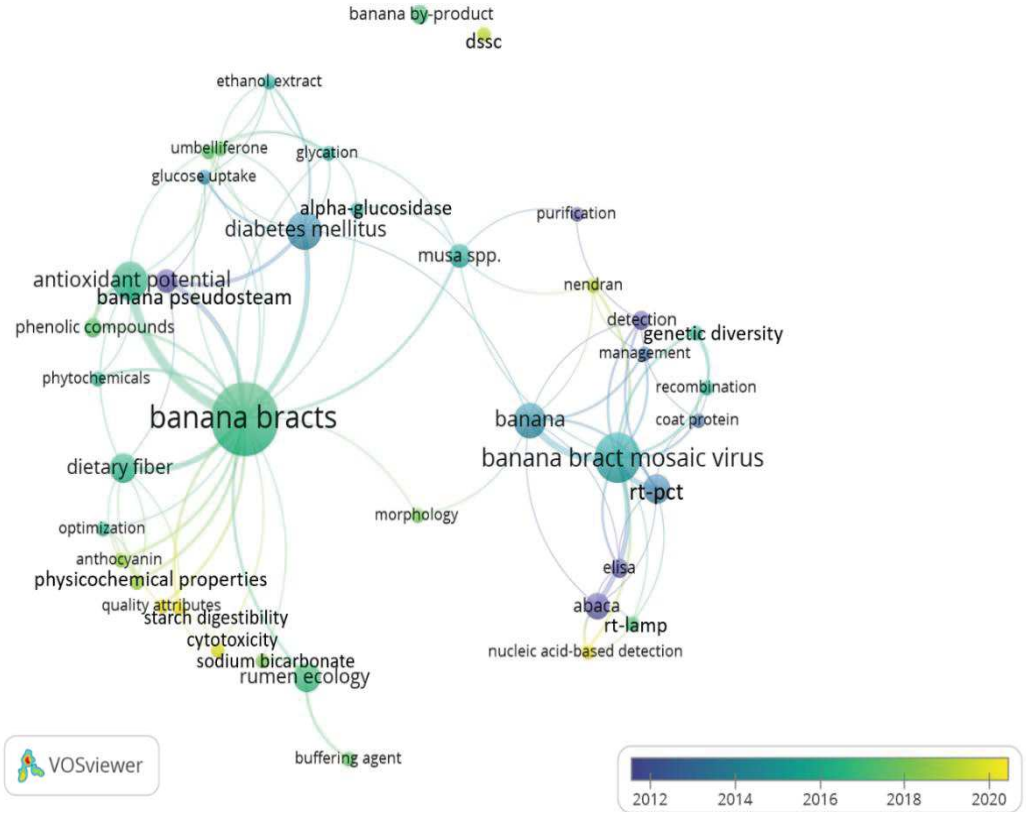

Figure 5. Map of the 40 most frequent keywords according to bibliometric analysis regarding the banana inflorescence in which the size of the circle represents the number of times the keywords were used and the color represents the average year of publication.

The terms ‘banana bracts’, ‘banana bract mosaic virus’, ‘antioxidant potential’, and ‘diabetes mellitus’ were the most cited AKs, occurring 26, 14, 9, and 9 times, respectively. These terms allow us to interpret that most of articles are focused on control of potyvirus and on the study of the bioactive properties of banana inflorescence. Moreover, we can also note that the VOSviewer organized the keywords at different clusters indicating the years that were used.

From the combinations of keywords obtained at the clusters, we can conclude that banana inflorescence is widely studied due to their health benefits, examples include effect on digestibility of nutrients, high antidiabetic and antioxidant potential due the content of dietary fiber and phenolic compounds.

Bibliometric analysis described above served as a basis for define the topics of highest priority concerning banana inflorescence. Current studies about the bioactive compounds, extraction, identification and its bioactivities will be discussed.

5. Extraction methods and beneficial bioactive properties of banana inflorescence

As mentioned previously on Figure 4, the greatest interest in the banana inflorescence is related to its bioactive compounds and associated bioactivities, mainly antioxidant and antidiabetic properties, according to information summarized (Table 3).

The antioxidant properties observed in banana inflorescence were attributed to its phenolic compounds (Arun et al., 2017; Bhaskar et al., 2012b; Ramu et al., 2017b). Antioxidants, in food science, have a very broader scope and can be defined as "substances that can protect, prevent and/or reduce the oxidative destruction of molecules" (Huang, Ou, & Prior, 2005). The beneficial role of phenolic compounds is attributed to their antioxidant properties that protects cells against damage by reactive oxygen species (ROS) by mechanisms such as the hydrogen donation and/or electron transfer to the free radical (Shahidi & Ambigaipalan, 2015). Antioxidants are categorized as primary and secondary antioxidants based on the mechanism of action. Primary antioxidant can neutralize free radicals by two mechanisms: i) hydrogen atom transfer (HAT); ii) single electron transfer (SET); phenolics are included in this category. The mechanism of secondary antioxidants consists on neutralization of prooxidant catalysts. In this category includes chelators of pro-oxidant metal ions Fe and Cu, for example (Zeb, 2020).

Bioactive compounds in plants are available in low concentrations, thereby, the development of proper method is one of the main challenges to extract biomolecules. The choice of technique depends on the food matrix, environmental concerns and extraction conditions (Azmir et al., 2013; Marathe, Jadhav, Bankar, & Singhal, 2017). Besides that, extraction process also depends on factors such as sample particle size, extraction time, temperature, pressure, and solvent (type, concentration and sample: solvent ratio) (Nadar, Rao, & Rathod, 2018). The growing interest in bioactive compounds has had a positive impact on studies about their recovery and several current studies have reported effective strategies for this.

Table 3. A summary of the most recent studies concerning bioactivities of banana inflorescence.

Bioactivities	Banana variety	Extraction method	Compounds	References
Antioxidant activity	*Musa paradisiaca* (Nendran variety) (inflorescence)	Ethyl acetate and methanol extracts	Polyphenols (major compounds: gallic acid, cinnamic acid, p-coumaric acid)	(Arun, Thomas, Reshmitha, Akhil, & Nisha, 2017)
Anti-microbial activity	Musa paradisiaca (inflorescence)	Supercritical CO_2 and compressed propane	-	(Correa et al., 2017)
Antioxidant activity	*Musa* sp. cv. Nanjangud rasa bale (inflorescence)	Ethanol, methanol and water extracts	Total phenolic compounds	(Ramu et al., 2017)
Antioxidant activity	*Musa balbisiana* (inflorescence)	Ethanolic extracts	Total phenolic compounds and flavonoids	(Revadigar et al., 2017)
Antidiabetic activity	*Musa* ssp. (inflorescence)	Ethanol extracts	Phytosterols (β-sitosterol, 31-norcyclolaudenone, 4-trimethyl-5a-cholesta-8, 25(27)-dien-3b-ol)	(Sheng et al., 2017)
Apoptosis-inducing and cytotoxic activities	*Musa paradisiaca* (Nendran variety) (inflorescence)	Ethyl acetate and methanol extracts	-	(Arun, Madhavan, Reshmitha, Thomas, & Nisha, 2018)
Antioxidant activity	*Musa balbisiana* (inflorescence)	Methanolic extracts	Total phenolic compounds and flavonoids	(Basumatary & Nath, 2018)
Antidiabetic activity	*Musa x paradisiaca*	Water and ultrasound assisted process	21 compounds and 17 metabolites glycosylated and acetylated phenylpropanoids of p-coumaric acid and caffeic acid, as well as a glycosylated flavonol and anthocyanins	(Vilhena et al., 2020)
Antioxidant activity	*Musa acuminate* (inflorescence)	Conventional extraction - ethanol (50, 75, 95%)	Lupeol and umbelliferone	(Chiang, Yang, Lai, & Chen, 2021)

In another study, researchers investigated the suitable extract conditions for the recovery of anthocyanins from banana bracts. Experiments were conducted to study solvents and distinct extract conditions (soaking, shaking, heating, micro-wave and ultrasonic

treatments) and response surface methodology design was used to select extraction variables (ethanol with citric acid (1, 2.5 and 5%), ethanol with acetic acid (1, 2.5 and 5%), acidified solvents with 0.5% HCl (hexane, ethyl acetate, methanol, ethanol, water), acidified solvents with 0.1% HCl (methanol, ethanol and water)) for the recovery of anthocyanins. This study also aimed to apply the silver nanoparticles (AgNPs) using anthocyanins extract (1 mg mL^{-1}) of banana bracts and evaluate their anti-proliferative potential (Susaritha, Prakash, & Vadivel, 2021). AgNPs are nanoparticles of noble metals (gold, platinum, silver) that have been shown distinct effects such as anti-inflammatory, anti-bacterial, anti-fungal, anti-proliferative and anti-tumor. By-products plant extracts could be used, in which phytochemicals can reduce the silver ions to AgNPs, which is a friendly green approach and cost-effective. Results showed that ethanol with 1% citric acid was a suitable and eco-friendly solvent system to extract anthocyanins from bracts (0.23 mg g^{-1}). The optimal conditions for the maximal recovery of anthocyanins (0.34 mg g^{-1}) were: citric acid concentration of 5% m: v, extraction time of 180 minutes and liquid to solid ratio of 15. Silver nanoparticles synthesized using anthocyanins showed good nano-characteristics with promising anti-proliferative activity against Dalton's lymphoma ascites (DLA) (cancer cells).

Ramírez-Bolaños, Pérez-Jiménez, Díaz, & Robaina (2021) reported results about the proximate composition of dried banana flower (DBF) and dried pseudo-stem (DBPS), and their phenolic compounds profile identified by HPLC-ESI-QTOF MS. DBF and DBPS were extracted at room temperature (under agitation) with methanol/water (50:50 v: v, pH 2) and then with acetone/water (70:30, v/v) for 1 hour. The two supernatants were combined, which corresponded to the extractable phenolic compounds fraction. The results showed that both had an interesting nutritional composition, especially regarding proteins (DBF: 13.59% and DBPS: 7.25%) and lipids (DBF: 8.66% and DBPS: 1.01%). Phenolic compounds in DBF emerged as a potentially relevant source of valuable compounds such as hydroxybenzoic acids (3,5-dihydroxybenzoic acid (4.4 µg g^{-1}), gentisic acid (4.4 µg g^{-1}), protocatechuic acid (4.3 µg g^{-1}), hydroxybenzoic acid (66.1 µg g-1), hydroxybenzaldehydes (4-hydroxybenzaldehyde (2.4 µg g^{-1}), benzoic acid (2.4 µg g^{-1}), hydroxycinnamic acids (caffeic acid (100 µg g^{-1}), p-coumaric acid (3.8 µg g^{-1}) and ferulic acid (16.4 µg g^{-1}) and flavonols (quercetin 3,4'-O-diglucoside (10.1 µg g^{-1}) and quercetin 3-O-sophoroside (10.1 µg g^{-1}) (Ramírez-Bolaños, Pérez-Jiménez, Díaz, & Robaina, 2021).

Although conventional extraction is the most widely used, different methods have been reported such as “non-conventional techniques" which are known as "green" or "clean"

methods. The objective of these techniques is to achieve a faster extraction rate, more effective energy use, increase the heat transfer and reduction of steps (Soquetta, Terra, & Bastos, 2018). The extraction of compounds involves complex mechanisms and it can be accomplished by manifold techniques. It is also important to improve the extraction yields and reduce environmental damage caused by toxic solvents. In this sense, the interest in technologies that are referred to as clean or green can reduce or eliminate the use of these solvents, and thus preserve the natural environment and its resources. In more recent studies concerning extraction of banana inflorescence some techniques such as enzyme-assisted extraction (EAE) and ultrasound assisted extraction were reported.

Extraction using ultrasound was applied to recovery dietary fiber (DF) from banana inflorescence. This technology involves two main types of physical phenomena: diffusion through the cell wall and rinsing the cell content after breaking the walls. For this, DF was extracted from banana bract, suspended in 0.5 mol L^{-1} NaOH at 50ºC under agitation (500 rpm) for 30 min, sonicated at 20 kHz at 77.6ºC for 14 min and 37% of sonication amplitude. Researchers formulated a dietary fiber (DF)-anthocyanin complex with different ratios of pigment-matrix where DF and anthocyanin were extracted from inflorescences. In this case, DF was used as a matrix to entrap anthocyanin in its fibrous structure. They reported that the pigment-matrix ratio (3:1) (DF-A3) was optimum for DF-anthocyanin formulation with improved antioxidant content (control without anthocyanin: 3.39 mg 100 g^{-1} and DF-A3: 41.64 mg 100 g^{-1}) and storage stability (during 87 days) (Begum & Deka, 2020). In a previous study by the same research group showed that the ultrasonic waves create bubbles that collapse, which increases the solvent penetration into the tissue resulting in the release of intracellular compounds (Begum & Deka, 2019a).

A comparative analysis between the enzymatic assisted extraction (EAE) and ultrasonic-assisted alkaline extraction (UAE) of proteins from banana inflorescence was reported by Sitthiya et al. (2018). The highest protein yield was reached with UAE (252.25 mg g^{-1}) under the following conditions: 30 min as extraction time, 50ºC, 1 mol L^{-1} NaOH and 24 kHz. These extracts generated high protein content when compared to EAE (102.98 mg g^{-1}) after 6 h incubation using pepsin. The UAE extracted proteins were characterized and showed the presence of tryptophan and tyrosine with antibacterial and anti-microbial effects (Sitthiya et al., 2018).

Apart from antioxidant properties, another interest in banana inflorescence is related to its antidiabetic properties. This association occurs due to a common practice in traditional

medicine reported in many cultures. In India, for instance, banana inflorescence was used for the treatment of diabetes through consumption of cooked inflorescence (Kumar, Bhowmik, Duraivel, & Umadevi, 2012). Some compounds identified including anthocyanins and phenolic acids have antidiabetic properties. Anthocyanins are involved in mechanisms that contribute to increased insulin sensitivity and reduced blood glucose levels (Guo & Ling, 2015). Phenolic acids showed inhibitory activity on α-amylase and aldose reductase, contributing to the reduction in glycemic levels (Alim, Kilinç, Şengül, & Beydemir, 2017). Therefore, the anti-diabetic effect of the inflorescence is attributed to the array of secondary metabolites inhibiting the enzymes, modulating multiple pathways and acting on various molecular targets involved in diabetes (Lau et al., 2020).

Recent studies also investigated this bioactivity on aqueous and methanolic extracts obtained from bracts and flowers of *Musa x paradisiaca* in streptozotocin (STZ)-induced diabetic rats followed by chemical characterization (Vilhena et al., 2020). The authors reported a decrease fasting glycemia for diabetic group treated with methanolic extract (107 mg dL^{-1}) as compared with the untreated diabetic group (209.3 mg dL^{-1}). When administered, the aqueous bract extract was highlighted to improve the post glucose load profile in diabetic rats as compared with the untreated group and methanolic bract extract. Furthermore, they also identified 21 compounds and 17 metabolites among which were glycosylated and acetylated phenylpropanoids of ρ-coumaric acid and caffeic acid, as well as a glycosylated flavonol and anthocyanins.

Banana inflorescence has been exhibited other biological activities. For instance, the anti-inflammatory and immunomodulatory effects of the hydroalcoholic extract of *Musa paradisiaca* inflorescence were reported in an experimental model of combined allergic rhinitis and asthma syndrome (CARAS) (Gadelha et al., 2021). The main results showed that the inflorescence inhibited airway syndrome by inhibiting type 2 immune cells by NF-κB pathway, which plays a key role in regulating the immune response to infection and modulated the human macrophages by decrease CD86 expression (member of the immunoglobulin superfamily) and HLA-DR (human leukocyte antigen complex).

It is important to conduct new studies about banana inflorescence in order to identify possible applications, in addition to carrying out further analysis regarding its bioactivity. Hence, further exploration should be encouraged to exploit their potential industrial applications.

6. Banana inflorescence as a novel ingredient and perspective for food industry

In the last few years, the demand towards functional food has substantially increased due to growing consumer concern about health. In this sense, the use of banana inflorescence can be an opportunity to increase value in this chain by using the extracts or components into different foods matrix. For this, it is necessary to analyze the proper levels since its addiction could affects the sensory attributes of food products. On the other hand, banana inflorescence consumption is not popular due to its cumbersome preparation process. Their development as a food ingredient will make feasibility its preparation by consumers. The dehydrated powder, for instance, can be easily incorporated into food products and also reduce agricultural waste. This technology promotes a reduction of available moisture that causes rotting and provides longer shelf life (Jha et al., 2021). In addition, in relation to the matrix, susceptibility to enzymatic browning can be a challenge to banana inflorescence uses (Jha et al., 2021). Researches evaluated the effect of different pretreatments to avoid these undesirable reactions. This research explored the pretreatment-induced changes on drying kinetics of sliced banana flower and the quality of dried banana flower powder sample. Samples were pretreated with blanching, citric acid solution, lemon juice, brine solution and rice rinse water. Results demonstrated that dipping banana flower slices in lemon juice and rice rinse water before drying operation could improve color parameters and enhance some functional and flow properties of banana flower powder. Hence, improvements in postharvest processing are necessary to overcome the limitations in this raw material.

An interesting initiative was taken in bakery products with a develop bread fortified with dietary fiber (DF) from banana bracts (Begum, Chakraborty, et al., 2020). The effect of DF addition on dough rheology, quality attributes and *in vitro* starch digestibility of bread was studied. Bread was prepared by incorporating DF (2–4 g per 100 g of flour mixture) with varying moisture (64–68 g per 100 g). Rheological study of dough showed an increase in dough stiffness and elasticity with higher incorporation of the DF. The improved textural property with acceptable sensory attributes was observed for bread with 2 g per 100 g DF of flour mixture and 66 g per 100 g moisture content. The incorporation of 4% of DF mixture (1.5 g DF and 0.5 g anthocyanin per 100 g of bread) showed a decrease of 42.71% in digestion rate of bread.

Another application is the use of banana bract flour in cookies and pizza dough (Queiroz et al., 2021). For cookie elaboration, cassava starch and bract flours were used to replace wheat flour. For pizza dough preparation, rice, sweet tapioca, and bract flours were used. The authors reported that both pizza and cookie doughs added with bract flour had a good

response from tasters, with an overall acceptance above 65%. The results showed that bract flour presents a high dietary fiber and minerals content and a low caloric value.

Furthermore, banana inflorescence (*kluthuk* type) has also been applied for beverage production. Their incorporation into fermented drink with probiotics was evaluated in formulations using different rations of banana flower: banana leaf: water (sample 1 (1: 0.5: 0.5), sample 2 (2: 1: 1), and sample 3 (3: 2: 2)). All samples were inoculated with *Lactobacillus paracasei* for 14 days under anaerobic conditions. The results showed that the amount of vitamin C obtained at sample 3 was 77.92 mg 100 g^{-1} with 22.10% antioxidant activity against DPPH-radicals. In conclusion, the combination of *kluthuk* banana flowers and leaves had a great potential to be developed into health functional food (Rompies et al., 2021).

Other similar food applications have also been evaluated in beverage for breastfeeding mothers (Amornlerdpison, Choommongkol, Narkprasom, & Yimyam, 2021). Compounds of banana inflorescence (*Musa x paradisiaca*) was extracted with different solvents to determine the bioactive compounds and antioxidant activity (ABTS radical scavenging). The results showed that a high-polarity solvent extraction (ethanol and water) resulted in a high recovering of total phenolic compounds and flavonoids. The bioactive compounds identified were β-sitosterol, flavonoids, saponin, and other phenolic compounds such as catechin and isoquercetin. The aqueous extract of banana inflorescence applied in beverage contained 392 mg GAE total phenolic compounds per serving size (350 mL/bottle). This beverage demonstrated the presence of phenolic compounds (392 mg GAE g^{-1}/bottle) which would benefit maternal breastfeeding.

Consumers are increasingly aware of the toxicological implications of the synthetic antioxidants, thereby there is an increasing demand for healthier products, which has led the meat industry to replace these compounds with natural antioxidants. Thus, extracts made with various parts of banana inflorescences (bracts, male flowers, rachis, and whole inflorescence) were applied in sausage formulations at different concentrations (from 0.5%, 1 to 2% v:m) (Rodrigues et al., 2020). The authors reported that the addition of banana inflorescence had a positive effect on the control of lipid oxidation during storage (28 days). In addition, the sensory attributes were not affected by addition of up to 2% of extract with no major changes on pH, water activity (aw) and color parameters. The addition of extract made with male flowers (EMF) to the sausage formulations had a positive effect on the control of lipid oxidation during storage. Another meat product application was reported by (Novidiyanto, Enardi, Devriany, Pratiwi, & Airuni, 2020). Banana flower with different levels (25, 50 and 75% m:m) was mixed

with chicken meat to produce shredded banana flower-chicken meat. The results showed that the product prepared with 25% of banana flower had the highest preferences in acceptability (color, aroma, taste, texture). It also presented the highest antioxidant capacity (53.74% of DPPH radical scavenging activity).

7. Conclusions

Based on literature there are well-established and consolidated studies concerning the bioactivities of the banana inflorescence with great potential for it to be utilized as an ingredient in food and nutraceutical industries. The bibliometric analysis indicated an increase in researches towards Food and Science Technology field revealing possibilities that has not yet been studied.

In this sense, further research that evaluates the extraction of compounds of interest and the identification of their profile in order to link chemical structures with their potential bioactivities is a relevant opportunity for banana inflorescence, since few studies report this approach. Strategies already well established for other plant matrices can also be explored, since they are scarce for banana inflorescence, such as encapsulation processes and bioaccessibility and bioavailability assays.

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CAPÍTULO II: Combination of solvents acted synergistically on the recovery of antioxidant compounds from banana bracts (*Musa acuminata*)

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Abstract

The aim of this study was to determine the best solvent mixture for the extraction of antioxidant compounds from three varieties of banana *(Musa acuminata)* bracts (Maçã, Nanica and Prata) using an experimental mixture design. For this, experiments were performed using pure, binary or ternary solvent mixtures containing water, methanol and acetone. The extraction of antioxidant compounds with water/acetone in equal proportions from the Maçã variety was the most appropriate condition and promoted increases of up to 6-fold in antioxidant activities evaluated by ABTS, DPPH and FRAP assays, respectively, compared to the extracts obtained using isolated solvents. For the Prata variety, the same combination of solvents resulted in increases of up to 8-fold evaluated by antioxidant methods compared to the extracts produced with the isolated solvents. The mixture of water (1/6), methanol (2/3) and acetone (1/6) was the most efficient for the Nanica variety and resulted in increases of up to 15-fold in antioxidant activities compared to isolated solvents. Moreover, the bioactive compounds identified by HPLC in banana bract extracts included rutin in three varieties and coumaric acid only in the Nanica variety. These results emphasize the importance of selecting the proper extraction solvent to obtain extracts with better antioxidant properties. This study reported for the first time a solvent evaluation for the recovery of phenolic compounds from bracts of three relevant Brazilian banana varieties.

Keywords: Banana flower, agricultural waste, conventional extraction, natural antioxidants, statistical mixture design.

1. Introduction

Banana (*Musa paradisiaca)* originates from the Southeast Asian region and is cultivated throughout the world, primarily in tropical and subtropical regions (Vu et al., 2019). It represents one of the most important and highly nutritional food crops consumed around the world, with an estimated consumption of approximately 90% as fresh fruit (Shahidi et al., 2019). Concerning global production estimated to be 116 million tons, the banana agroindustry generates large amounts of waste, including pseudostems, leaves, inflorescences and peels (FAO, 2019).

Plant-based agricultural wastes are considered one of the major sources of bioactive compounds, which may represent an opportunity for producers (Bandara and Chalamaiah, 2019). These wastes are generally discarded, but there is renewed interest due to the amount of compounds with potential health-promoting effects (Lau et al., 2020). Studies have suggested that the intake of bioactive compounds lowers the risk of diseases that are typically associated with oxidative stress, such as diabetes, cancer and cardiovascular disease (Alara et al., 2021; Câmara et al., 2020; Marathe et al., 2017; Rashmi and Negi, 2020).

An example of agricultural waste is banana inflorescence, also known as bract, flower or blossom, consisting of a rod that is located at the end of the stem. Banana inflorescence has attracted scientific interest due to its variety of bioactive compounds and its associated biological properties, including antioxidant, anti-diabetic and cardiovascular-protective activities (Arun et al., 2017; Ramu et al., 2017; Sheng et al., 2014).

One of the main critical points to obtain bioactive compounds is the extraction method since it defines the amount of compounds that will be transferred to the extract (Kajdžanoska et al., 2011). Although several methods can be used for extracting compounds, the choice of technique mainly depends on the raw material, environmental concerns, process conditions and applications of bioactives (Marathe et al., 2017). In addition, the extraction process also depends on factors such as the sample matrix, particle size, extraction time, temperature and solvent (type, concentration, sample: solvent ratio) (Garcia-Salas et al., 2010; Khoddami et al., 2013).

Among the extraction methods, solvent extraction is the oldest and most traditional technique in which a suitably sized raw material is exposed to different solvents that take up soluble components of interest (Kumar et al., 2017; Marathe et al., 2017). This technique has advantages such as low processing cost and easy operation (Chen and Wang, 2017). Commonly, solvents used for recovering polyphenols from agro-industrial wastes are polar, mainly

solutions containing ethanol, methanol and acetone or mixtures of them, as the presence of variable compounds with distinct structures and polarities may not be soluble in a pure solvent (Do et al., 2014; Socaci et al., 2018).

From this perspective, the evaluation of multicomponent systems containing solvents with different physicochemical properties increases the chances of promoting a more efficient recovery of the compounds of interest (Rasera et al., 2019). Based on these statements, this study focused on the investigation of the most adequate solvent mixture for the extraction of antioxidant compounds from banana *(Musa acuminata)* bracts (Maçã, Nanica and Prata varieties) (Fig. 1). Bioactive compounds (condensed tannins, flavonoids and total phenolic compounds) and antioxidant properties (ABTS, DPPH and FRAP assays) were used as responses to select the best extractor.

Figure 1. Brazilian banana inflorescence varieties Maçã (a), Nanica (b) and Prata (c) varieties used in the experiments.

2. Material and Methods

2.1 Material and reagents

Bracts of banana *(Musa paradisiaca)* Prata and Maçã varieties were collected from cultivated local farmland (20°33'56.5" south latitude, 46°05'21.1" west longitude) in Capitólio, state of Minas Gerais, Brazil. The Nanica variety was donated by Magário company, Jaíba, Minas Gerais, Brazil.

ABTS ([2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)]), DPPH (2,2-diphenyl-1-picrylhydrazyl), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic

acid), TPTZ (2,4,6-tripyridyl-s-triazine), Folin & Ciocalteau's phenol reagent, galic acid, catechin, and vanillin were purchased from Sigma–Aldrich (São Paulo, Brazil). All other chemicals were purchased in commercially available grade.

2.2 Experimental mixture design and obtaining banana bract extracts

To obtain the optimum mixture of the different solvents for maximum extraction of compounds and to identify the presence of synergistic or antagonistic effects between solvents, an experimental mixture design was applied (Rasera et al., 2019). In this model, vertices of a triangle correspond to the pure components that represent 100% of each solvent. The midpoints of the sides of the triangle represent the results for binary mixtures of solvents, and the central point (centroid) indicates ternary mixtures (de Castro et al., 2017). For this study, quadratic or cubic regression models were adjusted as a function of significant effects for the variations of all the responses ($p \leq 0.10$), considering acceptable determination coefficients above 70% ($R^2 > 0.70$). Eq. 1 represents these models as follows:

$$\boldsymbol{Yi} = \sum_{\boldsymbol{i=1}}^{\boldsymbol{q}} \boldsymbol{\beta_i X_i} + \sum\sum_{\boldsymbol{i<j}}^{\boldsymbol{q}} \boldsymbol{\beta_{ij} X_i X_j} + \sum \sum_{\boldsymbol{i<j<k}}^{\boldsymbol{q}} \sum \boldsymbol{\beta_{ijk} X_i X_j X_k} \quad (1)$$

where "Yi" represents the predicted response (total phenolic compounds, total flavonoid content, condensed tannins and antioxidant properties - ABTS, DPPH and FRAP); "q" is the number of independent variables (components) in the mixture; "Xi, Xj, Xk" indicate the coded independent variables; and "βi", "βij" and "βijk" are the model regression coefficients (linear, binary and ternary interaction, respectively) (de Castro et al., 2017).

The statistical significance of each regression coefficient was determined using Student's t-test ($p \leq 0.10$), and the polynomial regression model was evaluated by analysis of variance (ANOVA) ($p \leq 0.10$) using the software Statistica version 13.3 from TIBCO Software Inc. (Palo Alto, California, USA).

The recovery of antioxidant compounds was performed according to each experiment shown in Table 1. For this, bracts were cleaned, ground, frozen, freeze-dried and stored in vacuum packs at -18°C until analysis. To obtain extracts, 0.5 g of samples were mixed with 20 mL of solvent and maintained under stirring (100 rpm) for 1 hour in a shaker (Incubator shaker series 25, New Brunswick Scientific Co, USA). After the incubation time, the mixture was filtered through a filter membrane (Whatman® qualitative filter paper nº1) to obtain an extract

free of solid particles. The filtered material was collected and stored at -18°C for further analysis. All the experiments were performed in the absence of light.

Table 1. Matrix of the simplex centroid mixture design used for extraction of antioxidant compounds of banana bracts from Prata, Maçã and Nanica varieties using different solvents and their mixtures.

Run	Independent variables		
	Water (x_1)	Methanol (x_2)	Acetone (x_3)
1	1	0	0
2	0	1	0
3	0	0	1
4	1/2	1/2	0
5	1/2	0	1/2
6	0	1/2	1/2
7	1/3	1/3	1/3
8	2/3	1/6	1/6
9	1/6	2/3	1/6
10	1/6	1/6	2/3

2.3 Determination of bioactive compounds

2.3.1 Total phenolic compounds (TPC)

The total phenolic compounds were determined according to the method of Pereira et al., (2018) with slight modifications described by Magro and de Castro (2020). Aliquots of 25 µL of each extract (25 mg mL^{-1}) were mixed with 25 µL of Folin-Ciocalteau aqueous solution (50% v/v) and 200 µL of sodium carbonate solution (5% w/v). The reaction mixtures were incubated at 40°C in for 20 min, and the absorbance was determined at 760 nm in a microplate reader (Multiskan GO, Thermo Fisher Scientific, Finland). Quantification of the phenolic compounds was performed using a calibration curve of gallic acid (0 - 10 mg mL^{-1}). The results were expressed as mg of gallic acid equivalent per gram of freeze-dried sample (mg GAE g^{-1}).

2.3.2 Condensed tannins

The determination of the condensed tannin content was performed according to the method described by Arruda et al., (2018). Aliquots of 30 µL of extract (25 mg mL^{-1}) were mixed with 900 µL of a methanolic solution containing vanillin (4% m/v) and 450 µL of concentrated hydrochloric acid. The reaction mixtures were allowed to stand for 20 min at room temperature in the dark. Aliquots of 300 µL were added to a 96-well transparent microplate, and the absorbance was measured at 500 nm in a microplate reader (Multiskan GO, Thermo

Fisher Scientific, Finland). Catechin (0 – 1.0 mg mL^{-1}) was used for the calibration curve, and the results were expressed in mg of catechin equivalents per gram of freeze-dried sample (mg CE g^{-1}).

2.3.3 Total Flavonoid Content

Total flavonoids in the banana bract extracts were determined according to the method described by Zhishen et al. (1999). Briefly, 500 µL of sample (25 mg mL^{-1}) was homogenized with 150 µL of $NaNO_2$ (5% m/v), incubated for 5 min and added to 150 µL of $AlCl_3$ (10% m/v). After 6 min, aliquots of 1000 µL of NaOH (4% m/v) and 1200 µL ultrapure water were added to the mixture. Then, aliquots of 300 µL were transferred to 96-well transparent microplates, and the absorbance was monitored at 510 nm in a microplate reader (Multiskan GO, Thermo Fisher Scientific, Finland). Catechin (0 - 0.35 mg mL^{-1}) was used for the calibration curve, and the results were expressed as mg CE g^{-1}.

2.4 Measurement of the antioxidant properties

2.4.1 ABTS-radical cation scavenging activity

The ABTS assay was performed as described by Neta and de Castro (2020). The ABTS radical cation solution was prepared by mixing the aqueous ABTS working solution (7 mmol L^{-1}) with potassium persulfate solution (140 mmol L^{-1}) for 16 h in the dark at room temperature before use. The cation ABTS solution was diluted in distilled water until reaching an absorbance value of 0.70 ± 0.02 (734 nm). Aliquots of 20 µL of each extract (25 mg mL^{-1}) were added to 220 µL of ABTS radical cation solution, and the absorbance was read after 6 min at 734 nm using a microplate reader (Multiskan GO, Thermo Fisher Scientific, Finland). The control assay was prepared as described above but using distilled water instead of banana bract extracts. A standard curve was prepared using Trolox at different concentrations (0 – 400 µmol L^{-1}), and the results were expressed as µmol of Trolox equivalents per g of freeze-dried sample (µmol TE g^{-1}).

2.4.2 DPPH-radical scavenging activity

The DPPH assay was performed according to the method described by Rasera et al., (2019). Aliquots of 134 µL of DPPH ethanolic solution (150 µmol L^{-1}) were added to 66 µL of extracts (25 mg mL^{-1}) or standard (Trolox). After 45 min of the reaction at room temperature, the absorbance was measured at 517 nm using a spectrophotometer (Multiskan GO, Thermo

Fisher Scientific, Finland). Trolox was used as the standard with a calibration curve ranging from 0 to 125 μmol L^{-1}. The results were expressed as μmol TE g^{-1}.

2.4.3 Ferric Reducing Antioxidant Power (FRAP) assay

The FRAP assay was carried out according to the method described by Firuzi et al., (2005) with some adaptations proposed by Aguilar et al., (2018). The FRAP solution was prepared using 10 mL of 300 mmol L^{-1} acetate buffer (pH 3.6) mixed with 1 mL of 20 mmol L^{-1} ferric chloride hexahydrate dissolved in distilled water and 1 mL of 10 mmol L^{-1} TPTZ (2,4,6-tris(2-pyridyl)-s-triazine) dissolved in 40 mmol L^{-1} HCl. Using a 96-well transparent microplate, 25 μL of banana bract extracts (25 mg mL^{-1}) and 175 μL of FRAP solution freshly prepared and warmed at 37°C were mixed, and the absorbance was monitored in a microplate reader (Multiskan GO, Thermo Fisher Scientific, Finland) at 595 nm. The control assay was prepared as described above but using distilled water instead of samples. A standard curve was prepared using Trolox at different concentrations (0 – 250 μmol L^{-1}), and the results were expressed as μmol TE g^{-1}.

2.5 Identification of phenolic compounds by HPLC

Phenolic compound identification was performed according to the method described by Silva et al., (2021) with slight modifications. For that, an aliquot of 20 μL of extracts was injected into an analytical HPLC unit equipped with a Shimadzu ODS-A column (4.6 mm, 250 mm, 5 μm) and photodiode array detector (SPD-M10AVp, Shimadzu Co., Kyoto, Japan) at a flow rate of 1.0 mL min^{-1}. The mobile phase was composed of (A) water/formic acid (99.75/0.25, v/v) and (B) acetonitrile/formic acid/water (80/0.25/19.75, v/v). The gradient for chromatography separation started with 10% B and increased to 20% B at 10 min, 30% B at 20 min, 100% B at 30 min and 10% B at 35 min, finishing at 40 min. The column was maintained at 30°C during the analysis. The chromatograms were analyzed using Class-VP® software. The following standards (Sigma–Aldrich, St. Louis, MO, USA) were examined for phenolic acids (galic acid, 3,4-dihidroxibenzoic acid, vanillic acid, caffeic acid, coumaric acid, ferulic acid, and sinapic acid) and flavonoids (rutin and quercetin). Eq. (2) and (3) represent the limits of detection and quantification (LOD and LOQ) and were calculated as follows:

$$LOD = 3.3 \times s \div S \quad (2)$$

$$LOQ = 10 \times s \div S \quad (3)$$

where "*s*" is the estimated standard deviation of the linear coefficient of the equation and *"S"* is the slope of the analytical curve.

2.6 Calculations and statistics

The results were expressed as the mean values ± standard deviation (n = 3) and Minitab™19 software from Minitab Inc. (State College, Pennsylvania, USA) was used to verify whether there was a significant difference (p-value ≤ 0.05) between the values when analyzed by ANOVA followed by the Tukey test.

Pearson correlation was used to analyze the strength of linear dependence between phenolics and antioxidant activity. The correlation coefficient ranges from -1 to 1. A value of 1 implies a perfect and positive response, while a value of -1 indicates a negative correlation. A value of 0 implies that there is no linear correlation between the responses. When $p < 0.05$, the correlations between analyzed parameters were considered significant.

3. Results and discussion

3.1 Effect of the solvent composition on the recovery of antioxidant compounds

The results for bioactive compounds and antioxidant properties obtained for extracts produced with banana bracts from Maçã, Nanica and Prata varieties are displayed in Table 2. The highest content of bioactive compounds was detected for the extracts obtained from the Nanica variety (run 9), in which the TPC reached 24.95 mg GAE g^{-1} and the Maçã variety, which showed flavonoid and condensed tannin contents of 12.46 mg CE g^{-1} (run 3) and 16.68 mg CE g^{-1} (run 5), respectively. For antioxidant properties, the highlights were the extracts obtained from bracts of the Nanica variety, which reached 136.33 µmol TE g^{-1} for ABTS (run 9) and 144.57 µmol TE g^{-1} for FRAP (run 9), while the extract from Prata variety showed the greatest ability to inhibit DPPH radicals (52.54 µmol TE g^{-1} - run 2).

The use of a binary mixture of water and acetone (in equal proportions, run 5) resulted in banana bract extracts from Maçã and Prata varieties with maximum values of bioactive compounds (condensed tannins, flavonoids and total phenolic compounds) and antioxidant properties. However, the ternary mixture of water (1/6), methanol (2/3) and acetone (1/6) (run 9) resulted in the highest total bioactive compounds and antioxidant properties on extracts from the Nanica variety (Table 2).

This study showed that the mixture of solvents had synergistic and antagonistic effects on the antioxidant activities of banana bract extracts, contributing statistically significant ($p < 0.10$) effects on the responses. A synergistic effect between water and acetone (run 5) observed on banana bract extract from the Maçã variety showed increases of up to 1.4-fold evaluated by total phenolic compounds, total flavonoids and condensed tannins when compared to the

respective extracts produced with isolated solvents. This solvent mixture also promoted increases of up to 6-fold in antioxidant activities evaluated by ABTS, DPPH and FRAP assays, respectively, compared to the extracts obtained using individual solvents (runs 1 and 3). Similar results were observed for banana bract extract from Prata variety with same combination of solvents (run 5), which resulted in increases of up to 46-fold evaluated by total phenolic compounds and condensed tannins compared to the respective extracts produced with isolated solvents. This solvent combination also promoted increases of up to 8-fold in antioxidant activities evaluated by ABTS, DPPH and FRAP methods, compared to the extracts obtained with the isolated solvents (Table 2).

Table 2. Results for bioactive compounds (TPC, flavonoids and condensed tannins) and antioxidant activities (ABTS, DPPH and FRAP assays) in banana bract extracts.

Run	Responses					
	TPC (mg GAE g^{-1})	Flavonoids (mg CE g^{-1})	Condensed Tannins (mg CE g^{-1})	ABTS (µmol TE g^{-1})	DPPH (µmol TE g^{-1})	FRAP (µmol TE g^{-1})
Maçã variety						
1	11.46 ± 0.37 ^{cde}	4.27 ± 0.93 ^{e}	9.76 ± 0.43 ^{b}	36.72 ± 2.96 ^{f}	18.23 ± 0.57 ^{f}	37.54 ± 0.78 ^{d}
2	10.53 ± 0.40 ^{de}	6.58 ± 1.40 ^{cde}	9.81 ± 0.60 ^{b}	43.30 ± 1.90 ^{e}	19.7 ± 0.36 ^{f}	29.02 ± 1.76 ^{e}
3	8.52 ± 0.93 ^{e}	12.46 ± 0.56 ^{a}	10.32 ± 2.89 ^{b}	43.74 ± 1.17 ^{e}	17.85 ± 1.27 ^{f}	9.04 ± 0.87 ^{f}
4	13.73 ± 0.61 ^{bcd}	4.96 ± 1.37 ^{de}	10.50 ± 0.78 ^{b}	71.24 ± 1.14 ^{c}	25.44 ± 1.75 ^{de}	48.37 ± 2.31 ^{c}
5	16.55 ± 0.64 ^{ab}	10.51 ± 0.89 ^{ab}	16.68 ± 3.27 ^{a}	81.80 ± 1.83 ^{b}	33.72 ± 0.88 ^{ab}	65.36 ± 3.94 ^{b}
6	10.68 ± 0.31 ^{de}	7.15 ± 0.62 ^{cd}	12.23 ± 2.61 ^{ab}	49.54 ± 0.80 ^{e}	19.61 ± 1.30 ^{f}	40.77 ± 0.55 ^{d}
7	17.62 ± 0.59 ^{a}	7.92 ± 0.86 ^{bc}	14.16 ± 1.07 ^{ab}	96.05 ± 2.41 ^{a}	36.38 ± 1.26 ^{a}	78.41 ± 2.37 ^{a}
8	14.05 ± 0.76 ^{bc}	4.29 ± 0.15 ^{e}	9.86 ± 0.89 ^{b}	74.53 ± 1.00 ^{c}	28.60 ± 2.76 ^{cd}	53.20 ± 0.81 ^{c}
9	13.19 ± 2.23 ^{cd}	7.66 ± 0.30 ^{c}	12.04 ± 0.93 ^{ab}	71.64 ± 2.61 ^{c}	31.24 ± 1.72 ^{bc}	48.81 ± 0.06 ^{c}
10	14.05 ± 0.63 ^{bc}	8.27 ± 1.02 ^{bc}	11.35 ± 2.37 ^{ab}	58.46 ± 4.72 ^{d}	22.19 ± 1.82 ^{ef}	61.98 ± 4.28 ^{b}
Nanica variety						
1	13.77 ± 0.10 ^{de}	2.46 ± 0.41 ^{d}	0.06 ± 0.12 ^{f}	21.11 ± 1.39 ^{g}	9.39 ± 0.31 ^{f}	65.40 ± 1.67 ^{h}
2	17.51 ± 0.23 ^{bc}	2.59 ± 0.23 ^{d}	1.40 ± 0.03 ^{d}	60.14 ± 3.47 ^{f}	22.99 ± 0.95 ^{d}	90.00 ± 2.38 ^{f}
3	14.15 ± 0.09 ^{cde}	0.72 ± 0.10 ^{f}	0.70 ± 0.20 ^{e}	8.53 ± 3.38 ^{h}	3.34 ± 0.60 ^{g}	41.38 ± 3.57 ^{i}
4	9.92 ± 1.06 ^{f}	3.45 ± 0.01 ^{c}	2.16 ± 0.15 ^{c}	100.15 ± 0.69 ^{de}	27.15 ± 0.92 ^{c}	110.87 ± 2.06 ^{d}
5	11.91 ± 0.22 ^{ef}	3.17 ± 0.04 ^{c}	1.35 ± 0.10 ^{d}	103.63 ± 1.60 ^{cd}	21.65 ± 1.07 ^{d}	99.11 ± 2.94 ^{e}
6	13.78 ± 0.19 ^{de}	1.89 ± 0.04 ^{e}	1.51 ± 0.07 ^{d}	51.62 ± 3.40 ^{f}	18.00 ± 0.76 ^{e}	73.91 ± 2.20 ^{g}
7	16.47 ± 0.73 ^{cd}	4.96 ± 0.02 ^{a}	3.52 ± 0.43 ^{b}	119.85 ± 1.67 ^{b}	30.96 ± 1.57 ^{b}	134.90 ± 3.61 ^{b}
8	16.57 ± 0.70 ^{cd}	2.57 ± 0.07 ^{d}	1.03 ± 0.18 ^{de}	91.96 ± 2.50 ^{e}	20.82 ± 0.63 ^{d}	93.94 ± 1.13 ^{ef}
9	24.95 ± 1.36 ^{a}	4.58 ± 0.02 ^{a}	4.17 ± 0.17 ^{a}	136.33 ± 3.06 ^{a}	33.75 ± 0.32 ^{a}	144.57 ± 1.12 ^{a}
10	20.09 ± 3.00 ^{b}	4.01 ± 0.04 ^{b}	3.80 ± 0.26 ^{ab}	111.15 ± 0.65 ^{c}	31.55 ± 1.15 ^{ab}	123.65 ± 2.90 ^{c}
Prata variety						
1	3.40 ± 0.33 ^{e}	*Not detected*	0.15 ± 1.41 ^{b}	31.97 ± 1.33 ^{c}	19.79 ± 0.81 ^{e}	18.57 ± 2.47 ^{e}
2	8.42 ± 0.45 ^{d}	5.16 ± 0.65 ^{a}	5.84 ± 1.93 ^{ab}	62.69 ± 6.13 ^{ab}	52.54 ± 0.33 ^{a}	75.26 ± 1.90 ^{ab}
3	1.51 ± 0.45 ^{f}	*Not detected*	3.57 ± 4.09 ^{ab}	15.27 ± 2.01 ^{d}	12.31 ± 1.18 ^{f}	8.29 ± 1.01 ^{f}
4	8.63 ± 0.40 ^{cd}	4.21 ± 6.47 ^{a}	5.89 ± 3.22 ^{ab}	56.52 ± 0.42 ^{ab}	41.65 ± 1,13 ^{c}	67.10 ± 5,80 ^{bc}
5	14.93 ± 1.02 ^{a}	4.95 ± 0.97 ^{a}	7.09 ± 1.65 ^{a}	67.75 ± 11.59 ^{a}	47.38 ± 1.00 ^{b}	75.77 ± 0.37 ^{ab}
6	8.46 ± 0.56 ^{d}	5.85 ± 0.59 ^{a}	4.44 ± 0.13 ^{ab}	56.74 ± 0.43 ^{ab}	48.25 ±0.35 ^{b}	77.73 ± 2.35 ^{a}
7	11.50 ± 0.45 ^{b}	2.14 ± 0.62 ^{a}	7.53 ± 0.65 ^{a}	53.10 ± 11.14 ^{ab}	43.86 ± 0.17 ^{c}	71.92 ± 2.35 ^{abc}
8	8.48 ± 0.36 ^{cd}	1.71 ± 1.71 ^{a}	3.53 ±1.28 ^{ab}	57.21 ± 2.31 ^{ab}	42.11 ± 0.73 ^{c}	65.99 ± 1.09 ^{cd}
9	10.14 ± 0.39 ^{bc}	2.62 ± 0.38 ^{a}	7.62 ± 2.51 ^{a}	64.82 ± 2.72 ^{ab}	48.14 ± 0.88 ^{b}	64.39 ± 7.22 ^{cd}
10	15.04 ± 0.63 ^{a}	1.80 ± 0.78 ^{a}	5.58 ± 1.13 ^{ab}	49.83 ± 0.59 ^{b}	37.48 ± 0.39 ^{d}	57.12 ± 0.79 ^{d}

Values were expressed as the mean (triplicate) ± standard deviation. Different lowercase letters in the same column indicate statistical difference ($p < 0.05$) between the results by Tukey test for each determination and banana bract variety.

On the other hand, the combination of water, methanol and acetone (run 9) was the most appropriate to recover antioxidant compounds from bracts of the Nanica variety. This combination of solvents resulted in increases of up to 68-fold evaluated by total phenolic compounds, total flavonoids and condensed tannins compared to the respective extracts produced with isolated solvents. This mixture also resulted in increases up to 15-fold in antioxidant activities analyzed by ABTS, DPPH and FRAP assays, respectively, compared to the extracts obtained using individual solvents (runs 1 to 3).

Most of the mathematical models showed R^2 values greater than 0.70, and the F values calculated were higher than F-tabulated with statistical significance ($p \leq 0.10$) according to ANOVA (Table 3). For responses where the statistical parameters were not satisfactory, the mathematical models (equations) were not generated.

The variations in bioactive compounds and antioxidant properties of the extracts obtained from Maçã, Nanica and Prata varieties were also depicted using mixture contour plots (Figures 2-4). The interpretation of contour plots confirmed that the best extraction solvent was the binary mixture between water and acetone for both Prata (Figure 4) and Maçã (Figure 2) banana bracts. For banana bracts from the Nanica variety, interpretation of the contour plot (FRAP) showed a strong synergistic effect between the ternary mixture of water, methanol and acetone (Figure 3), which resulted in the highest values of antioxidant activity of the extracts.

Table 3. Analysis of variance (ANOVA), including models, R^2 and probability values for the final reduced models for TPC, flavonoids, condensed tannins and antioxidant activity (ABTS, DPPH and FRAP) from the extract of banana bracts (Prata, Maçã and Nanica varieties).

Responses	Model	Equations	F calculated	F tabulated	R^2	p-value
Banana bract (Maçã variety)						
TPC	Quadratic	$Y = 10.82\ x_1 + 11.18\ x_2 + 9.33\ x_3 + 13.37\ x_1x_2 + 28.99\ x_1x_3$	6.97	3.52	0.85	0.020
Total flavonoids	Not valid	-	3.02	5.28	0.85	0.190
Condensed tannins	Quadratic	$Y = 9.21\ x_1 + 10.99\ x_2 + 10.66\ x_3 + 22.50\ x_1x_3$	3.66	3.29	0.70	0.080
ABTS	Quadratic	$Y = 41.16\ x_1 + 48.34\ x_2 + 44.18\ x_3 + 137.66\ x_1x_2 + 169.83\ x_1x_3$	6.10	3.52	0.83	0.030
DPPH	Quadratic	$Y = 17.28\ x_1 + 22.03\ x_2 + 17.13\ x_3 + 37.66\ x_1x_2 + 68.37\ x_1x_3$	4.12	3.52	0.76	0.070
FRAP	Quadratic	$Y = 26.92\ x_1 + 77.85\ x_2 + 8.60\ x_3 + 225.61\ x_1x_3 + 108.54\ x_2x_3$	5.08	3.52	0.80	0.050
Banana bract (Nanica variety)						
TPC	Not valid	-	0.65	5.28	0.56	0.690
Total flavonoids	Not valid	-	1.70	5.28	0.77	0.350
Condensed tannins	Not valid	-	1.54	5.28	0.75	0.380
ABTS	Not valid	-	1.69	3.29	0.45	0.260
DPPH	Not valid	-	2.49	3.29	0.55	0.150
FRAP	Quadratic	$Y = 11.65\ x_1 + 79.29\ x_2 + 25.19\ x_3 + 285.38\ x_1x_2 + 397.53\ x_1x_3$	5.13	3.52	0.80	0.050
Banana bract (Prata variety)						
TPC	Quadratic	$Y= 3.1\ 1x_1 + 8.21x_2 + 1.96x_3 + 25.47x_1x_3$	6.38	3.29	0.76	0.020
Total flavonoids	Quadratic	$Y= 22\ x_1 + 26.22x_2 + 24.87x_3 – 6.87x_1x_2 + 6.71x_2x_3$	7.97	4.05	0.90	0.030
Condensed tannins	Quadratic	$Y= -0.34\ x_1 + 6.22x_2 + 3.49x_3 + 12.17x_1x_2 + 20.60x_1x_3$	11.28	3.52	0.90	0.010
ABTS	Cubic	$Y= 32.45\ x_1 + 63.45x_2 + 15.23x_3 + 39.23x_1x_2 + 177.39x_1x_3 + 72.48x_2x_3 – 336.89x_1x_2x_3$	5.60	3.52	0.81	0.040
DPPH	Cubic	$Y= 20.72\ x_1 + 52.05x_2 + 12.05x_3 + 22.82x_1x_2 + 126.66x_1x_3 + 61.81x_2x_3 – 198.24x_1x_2x_3$	57.21	5.28	0.99	0.003
FRAP	Quadratic	$Y = 26.92\ x_1 + 77.85\ x_2 + 8.60\ x_3 + 225.61\ x_1x_3 + 108.54\ x_2x_3$	11.31	3.52	0.90	0.010

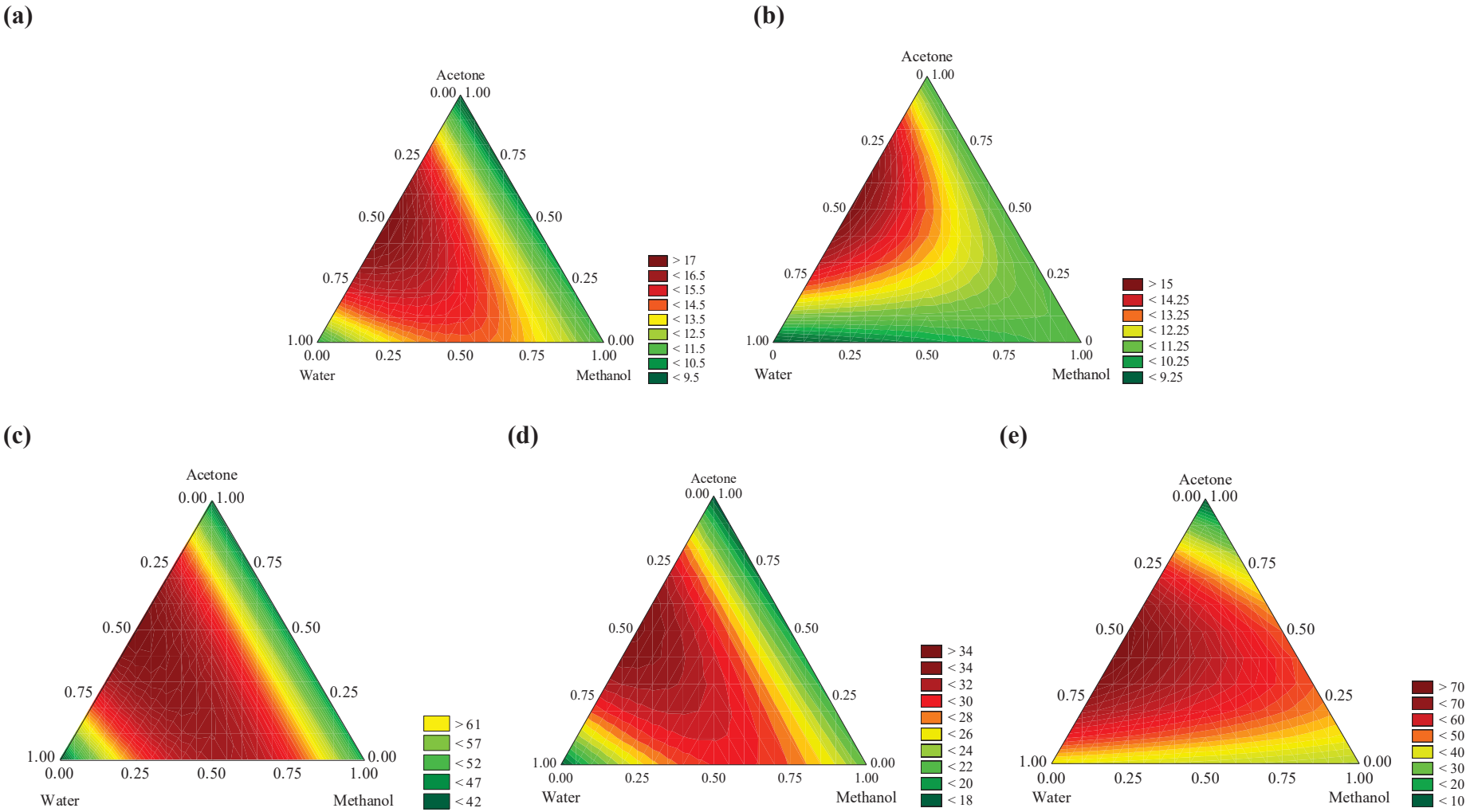

Figure 2. Contour plots for total phenolic compound (TPC) (a), condensed tannins (b) and antioxidant activity evaluated by ABTS (c), DPPH (d) and FRAP (e) methods, respectively, for banana bract extracts (Maçã variety).

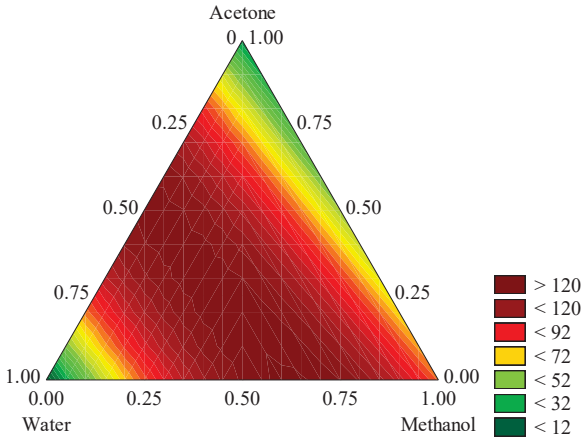

Figure 3. Contour plot for antioxidant activity evaluated by the FRAP method for banana bract extracts (Nanica variety).

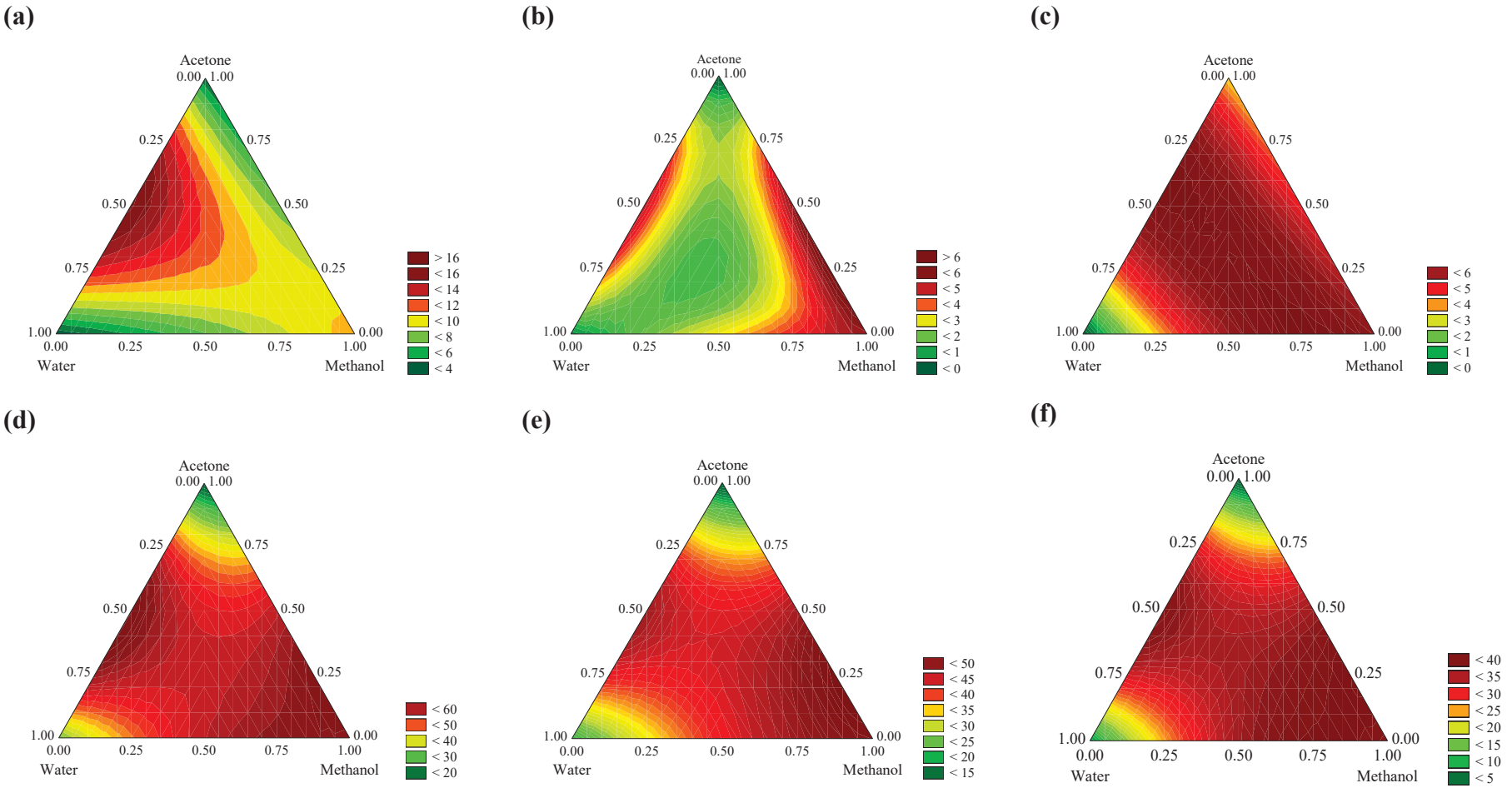

Figure 4. Contour plots for total phenolic compound (TPC) (a), total flavonoids (b), condensed tannins (c), and antioxidant activity evaluated by ABTS (d), DPPH (e) and FRAP (f) methods, respectively, for banana bract extracts (Prata variety).

Most soluble phenolics are located in the vacuoles of plant cells, and insoluble-bound phenolics are located in the cell wall matrix of plant cells (Shahidi and Yeo, 2016). Generally, these compounds are available in plants at low concentrations; consequently, it is necessary to extract them from plants (Nadar et al., 2018). Among several types of extraction, in this study, solid–liquid extraction was explored. In this case, solvent polarity has a major impact on the selectivity of the solutes extracted, which is related to the chemical structure of the compounds of interest. Modeling compound/solvent interactions is a challenge to favor the choice of the appropriate extracting liquid (Lefebvre et al., 2021).

Polar solvents such as aqueous mixtures containing methanol, ethanol, acetone and ethyl acetate are frequently used for recovering polyphenols from agro-industrial wastes (Do et al., 2014; Fierascu et al., 2019; Lohvina et al., 2022). In particular, methanol has been found to be more efficient in the extraction of sugars, organic acids and phenolic compounds with low molecular weight, while flavanols with higher molecular weight are better extracted with aqueous acetone solutions (Altemimi et al., 2017; Dai and Mumper, 2010; Salih et al., 2021).

The different polarities of each solvent applied to banana bracts support the results observed in our study. For most runs, the combination of the solvents (water, methanol and acetone), particularly in binary and ternary mixtures, resulted in extracts with higher recovery of bioactive compounds and, consequently, in extracts with high antioxidant activity. These results indicated that the antioxidant compounds of banana bracts (Maçã, Nanica and Prata varieties) have good affinity for these solvents. Moreover, it is worth noting that the mixtures with water resulted in increased extraction of compounds.

According to the literature, the combination of water and organic solvents creates a more polar condition, which can facilitate the extraction of soluble phenolic compounds (Meneses et al., 2013; Socaci et al., 2018). However, the efficiency of pure water as an extraction solvent is reduced because phenolic compounds exhibit a wide range of solubilities and are often more soluble in solvents less polar than water (Socaci et al., 2018; Zuorro et al., 2019).

Findings with regard to the efficiency of other solvents have also been reported in the literature concerning banana inflorescence. Extracts obtained from the buds and bracts of Mysore banana (Musa paradisiaca cv. Mysore) using several solvents (petroleum ether, hexane, chloroform, ethyl acetate, acetone, isopropanol, ethanol, methanol and water) were investigated for their antioxidant properties. In general, extracts produced with more polar solvents showed the highest values of antioxidant activity. Methanolic extracts of buds showed the most

prominent results, reaching 77.8% DPPH-radical scavenging, 67.2% lipid peroxidation inhibition and TEAC, FRAP and TPC values of 137.71 μmol TE g^{-1}, 2114.70 μmol Fe^{2+} g^{-1} and 122.03 mg GAE g^{-1}, respectively. The extracts produced with the bracts followed a similar trend but with lower values of antioxidant activity and content of phenolic compounds (Padam et al., 2012).

The highest efficiency of the water: acetone combination in compound extraction was also found by other authors. Extracts from brewer's spent grain were investigated in relation to bioactive compounds and their antioxidant, antimicrobial and antimutagenic activities. The most efficient solvent was the combination of acetone and water (60/40, v/v), which resulted in extracts with 120 mg GAE/100 g of total phenolic compounds, 50 mg of quercetin equivalent (QE)/100 g of total flavonoids, 1.2 μM TE/100 g of ABTS and 40% inhibition of DPPH radicals. All extracts showed antimicrobial activity, and hexane extracts, with low phenolic content and low antioxidant activity, showed similar or higher antimicrobial and antimutagenic properties (Socaci et al., 2018).

Extracts from apple pomace of the Gala variety *(Malus domestica*) using acetone, ethanol and methanol were studied for their phenolic compounds with antibacterial activity. The results showed that the extraction of isolated phenolic compounds was increased when 53.5% acetone was used as the solvent in relation to the mixture of tested variables. They also demonstrated that ethanol and acetone had nonsignificant differences in phenolic compound values, and extracts obtained with acetone as the solvent showed the maximum phenolic recovery. The optimization process resulted in the recovery of 9.86 g of chlorogenic acid equivalents (CAE) kg^{-1} of phenolic compounds using the following parameters: particle size of 30 mesh, ratio pomace/solvent 1:80 w/v and solvent concentration (acetone) of 53.50% v/v. The extract obtained with acetone also presented the highest percentage of inhibition for all the evaluated strains (Zardo et al., 2021).

The correlation analysis performed to verify the interdependence between the content of phenolic compounds (total, condensed tannins and flavonoids) and the antioxidant activities (ABTS, DPPH and FRAP) is displayed in Table 4. In most cases, the results showed that the significant ($p < 0.05$) and positive correlations ranged from moderate to strong (Pearson coefficient ranging from 0.63 to 0.97), which reinforces the importance of recovering phenolic compounds in greater amounts to obtain extracts with high antioxidant activity.

Table 4. Correlation analysis between the bioactive compounds and antioxidant activity responses for the banana bract extracts obtained using different solvents and their mixtures.

Parameters	Banana bract variety					
	Maçã		Nanica		Prata	
	Pearson coefficient	p-value*	Pearson coefficient	p-value*	Pearson coefficient	p-value*
TPC vs. ABTS	0.90	<0.001	0.44	0.201	0.75	0.012
TPC vs. DPPH	0.89	<0.001	0.50	0.138	0.70	0.025
TPC vs. FRAP	0.96	<0.001	0.54	0.106	0.74	0.014
Flavonoids vs. ABTS	0.05	0.894	0.89	<0.001	0.76	0.011
Flavonoids vs. DPPH	0.07	0.857	0.91	<0.001	0.82	0.004
Flavonoids vs. FRAP	0.00	0.628	0.97	<0.001	0.83	0.003
Condensed tannins vs. ABTS	0.65	0.040	0.83	0.003	0.66	0.037
Condensed tannins vs. DPPH	0.71	0.022	0.88	0.001	0.69	0.026
Condensed tannins vs. FRAP	0.63	0.049	0.89	0.001	0.69	0.026

* Significant at $p \leq 0.05$.

The results obtained in our study are in agreement with those reported by Chen et al., (2017), Tan et al., (2017) and Vijayalaxmi et al., (2015), in which a strong correlation between antioxidant activity and TPC was observed, suggesting that phenolics could be the major contributor to their antioxidant properties. It is worth noting that the antioxidant activity of each phenolic compound mainly depends on the number of aromatic and hydroxyl groups and the positioning of these groups (Sumczynski et al., 2017). However, there is no information in the literature concerning the contribution of isolated phenolic compounds to their overall antioxidant activity in banana inflorescence. Thus, correlation analysis was performed to determine the major compounds contributing to the antioxidant activity of the banana bract extracts (Table 4).

3.2 Identification of phenolic compounds by HPLC

The identification of phenolic compounds was performed using the banana bract extract obtained with the binary mixture of water: acetone in equal proportions (run 5) for the Maçã and Prata varieties, and the ternary mixture of water (1/6), methanol (2/3) and acetone (1/6) (run 9) was used for the Nanica variety. Rutin was the main phenolic compound identified in all analyzed varieties, while coumaric acid was detected only in extracts from the Nanica variety (Table 5).

Table 5. Quantification of phenolic compounds from banana bracts by HPLC.

Banana bract extract (variety)				Validation parameters		
Concentration (μg g^{-1} sample)						
Compound	**Maçã**	**Nanica**	**Prata**	**LOD (μg)[2]**	**LOQ (μg)[3]**	**Linearity (R^2)**
Coumaric acid	n.d.[1]	124.84 ± 0.97	n.d.[1]	0.0023	0.0070	0.9994
Rutin	3049.19 ± 0.12 [a]	623.47 ± 0.01 [c]	2370.24 ± 0.01 [b]	0.0014	0.0042	0.9745

Values were expressed as the mean (triplicate) ± standard deviation.

Different lowercase letters in the same line indicate statistical difference ($p < 0.05$) between the results by Tukey test.

[1] n.d: not detected

[2] LOD: detection limit

[3] LOQ: limit of quantification

Considering the relationship between extraction capacity and the composition of the solvents applied, it is worth noting the high efficiency of recovery of coumaric acid, a low molecular weight compound, by methanol, present in the extracting solvent mixture used for the Nanica variety. On the other hand, the presence of acetone in Maçã and Prata extracts favored the recovery of rutin, a flavonoid with high molecular weight. In addition, it is necessary to consider the amounts of these compounds naturally present in each banana variety, which can vary considerably regardless of the extracting solution used.

Studies on the phenolic profile of banana inflorescence were reported in 2012 (Bhaskar et al., 2012) for the first time, with few publications until now, revealing possibilities that have not yet been studied.

Bound phenolics from the hemicellulose A and B fractions of banana inflorescence and pseudostem (*Musa* sp. cv. Elakki bale) were investigated. Phenolics were extracted with 70% ethanol and hexane, and the residue was extracted with NaOH. The polyphenol profile identified by HPLC showed that the major phenolic acids in extracts were gallic acid, epicatechin, syringic acid and ρ-coumaric acid (Bhaskar et al., 2012).

The polyphenol profile and quantification of dried banana inflorescence extracts (Nendran variety) were reported by Arun et al. (2017). The HPLC analysis showed a total of 10 phenolics in the ethyl acetate extract from banana inflorescence, with the major compounds identified as cinnamic acid, catechol, myricetin and ellagic acid. In the methanolic extract, 12 phenolics were identified, among which gallic acid, catechol, syringic acid, chlorogenic acid and ρ-coumaric acid were the predominant (Arun et al., 2017). In this study, methanol was the most adequate solvent to recover antioxidant compounds.

The phenolic composition of dried banana inflorescence (*Musa paradisiaca*) from Thailand was identified by LC–MS, and 6 compounds were detected: gallic acid, quercetin, rutin, tannic acid and the major phenolics were catechin and isoquercetin (Amornlerdpison et al., 2021). Another study reported the polyphenol profile of banana inflorescence using HPLC and ESI-MS analysis and showed the presence of gallic acid (4.49% w/w) and quercetin (1.13% w/w) (Nisha and Mini, 2013).

Baxijiao, a variety of banana inflorescences from China, was studied to determine their phytochemical profile using NMR spectroscopy analysis. Several compounds were identified, such as vanillic and ferulic acid (organic acids), β-sitosterol, daucosterol (sterols) and 9-(4′-hydroxyphenyl)-2-methoxyphenalen-1-one (phenylphenalenone) (Sheng et al., 2014). The polyphenol profiles of the outer and inner bracts of banana inflorescence were identified by HPLC and showed the presence of 6 free phenolics in the outer bracts (ρ-coumaric acid, salicylic acid, quercetin, quinic acid, ferulic acid, vanillic acid) and 8 free phenolics in the inner bracts (quinic acid, chlorogenic, salicylic, ρ-coumaric, ferulic, catechin, quercetin, syringic). In addition, significant amounts of bound phenolics ferulic and salicylic acid were the major ones found in outer and inner bracts (Begum and Deka, 2019).

It is interesting to note that the two major compounds detected in banana bract extracts in our study, namely, ρ-coumaric acid and rutin, have already been reported to have several types of positive effects on the health of those who consume them, especially related to their antioxidant properties. These phenolics prevent oxidative stress-related disorders due to their high effectiveness as reducing agents and scavenging free radicals (Chen et al., 2017; Shahidi et al., 2019).

ρ-Coumaric acid (4-hydroxycinnamic acid), particularly, is a phenolic acid found ubiquitous in plants and mushrooms in free form (at low concentrations) and in bound (conjugated) forms (at high concentrations) (Shen et al., 2019). Investigations have shown that conjugates of coumaric acid exhibit various bioactivities, including antioxidant, anti-inflammatory, antimutagenic, anti-ulcer, antiplatelet and antitumor activities, which greatly increase after the formation of conjugates (Pei et al., 2016). The antioxidant activity of coumaric acid can be attributed to the presence of a phenolic hydroxyl in its structure (Liu et al., 2020; Shen et al., 2019).

Rutin, a flavonol glycoside, has a wide range of biological activities, including anti-inflammatory, antimicrobial and anticancer activities, mainly attributed to its antioxidant properties as a free radical scavenger (Gullón et al., 2017). The antioxidant activity of rutin is

due to the presence of phenolic rings and free hydroxyl groups in its chemical structure. These free hydroxyl groups can donate hydrogen atoms to prevent further oxidation (Chua, 2013). It has been shown that rutin counteracts some flavonoids that exert prooxidant activities by catalyzing oxygen radical generation (Negahdari et al., 2021).

Phytochemical studies dedicated to the isolation and elucidation of the structures of compounds in banana inflorescence are scarce when compared to other parts of the banana plant. This identification will be necessary to assess the correlation between the chemical structure and the antioxidant activity of the compounds, which have relevant potential to be incorporated into food and/or nutraceuticals.

These results observed in the literature showed possibilities not yet studied. Regarding conventional extraction, it is necessary to investigate the chemical nature of the extraction solvent, as this choice will govern extraction recovery and extraction selectivity as well as different methods and their parameters to examine the role of each solvent. Considering the diversity of banana variety compositions, studies concerning solvent extraction of bioactive compounds are extremely relevant.

4. Conclusion

The extraction of antioxidant compounds from Maçã and Prata extracts showed that the combination of water and acetone, in equal proportions, was the best solvent, while the ternary mixture of water (1/6), methanol (2/3) and acetone (1/6) was efficient for the recovery of compounds from Nanica extracts. Under these conditions, rutin was identified in all analyzed varieties, and ρ-coumaric acid was found in the Nanica variety. These results can support future research, since they contribute information about the bioactive potential of bracts of three banana varieties highly consumed in Brazil. These findings emphasize the need to utilize agricultural banana waste, a quite abundant resource with a rich source of bioactive compounds.

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CAPÍTULO III: Bioactive compounds from banana bracts: a study based on the optimization of extraction parameters, antioxidant properties and identification of phenolics

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Abstract

Banana is one of the most important and highly nutritional fruit crops cultivated around the world, mainly in Brazil, which is one of the five largest producers. Due to the large production, it is evident that the banana agroindustry generates large quantities of waste, such as bracts. In our study, we aimed to evaluate the influence of different extraction variables (temperature, agitation and solid-to-solvent ratio) on the recovery of bioactive compounds from two varieties (Maçã and Prata) of banana *(Musa acuminata)* bracts. The extraction parameters defined for all banana bract varieties to increase their antioxidant properties were: incubation temperature of 37.5°C, agitation of 100 rpm and solid-to-solvent ratio of 0.50% m/v (run 13). At these parameters, the highlights were: the highest total phenolic compound (35.30 mg GAE g^{-1}), DPPH (93.72 μmol TE g^{-1}) and ferric reducing power (FRAP) (149.68 μmol TE g^{-1}) was detected for the extracts obtained from the Maçã variety, while, ABTS (554.00 μmol TE g^{-1}) were the best result observed in Prata variety extracts. Furthermore, the bioactive compounds identified and quantitated by HPLC in banana bract extracts included rutin in all varieties. This study reported for the first time the extraction parameters for the recovery of phenolic compounds from two relevant Brazilian varieties of banana bracts. It could be considered a potential raw material for the extraction of bioactive compounds for food applications.

Keywords: Banana flower, agricultural waste, conventional extraction, phenolic compounds.

1. Introduction

Banana has been considered a “superfruit” due to its rich source of important phytonutrients, including vitamins, phenolics and several minerals (Singh et al., 2016). It is one of the most popular fruits and the fifth most important agricultural food crop in terms of world trade (Singh et al., 2016; Vu et al., 2018). A high growth rate of world banana production and consumption leads to a huge amount of valuable byproducts, including inflorescences, peel, leaves, pseudostems and stalks (Padam et al., 2014).

Bracts, a part of banana inflorescence, also known as flower or blossom, are a dark purple-red heart-shaped structure located at the end of the peduncle of plantain (Lau et al., 2020). This structure is disposed of during banana harvesting or is left in the plantations used as a soil cover, and farmers discard wastes into rivers or on roadsides, causing serious environmental problems (Chai et al., 2018; Schmidt et al., 2015). This is a common problem that involves agricultural byproducts and wastes, which are commonly discarded, without proper treatment. Although, there has been a growing interest in converting wastes into value-added compounds with high bioactivity (Faustino et al., 2019; Lau et al., 2020).

Byproducts and wastes from agroindustrial activities are being increasingly recognized due to their source of bioactive compounds, mainly phenolics, which are associated with antioxidant activities that are effective in protecting the human body against various oxidative stresses (Shahidi et al., 2019; Taamalli et al., 2019). The recovery of these compounds from agricultural wastes is a sustainable and great strategy to reduce environmental pollution in the short and long term (Nguyen, 2017). Banana bract, for instance, has a variety of phenolics with a broad range of bioactivities, including antioxidant properties and antidiabetic and cardiovascular-protective activities (Arun et al., 2018; Ramu et al., 2017; Sheng et al., 2014). Thus, to utilize bound phenolic compounds, methods to release and extract the phenolics from agricultural wastes must be devised (Acosta-Estrada et al., 2014). For this, extraction represents one of the most relevant stages to obtain extracts rich in bioactive compounds, exerting a significant and crucial role in the final result. In addition, selection of the proper extraction method is essential to maximize the yield from food matrices (Azmir et al., 2013; Jha and Sit, 2021). However, efficient extraction is governed by other parameters, including the plant component matrix, sample particle size, solid-to-solvent ratio, extraction solvent, temperature, pressure, and time (Alara et al., 2021). It is important to select the factors that have the greatest impact on the food matrix and methods and to select the optimum conditions for these parameters while also studying their interactions (Hassan et al., 2021). Therefore, the aim of

this study was to evaluate the effect of process parameters (temperature, agitation and solid-to-solvent ratio) on the recovery of compounds with antioxidant properties from banana bracts.

2. Material and Methods

2.1 Material and reagents

Bracts of banana *(Musa paradisiaca)* Prata and Maçã varieties were collected from cultivated local farmland (20°33'56.5" south latitude, 46°05'21.1" west longitude) in Capitólio, state of Minas Gerais, Brazil. ABTS ([2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)]), DPPH (2,2-diphenyl-1-picrylhydrazyl), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), TPTZ (2,4,6-tripyridyl-s-triazine), Folin & Ciocalteau's phenol reagent, galic acid, catechin, and vanillin were acquired from Sigma–Aldrich (São Paulo, Brazil). All other chemicals were acquired in commercially grade.

2.2 Experimental design and obtaining banana bract extracts

A Central Composite Rotatable Design (CCRD) with eight factorial points, three replicates at the central point and six axial points (17 runs) was applied to study the recovery of antioxidant compounds from banana bracts. The three independent variables used in this study were temperature (°C) (x_1), agitation (rpm) (x_2) and solid-to-solvent ratio (%) (x_3), while the dependent variables were the total phenolic compound content and the antioxidant properties evaluated by ABTS, DPPH and FRAP assays.

A second-order model equation was used to define the mathematical models, as represented below:

$$\boldsymbol{Y} = \beta_0 + \sum_{\boldsymbol{i=1}}^{\boldsymbol{n}} \boldsymbol{\beta_i X_i} + \sum_{\boldsymbol{i=1}}^{\boldsymbol{n-1}} \sum_{\boldsymbol{j=i+1}}^{\boldsymbol{n}} \boldsymbol{\beta_{ij} X_i X_j} \quad (1)$$

where *Y* is the estimated response, *i* and *j* have values from 1 to the number of variables (n), β_0 is the intercept term, β_i is the linear coefficient, β_{ij} is the quadratic coefficient, and *xi* and *xj* are the coded independent variables. To evaluate the quality of fit of the experimental design, the followed determinations were considered: i) the significance ($p \leq 0.10$) of the regression coefficients was obtained using Student's t test, ii) the coefficient of determination (R^2) was considered acceptable when greater than or equal to 75% ($R^2 \geq 0.75$) and iii) the Fisher's test (analysis of variance-ANOVA) was used to verify the significance of the second-order model equations at 95% confidence level ($p \leq 0.05$). The software Statistica version 13.3 from TIBCO Software Inc. (Palo Alto, California, USA) was used to generate the experimental design and

to statistical analysis. The recovery of compounds was carried out under the defined conditions for each run of the CCRD (Table 1). Banana bracts were removed from its inflorescence, cleaned, ground, frozen, freeze-dried and stored in vacuum packs at -18°C. To obtain the extracts, Maçã and Prata bracts were mixed with 20 mL of solvent (water and acetone in equal proportions). These solvent combinations were chosen based on previous studies performed by our research group. After this, the samples were stirred for 1 h in a thermostatic water bath (Fanem Model 145, São Paulo, Brazil), and the mixtures were filtered through a filter membrane (Whatman® qualitative filter paper nº1) resulting in an extract free of insoluble particles. The filtered substance was collected and stored at -18°C for further analysis.

Table 1. Matrix of the CCRD used to study the effects of the independent variables temperature, agitation and solid-to-solvent ratio on the antioxidant properties of the banana bract extracts.

Run	Independent variables					
	Coded variables			Real variables		
	x_1	x_2	x_3	Temperature (°C)	Agitation (rpm)	Solid-to-Solvent ratio (%) (mg mL^{-1})
1	-1	-1	-1	30	70	1 (10)
2	1	-1	-1	45	70	1 (10)
3	-1	1	-1	30	130	1 (10)
4	1	1	-1	45	130	1 (10)
5	-1	-1	1	30	70	2.50 (25)
6	1	-1	1	45	70	2.50 (25)
7	-1	1	1	30	130	2.50 (25)
8	1	1	1	45	130	2.50 (25)
9	-1.68	0	0	25	100	1.75 (17.5)
10	1.68	0	0	50	100	1.75 (17.5)
11	0	-1.68	0	37.5	50	1.75 (17.5)
12	0	1.68	0	37.5	150	1.75 (17.5)
13	0	0	-1.68	37.5	100	0.50 (5)
14	0	0	1.68	37.5	100	3.00 (30)
15	0	0	0	37.5	100	1.75 (17.5)
16	0	0	0	37.5	100	1.75 (17.5)
17	0	0	0	37.5	100	1.75 (17.5)

2.3 Total phenolic compounds (TPC)

The total phenolic compounds were executed according to the method described by Magro and de Castro, (2020). Aliquots of 25 µL extracts, 25 µL of Folin-Ciocalteau solution (50% v/v) and 200 µL of sodium carbonate (5% w/v) were mixed and then, incubated at 40°C in the dark for 20 min. The absorbance was measured at 760 nm on a spectrophotometer (Multiskan GO, Thermo Fisher Scientific, Finland). For the calibration curve, gallic acid was used with a concentration ranging from 0 to 10 mg mL^{-1} and the results are expressed as mg of gallic acid equivalent per gram of freeze-dried sample (mg GAE g^{-1}).

2.4 Measurement of the antioxidant properties

2.4.1 ABTS-radical cation scavenging activity

The ABTS assay was performed as described by Neta and de Castro, (2020). The ABTS aqueous solution (7 mmol L^{-1}) and potassium persulfate (140 mmol L^{-1}) were mixed and maintained for 16 h at room temperature and in the dark to generate de free radical. After this incubation period, the absorbance was adjusted to 0.70 ± 0.02 with deionized water. Aliquots of 20 µL of extracts were mixed with 220 µL of ABTS solution, and the absorbance measurements were determined after 6 min of reaction at 734 nm using a spectrophotometer (Multiskan GO, Thermo Fisher Scientific, Finland). Trolox was used for the calibration curve (0 – 400 µmol L^{-1}) and the results were expressed as µmol of Trolox equivalents per g of freeze-dried sample (µmol TE g^{-1}).

2.4.2 DPPH-radical scavenging activity

The DPPH assay was performed according to the method described by Rasera et al., (2019). Aliquots of 134 µL of DPPH ethanolic solution (150 µmol L^{-1}) were added to 66 µL of extracts (25 mg mL^{-1}) or standard (Trolox). After 45 min of the reaction at room temperature, the absorbance was measured at 517 nm using a spectrophotometer (Multiskan GO, Thermo Fisher Scientific, Finland). Trolox was used as the standard with a calibration curve ranging from 0 to 125 µmol L^{-1}. The results were expressed as µmol TE g^{-1}.

2.4.3 Ferric Reducing Antioxidant Power (FRAP) assay

The FRAP assay was executed in accordance with the method reported by Firuzi et al., (2005) with some modifications proposed by Aguilar et al., (2018). The FRAP solution was composed of 300 mmol L^{-1} acetate buffer (pH 3.6), 20 mmol L^{-1} ferric chloride hexahydrate dissolved in distilled water and 10 mmol L^{-1} TPTZ (2,4,6-tris(2-pyridyl)-s-triazine) diluted in

40 mmol L^{-1} HCl (10:1:1, v/v/v). Aliquots of 25 µL of banana bract extracts (25 mg mL^{-1}) and 175 µL of freshly prepared FRAP solution were mixed and absorbance measurements were obtained after 30 min of reaction at 595 nm using a spectrophotometer (Multiskan GO, Thermo Fisher Scientific, Finland). Trolox (0 - 250 µmol L^{-1}) was used as the standard, and the results were expressed as µmol TE g^{-1}.

2.5 Identification of phenolic compounds by High Performance Liquid Chromatography (HPLC)

Phenolic compounds identification was performed according to the method described by Silva et al., (2021) with slight modifications. The separation of compounds by HPLC was performed using a Shimadzu ODS-A column (4.6 mm, 250 mm, 5 µm) in a thermostated oven at a constant temperature of 30ºC, an injection volume of 20 µL and a photodiode array detector (SPD-M10AVp, Shimadzu Co., Kyoto, Japan). The mobile phase consisted of (eluent A) water/formic acid (99.75/0.25, v/v) and (eluent B) acetonitrile/formic acid/water (80/0.25/19.75, v/v) at a flow rate of 1.0 mL min^{-1}. The gradient conditions were as follows: 10% (B), increased to 20% (B) at 10 min, 30% (B) at 20 min, 100% (B) at 30 min and 10% (B) at 35 min. The analysis was completed in 40 min and the chromatograms were analyzed with Class-VP® software. Each compound peak was identified based on a comparison of the retention time, spectral data and peak area. Phenolic acid standards (galic acid, 3,4-dihydroxybenzoic acid, vanillic acid, caffeic acid, coumaric acid, ferulic acid, and sinapic acid) and flavonoids (rutin and quercetin) were used to quantify the individual compounds. The standard curves (0.03 – 0.20 µg mL^{-1} concentrations) were determined using the chromatographic parameters previously mentioned. The limit of detection (LOD) and limit of quantification (LOQ) (Equations 2 and 3) were calculated as follows:

$$LOD = 3.3 \times s \div S \quad (2)$$

$$LOQ = 10 \times s \div S \quad (3)$$

where *'s'* is the standard deviation of the linear coefficient of the equation and *'S'* is the slope of the standard curve.

2.6 Calculations and statistics

The results were reported as the mean values ± standard deviation (n = 3) and Minitab software, version 19 (Minitab Inc., USA) was used to verify whether there was a significant difference (p-value $\leq$ 0.05) between the means when calculated by analysis of variance (ANOVA) followed by Tukey´s test.

3. Results and discussion

3.1 Effects of process parameters on the recovery of antioxidant compounds

The results of the experimental design (CCRD) for the extraction of bioactive compounds from banana bract extracts under different conditions of temperature, agitation and solid-to-solvent ratio are displayed in Table 2.

The highest content of bioactive compounds was detected for the extracts obtained from the Maçã variety (run 13), in which the TPC reached 35.30 mg GAE g^{-1}. For antioxidant properties, the highlights were the extracts obtained from bracts of the Prata variety, which reached 554.00 μmol TE g^{-1} for ABTS (run 13), while the extract from the Maçã variety showed the greatest ability to inhibit DPPH radicals (93.72 μmol TE g^{-1}, run 13) and ferric reducing power (149.68 μmol TE g^{-1} for FRAP, run 13). Thus, the parameters investigated with an incubation temperature of 37.5°C, agitation of 100 rpm and solid-to-solvent ratio of 0.50% (run 13) was selected to find the best extraction parameters to obtain extracts with greater antioxidant properties in all banana bract varieties.

The results showed that the independent variables (temperature, agitation and solid-to-solvent ratio) had distinct effects on the extraction process, with great variation in the antioxidant activities in relation to each condition. This variation may be attributed to some factors: (1) the chemical nature of recovered phenolic compounds, (2) the composition naturally present in each variety of banana inflorescence can vary considerably, and (3) the antioxidant methods differ from each other in terms of reaction mechanisms (Gulcin, 2020; Lau et al., 2020; Silva et al., 2021). In addition to these factors, it is important to discuss the possible phenomena involved in the application of the different extraction parameters that were evaluated by the CCRD. The analysis of the independent variable effects and their interactions on the TPC and antioxidant properties evaluated in the CCRD are shown in Table 3. This verification allows us to determine whether the parameters investigated had a significant or non-significant effect, which may be positive or negative. Regression coefficients that exhibit the magnitude and significance of variables are used to construct equations and predict experimental responses at different conditions (Oliveira and de Castro, 2020).

Table 2. TPC and antioxidant activities (ABTS, DPPH and FRAP assays) in banana bract extracts.

Run	TPC (mg GAE g^{-1})	ABTS (µmol TE g^{-1})	DPPH (µmol TE g^{-1})	FRAP (µmol TE g^{-1})
		Maçã variety		
1	13.63 ± 4.01 ^{efghi}	105.31 ± 3.12 ^{b}	47.89 ± 3.76 ^{cd}	96.74 ± 4.58 ^{c}
2	22.67 ± 0.70 ^{b}	130.82 ± 4.66 ^{a}	75.08 ± 1.78 ^{b}	113.46 ± 0.77 ^{b}
3	17.23 ± 0.12 ^{cde}	82.74 ± 5.34 ^{bc}	47.05 ± 2.18 ^{cde}	88.76 ± 2.84 ^{d}
4	18.83 ± 1.67 ^{bc}	112.08 ± 5.29 ^{b}	70.41 ± 4.82 ^{b}	116.49 ± 1.91 ^{b}
5	9.38 ± 1.74 ^{i}	55.87 ± 1.06 ^{de}	23.53 ± 2.68 ^{hi}	53.72 ± 0.76 ^{g}
6	14.47 ± 0.42 ^{cdefg}	69.81 ± 8.19 ^{cd}	39.83 ± 1.03 ^{ef}	77.71 ± 0.80 ^{e}
7	10.96 ± 0.42 ^{ghi}	54.68 ± 1.66 ^{e}	26.86 ± 1.04 ^{ghi}	54.87 ± 2.40 ^{g}
8	12.76 ± 1.19 ^{fghi}	86.42 ± 1.25 ^{bc}	41.60 ± 2.84 ^{def}	78.72 ± 2.25 ^{e}
9	12.71 ± 0.01 ^{fghi}	59.47 ± 1.93 ^{de}	33.91 ± 0.78 ^{fg}	87.31 ± 1.22 ^{d}
10	18.07 ± 1.05 ^{cd}	89.66 ± 8.61 ^{bc}	50.28 ± 2.45 ^{c}	69.07 ± 0.09 ^{f}
11	16.00 ± 0.28 ^{cdef}	97.36 ± 6.18 ^{bc}	54.02 ± 3.09 ^{c}	91.34 ± 1.01 ^{cd}
12	18.12 ± 1.18 ^{cd}	89.43 ± 4.25 ^{bc}	49.92 ± 0.84 ^{c}	79.67 ± 1.12 ^{e}
13	35.30 ± 0.79 ^{a}	94.25 ± 8.21 ^{bc}	93.72 ± 1.85 ^{a}	149.68 ± 1.98 ^{a}
14	10.02 ± 0.48 ^{hi}	45.78 ± 4.93 ^{e}	20.17 ± 2.31 ^{i}	45.90 ± 0.10 ^{h}
15	14.20 ± 0.58 ^{defgh}	55.71 ± 6.07 ^{de}	34.23 ± 4.76 ^{fg}	63.95 ± 1.29 ^{f}
16	9.47 ± 4.36 ^{i}	55.68 ± 2.93 ^{de}	29.74 ± 0.84 ^{gh}	63.62 ± 0.92 ^{f}
17	12.28 ± 0.40 ^{fghi}	39.25 ± 8.14 ^{e}	29.82 ± 0.84 ^{gh}	54.76 ± 1.89 ^{g}
		Prata variety		
1	8.02 ± 0.49 ^{efg}	319.30 ± 9.18 ^{b}	62.15 ± 3.30 ^{b}	89.91 ± 6.74 ^{bc}
2	21.03 ± 3.44 ^{a}	308.89 ± 8.19 ^{b}	68.50 ± 8.00 ^{ab}	101.61 ± 1.82 ^{ab}
3	16.92 ± 3.28 ^{ab}	314.32 ± 4.19 ^{b}	62.67 ± 0.73 ^{b}	88.31 ± 8.12 ^{bc}
4	20.46 ± 1.75 ^{a}	305.80 ± 5.64 ^{b}	48.64 ± 2.02 ^{d}	78.62 ± 6.14 ^{cdef}
5	9.26 ± 0.53 ^{cdefg}	156.18 ± 1.78 ^{d}	44.66 ± 1.06 ^{de}	76.09 ± 0.82 ^{cdef}
6	13.59 ± 2.25 ^{bcd}	162.62 ± 0.79 ^{d}	45.74 ± 2.81 ^{de}	79.15 ± 3.56 ^{cdef}
7	11.31 ± 0.05 ^{cdef}	154.30 ± 3.89 ^{d}	38.25 ± 1.31 ^{ef}	65.52 ± 3.23 ^{fgh}
8	14.14 ± 0.31 ^{bc}	157.74 ± 0.39 ^{d}	37.14 ± 1.02 ^{ef}	60.87 ± 2.72 ^{gh}
9	7.45 ± 0.15 ^{fg}	209.11 ± 1.37 ^{c}	49.62 ± 0.94 ^{d}	79.76 ± 1.36 ^{cde}
10	13.63 ± 0.58 ^{bcd}	216.29 ± 3.50 ^{c}	59.60 ± 4.25 ^{bc}	87.39 ± 4.12 ^{cd}
11	13.33 ± 1.48 ^{bcde}	218.10 ± 2.15 ^{c}	52.50 ± 2.10 ^{cd}	87.97 ± 6.00 ^{bcd}
12	20.48 ± 0.47 ^{a}	210.98 ± 0.99 ^{c}	44.86 ± 3.12 ^{de}	76.80 ± 1.76 ^{cdef}
13	5.22 ± 0.05 ^{g}	554.00 ± 13.78 ^{a}	71.88 ± 4.04 ^{a}	107.84 ± 1.74 ^{a}
14	10.26 ± 0.55 ^{cdefg}	133.78 ± 1.66 ^{e}	35.13 ± 2.17 ^{f}	55.43 ± 2.72 ^{h}
15	10.56 ± 1.40 ^{cdefg}	211.73 ± 1.50 ^{c}	47.57 ± 0.19 ^{d}	73.83 ± 0.65 ^{defg}
16	8.36 ± 0.56 ^{defg}	204.59 ± 1.27 ^{c}	44.82 ± 1.08 ^{de}	67.86 ± 0.33 ^{efgh}
17	11.89 ± 0.13 ^{bcdef}	212.46 ± 5.08 ^{c}	46.01 ± 1.56 ^{de}	76.27 ± 1.11 ^{cdef}

Values were expressed as the mean (triplicate) ± standard deviation. Different lowercase letters in the same column indicate statistical difference ($p < 0.05$) between the results for each banana variety evaluated in the same antioxidant method by Tukey test.

The estimated effects were analyzed according to each variety; the effects obtained in TPC (Maçã variety) did not show statistical significance ($p \geq 0.10$); therefore, they were not presented.

According to CCRD analysis for the Maçã variety, which are inferred in the equations (Table 3), the estimated regression coefficients demonstrated positive and significant effects ($p \leq 0.10$) of temperature and agitation which represents that the increase of this parameters resulted in a greater recovery of compounds. In addition, the solid-to-solvent ratio showed a linear term with a negative effect and the quadratic a positive effect, which means that the

increase in this variable is negative up to a certain point and then becomes a positive, increasing the extraction of antioxidant compounds.

For Prata variety (Table 3), generally, the temperature had a positive and significant effect ($p \leq 0.10$) on the recovery of antioxidant compounds, while the agitation and solid-to-solvent ratio had negative effects, indicating that as these variables increased, the extracts produced had lower antioxidant properties. In general, the parameters that most affected our results were temperature and solid-to-solvent ratio.

Previous studies have also investigated the effect of different extraction parameters on the recovery of phenolic compounds and antioxidant activity from plant matrices. The antioxidant properties of *Limonium sinuatum* (an edible flower) extracts using ultrasound-assisted extraction (UAE) were compared with solvent extraction (also namely 'conventional method') and Soxhlet. The selected conditions were: temperature of 40ºC (30 – 80ºC, range studied), ethanol concentration 60%, ultrasonication time of 9.8 min and ratio of solvent to solid, 56.9:1 mL g^{-1}. With these selected parameters, the flower extracts showed TEAC value of 483.01 μmol TE g^{-1}. A declined in responses was observed when the temperature was higher than 40ºC, indicating a degradation of compounds (Xu et al., 2017).

The extraction conditions of phenolics in dates seeds (*Phoenix dactylifera L.*) were evaluated and their optimal conditions were: extraction at 45ºC (25ºC - 65ºC, range studied) and solvent-to-solid ratio of 60:1 with acetone/water (in equal proportions). Under the optimal parameters, date seeds extracts showed 100 mg g^{-1} of total phenolics. In addition, it was observed that above 45ºC occurred a decrease efficiency on extraction of phenolic compounds. In this study authors also concluded that with the increase in temperature occurred an increase in diffusion coefficient and the solubility of the system. However, the limiting factor was the acetone aqueous solution, with boiling point of 56.2ºC, under these circumstances, acetone evaporation could change the solution ratio (Al-Farsi and Lee, 2008).

Table 3. Analysis of variance (ANOVA), including models, R^2 and probability values for the final reduced models for TPC and antioxidant activity (ABTS, DPPH and FRAP) from the extracts of banana bracts (Maçã and Prata varieties).

Responses	Model	Equations	F calculated	F tabulated	R^2	p-value
Maçã variety						
TPC	-	Statistically invalid model	-	-	0.30	-
ABTS	Cubic	$Y = 49.80 + 11.08\ x_1 + 9.98\ x_1^2 + 16.65\ x_2^2 - 17.99\ x_3 + 8.36\ x_3^2 + 7.09\ x_2x_3$	28.62	2.46	0.93	<0.001
DPPH	Quadratic	$Y = 31.60 + 7.99\ x_1 + 6.22\ x_2^2 - 17.01\ x_3 + 7.98\ x_3^2$	30.46	2.39	0.90	<0.001
FRAP	Quadratic	$Y = 61.03 + 7.92\ x_2^2 - 23.80\ x_3 + 12.27\ x_3^2$	18.64	2.56	0.80	<0.001
Prata variety						
TPC	Quadratic	$Y = 10.10 + 2.50\ x_1 + 2.91\ x_2^2$	7.75	2.73	0.75	0.006
ABTS	Quadratic	$Y = 211.21 - 96.96\ x_3 + 41.92\ x_3^2$	106.47	2.73	0.94	<0.001
DPPH	Cubic	$Y = 46.92 + 0.66\ x_1 + 2.47\ x_1^2 - 3.45\ x_2 - 10.10\ x_3 - 2.82\ x_1x_2$	21.18	2.46	0.93	<0.001
FRAP	Linear	$Y = 79.60 - 5.29\ x_2 - 12.08\ x_3$	29.84	2.73	0.80	<0.001

The coded values in model equations represent the independent variables and their interactions: x_1 = temperature; x_2 = agitation and x_3 = solid-to-solvent ratio (%).

The effects of process parameters time, temperature, solid-liquid ratio and ethanol concentration (and their interactions) was investigated for the extraction of phenolic compounds and antioxidant properties of *Eleutherine bulbosa* bulb (herbaceous plant). Authors reported that the optimal conditions were: temperature of 45ºC (40ºC – 70ºC, range studied), solid-liquid ratio of 10:146 (w/v), 70 min as extraction time and ethanol concentration of 90%. Under these conditions, the TPC reaching 82.52 mg GAE g^{-1}, total flavonoids content was 32.01 mg GAE g^{-1}, DPPH- and ABTS- radicals scavenging activity reached 75.23% and 74.86%, respectively. Researchers highlighted that prolonged extraction time and the increase of temperature reduced the recovery of flavonoids (Kamarudin et al., 2020).

Temperature has been reported to improve the recovery of phenolics, due to several aspects, as follows: (i) its activation starts the diffusion of the phenolics, with the support of solvent extraction; (ii) the mass transport (or diffusion coefficient) of compounds, which can reduce solvent viscosity and surface tension; (iii) the softening of the plant tissues, which contribute to increase the permeability (Al-Farsi and Lee, 2008; Frempong et al., 2021).

Despite the positive effects of temperature on phenolic extraction, it is necessary to investigate the proper temperature range for each type of plant matrix to maximize the

extraction of bioactive compounds. An upper limit of temperature extraction could be applied in order to avoid thermal or chemical degradation, volatilization of solvent and reactions between food matrix compounds (Al-Farsi and Lee, 2008; Arruda et al., 2017; Zainal-Abidin et al., 2017).

The solid-to-solvent ratio exhibited an important effect on the evaluated responses and it was crucial in this study, since the extraction of antioxidant compounds from bracts using lower amount of solids (solid-to-solvent ratio 0.50%) in relation to the solvent resulted in greater responses.

Similar results concerning the effect of this parameter on the extraction of phenolics were also reported for brown seaweed *(Padina australis)*. The selected ultrasound-assisted extraction (UAE) conditions were: temperature of 60ºC, extraction time of 60 min, solid-to-solvent ratio of 1 g/100 mL (range studied of 1/20, 1/40, 1/60, 1/80 and 1/100) and solvent concentration of 60% (v/v) aqueous ethanol solution. Under these conditions, resulted in 5.07 mg GAE g^{-1} for TPC, 8.35 mg TE g^{-1} for radical scavenging activity (DPPH) and 5.70 mg TE g^{-1} for FRAP. In particular, it was observed that the increasing in solid-to-solvent ratio showed a decrease in responses (TPC and antioxidant properties), similar to the observed in our work (Hassan et al., 2021).

This trend is in agreement with that reported for the influence of solvent-to-solid ratio on extraction of *Achillea kellalensis* extract (medicinal plant). The optimum UAE results was found to be solvent-to-solid ratio of 30.95, ethanol concentration of 46.15%, ultrasonic time of 18.96 min and sonication amplitude of 89.73%. They remarked that an increase in solvent-to-solid ratio up to 25% was accomplished by an improvement in extraction yield (Yancheshmeh et al., 2021).

These results can be elucidated by some considerations: during extraction, the driving force that occurs during mass transfer within the solid is considered the concentration gradient that is greater when a higher solvent-to-solid ratio is used, this is in accordance with mass transfer principles (Pinelo et al., 2005).When a higher solvent-to-solid ratio is applied increases the concentration gradient in consequence, increases the rate of diffusion of sample compounds, resulting in a efficiency extraction process (Hassan et al., 2021). On the other hand, the indiscriminate increase of this proportion can negatively affect the extraction. This result was reported in a study about extraction of pectin from waste of *Artocarpus heterophyllus* fruit, in which was observed a decrease in extraction yield using the proportion above 25 mL g^{-1} (Moorthy et al., 2017).

Dietary fibre (DF) is the main constituent of bracts (61 - 66%) which has high hydration properties such as water holding capacity (WHC) and water swelling capacity (WSC), functional properties related to viscosity and water available. By using a comparison, these properties are significantly higher as compared to cellulose (WHC from bracts was 12 g water g^{-1} and cellulose, 5 g water g^{-1}; WSC value was 15 mL g^{-1} and cellulose, 4.5 mL g^{-1}) (Begum and Deka, 2019). A high fibre content increases the viscosity of the system restricting the water availability (Begum et al., 2020). In this context, if the availability of water is reduced it can affect the extraction because the combination of water and organic solvent facilitates the extraction of phenolic compounds by offering a more polar medium. Thus, the adequate ratio between solid (sample) and solvent (or their mixtures) must be considered in the extraction process (Socaci et al., 2018).

Agitation also promoted an important role in extraction. It enhances the mass transfer rate, improves mass and oxygen transfer, removes the concentrated solution from the surface of the sample and maintains homogeneous chemical and physical conditions in the process (Azmir et al., 2013; Zainal-Abidin et al., 2017; Zhou et al., 2018). This parameter showed positive effects in all assays for the Maçã variety. It was possible to conclude that agitation must be adjusted according to the matrix. Thus, it is important to establish extraction parameters to increase the recovery of compounds from the food matrix, aiming to exploit the maximum for its beneficial effects, mainly for new sources.

Most mathematical models showed R^2 greater than 0.75 and the F calculated values were higher than the F tabulated values with statistical significance ($p \leq 0.10$) according to ANOVA (Table 3). Responses with unsatisfactory statistical parameters did not generate mathematical models (equations).

Experimental data were depicted into a contour plot for each assay (Figures 1 to 4) and indicated that the highest recovery of compounds in all analyzed varieties was reached at the followed conditions: incubation temperature of 37.5°C, agitation of 100 rpm and solid-to-solvent ratio of 0.50% (run 13). In contrast, the lowest values were observed in run 14 when the extraction was conducted with 37.5°C as an incubation temperature, agitation of 100 rpm and solid-to-solvent ratio of 3%. The low level of variation for most of the central points (runs 15-17) indicated good reproducibility of the experimental data.

Previous studies performed by our research group evaluated the combination of solvents (water, acetone and methanol) on recovery of antioxidant compounds from banana bracts. The extraction conditions were: 1h as extraction time, temperature of 25°C and solid-

to-solvent ratio of 2.5%. Results showed that the mixture of water and acetone (in equal proportions) efficiently recovered compounds from Maçã and Prata varieties. These results (runs M and P) were compared with the values obtained in the current study (runs M13 and P13) using the same solvents, with the parameters chosen by CCRD (Table 4).

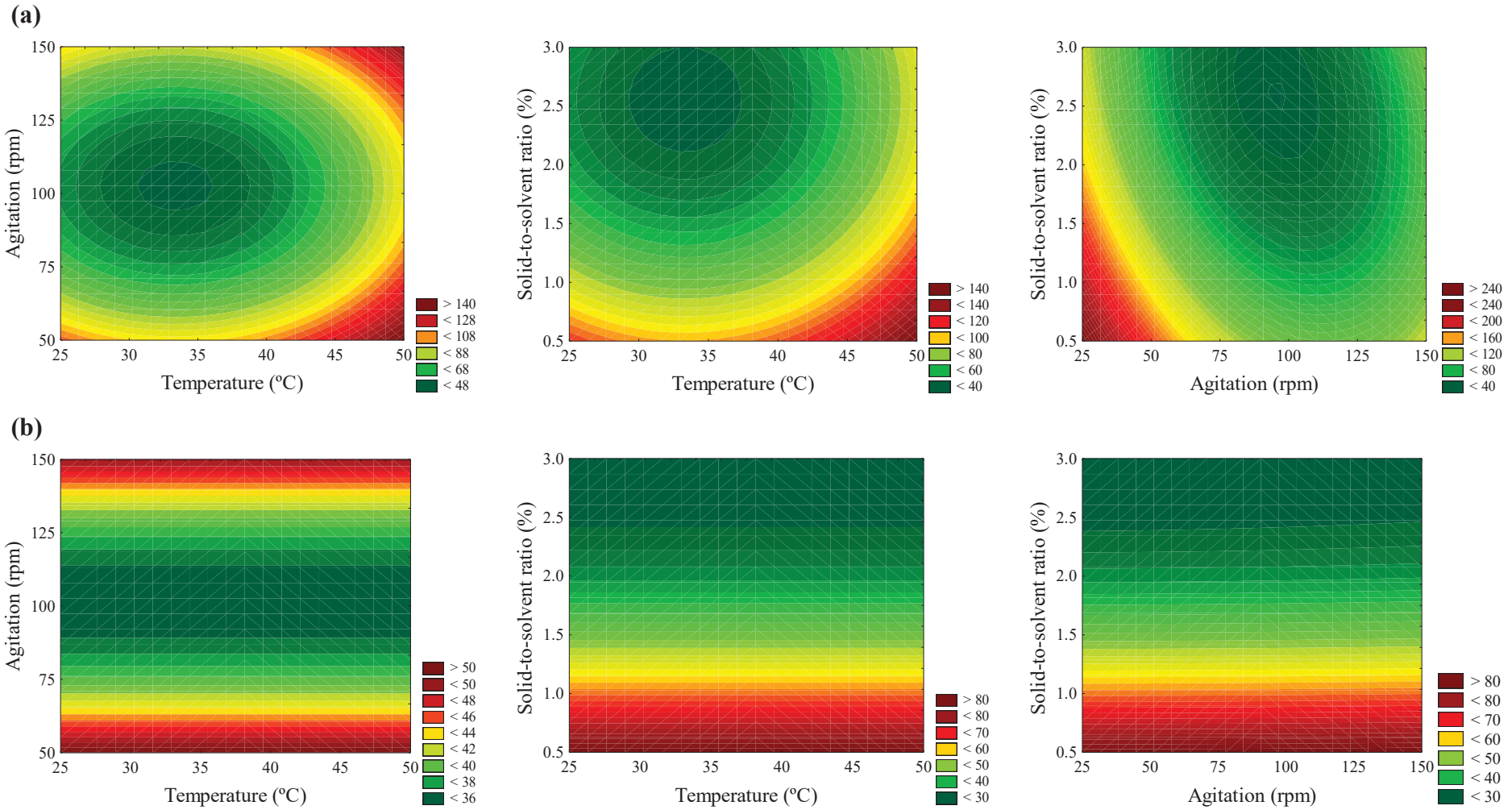

Figure 1. Contour plots for antioxidant activity evaluated by ABTS (a) and DPPH (b) assays for banana bract extracts (Maçã variety).

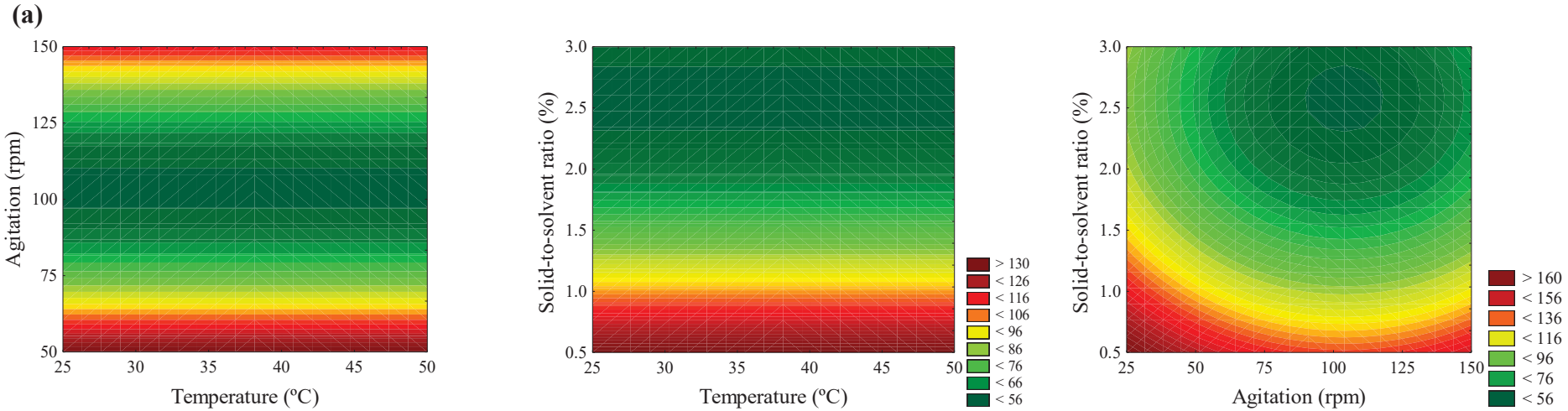

Figure 2. Contour plots for antioxidant activity evaluated by FRAP (a) assay for banana bract extracts (Maçã variety).

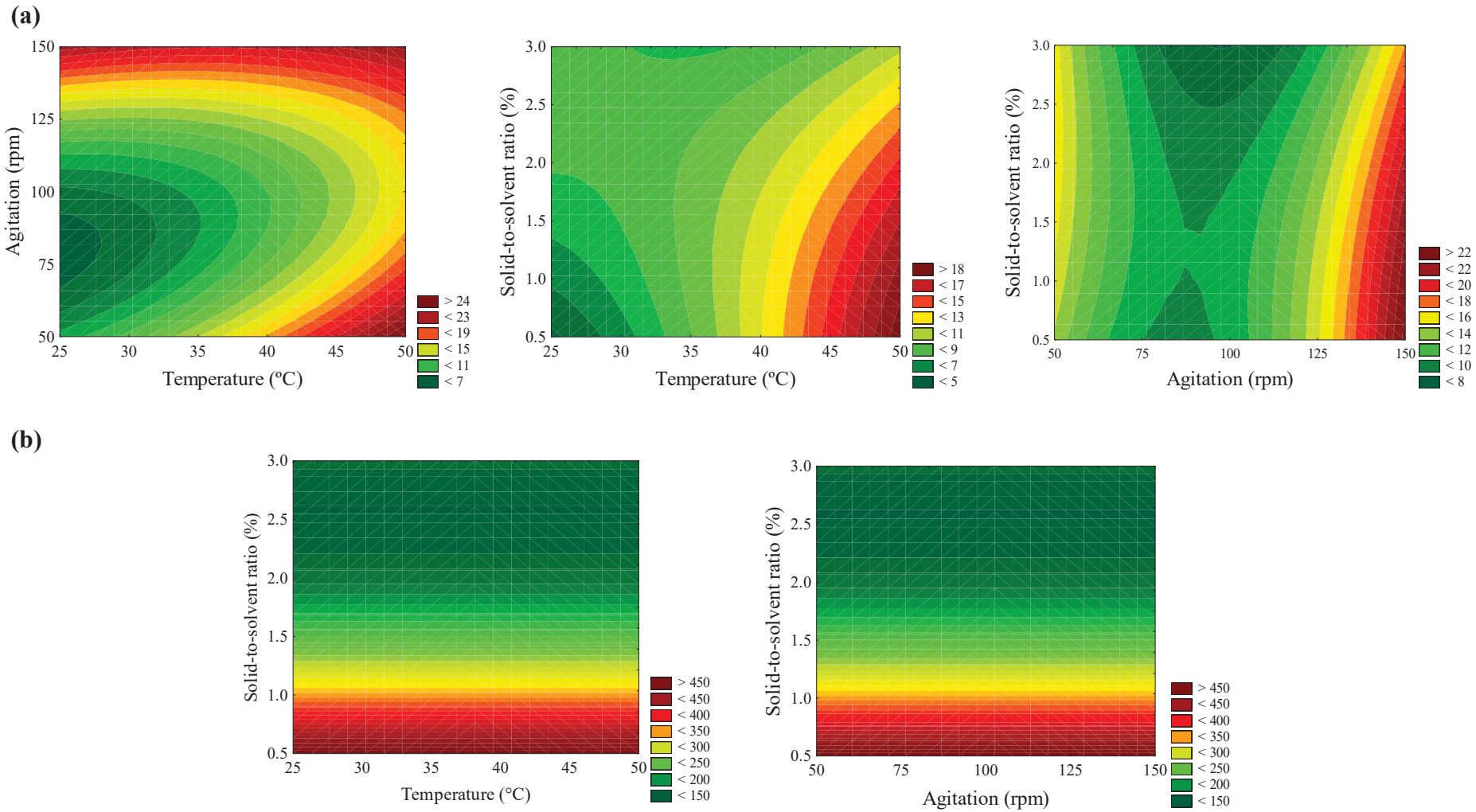

Figure 3. Contour plots for total phenolic compound (TPC) (a) and antioxidant activity evaluated by ABTS (b) assay for banana bract extracts (Prata variety).

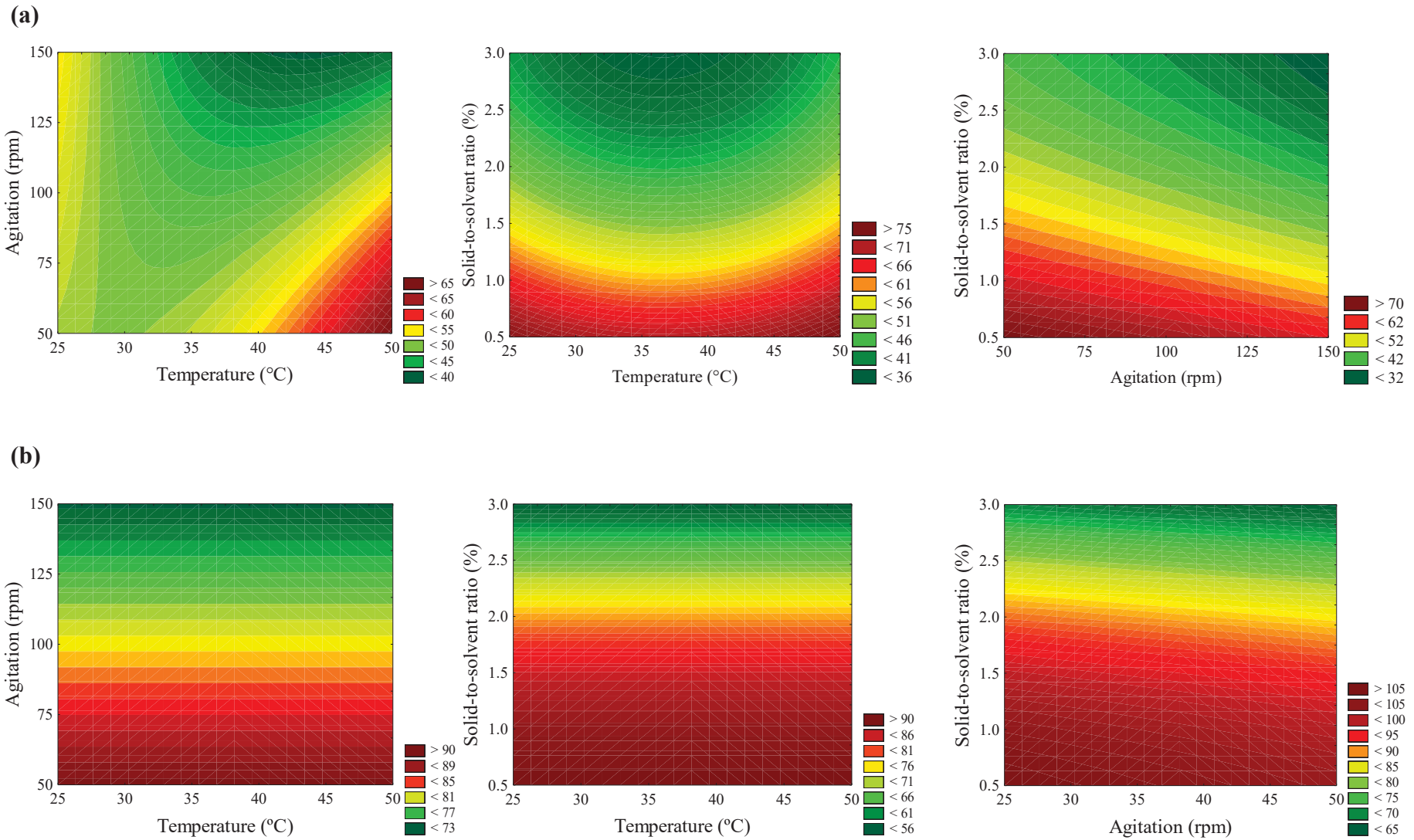

Figure 4. Contour plots for antioxidant activity evaluated by DPPH (a) and FRAP (b) assays for banana bract extracts (Prata variety).

Table 4. Comparative analysis of means obtained in a previous study of non-optimized extraction parameters (runs M and P) versus optimized extraction parameters (present study) (runs M13 and P13) based on antioxidant properties measured by ABTS, DPPH and FRAP assays.

Run	ABTS (µmol TE g^{-1})	Variation (%)[1]	DPPH (µmol TE g^{-1})	Variation (%)[1]	FRAP (µmol TE g^{-1})	Variation (%)[1]
			Maçã variety			
M	81.80 ± 1.83 [a]	15.22	33.72 ± 0.88 [b]	177.93	65.36 ± 3.94 [b]	129.00
M13	94.25 ± 8.21 [a]		93.72 ± 1.85 [a]		149.68 ± 1.98 [a]	
			Prata variety			
P	67.75 ± 11.59 [b]	717.71	47.38 ± 1.00 [b]	51.70	75.77 ± 0.37 [b]	42.32
P13	554.00 ± 13.78 [a]		71.88 ± 4.04 [a]		107.84 ± 1.74 [a]	

Results were expressed as the mean (triplicate) ± standard deviation. Different lowercase letters in the same column indicate statistical difference ($p < 0.05$) between the results for each banana variety evaluated in the same antioxidant method by Tukey test.

[1]Value calculated through the comparative evaluation of the variation of antioxidant activity results obtained in the present study (samples M13 and P13) in relation to those obtained in the study of solvent mixtures (samples M and P).

This comparison indicated that the investigation based on the optimization of extraction parameters improved the antioxidant activities in most of assays. It was observed an increase between 15.22% and 177.93% in ABTS, DPPH and FRAP assays in responses from Maçã variety compared to the study concerning mixture of solvents. The Prata variety showed increases between 42.32% and 717.71% in antioxidant activities compared to the previous study. These results reinforce that it is crucial to develop and optimize an extraction process for each studied matrix.

3.2 Identification and quantification of phenolic compounds from banana bract extracts by HPLC

The identification of compounds from all banana bract varieties was performed using extracts obtained with an incubation temperature of 37.5°C, agitation of 100 rpm and solid-to-solvent ratio of 0.50% (run 13). This condition was chosen to find the best extraction parameters to obtain extracts with higher antioxidant properties. Rutin was found to be as the main phenolic compound in Maçã and Prata bracts extracts (Table 5).

Table 5. Quantification of phenolic compounds from banana bract extracts by high-performance liquid chromatography (HPLC).

	Banana bract extract (variety) Concentration (μg g^{-1})		Validation parameters		
Compound	**Maçã**	**Prata**	**LOD (μg)[1]**	**LOQ (μg)[2]**	**Linearity (R^2)**
Rutin	1305.13 ± 0.03 ^{c}	4663.28 ± 0.10 ^{a}	0.0014	0.0042	0.975

Values were expressed as the mean (triplicate) ± standard deviation.

Different lowercase letters in the same line indicate statistical difference ($p < 0.05$) between the results by Tukey test.

[1] LOD: detection limit

[2] LOQ: limit of quantification

Identification of phenolic compounds in extracts from banana inflorescence has already been reported in some studies. Muchahary and Deka (2021) studied different methods to extract phytochemical constituents from bhimkol banana inflorescence, as follows: ultrasound-assisted extraction (UAE), supercritical fluid extraction (SCFE), and conventional extraction method (70% ethanol as solvent extraction, solid: solvent ratio 2:30, 30ºC as incubation). The polyphenol profiles of extracts were identified by RP-HPLC and showed compounds with distinct concentrations according to the extraction applied (SCFE, ultrasound and conventional methods). Extraction by SCFE was found to be most efficient method than UAE and conventional extraction based on extraction yield (10.10, 9.98 and 7%, respectively), TPC (2,750.37, 1,898.20 and 1,870.21 mg GAE 100 g^{-1}, respectively) and DPPH inhibition assay (79.41, 80.13 and 80.00%, respectively). The extracts obtained using SCFE revealed higher phenolic contents as compared to those obtained by other methods (ultrasound and ethanol extraction). The quantification of compounds in extracts obtained using SCFE, UAE and conventional methods showed the presence of rutin (23.23, 20.70 and 20.26 mg g^{-1}, respectively), ferulic acid (16.05, 15.82 and 15.69 mg g^{-1}, respectively) and quercetin (303.39, 285.58 and 273.22 mg g^{-1}, respectively) were the predominant (Muchahary and Deka, 2021). From our extraction method, rutin content (Table 5) found in banana bract extracts (Maçã and Prata varieties) exhibited an increase between 4 and 19-fold higher than the aforementioned study.

The nutraceutical properties of inflorescence extracts of *Musa paradisiaca* (Nendran variety) were investigated by Arun et al., (2017). From the methanolic extract were identified twelve phenolics among which catechol (323 μg mL^{-1}), chlorogenic acid (115 μg mL^{-1}), ρ-coumaric acid (108 μg mL^{-1}), ferulic acid (19 μg mL^{-1}) and gallic acid (1290 μg mL^{-1}) were the predominant.

Phenolic compounds from dried banana inflorescence (*Musa acuminata cavendish*) were identified by HPLC-MS and the major compounds caffeic acid (100 μg g^{-1}), hydroxybenzoic acid (66.1 μg g^{-1}), ferulic acid (16.4 μg g^{-1}), quercetin 3,4'-O-diglucoside (10.1 μg g^{-1}), and ρ-coumaric (3.8 μg g^{-1}) acid were detected. The relevant fraction of phenolic compounds in dried banana was associated with hemicellulose A, therefore corresponding to the hydrolysable polyphenol fractions (Ramírez-Bolaños et al., 2021).

It is noteworthy that these findings (ρ-coumaric acid, ferulic acid and rutin) have already been reported to have several health benefits related to their antioxidant properties. The main point of attraction of antioxidants is their preventive role in various conditions related to oxidative stress due to their high efficacy as reducing agents and free radical scavengers (Alara et al., 2021; Chen et al., 2017; Shahidi et al., 2019)

Rutin, a flavonol abundantly found in plants, has also been reported to have a number of biological activities, including antioxidant, cytoprotective, vasoprotective, anticarcinogenic, neuroprotective and cardioprotective activities (Ganeshpurkar and Saluja, 2017). However, the major disadvantage of rutin is its poor bioavailability because of its low aqueous solubility and limited membrane permeability. To overcome this barrier, further studies are needed on the mechanisms used to increase rutin solubility in both aqueous and lipid phases. Since the demand for rutin has a rising tendency, it is important to investigate the most recent extraction and purification methods of this compound (Gullón et al., 2017).

According to our results, banana bracts showed several opportunities to explore. The extracts showed high content of phenolic compounds with antioxidant properties since the results found in identification step were higher than aforementioned values in the literature. There is a constant and growing demand for natural resources to replace synthetic compounds and the development of novel functional food ingredients. For this, it is essential to investigate a suitable extraction method, determine the main parameters that have an impact on the food matrix, choose the conditions for these parameters and understand their interactions; such aspects were investigated in this study.

4. Conclusion

The results obtained showed that banana bracts from different sources have large amounts of bioactive compounds. Some process conditions were studied and showed that extracts from Maçã and Prata varieties obtained using incubation temperature of 37.5°C, agitation of 100 rpm and solid-to-solvent ratio of 0.50% showed better antioxidant properties. From our extraction method, rutin amount found in extracts exhibited values between 4 and 19-

fold higher than that previously found in the literature. These results can support future researches with possibilities not yet studied and advancing studies on banana inflorescence. The utilization of agricultural banana waste proved to be a potential ingredient for food industries and consequently provided several benefits to human health.

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CAPÍTULO IV: Synergistic action of multiple enzymes resulted in efficient hydrolysis of banana bracts and products with improved antioxidant properties

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Abstract

This study investigated the effect of enzymatic hydrolysis of banana bracts from three varieties (Maçã, Nanica and Prata) using pectinase, protease and cellulase (singly or in combinations) on their antioxidant properties. The results showed that the antioxidant properties and total phenolic compounds (TPC) of banana bract extracts increased after the enzymatic treatment with a clear synergistic effect between the different enzymes. The ternary mixture of pectinase, protease and cellulase resulted in increases of 458% and 678% in TPC content for extracts obtained from Maçã and Nanica varieties and up to 65% in antioxidant properties of those produced from Prata variety compared to the control (non-hydrolyzed samples). In general, the extracts obtained from the Prata variety showed the highest levels of phenolic compounds, as well as antioxidant activity, as follows: 14.70 mg GAE g^{-1} for TPC, 82.57 μmol TE g^{-1} for ABTS, 22.26 μmol TE g^{-1} for DPPH and 47.09 μmol TE g^{-1} for FRAP. Phenolic compounds identified by HPLC in banana bract extracts included ρ-coumaric acid, ferulic acid, sinapic acid, vanillic acid and rutin. This study reported for the first time the enzymatic treatment applied on banana bracts as a promising method to release antioxidant compounds. This offers a new opportunity to exploit these wastes through an environmentally friendly, fast, safe and efficient process.

Keywords: Banana flower, green extraction methods, agricultural waste, natural antioxidants.

1. Introduction

Banana (*Musa acuminata*) is considered one of the most consumed fruits in the world due to its highly nutritional value and it has a relevant economic interest. In 2020, 119 million tons of banana were produced in more than 150 countries which India, China, Brazil, Ecuador and Philippines are the major producers (FAO, 2020).

Concerning this chain production, banana industry generates large amount of agricultural waste with high value-added. All banana plant structures such as fruit, pseudostem, leaves, inflorescences and peels have been reported to have medicinal properties (Mathew and Negi, 2017). Bract, a part of banana inflorescence, also known as flower or blossom, are typically treated as an agricultural waste, although it is widely used by different regions as a culinary purposes and folk medicine (Sitthiya et al., 2018). Banana inflorescence has gained scientific relevance due to its variety of bioactive compounds and its associated beneficial health properties, including antioxidant, anti-diabetic, anti-microbial, anti-inflammatory and cardiovascular-protective activities (Arun et al., 2017; Basumatary and Nath, 2018; Bhaskar et al., 2012; Padam et al., 2012; Prakasan and Saraswathy, 2013; Ramu et al., 2017; Sheng et al., 2014).

In last few years, the demand for functional ingredients has been increased as well as the interest in agricultural wastes due to its great source of bioactive compounds (Bandara and Chalamaiah, 2019). Several researches have shown that the daily intake of plant-based such as vegetables, fruits and whole grains is an essential factor to protective effects on human health. The antioxidant properties of these foods have been related with the presence of bioactive compounds, such as polyphenols, which may have preventive effects for obesity, cancer, cardiovascular and neurodegenerative diseases (Cory et al., 2018; Marathe et al., 2017). The protective role of phenolic compounds can be attributed to their antioxidant properties that prevent the formation of reactive species and stabilizes free radicals through the transfer of electrons and hydrogen ions (Olszowy, 2019).

Bioactive compounds in plants are available in low concentrations, thereby, the development of a proper method is one of the main challenges to extract these biomolecules (Azmir et al., 2013; Marathe et al., 2017). Currently, several strategies have been investigated to encourage sustainable methods, as an alternative to conventional extraction using organic solvents due to low environmental impact and higher efficiency (De la Peña-Armada et al., 2020; Soquetta et al., 2018). Examples of these methods include high pressure, pressurized liquids, pulsed electric fields, microwaves and enzyme-assisted extraction (EAE). EAE consists

of the disruption of structures of the cell wall (cellulose, hemicellulose, lignin, pectin and proteins) by hydrolysis using an enzyme as a catalyst with optimum experimental conditions, in order to liberate the intracellular constituents, such as phenolic compounds (Nadar et al., 2018). Thus, the addition of specific hydrolytic enzymes in the system allows to recovery several components. The use of enzymes, such as cellulases, pectinases and proteases, has already been reported to be efficient for hydrolysis of plant substrates and recovery of bioactive compounds (Casas and Domínguez González, 2017; Danalache et al., 2018; Gligor et al., 2019). Furthermore, it is a technology with low environmental impact and high recovery, due to the specificity and regioselectivity of the enzymes (Liu et al., 2016; Mu et al., 2020).

In this context, the aim of this study was to investigate the isolated and combined use of different enzymes on the hydrolysis of three varieties of banana bracts (Maçã, Nanica and Prata) and their effects on the recovery of compounds with antioxidant properties.

2. Material and Methods

2.1 Material and reagents

Bracts of banana *(Musa paradisiaca)* Maçã and Prata varieties were collected from cultivated local farmland (20°33'56.5" south latitude, 46°05'21.1" west longitude) in Capitólio, state of Minas Gerais, Brazil. The Nanica variety was donated by Magário company, Jaíba, Minas Gerais, Brazil.

ABTS ([2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)]), DPPH (2,2-diphenyl-1-picrylhydrazyl), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), TPTZ (2,4,6-tripyridyl-s-triazine), Folin & Ciocalteau's phenol reagent, gallic acid, catechin, vanillin and the commercial enzymes Flavourzyme™ (protease), Celluclast™ 1.5 L (cellulase) and Pectinex™ Ultra Pulp (pectinase) were acquired from Sigma-Aldrich (São Paulo, Brazil). All other chemicals were purchased in analytical available grade.

2.2 Enzymatic hydrolysis and obtaining banana bract extracts

Banana bract was separated from inflorescence, cleaned, ground, frozen, freeze-dried and the powdered sample was stored in vacuum packs at -18°C. For enzymatic hydrolysis, the substrate solution was prepared using 0.25 g of banana bracts and 25 mL of phosphate buffer (100 mmol L^{-1} pH 5.0). Three different commercial enzymes preparations namely: Pectinex™ Ultra Pulp (blend of pectinases, hemicellulases and beta-glucanases), Flavourzyme™ (proteases from *Aspergillus oryzae*) and Celluclast™ 1.5 L (cellulase derived from *Trichoderma reesei* ATCC 26921) were applied singly or in binary/ternary mixtures on

substrate solution at a final concentration of 1% (v/v) (Table 1). Non-hydrolyzed samples (control) were also prepared as mentioned above, without the addition of enzymes. All the mixtures were kept at 50°C during 2 h under agitation of 100 rpm, followed by cooling for 10 min to enzymatic activity cessation and stored under freezing until analysis.

Table 1. Matrix of experimental mixture design used for extraction of antioxidant compounds of banana bracts from Maçã, Prata and Nanica varieties using different enzymatic preparations.

Run	Independent variables					
	Coded variables			Real variables (mL)		
	x_1	x_2	x_3	Pectinex™	Flavourzyme™	Celluclast™
Control	0	0	0	0	0	0
1	1	0	0	0.250	0	0
2	0	1	0	0	0.250	0
3	0	0	1	0	0	0.250
4	1/2	1/2	0	0.125	0.125	0
5	1/2	0	1/2	0.125	0	0.125
6	0	1/2	1/2	0	0.125	0.125
7	1/3	1/3	1/3	0.083	0.083	0.083
8	2/3	1/6	1/6	0.165	0.042	0.042
9	1/6	2/3	1/6	0.042	0.165	0.042
10	1/6	1/6	2/3	0.042	0.042	0.165

To determine the most adequate enzymes or mixtures of them for maximum antioxidant compounds recovery from banana bracts, a statistical mixture design was employed (Neto et al., 2010). Each enzyme preparation was evaluated at six levels: 0 (0%), 1/6 (16%), 1/2 (50%), 1/3 (33%), 2/3 (66%) and 1 (100%), totalizing 10 runs (extracts) (Table 1). All the runs were evaluated comparatively with non-hydrolyzed extracts (control). For this study, quadratic and/or cubic models were employed to fit variations of all investigated responses ($p \leq 0.10$) as a function of interaction effects between the proportions of pectinase, protease and cellulase, with acceptable determination coefficients greater than 70% ($R^2 > 0.70$) represented by Eq. (1):

$$\boldsymbol{Yi} = \sum_{i=1}^{q} \boldsymbol{\beta}_i \boldsymbol{X}_i + \sum \sum_{i<j}^{q} \boldsymbol{\beta}_{ij} \boldsymbol{X}_i \boldsymbol{X}_j + \sum \sum_{i<j<k}^{q} \sum \boldsymbol{\beta}_{ijk} \boldsymbol{X}_i \boldsymbol{X}_j \boldsymbol{X}_k \quad (1)$$

where ′Yi′ corresponds to predicted response (total phenolic compounds and antioxidant properties - ABTS, DPPH and FRAP); ′q' represents the independent variables (components) in the system; "Xi, Xj, Xk" represent the coded components; and "βi", "βij" and "βijk" corresponds to the regression coefficients (binary and ternary interaction, respectively)

(de Castro et al., 2017). The software Statistica 13.3 TIBCO Software Inc. (Palo Alto, California, USA) was used for Student's t-test ($p \leq 0.10$) and model building by analysis of variance (ANOVA) ($p \leq 0.10$).

2.3 Determination of total phenolic compounds (TPC)

The total phenolic compounds were determined according to the method described by (Magro & de Castro, 2020). Aliquots of 25 µL of diluted extracts (25 mg mL^{-1}), 25 µL of Folin-Ciocalteau solution (50% v/v) and 200 µL of sodium carbonate (5% w/v) were mixed and then, incubated at 40°C in the dark for 20 min. The absorbance was obtained at 760 nm on a spectrophotometer (Multiskan GO, Thermo Fisher Scientific, Finland). For the calibration curve, gallic acid was used with a concentration range from 0 to 10 mg mL^{-1} and the results were expressed as mg of gallic acid equivalent per gram of freeze-dried sample (mg GAE g^{-1}).

2.4 Measurement of the antioxidant properties

2.4.1 ABTS-radical cation scavenging activity

The ABTS-radical cation scavenging activity was determined as described by Neta and de Castro (2020). The ABTS aqueous solution (7 mmol L^{-1}) and potassium persulfate (140 mmol L^{-1}) were mixed and maintained for 16 h at room temperature, without light exposure, to generate the free radical. After this incubation period, the absorbance was adjusted to 0.70 ± 0.02 with deionized water. Aliquots of 20 µL of extracts (25 mg mL^{-1}) were mixed with 220 µL of ABTS solution and the absorbance measurements were determined after 6 min of reaction at 734 nm using a spectrophotometer (Multiskan GO, Thermo Fisher Scientific, Finland). Trolox was used for the calibration curve (0 – 400 µmol L^{-1}) and the results were expressed as µmol of Trolox equivalents per g of freeze-dried sample (µmol TE g^{-1}).

2.4.2 DPPH-radical scavenging activity

The DPPH assay was performed according to the method described by Rasera et al., (2019). Aliquots of 134 µL of DPPH ethanolic solution (150 µmol L^{-1}) were added to 66 µL of diluted extracts (25 mg mL^{-1}) or standard (Trolox). After 45 min of the reaction without light exposure at room temperature, the absorbance measurements were determined at 517 nm against a blank (ethanol) using a spectrophotometer (Multiskan GO, Thermo Fisher Scientific, Finland). Trolox was used as the standard with a calibration curve ranging from 0 to 125 µmol L^{-1}. The results were expressed as µmol TE g^{-1}.

2.4.3 Ferric Reducing Antioxidant Power (FRAP)

The FRAP assay was performed according to the method described by Firuzi et al., (2005) with some modifications proposed by Aguilar et al., (2018). The FRAP solution was composed of acetate buffer (300 mmol L^{-1}, pH 3.6), ferric chloride hexahydrate (20 mmol L^{-1}) dissolved in distilled water and TPTZ (2,4,6-tris(2-pyridyl)-s-triazine) (10 mmol L^{-1}) diluted in HCl (40 mmol L^{-1}) (10:1:1, v:v:v). Aliquots of 25 µL of banana bract extracts (25 mg mL^{-1}) and 175 µL of freshly prepared FRAP solution were mixed and absorbance measurements were collected after 30 min of reaction at 595 nm using a spectrophotometer (Multiskan GO, Thermo Fisher Scientific, Finland). Trolox (0 - 250 µmol L^{-1}) was used as the standard, and the results were expressed as µmol TE g^{-1}.

2.5 Identification of phenolic compounds by HPLC

Phenolic compounds identification was performed according to the method described by Silva et al., (2021) with slight modifications. The separation of compounds by HPLC was performed using a Shimadzu ODS-A column (4.6 mm, 250 mm, 5 µm) in a thermostated oven at a constant temperature of 30ºC, an injection volume of 20 µL and a photodiode array detector (SPD-M10AVp, Shimadzu Co., Kyoto, Japan). The mobile phase consisted of eluent A, water/formic acid (99.75/0.25, v/v) and eluent B, acetonitrile/formic acid/water (80/0.25/19.75, v/v) at a flow rate of 1.0 mL min^{-1}. The gradient conditions were as follows: 10% (B), increased to 20% (B) at 10 min, 30% (B) at 20 min, 100% (B) at 30 min and 10% (B) at 35 min. The analysis was completed in 40 min and the chromatograms were analyzed with Class-VP® software. Each compound peak was identified based on a comparison of the retention time, spectral data and peak area. Phenolic acid standards (gallic acid, 3,4-dihydroxybenzoic acid, vanillic acid, caffeic acid, coumaric acid, ferulic acid and sinapic acid) and flavonoids (rutin and quercetin) were used to quantify the individual compounds. The standard curves (0.03 – 0.20 µg mL^{-1} concentrations) were determined using the chromatographic parameters previously mentioned. The limit of detection (LOD) and limit of quantification (LOQ) (Equations 2 and 3) were calculated as follows:

$$LOD = 3.3 \times s \div S \quad (2)$$

$$LOQ = 10 \times s \div S \quad (3)$$

where "*s*" is the standard deviation of the linear coefficient of the equation and "*S*" is the slope of the standard curve.

2.6 Calculations and statistics

The results were reported as the mean values ± standard deviation (n = 3) and Minitab software, version 19 (Minitab Inc., USA) was used to verify if there was a significant difference (p-value ≤ 0.05) between the means calculated by analysis of variance (ANOVA) followed by Tukey´s test.

3. Results and discussion

3.1 Effect of enzymatic hydrolysis on the recovery of antioxidant compounds

The experimental values under distinct combinations of enzymatic preparations are displayed in Table 2. The highest TPC content (15.87 mg GAE g^{-1}) was detected for the extract obtained from the Maçã variety (run 10). For antioxidant properties, the highlights were the extracts obtained from bracts of the Prata variety, that reached 82.57 μmol TE g^{-1} for ABTS (run 9) and 47.09 μmol TE g^{-1} for FRAP (run 4), while the extract from Maçã variety showed the greatest ability to inhibit DPPH radicals (30.52 μmol TE g^{-1} - run 7).

Ternary mixture of the enzymes (Pectinex™, Flavourzyme™ and Celluclast™, in equal proportions, run 7) was responsible by obtaining of banana bract extracts from Maçã and Prata varieties with maximum values of TPC and antioxidant activities. Maçã variety showed increases ranging from 30% to 458% in all analysis when compared to the control (non-hydrolyzed). Similar results were obtained for banana bract extract from Prata variety, in which increases greater than 65% were detected for TPC and antioxidant activities compared to the control. For the Nanica variety, the ternary mixture was also very efficient, however the proportion between the three enzymes was different; in this case, the mixture of Pectinex™ (1/6), Flavourzyme™ (2/3) and Celluclast™ (1/6) (run 9) resulted in the highest TPC content and antioxidant properties, reaching increases ranging from 13% to 678% compared to the control (Table 2). Most of the mathematical models proposed for Nanica and Prata varieties showed R^2 values greater than 0.74, and the F values calculated were higher than F-tabulated with statistical significance (p ≤ 0.10) according to ANOVA (Table 3).

Table 2. Results for total phenolic compounds (TPC) and antioxidant activities (ABTS, DPPH and FRAP assays) in banana bract extracts obtained by use of different enzymes (pectinase, protease and cellulase) and combination of them.

Run	TPC (mg GAE g^{-1})	ABTS (µmol TE g^{-1})	DPPH (µmol TE g^{-1})	FRAP (µmol TE g^{-1})
Maçã variety				
Control	2.69 ± 0.76 ^{f}	Not detected	17.43 ± 0.09 ^{ef}	3.38 ± 0.25 ^{f}
1	5.49 ± 0.71 ^{de}	9.45 ± 3.97 ^{d}	23.65 ± 2.19 ^{bc}	3.70 ± 0.31 ^{def}
2	8.89 ± 0.33 ^{c}	3.50 ± 0.38 ^{d}	18.71 ± 1.39 ^{def}	3.67 ± 0.28 ^{def}
3	10.24 ± 0.24 ^{c}	37.30 ± 0.31 ^{abc}	15.55 ± 1.66 ^{f}	3.64 ± 0.30 ^{ef}
4	6.83 ± 0.13 ^{d}	8.84 ± 0.74 ^{d}	20.03 ± 1.57 ^{cde}	3.91 ± 0.46 ^{cdef}
5	4.68 ± 0.05 ^{e}	47.25 ± 3.33 ^{a}	20.01 ± 1.03 ^{cde}	3.75 ± 0.27 ^{def}
6	6.55 ± 0.29 ^{d}	31.05 ± 1.29 ^{bc}	24.88 ± 0.68 ^{b}	5.10 ± 0.43 ^{b}
7	15.00 ± 0.36 ^{a}	37.58 ± 2.60 ^{abc}	30.52 ± 1.86 ^{a}	4.40 ± 0.03 ^{bcde}
8	12.98 ± 0.57 ^{b}	39.80 ± 1.59 ^{ab}	22.26 ± 0.75 ^{bcd}	4.48 ± 0.19 ^{bcd}
9	6.54 ± 0.17 ^{d}	36.11 ± 3.13 ^{abc}	20.46 ± 1.58 ^{cde}	4.66 ± 0.08 ^{bc}
10	15.87 ± 0.59 ^{a}	24.48 ± 4.74 ^{c}	19.66 ± 0.47 ^{de}	7.42 ± 0.09 ^{a}
Nanica variety				
Control	0.77 ± 0.22 ^{d}	14.54 ± 0.65 ^{f}	12.77 ± 0.14 ^{b}	18.21 ± 1.23 ^{abc}
1	2.03 ± 0.06 ^{d}	34.73 ± 1.44 ^{de}	15.87 ± 1.47 ^{ab}	16.02 ± 0.18 ^{c}
2	3.76 ± 0.17 ^{c}	49.16 ± 0.23 ^{bc}	16.72 ± 0.75 ^{bc}	16.70 ± 0.72 ^{bc}
3	4.77 ± 0.25 ^{abc}	30.15 ± 1.45 ^{ef}	15.64 ± 0.75 ^{ab}	18.30 ± 2.28 ^{abc}
4	4.66 ± 0.04 ^{bc}	49.56 ± 1.37 ^{bc}	12.62 ± 2.42 ^{b}	17.24 ± 0.84 ^{abc}
5	4.28 ± 0.52 ^{c}	44.94 ± 1.06 ^{cd}	14.96 ± 0.54 ^{ab}	17.97 ± 0.33 ^{abc}
6	4.59 ± 0.17 ^{bc}	53.02 ± 1.89 ^{abc}	13.08 ± 0.10 ^{ab}	16.64 ± 0.68 ^{bc}
7	5.00 ± 0.27 ^{abc}	59.73 ± 2.60 ^{ab}	18.74 ± 0.04 ^{ab}	19.53 ± 0.35 ^{abc}
8	3.81 ± 0.36 ^{c}	49.47 ± 0.90 ^{bc}	22.02 ± 3.05 ^{ab}	17.47 ± 0.50 ^{abc}
9	5.99 ± 0.80 ^{a}	62.44 ± 4.43 ^{a}	24.10 ± 0.43 ^{a}	20.62 ± 1.18 ^{a}
10	5.67 ± 0.39 ^{ab}	51.46 ± 0.78 ^{bc}	13.37 ± 0.51 ^{ab}	19.85 ± 1.02 ^{ab}
Prata variety				
Control	*Not detected*	20.77 ± 2.41 ^{d}	11.81 ± 1.01 ^{d}	28.06 ± 3.84 ^{bcd}
1	8.51 ± 0.38 ^{e}	43.41 ± 3.58 ^{cd}	18.54 ± 1.63 ^{abc}	43.15 ± 4.72 ^{ab}
2	14.70 ± 0.23 ^{a}	77.92 ± 0.89 ^{ab}	17.30 ± 0.53 ^{abc}	34.43 ± 2.83 ^{abc}
3	1.68 ± 0.25 ^{g}	52.95 ± 6.84 ^{bc}	16.60 ± 1.34 ^{bcd}	13.37 ± 3.07 ^{d}
4	1.81 ± 0.15 ^{g}	80.18 ± 2.51 ^{ab}	4.29 ± 3.43 ^{e}	47.09 ± 0.79 ^{a}
5	0.75 ± 0.12 ^{h}	63.78 ± 2.81 ^{abc}	5.36 ± 0.75 ^{e}	38.27 ± 5.07 ^{ab}
6	12.56 ± 0.44 ^{b}	79.17 ± 7.07 ^{ab}	17.85 ± 1.98 ^{abc}	19.85 ± 1.78 ^{cd}
7	9.51 ± 0.22 ^{d}	66.22 ± 8.39 ^{abc}	22.26 ± 0.92 ^{a}	46.29 ± 3.60 ^{a}
8	9.40 ± 0.41 ^{de}	73.93 ± 1.34 ^{ab}	20.51 ± 1.58 ^{ab}	38.08 ± 4.02 ^{ab}
9	5.20 ± 0.06 ^{f}	82.57 ± 1.21 ^{a}	15.32 ± 1.27 ^{cd}	18.39 ± 5.64 ^{cd}
10	10.85 ± 0.13 ^{c}	76.76 ± 11.87 ^{ab}	19.44 ± 1.14 ^{abc}	33.28 ± 3.01 ^{abc}

Values were expressed as the mean (triplicate) ± standard deviation. Different lowercase letters in the same line indicate statistical difference ($p < 0.05$) between the results by Tukey test.

Table 3. Analysis of variance (ANOVA), including models, R^2 and probability values for the final reduced models for TPC and antioxidant activity (ABTS and DPPH) from banana bract extracts (Nanica and Prata varieties).

Responses	Model	Equations	Fcalculated	Ftabulated	R^2	p-value
		Nanica variety				
TPC	Quadratic	$Y = 2.20\ x_1 + 4.18\ x_2 + 5.51\ x_3 + 7.95\ x_1x_2$	5.67	3.29	0.74	0.035
ABTS	Cubic	$Y = 34.18\ x_1 + 50.10\ x_2 + 30.25\ x_3 + 31.25\ x_1x_2 + 49.10\ x_1x_3 + 55.54\ x_2x_3 + 214.21\ x_1x_2x_3$	24.49	5.28	0.98	0.012
		Prata variety				
ABTS	Quadratic	$Y = 50.46\ x_1 + 81.48\ x_2 + 64.86\ x_3 + 66.92\ x_1x_2$	4.09	3.26	0.77	0.070
DPPH	Cubic	$Y= 19.91\ x_1 + 16.38\ x_2 + 16.77\ x_3 - 54.14\ x_1\ x_2 - 46.28\ x_1\ x_3 + 423.52\ x_1x_2\ x_3$	6.67	4.05	0.89	0.040

The coded values in model equations represent the independent variables and their interactions: x_1 = Pectinex™; x_2 = Flavourzyme™ and x_3 = Celluclast™.

For responses in which the statistical parameters were not satisfactory, the mathematical models (equations) were not generated (Table 4).

Table 4. Statistical parameters including F-test, R^2 and probability values for TPC and antioxidant activities (ABTS, DPPH and FRAP) from the extract of banana bract extracts (Maçã, Nanica and Prata varieties), that did not generate statistically valid models.

Responses	Fcalculated	Ftabulated	R^2	p-value
	Maçã variety			
TPC	3.40	3.29	0.63	0.09
ABTS	1.13	5.28	0.68	0.49
DPPH	2.74	3.29	0.58	0.13
FRAP	0.46	3.26	0.11	0.65
	Nanica variety			
DPPH	1.28	3.26	0.26	0.330
FRAP	0.66	3.26	0.15	0.540
	Prata variety			
TPC	1.64	3.29	0.45	0.270
FRAP	4.17	3.26	0.54	0.070

The coded values in model equations represent the independent variables and their interactions: x_1 = Pectinex™; x_2 = Flavourzyme™ and x_3 = Celluclast™.

The variations in TPC content and antioxidant assays of the analyzed banana bract extracts were also represented by mixture contour plots (Figures 1 and 2).

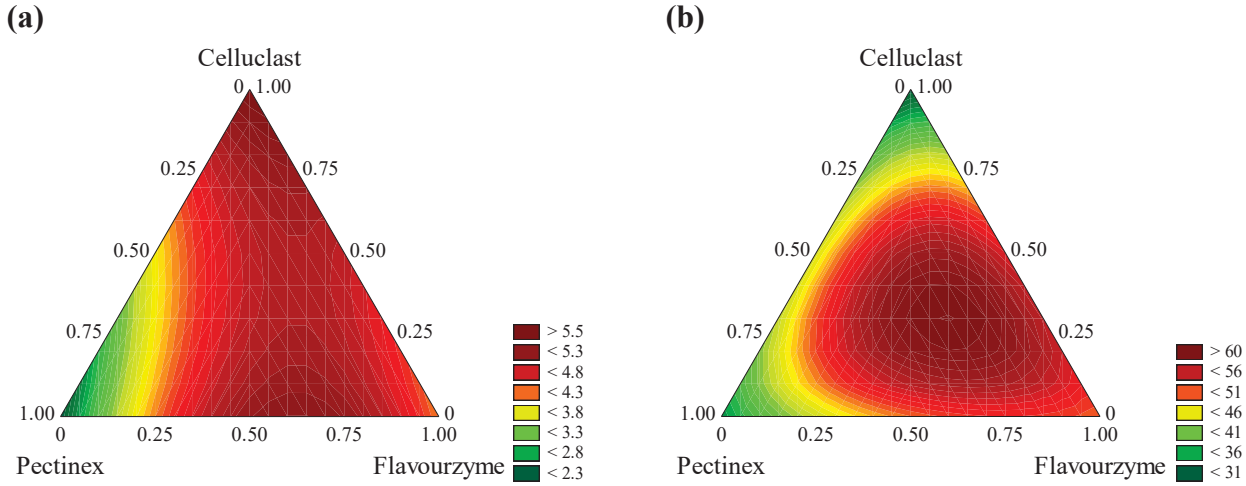

Figure 1. Contour plots for total phenolic compound (TPC) (a) and antioxidant activity evaluated by ABTS (b) method for banana bract extracts (Nanica variety).

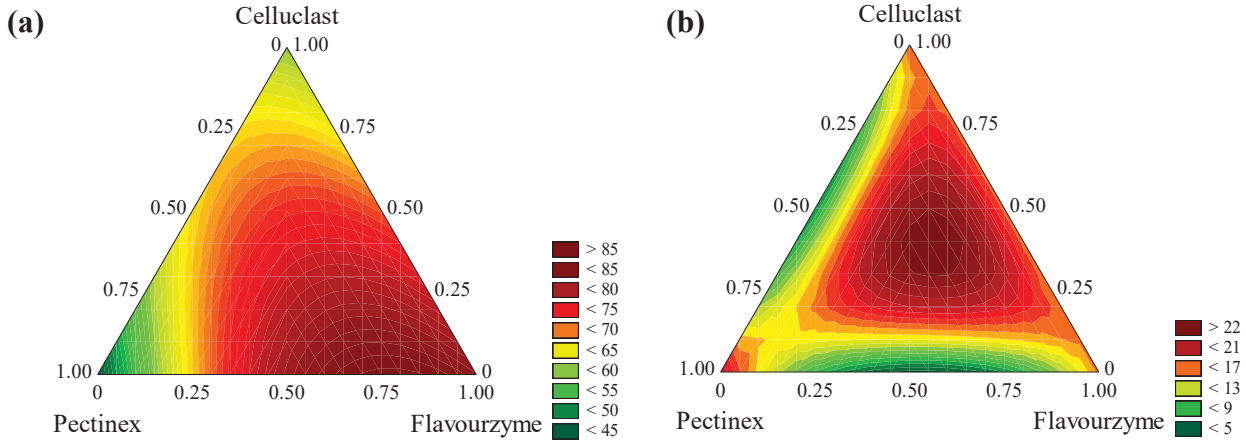

Figure 2. Contour plots for antioxidant activity evaluated by ABTS (a) and DPPH (b) methods, respectively, for banana bract extracts (Prata variety).

The interpretation of the contour plots just confirmed that the ternary combination Pectinex™, Flavourzyme™ and Celluclast™ was the most suitable to obtain extracts from banana bracts with higher concentration of TPC and antioxidant properties. According to the Fig. 1a, we can see a synergic effect improving the TPC content of Nanica variety when the enzymes Pectinex™ and Flavourzyme™ were used in binary combination (run 4) with increases between 24 and 130% as compared to the single enzymes (runs 1 and 2), respectively. A synergistic effect between the ternary mixture of Pectinex™ (1/6), Flavourzyme™ (2/3) and Celluclast™ (1/6) (run 9) was also detected; under this condition, increases of 80, 27 and 107% in ABTS assay were reached when compared to the extracts obtained by the use of single enzymes (runs 1, 2 and 3), respectively (Fig. 1b). Similar synergistic effect was observed in Prata variety for the binary mixture composed of Pectinex™ and Flavourzyme™ (run 4) with increases between 3% and 85% in ABTS assay when compared to the individual enzymes (runs 1 and 2), respectively (Fig. 2a). On the other hand, the ternary combination of Pectinex™ (1/3), Flavourzyme™ (1/3) and Celluclast™ (1/3) (run 7) showed a synergic effect improving the DPPH assay with increases of 20, 29 and 34% when compared to the isolated enzymes (run 1, 2 and 3), respectively (Fig. 2b).

Plant cell wall consisted of a well-organized matrix of carbohydrates (cellulose, hemicellulose and pectin) composed by sugar-alcohols bonds. The majority of bioactive compounds are enclosed to the cell wall polysaccharides by hydrogen bonds and hydrophobic linkages. Phenolic acids, for example, are associated to the cell wall by hydrogen and hydrophobic linkages and by ether bonds between proteins and carbohydrates (Marathe et al., 2019; Patil et al., 2021).

Banana bracts, for instance, have cell wall rich in cellulose (5.48-13.19%), hemicellulose (14.36-18.83%), pectin (3.97-5.31%) and other macronutrients such as proteins (2.06-2.55%) and a fiber-rich content (61.13-62.22%) (Begum & Deka, 2019). To release this complex, enzyme assisted extraction was applied to facilitate the recovery of compounds through the degradation of plant cell structure and, consequently, biomolecules will be more available for extraction (Nadar et al., 2018). For better yields in the recovery of bioactive compounds, the enzymatic preparation mixtures were used due to broad spectrum of activity that enables the hydrolysis of distinct constituents of the plant cell wall (Costa et al., 2020).

In view of the complexity of the composition of banana bracts, it is evident why the enzyme combinations and their significant interaction effects were more efficient in the hydrolysis and recovery of antioxidant compounds from this material. Pectinex™ is composed

of pectinases, hemicellulases and beta-glucanases and acts mainly on hydrolyze the pectic substances and also glycosidic bonds along the carbon chain (Liu and Kokare, 2017). It also has been proved to be particularly efficient for phenolic compounds extraction (Danalache et al., 2018). The use of hemicellulase together with pectinases allows the complete disintegration of the cell wall and improve the recovery of biomolecules (Mehta and Sehgal, 2019). Flavourzyme™ is a blend of proteases which broader specificity and has two mechanisms of action: the endopeptidase activity is responsible for the cleavage of peptide bonds inside the polypeptide chain and the exopeptidase activity catalyzes the hydrolysis of a peptide bond from the N- or C-terminal of a polypeptide chain, (Ohara et al., 2021). Celluclast™, is mostly constituted by cellulases (endo-glucanases) that hydrolyses the cellulosic chain and converts them to oligosaccharides, cellobiose and glucose (De la Peña-Armada et al., 2020).

Therefore, the cooperative effects between enzyme preparations with different specificities resulted in a cell wall degradation and consequent release of bioactive compounds. Thus, the diversity of compounds is greater when enzyme blends are used, increasing the antioxidant properties of the extracts in relation to the extracts produced from non-hydrolyzed samples (control).

Studies regarding banana inflorescence were reported since 1979, however, the potential application of enzymatic hydrolysis are still under explored. The only study found in literature reported a comparative analysis between the enzyme-assisted extraction (EAE) and ultrasonic-assisted alkaline extraction (UAE) of proteins from banana inflorescence. The greatest protein yield was reached with UAE (252.25 mg g^{-1}) under the following conditions: 30 min as extraction time, 50ºC, 1 mol L^{-1} NaOH and 24 kHz. These extracts generated high protein content when compared to EAE (102.98 mg g^{-1}) after 6 h incubation using pepsin. The UAE extracted proteins were characterized and showed the presence of tryptophan, tyrosine and amide bonds with antibacterial and anti-microbial effects (Sitthiya et al., 2018).

In a more recent studies, this technology was applied in other banana agroindustrial wastes such as peel, peduncle and pseudostem. The cellulose fiber from dried banana peel was investigated and the product obtained was a potential prebiotic fiber. For this, hemicellulose and lignin were removed by alkaline pre-treatment followed by two methods: an enzymatic hydrolysis with Celluclast™ 1.5 L and diluted acid hydrolysis. The results showed that enzymatic treatment was better than dilute-acid hydrolysis due to higher content of water-soluble cellulose and cellodextrins, which promoted the growth of probiotics (Phirom-on and Apiraksakorn, 2021).

The effect of xylooligosaccharides (XOS) from banana pseudostem xylan was investigated and evaluated for their prebiotic activity. Xylan was solubilized with 6% H_2O_2 followed by an enzymatic treatment performed using endoxylanase from *Aspergillus versicolor* and the obtained xylan was alkali extracted. The results showed a good yield and content of XOS (61% and 11 g L^{-1}, respectively). The media with high degree of polymerization XOS evidenced their prebiotic property (de Freitas et al., 2021). The extraction of cellulose from banana peduncle by combination of mild acid treatment followed by enzymatic hydrolysis for glucose production was studied. Results showed that the pre-treatment facilitated the cellulose yield (with low degree of polymerization) and increasing the enzymatic hydrolysis with a commercial cellulase enzyme from *Aspergillus niger*. The maximal glucose yield (97%) was detected with 50 mg mL^{-1} of substrate, 30 FPU g^{-1} (filter paper units per gram of enzymatic solution) of enzyme, 5 mg mL^{-1} of surfactant and 96 h incubation time. Under these conditions this process also eliminated lignin and hemicellulose (Baruah et al., 2022). In general, further studies related to banana inflorescence are encouraged due to its higher concentration of bioactive compounds (polyphenols) than other fractions of banana plantain (Lau et al., 2020). Previous studies reported this substantially difference in nutritional composition of the banana inflorescence (*Musa* sp. cv. Nanjangud rasa bale) and pseudostem. Phytochemical constituents as phenols showed values of 2.01 and 1.88 mg g^{-1} and flavonoids exhibited values of 0.83 and 0.78 mg g^{-1}, respectively (Ramu et al., 2017). In another study, banana inflorescence (*Musa* sp cultivar Elakki bale) was found to be a better and rich source of dietary fibre (65.6 %) when compared to banana pseudostem (28.8 %) (Bhaskar et al., 2012).

3.2 Identification of phenolic compounds by HPLC

The identification of phenolic compounds was carried out with the extracts that showed the best results for antioxidant properties, as follows: i) for the Maçã and Prata varieties, the extracts produced using the ternary mixture of Pectinex™, Flavourzyme™ and Celluclast™, in equal proportions (run 7) and ii) for the Nanica variety, the extracts produced using the ternary mixture of Pectinex™ (1/6), Flavourzyme™ (2/6) and Celluclast™ (1/6) (run 9). According to our results, most of the runs revealed an increase in recovery of phenolic compounds after the enzymatic hydrolysis compared to the control (non-hydrolyzed extracts). A total of five compounds were detected in the extracts: ρ-coumaric acid, ferulic acid, sinapic acid, vanillic acid (phenolic acids) and rutin (flavonoid) (Table 4).

Table 4. Quantification by HPLC of phenolic compounds from banana bracts extracts after enzymatic hydrolysis using the ternary mixture of pectinase, protease and cellulase.

Compound	Variety	Concentration (μg g^{-1})		Validation parameters		
		Non-hydrolyzed samples (control)	Hydrolyzed samples	LOD (μg)[1]	LOQ (μg)[2]	Linearity (R^2)
Coumaric acid	Maçã	n.d.	n.d.	0.0023	0.0070	0.9994
	Nanica	n.d.	76.46 ± 5.38			
	Prata	n.d.	46.97 ± 0.09			
Ferulic acid	Maçã	n.d.	36.17 ± 0.60	0.0015	0.0046	0.9985
	Nanica	7.20 ± 0.18 [b]	44.22 ± 2.48 [a]			
	Prata	n.d.	78.98 ± 0.24			
Sinapic acid	Maçã	n.d.	n.d.	0.0262	0.0792	0.9907
	Nanica	n.d.	22.77 ± 0.13			
	Prata	n.d.	n.d.			
Vanillic acid	Maçã	4.64 ± 0.31 [b]	6.81 ± 0.62 [a]	0.0022	0.0066	0.9979
	Nanica	n.d.	n.d.			
	Prata	5.85 ± 0.11 [b]	13.07 ± 0.18 [a]			
Rutin	Maçã	n.d.	n.d.	0.0014	0.0042	0.9745
	Nanica	n.d.	n.d.			
	Prata	27.01 ± 0.01 [b]	33.56 ± 0.01 [a]			

Values were expressed as the mean (triplicate) ± standard deviation. Different lowercase letters in the same line indicate statistical difference ($p < 0.05$) between the results by Tukey test.

n.d: not detected; [1] LOD: detection limit; [2] LOQ: limit of quantification

Coumaric acid was the main compound identified in banana bract extracts, while sinapic acid was observed only in hydrolyzed extracts from the Nanica variety and rutin was found only in hydrolyzed and non-hydrolyzed extracts from the Prata variety (Table 4).

These increment in recovery of biomolecules is associated with the increase of phenolic acids (available in insoluble form) that are bounded covalently with constituents of cellular walls (cellulose, hemicellulose, lignin and pectin. Thus, enzymes (and their combinations) aid the cell wall solubilization, releasing the bioactive compounds (Nadar et al., 2018).

EAE has been considered as an excellent method to promote extracts with higher biological activities. Among them, it is important to highlight those compounds detected in banana bract extracts (ρ-coumaric acid, ferulic acid, sinapic acid, vanillic acid and rutin) were reported to promote several health benefits associated to antioxidant activities. The beneficial effects of antioxidant are related to reduction of oxidative stress that acts as free radical scavengers and reducing agents (Leichtweis et al., 2021).

The hydroxycinnamic acids, such as ρ-coumaric acid, ferulic acid and sinapic acid, have wide array of biological activities including antioxidant, anti-inflammatory, anticancer

and neuroprotective effects. Among these, the mechanism of antioxidant effect is based on the ability for neutralization of free radical with generation of more stable phenoxyl radicals (Torrisi et al., 2021). Vanillic acid, belonging to the hydroxybenzoic acids, also having a numerous biological activities including antioxidant, anti-inflammatory, anticancer and neuroprotective activities (Punvittayagul et al., 2021; Ullah et al., 2021).

The biological properties of rutin, a flavonol glycoside, comprises of antioxidant, anti-inflammatory, antimicrobial, anticancer activities, neuroprotective and cardioprotective activities, are mostly associated to its antioxidant activities as a free radical scavenger (Gullón et al., 2017). The antioxidant capacity is due to the presence of phenolic rings and free hydroxyl groups which can donate hydrogen atoms to prevent further oxidation (Chua, 2013).

The ethanolic extract from banana inflorescence (*Musa* sp. cv. Nanjangud rasa bale) was investigated for its antihyperglycaemic effects. The results showed that ethanol extract of banana inflorescence inhibited α-glucosidase enzyme in comparison with drug acarbose (positive control) with IC_{50} values of 7.79 μg mL^{-1} and 9.68 μg mL^{-1}, respectively. The main compounds present in extracts were umbelliferone (7.08 μg mL^{-1}) and lupeol (7.18 μg mL^{-1}), belonged to the coumarin and triterpenoids, respectively. These compounds isolated from banana inflorescence proved to be α-glucosidase inhibitors and they presented a higher free radical scavenging activity which protective role against free radicals. Umbelliferone is recognized for its bioactivities including antirheumatic, analgesic effect and antipyretic, whereas, lupeol were reported as anti-inflammatory and anticancer effects (Ramu et al., 2014).

Colorimetric methods, such as the ABTS, DPPH and FRAP assays, are widely used for first-level screening to assess the potential bioactivity of plant substrates (de Camargo et al., 2019). However, it is important to recognize that these methods have some limitations and that further analyses involving the understanding of the antioxidant action of bioactive compounds are crucial to confirm their biological effects. Therefore, the determination of bioactive compounds by chromatographic techniques to establish the structure–activity relationship and application in at least one in vitro biological test (i.e., cell lines and simulated digestion) or, preferably, in vivo evaluation using animal models are strongly encouraged as complementary analyses to prove the effects of these compounds (Granato et al., 2018).

4. Conclusion

The enzymatic hydrolysis of banana bracts using a mixture of pectinase, protease and cellulase improved the recovery of bioactive compounds with antioxidant properties of banana bracts from different varieties. The identification of several phenolics confirmed the potential

use of banana bracts as a source of bioactive compounds strongly associated with health promotion. Thus, the results obtained in our study can support future opportunities for the development of functional ingredients for food industry using a fast, efficient and safe process, such as enzymatic hydrolysis.

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Discussão geral

Os resultados obtidos na primeira etapa do projeto envolveram o estudo de extração de compostos bioativos com o uso de água e solventes orgânicos. A combinação de solventes demonstrou que as misturas binárias e ternárias resultaram em extratos com maior recuperação de compostos e, consequentemente em extratos com elevada atividade antioxidante. A extração de compostos antioxidantes dos extratos de brácteas de banana das variedades Maçã e Prata mostrou que a combinação de água e acetona, em proporções iguais, foi o melhor solvente, enquanto a mistura ternária de água (1/6), metanol (2/3) e acetona (1/6) foi eficiente para a recuperação de compostos a partir da variedade Nanica. Nessas condições, a rutina foi identificada em todas as variedades analisadas e o ácido ρ-cumárico foi encontrado na variedade Nanica. Dentre tais combinações, as misturas aquosas apresentaram destaque devido a uma condição mais polar que pode facilitar a extração de compostos fenólicos solúveis. Vale destacar também que a eficiência da água como solvente de extração é reduzida porque os compostos fenólicos possuem variadas solubilidades e geralmente são mais solúveis em solventes menos polares do que a água.

Na avaliação dos parâmetros relacionados à extração convencional, os resultados mostraram que as variáveis independentes (temperatura, agitação e a razão sólido-solvente) apresentaram efeitos distintos com grande variação nas atividades antioxidantes relacionadas a cada condição analisada. Dessa forma, os parâmetros escolhidos para as variedades Maçã e Prata foram: temperatura de 37,5ºC, agitação de 100 rpm e o ratio sólido-solvente de 0,50% m/v. Sob estes parâmetros, os destaques foram: maior teor de compostos fenólicos totais (35,30 mg GAE g^{-1}), DPPH (93,72 µmol TE g^{-1}) e poder redutor de ferro (FRAP) (149,68 µmol TE g^{-1}) detectados para o extratos obtidos da variedade Maçã, enquanto que para a análise ABTS (554,00 µmol TE g^{-1}) o melhor resultado foi observado nos extratos da variedade Prata. Além disso, os compostos bioativos identificados e quantificados por HPLC em extratos de brácteas de bananeira incluíram o flavonoide rutina em ambas variedades. Tais resultados demonstraram a importância na escolha de um método apropriado e a investigação dos parâmetros de processo visando maximizar a recuperação de compostos da matriz alimentar e explorar seus efeitos benéficos.

A hidrólise enzimática demonstrou que é um processo promissor, garantindo a recuperação eficiente dos compostos de interesse. Os resultados realizados nesta etapa mostraram que o teor de compostos fenólicos e as propriedades antioxidantes dos extratos de brácteas de bananeira foram superiores após o tratamento enzimático, independente da enzima

utilizada de forma isolada ou combinada para a recuperação de compostos. A mistura ternária de pectinase, protease e celulase (em proporções iguais) resultou em aumentos de 30 a 458%% em todas as análises para os extratos obtidos das variedades Maçã e aumentos acima de 65% no teor de compostos fenólicos e propriedades antioxidantes da variedade Prata em relação ao controle (amostras não hidrolisadas). A mistura ternária de pectinase (1/6), protease (2/6) e celulase (1/9) promoveram aumentos de 13% a 678% no teor de compostos fenólicos e propriedades antioxidantes quando comparados com o controle. Em geral, os extratos obtidos das brácteas da variedade Prata apresentaram os maiores teores de compostos fenólicos, assim como atividade antioxidante, sendo: 14,70 mg AGE g^{-1} para TPC, 82,57 μmol TE g^{-1} para ABTS, 22,26 μmol TE g^{-1} para DPPH e 47,09 μmol TE g^{-1} para FRAP.

Os compostos fenólicos identificados por HPLC nos extratos de brácteas de bananeira incluíram ácido ρ-cumárico, ácido ferúlico, ácido sinápico, ácido vanílico e rutina. Dessa forma, o processo de extração compõe uma das fases mais críticas para a obtenção de biomoléculas, as quais podem ser utilizadas para direcionar a aplicação de compostos como princípios ativos ou precursores de outras biomoléculas. Os avanços no desenvolvimento e o aprimoramento de tecnologias para a identificação de compostos devem ser vistos como uma ferramenta para uma melhor compreensão dos fenômenos biológicos.

Diante de diversos fatores que resultaram no crescimento populacional de forma exacerbada, dentro de um contexto de crescente produção global de alimentos e agravamento de problemas ambientais, é de suma importância a utilização de processos sustentáveis como a reutilização de resíduos agroindustriais. Neste sentido, este estudo permeou importantes pilares como o aspecto ambiental e social para agregar valor a um resíduo da colheita de banana o qual poderá incrementar a fonte de renda de pequenos e médios produtores rurais, contribuindo assim na oferta de ingredientes que promovam a saúde e o bem-estar dos consumidores.

Conclusão geral

Baseado nos resultados adquiridos foi possível demonstrar a relevância das brácteas de bananeira das variedades Maçã, Nanica e Prata como um recurso abundante e uma fonte potencial de compostos bioativos que promovem diversos benefícios à saúde humana. O estudo de diferentes técnicas de extração e fatores relacionados ao processo oferece diversas oportunidades de exploração da matéria-prima para obtenção de compostos antioxidantes, que vão desde os métodos convencionais e clássicos até métodos ambientalmente amigáveis. É válido ainda ressaltar como o estudo individualizado e particularizado de matérias-primas que possuem uma origem comum, mas pertencentes a diferentes variedades, como no caso das brácteas de bananeira avaliadas neste estudo, é de suma importância para identificar os processos e os parâmetros associados mais adequados para recuperação dos compostos de interesse.

Com isso, a principal contribuição científica deste trabalho visou o aproveitamento de uma matriz extensivamente produzida e distribuída mundialmente, de baixo custo e com o uso de procedimentos eficientes, que podem garantir o uso sustentável de um resíduo proveniente da bananicultura.

Perspectivas futuras

A utilização de brácteas de bananeira como fonte de compostos bioativos com propriedades antioxidantes revelou inúmeras oportunidades de investigação. Uma delas consiste no estudo das tecnologias mais adequadas para o processamento de inflorescência de bananeira para a preservação de seus compostos. Neste sentido, a compreensão das propriedades físico-químicas e tecnológicas da inflorescência também é de suma importância para identificar as melhores aplicações em produtos alimentícios.

Os extratos produzidos a partir desta matriz vegetal possuem uma complexidade de biomoléculas bioativas promissoras que exigem um nível aprofundado de identificação química exigindo a utilização de equipamentos e ensaios sensíveis. É válido ainda ressaltar que vincular uma estrutura química com a sua bioatividade não é um processo direto e simples. Essa correlação é apontada como um dos principais desafios no qual ainda há necessidade de uma pesquisa sistemática para melhoria da compreensão das propriedades antioxidantes de compostos naturais.

Tendo em vista que a quantidade total de bioativos presentes em matrizes alimentares não reflete necessariamente a quantidade absorvida e metabolizada pelo organismo humano, pesquisas sobre a digestão gastrointestinal simulada e sua biodisponibilidade no corpo humano é uma outra vertente de investigação. Em consonância com este tema, a utilização de modelos de absorção intestinal (culturas de células) associados a modelos de digestão in vitro para identificar com maior precisão os sistemas baseados em carreadores mais promissores para administração oral também serão potenciais avanços nesta área de pesquisa.

Contudo, potenciais avanços na identificação de biomoléculas e o conhecimento de suas atividades biológicas deve ser vislumbrado como uma perspectiva essencial no desenvolvimento e aplicação de novos ingredientes/produtos como fonte de antioxidantes naturais.

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Anexo - Comprovante de Cadastro de Acesso (SisGen)

Ministério do Meio Ambiente
CONSELHO DE GESTÃO DO PATRIMÔNIO GENÉTICO

SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO

Certidão

Cadastro nº AE04421

Declaramos, nos termos do art. 41 do Decreto nº 8.772/2016, que o cadastro de acesso ao patrimônio genético ou conhecimento tradicional associado, abaixo identificado e resumido, no Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado foi submetido ao procedimento administrativo de verificação e não foi objeto de requerimentos admitidos de verificação de indícios de irregularidades ou, caso tenha sido, o requerimento de verificação não foi acatado pelo CGen.

Número do cadastro:	**AE04421**
Usuário:	**UNICAMP**
CPF/CNPJ:	**46.068.425/0001-33**
Objeto do Acesso:	**Patrimônio Genético/CTA**
Finalidade do Acesso:	**Pesquisa**

Espécie

Musa acuminata

Fonte do CTA

CTA de origem não identificável

Título da Atividade:	**Brácteas de bananeira como fonte de substâncias biologicamente ativas: uma avaliação comparativa entre métodos de extração convencionais e hidrólise enzimática para recuperação de compostos bioativos**

Equipe

Ruann Janser Soares de Castro	**UNICAMP**
Karen Linelle de Oliveira Santos	**Unicamp**

Data do Cadastro:	**13/03/2019 16:01:08**
Situação do Cadastro:	**Concluído**

Conselho de Gestão do Patrimônio Genético
Situação cadastral conforme consulta ao SisGen em **18:03** de **26/07/2022**.

SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO - SISGEN