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A Review of Anthocyanin Extraction and Bioethanol Production from Fruit Residues

Extração de antocianinas e produção de bioetanol de resíduos de frutas: uma revisão

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ABSTRACT

The growth in fruit consumption worldwide has generated an increase in waste. One way to value this residue and reduce future environmental problems is by using some of the available bioactive compounds. Among the compounds found in this kind of waste are cellulose, hemicellulose, soluble sugars, reducing sugars, organic acids, and polyphenols, that is, biocompounds with potential industrial applications. Conventional or unconventional extraction techniques can recover these added-value compounds, such as anthocyanins, that act as natural dyes. Other processes can be applied to this residue, such as hydrolysis and fermentation, to obtain reducing sugars and produce biofuels. In this context, this review provides information about using fruit waste through anthocyanin extraction techniques and reducing sugars in bioethanol production, considering the importance of using biorefinery integrated into constructing a circular economy.

Keywords: value-added products, phenolic compounds, reducing sugars, biofuels, integrated biorefinery.

RESUMO

O crescimento do consumo de frutas no mundo tem gerado um umento na quantidade de resíduos. Uma forma de valorizar esse resíduo e reduzir futuros problemas ambientais é pelo aproveitamento de alguns compostos bioativos disponíveis. Entre os compostos encontrados nesse tipo de resíduo estão a celulose, a hemicelulose, os açúcares solúveis, os açúcares redutores, os ácidos orgânicos e os polifenóis, ou seja, biocompostos com potencial aplicação industrial. Diferentes técnicas de extração, convencionais ou não convencionais, podem ser usadas para recuperar esses compostos com valor agregado, como as antocianinas, que atuam como corantes naturais. Outros processos podem ser aplicados a esse resíduo, como hidrólise e fermentação, na obtenção de açúcares redutores e produção de biocombustíveis. Nesse contexto, este trabalho de revisão traz informações sobre o aproveitamento de resíduos de frutas por meio de técnicas utilizadas na extração de antocianinas e também sobre o uso dos açúcares redutores na produção de bioetanol, considerando a importância do uso da biorrefinaria integrada na construção de uma economia circular.

Palavras-chave: produtos com valor agregado; compostos fenólicos; açúcares redutores; biocombustíveis; biorrefinaria integrada.

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Introduction

Population growth and the search for better eating habits have required expanding fruit and vegetable production, especially in urban areas (Ganesh et al., 2022). With the increase in consumption and processing of these fruits and vegetables, the amount of agricultural waste generated also increases, which can result in an environmental problem if this waste does not receive adequate treatment and disposal (Liu, 2023).

Among the alternatives to using these agricultural residues is obtaining value-added products rich in organic compounds such as cellulose, soluble sugars, acids, and polyphenols (Magama et al., 2022). Some natural dyes, such as anthocyanins, can be extracted from these wastes and used as substitutes for synthetic dyes in the food and cosmetic industries (Belwal et al., 2019; Albuquerque et al., 2020). It is also possible to use organic compounds in these biomasses, such as soluble sugars, to produce biofuels (Casabar et al., 2020).

Anthocyanins, responsible for the blue, purple, and red colors in fruits and flowers, are pigments from the flavonoid classes and are essential in industry due to their antioxidant properties (Welch et al., 2008). The extraction of these anthocyanins from biomass can be done through different processes, such as solvent extraction (SE) (Galvão et al., 2020), pressurized liquid extraction (PLE) (Farooq et al., 2020), ultrasound-assisted extraction (UAE) (Xu et al., 2022), microwave-assisted extraction (MAE) (Kaderides et al., 2019), and pulsed electric field extraction (PEFE) (Bocker and Silva, 2022).

The large amount of sugars present in these residues also makes them second-generation biomass with potential use in the production of biofuels. Several studies report using these sugars as a substrate in fermentative processes that aim to produce alternative energy sources such as biogas and bioethanol (Allison and Simmons, 2018; Casabar et al., 2020; Chowdhary et al., 2021; Ganesh et al., 2022).

Using fruit and vegetable waste appears to be a sustainable approach to obtaining added-value products, thus fitting into a circular economy context. This review compiles studies that show the use of fruit biomass through the main processes currently used for the extraction of anthocyanins and the use of sugars in the production of biofuels.

Fruits as a source of biomass

The consumption of red, pink, purple, and blue fruits has become popular worldwide, partly due to their pleasant appearance and taste but also due to biocompounds beneficial to human health (Martinsen et al., 2020). The industry sells these fruits in the form of different food products (jellies, juices, ice creams, cereal bars, dietary supplements, and fermented products) (Bortolini et al., 2022) and also in the form of cosmetic products (soaps, exfoliating jellies, sunscreens, moisturizing creams, shampoos, and conditioners) (Cefali et al., 2019).

Fruits such as grapes (Allison and Simmons, 2018; Chowdhary et al., 2021), cherries (Uribe et al., 2023), raspberries (Xu et al., 2022), jabuticaba (Albuquerque et al., 2020; Galvão et al., 2020), mulberry (Guo et al., 2019), among others, are composed of a range of nutrients, being rich in phenolic compounds, such as phenolic acids (hydroxycinnamic and hydroxybenzoic acids), flavonoids (flavanols, flavonols, and anthocyanins), and stilbenes (resveratrol), found in varying concentrations in different portions of fruits (Albuquerque et al., 2020).

As a result of the industrial processing of these fruits, there is a large amount of residue formed by peels, pulps, and seeds, which, even after disposal, presents several value-added products such as cellulose, soluble sugars, organic acids, and phenolic compounds (Magama et al., 2022). The valorization of this fruit residue can occur through its use as a source of biomass, either for the extraction of biocompounds of industrial interest, such as anthocyanins, or in the use of sugars in the production of biofuels.

An alternative that makes the use of this large amount of residue viable is integrated biorefinery, which is based on the use of a sequence of treatments to obtain different helpful chemical compounds and has an essential role in the development of greener technologies, sustainability, and the concept of circular economy (Campos et al., 2020; Ferri et al., 2020; Paini et al., 2022) (Figure 1).

Anthocyanins

Anthocyanins are pigments from the flavonoid class, a subgroup of phenolic compounds (Welch et al., 2008). They are water-soluble anthocyanidin glycosides, which have two aromatic rings (A and B) in their structure, separated by a six-membered ring containing oxygen (C) (Zhao et al., 2017). These anthocyanidins are unstable in free form, so they are usually found with sugars such as glucose, galactose, arabinose, rhamnose, and xylose, linked at position 3 of the C ring (Ongkowijoyo et al., 2018). Around 150 structures are known, generally with hydroxyl or methoxyl groups occupying the R_1 and R_2 positions.

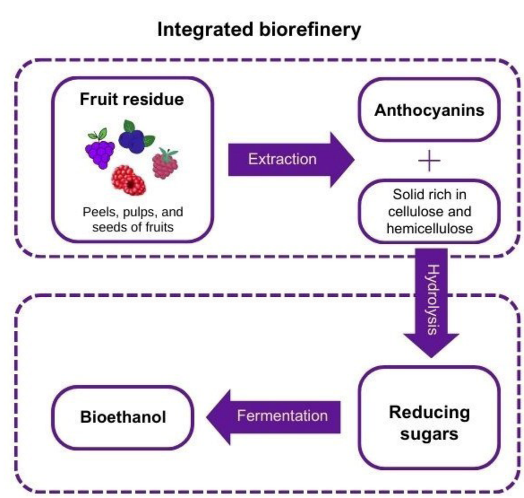


Figure 1 – Description of an integrated biorefinery process applied to fruit residue valorization.

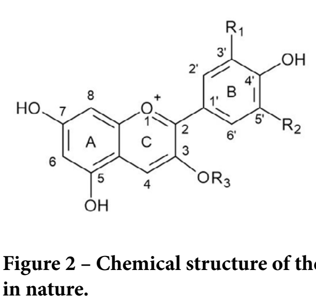
The most common of which are cyanidin, pelargonidin, peonidin, petunidin, delphinidin, and malvidin (Zhao et al., 2017; López et al., 2018) (Figure 2).

In nature, these pigments, secondary plant metabolites, are present in various fruits and vegetables and responsible for the blue, purple, and red colors (Flores et al., 2016). The color presented by anthocyanin is related to the number of hydroxyl and methoxyl groups in the R_1 and R_2 positions. Blue shades indicate the presence of more hydroxyl groups, while reddish shades indicate the presence of more methoxy groups. The pH also affects the color of anthocyanins, with shades ranging from red, purple, and blue in acidic, neutral, and essential solutions, respectively (Mortensen, 2006; Martin et al., 2017). Regarding stability, solutions with lower pHs present higher concentrations of anthocyanins because, in this pH range, anthocyanins are found in the form of the flavylium cation (Ahmadiani et al., 2019).

Anthocyanins are known for their antioxidant capacity, which is related to their chemical structure. The presence of a positive charge and electron-donating substituents makes anthocyanins suitable donors of hydrogen for free radicals, reducing the number of these radicals, preventing the formation of new ones, and thus acting to protect various biomolecules such as proteins, lipids, and DNA (Martin et al., 2017).

The concentration of these anthocyanins in fruits and vegetables varies according to how they are grown and processed. After extraction from nature, its color and stability are affected by light, pH, temperature, enzymes, water activity, oxygen, and other pigments (Weber et al., 2017). This instability of anthocyanins is one of the difficulties related to their use. Different processes, such as acylation, have been studied to solve the problem of industrial applications of these compounds (Luo et al., 2022).

Finally, anthocyanins have been widely used in the pharmaceutical industry due to the positive effects they bring to human health based on their antioxidant, anti-inflammatory, antibiotic, antidiabetic, and chemoprotective properties; in the food industry, they are widely used as natural colorants and also for their antioxidant effect (Weber et al., 2017; Ongkowijoyo et al., 2018).



Anthocyanin	R_1	R_2
Pelargonidin	H	H
Cyanidin	OH	H
Delphinidin	OH	OH
Peonidin	OCH_3	H
Petunidin	OCH_3	OH
Malvidin	OCH_3	OCH_3

Figure 2 – Chemical structure of the most common anthocyanins present in nature.
Source: adapted from Zhao et al. (2017).

Anthocyanin extraction

The extraction of bioactive compounds from fruits, such as anthocyanins, is an essential process for the subsequent analysis and use of these compounds. Various conventional and alternative extraction techniques are used worldwide to take advantage of the most varied matrices, such as solvent extraction, pressurized liquid extraction, ultrasound-assisted extraction, microwave-assisted extraction, and pulsed electric field extraction.

Solvent extraction

Solvent or solid–liquid extraction is a simple and cheap conventional technique widely used to obtain anthocyanins from fruits and vegetables (Galvão et al., 2020). In this technique, the sample is generally placed in an extraction thimble and inserted into a Soxhlet in the presence of the solvent of interest. During the process, the solvent passes through the biomass, thus promoting the extraction of phenolic compounds (Tan et al., 2022). The choice of solvent is made considering the characteristics of the analyte to be extracted. The most commonly used anthocyanin solvents are methanol, ethanol, acidified water, and acidified ethanol. Under these extraction conditions, the acidity of the medium helps in the penetration of the solvent into the tissues of fruits and vegetables and also in the stabilization of the extract containing anthocyanins, which are found in the form of the flavylium cation, providing greater yields (Ahmadiani et al., 2019; Galvão et al., 2020; Tan et al., 2022). Ferreira et al. (2020) report the extraction of anthocyanins from blueberry pomace using water acidified with 1% by weight of citric acid, with an anthocyanin yield of 171.7±0.70 mg/100 g, under conditions of 50°C for 5 min. Benchikh et al. (2021) describe using water acidified with 15% HCl 0.1 M for 9.36 min to obtain 38.04 mg/100 g of anthocyanins. According to Walkowiak-Tomczak and Czapski (2007), pHs above 3 can result in the degradation of anthocyanins due to instability, which may explain the lower yield found by Benchikh et al. (2021).

Pressurized liquid extraction

Pressurized liquid extraction, also known as accelerated solvent extraction, is a technique commonly used to obtain phenolic compounds, such as anthocyanins, and can serve as an alternative to solvent extraction (Setyaningsih et al., 2016; Farooq et al., 2020). Here, the solvents, generally the same ones used for SE, are subjected to temperatures ranging from 40 to 200°C and high pressures, 4 to 20 MPa, which keep the solvents in a liquid state even at temperatures above their boiling point. High pressures favor the diffusion of the solvent into the biomass tissues through the pores, increasing the contact of the solvent with the biocompounds and increasing recovery rates (Farooq et al., 2020). Pereira et al. (2019) used extraction with pressurized liquids to obtain 10.21 mg/g of anthocyanins from dried grape pomace, using as solvent a mixture of ethanol/water (50% (m/m)) and an extraction temperature of 40°C. A yield of 45.0 mg/g of anthocyanins from jambolan was obtained by Sabino et al. (2021) when the extraction was done using 80% v/v ethanol and 90°C for 10 min, corroborating what was said by Farooq et al. (2020) that higher pressures, in this case, arising from a higher temperature, favor the extraction of the biocompounds.

Ultrasound-assisted extraction

Ultrasound-assisted extraction has some advantages when compared to conventional anthocyanin recovery techniques. The factors that can lead to this technique being considered a green process are shorter extraction time, reduced solvents, and increased yield (Pinela et al., 2019). The phenomenon involved in ultrasound-assisted extraction consists of bubble cavitation, that is, the formation, growth, and collapse of these bubbles, resulting in a shear force. The energy generated in this process breaks the cell walls of the biomass, facilitating solvent penetration, improving solid–liquid mass transfer, and increasing the release of compounds present in this biomass (Backes et al., 2018; Bortolini et al., 2022). The effect of cavitation can lead to the degradation of anthocyanins, so for ultrasound-assisted extraction to be efficient, some variables such as ultrasound power, solid–liquid ratio, temperature, and extraction time must be observed and controlled during the process (Tan et al., 2022).

Fernandes et al. (2020) used jaboticaba peel to compare the extraction of phenolic compounds with and without ultrasound assistance. The highest yield of anthocyanins (7.9 mg/g) was obtained in ultrasound-assisted extraction using water acidified with HCl at 25 kHz for 20 min. The authors also reported a decrease in anthocyanin content between 20 and 40 min in extraction without ultrasound assistance, which was not noted in ultrasound-assisted extraction.

Liao et al. (2022) evaluated the extraction of anthocyanins using ultrasound frequencies ranging from 18 to 90 kHz. The highest yield, 796.9 μg/g of anthocyanins, was obtained in the ultrasound frequency range of 62–64 kHz. The assays using frequencies in the range of 78–90 kHz obtained a lower yield of anthocyanins, showing that using higher frequencies can lead to the degradation of these biocompounds.

Microwave-assisted extraction

Among the alternative techniques used for the extraction of biocompounds, microwave-assisted extraction has stood out. Reduction in solvent volume, shorter extraction time, higher recovery rates, good reproducibility, and lower cost-benefit are among the factors that have made this technique increasingly widespread (Alara and Abdurahman, 2019; Churyumov et al., 2021). Microwave-assisted extraction can be carried out in a closed container under controlled pressure and temperature or in an open container under atmospheric pressure and a maximum temperature defined according to the solvent used (Belwal et al., 2018). In this type of extraction, microwave irradiation induces the rotation of the solvent's polar molecules, generating rapid heating and collisions with the matrix, facilitating the separation of compounds from this matrix (Churyumov et al., 2021). Microwave power, irradiation time, temperature, and solvent concentration are generally the factors considered in this extraction technique (Alara et al., 2019). Da Rocha and Noreña (2020) reported the extraction of anthocyanins from grape pomace, using an acidic aqueous solution with 2% citric acid, with the optimal extraction conditions at 800 W for 7 min.

Pulsed electric field extraction

Pulsed electric field extraction is a non-thermal technique that consists of applying, for a short period, a low electric field (<10 kV/cm) and low energy (<10 kJ/kg), causing electroporation of cell membranes of biomass (Puértolas et al., 2013; Bobinaitė et al., 2015). This phenomenon makes the biomass matrix more permeable, facilitating the extraction of anthocyanins (Barba et al., 2016; Bocker and Silva, 2022). In addition to reducing the need to use large amounts of solvent, this technique also facilitates the use of high temperatures, contributing to the stability of anthocyanins during the extraction process (Bocker and Silva, 2022). Exposure time, electric field intensity, and total pulse energy input are generally the variables evaluated when optimizing the extraction of biocompounds using this method (Tena and Asuero, 2022). Carpentieri et al. (2023) describe the increase in the permeabilization of the cell membrane of the tissues that make up the red grape pomace through the optimization of extraction assisted by a pulsed electric field, resulting in a 23% increase in the content of anthocyanins extracted when compared to the control method.

In this context, Table 1 summarizes recent studies in anthocyanin extraction with different fruit residues as a source of biomass, extraction techniques, extraction conditions, and yields of anthocyanins obtained.

Utilization of solids after anthocyanin extraction

Within the integrated biorefinery concept, the extraction stage aims to facilitate access to target molecules, such as anthocyanins, increasing yield and promoting isolation without causing damage to the structure of these biocompounds using techniques such as those mentioned in this article, which include ultrasound-assisted extraction, microwave-assisted extraction, and pressurized liquid-assisted extraction. These techniques represent innovation and industrial maturity for this purpose. After extraction, the solid fraction can be valorized through hydrolysis to produce reducing sugars from cellulose and hemicellulose, biochemical conversions such as anaerobic digestion and fermentation for the production of fertilizers and biofuels respectively, or hydrothermal processes such as hydrothermal carbonization for the production of hydrochar (Paini et al., 2022).

Grape pomace, for example, is among the most studied raw materials (Paini et al., 2022), being considered a high-quality biodegradable residue with significant amounts of phenols, flavonols, and anthocyanins. Today, the extraction of bioactive compounds from this waste stands out as an alternative to using raw waste to produce biofuels, biocarbons, biopolymers, and biofertilizers (Sirohi et al., 2020).

In its context, the red fruit residue contains considerable amounts of cellulose, hemicellulose, lignin, and phenolic compounds. These cellulose and hemicellulose fractions can be converted into sugars that serve as substrates in fermentative processes, making fruit residue an attractive biomass for the rapid and satisfactory production of second-generation bioethanol (Mishra et al., 2022; Panahi et al., 2022).

So that these polysaccharides can be used as a source of sugar in the fermentation process, physical, physicochemical, chemical, or biological pretreatments are applied to break down the lignin present in the biomass matrix and release the polysaccharides. Afterward, hydrolysis processes, such as enzymatic hydrolysis, are necessary to convert polysaccharides into smaller sugars, such as glucose, xylose, arabinose, mannose, galactose, and rhamnose (Fakayode et al., 2021; Panahi et al., 2022). Finally, sugars are converted into CO_2 and bioethanol in the fermentation stage. Ethanol production uses yeast or some strains of bacteria, with *Saccharomyces cerevisiae* being the most used microorganism (Roukas and Kotzekidou, 2022). Table 2 shows examples of the use of different fruit residues in obtaining sugars by hydrolysis and the subsequent production of bioethanol by fermentation.In addition, Nguyen et al. (2022) studied bioethanol production from hydrolysis with fruit waste using hydrothermal pretreatment in an autoclave, followed by enzymatic enzyme cellulase and fermentation with *S. cerevisiae*, and the best bioethanol yield achieved 16.74 g/L in 24 h of fermentation. Similarly, Ratnadewi et al. (2023) used red dragon fruit peel as a source of glucose, obtained from cellulose hydrolysis with sulfuric acid, to produce bioethanol by fermentation.

Demiray et al. (2024) described the sequential anthocyanin extraction and bioethanol production from eggplant peel. Extraction was done using solvent and an ultrasound bath with a yield of 2306.1±3.5 mg/kg. In contrast, fermentation was carried out with *K luyveromyces marxianus* and *S. cerevisiae*, reaching yields of 26.6 and 27.5 g/L, respectively.

Table 1 – A review of recent studies exploring anthocyanin extraction from fruit residues.

Biomass	Extraction technique	Extraction conditions	Yield productivity of anthocyanins	References
Blueberry pomace	SE	Water acidified with 1% by mass of citric acid, 100°C, and 5 min	171.7±0.7 mg/100 g pomace	Ferreira et al. (2020)
Purple passion fruit peel	SE	pH 2.0, solid–liquid ratio 29.79 g of solvent per gram of raw material, 52.03°C, and 180 min	577.59 mg/100 g dry peel	Herrera-Ramirez et al. (2020)
Strawberry	SE	pH 1.3, water acidified with 15 % of HCl 0.1 M, 586 rpm, solid–liquid ratio 1.26 g/40 mL, and 9.36 min	38.04 mg/100 g FW	Benchikh et al. (2021)
Grape marc	PLE	pH 2.0, ethanol–water 50% w/w, 40°C, and 40 min	10.21 mg/g dried grape marc	Pereira et al. (2019)
Jambolan	PLE	Ethanol 80% v/v, 90°C, 10 min, and 2 extraction cycles	45.0 mg/g DF	Sabino et al. (2021)
Jabuticaba peel	UAE	pH 1.5, water acidified with HCl, 25 kHz, and 20 min	7.9 mg/g dry peel	Fernandes et al. (2020)
Mulberry skin residue	UAE	305 W, 45°C, and 40 min	5.212 mg/g extract	Li et al. (2020)
Blueberry pomace	UAE	400 W, 40°C, and 40 min	108.23 mg/100 g DW	Zhang et al. (2022)
Blueberry powder	MAE	Ethanol 60%, solid–liquid ratio 1:30, 800 W (1st stage), and 280 W (2nd stage)	84.82%	Liu et al. (2019)
Grape pomace	MAE	Water acidified with 2% citric acid, 1,000 W, and 10 min	45%	Da Rocha and Noreña (2020)
Sour cherry peel	MAE	Ethanol 80%, 500 W, and 90 s	12.47 mg/g FM	Şahin et al. (2021)
Jabuticaba peel	MAE	pH 6, methanol 30%, solid–liquid ratio 4.7 mg/mL, 81°C, 800 W, and 10 min	9.7±0.28 mg/g jabuticaba peel	Barroso et al. (2023)
Blackcurrant	PEFE	1318 V/cm and 315 pulses (100 ms for each pulse)	1.38 mg/g extract	Gagneten et al. (2019)
Grape pomace	SE + PEFE	Ethanol 50%, 50°C, and 300 min 4.6 kV/cm – 20 kJ/kg and pulse width of 20 s	1.03 mg/g DM	Carpentieri et al. (2023)

SE: solvent extraction; PLE: pressurized liquid extraction; UAE: ultrasound-assisted extraction; MAE: microwave-assisted extraction; PEFE: pulsed electric field extraction.

Table 2 – Production of bioethanol from fruit waste.

Biomass	Hydrolysis	Microorganism	Bioethanol yield	References
Banana peduncle	Dilute sulfuric acid	*Kluyveromyces marxianus*	21.89 g/L	Sathendra et al. (2019)
Pineapple peels	Cellulase and *T richoderma harzianum* inoculum	*Saccharomyces cerevisiae*	197.6±9.9 g/L	Casabar et al. (2020)
Apple pomace	Cellulase and xylanase	*S. cerevisiae* and isolate APW-12	49.6 g/L	Kumar et al. (2020)
Coconut meal	Cellulase	*S. cerevisiae*	8.5 g/L	Sangkharak et al. (2020)
Pomegranate peels	Cellulase	*S. cerevisiae*	12.9 g/L	Mazaheri et al. (2021)
Banana	Enzimatic (Pectinex Ultra SP-L)	*S. cerevisiae Angel*	31.87 g/L	Favaretto et al. (2023)
Citrus peels	Viscozyme®	*S. cerevisiae*	66%	Teke et al. (2023)

Few works in the literature, such as the one mentioned earlier, apply the integrated biorefinery concept to obtain anthocyanins and produce bioethanol. Thus, the authors highlight the need to expand studies in the sequential obtaining of biocompounds, such as anthocyanins and bioethanol, especially from fruit waste, aiming at the importance of an integrated biorefinery in developing a circular economy.

Conclusion

Fruit pomace is a source of value-added compounds such as anthocyanins and sugars. Several studies show that the valorization of this residue can occur through the extraction of biocompounds using conventional techniques, such as solvent extraction, and through unconventional methods, such as extraction with pressurized liquids, ultrasound-assisted extraction, microwave-assisted extraction, and pulsed electric field extraction. The integrated biorefinery also shows that fruit pomace can be used after the extraction of anthocyanins as an essential source of sugars for the fermentable production of biofuels, especially bioethanol. In conclusion, there is still a lot to be explored. At present, fruit biomass is essential in the context of the circular economy, as well as in developing ways to value this waste, whether generating business opportunities or contributing to resolving environmental and economic issues.

Contribution of authors:

SALDANHA, L.;F.: conceptualization, data curation, formal analysis, funding, acquisition, investigation, methodology, project administration, resources, software, validation, visualization, writing — original draft, and writing — review and editing. TREICHEL, H: conceptualization, data curation, formal analysis, funding, acquisition, investigation, methodology, project administration, resources, software, supervision, validation, visualization, writing — original draft, and writing — review and editing.

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