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USE OF PASSION FRUIT PEEL (PFP) HYDROLYSATE AS SUBSTRATE FOR BIOTECHNOLOGICAL XYLITOL PRODUCTION

Aida Aguilera Infante Neta[1*], Alan Portal D'Almeida[2], Tiago Lima de Albuquerque[1]

*[1]Department of Food Engineering, Center for Agricultural Sciences, Federal University of Ceará, Fortaleza, Brazil. (*aidaaguilera@alu.ufc.br)*
[2]Department of Chemical Engineering, Center for Technology, Federal University of Ceará, Fortaleza, Brazil.

ABSTRACT: Brazil, a global agribusiness powerhouse, is the largest producer of passion fruit (Passiflora edulis). The majority is used in the food industry, resulting in a staggering 523,394 tons of waste (2022), with the peel being the primary component. This research takes a novel approach by exploring the use of this lignocellulosic material for the generation of new products, a promising avenue for waste management. The study focuses on the use of passion fruit peel for the release of fermentable sugars that can be converted into xylitol, a sugar substitute in foods. The peel was meticulously characterized, and its hemicellulosic fraction was hydrolyzed to produce xylose, the main sugar used in xylitol generation. The hydrolysis process yielded 19 g/L of xylose, which translated to a production of 0.69 g/L in the neutralized medium. However, after a detoxification process that removed impurities, xylitol production skyrocketed by 53.1%. This innovative research demonstrates the potential of producing a high-value sweetener from a commonly discarded material, setting the stage for further studies and the development of new techniques to utilize lignocellulosic waste for polyol production.

KEYWORDS: Passion fruit peel; Acid hydrolysis; *Kluyveromyces*; Xylitol; Sustainable.

1 INTRODUCTION

Brazil stands out as the world's largest producer of yellow passion fruit (*Passiflora edulis*), with its pulp highly appreciated in both international and domestic cuisine for the production of juices, sweets, and sauces (Ferreira et al., 2022 e Santos et al., 2023). In 2022, the national production reached 697,859 tons, with the states of Ceará, Bahia, and Santa Catarina standing out, representing 25.8%, 25.4%, and 5.6% of the national production value, respectively (IBGE, 2022). The passion fruit processing industry generates a significant amount of waste, composed of pulp, seeds, and primarily passion fruit peel (PFP), which account for about 75% of the fruit's mass, totaling 523,394 tons of waste generated industrially in 2022, for example (Francischini *et al.*, 2020).

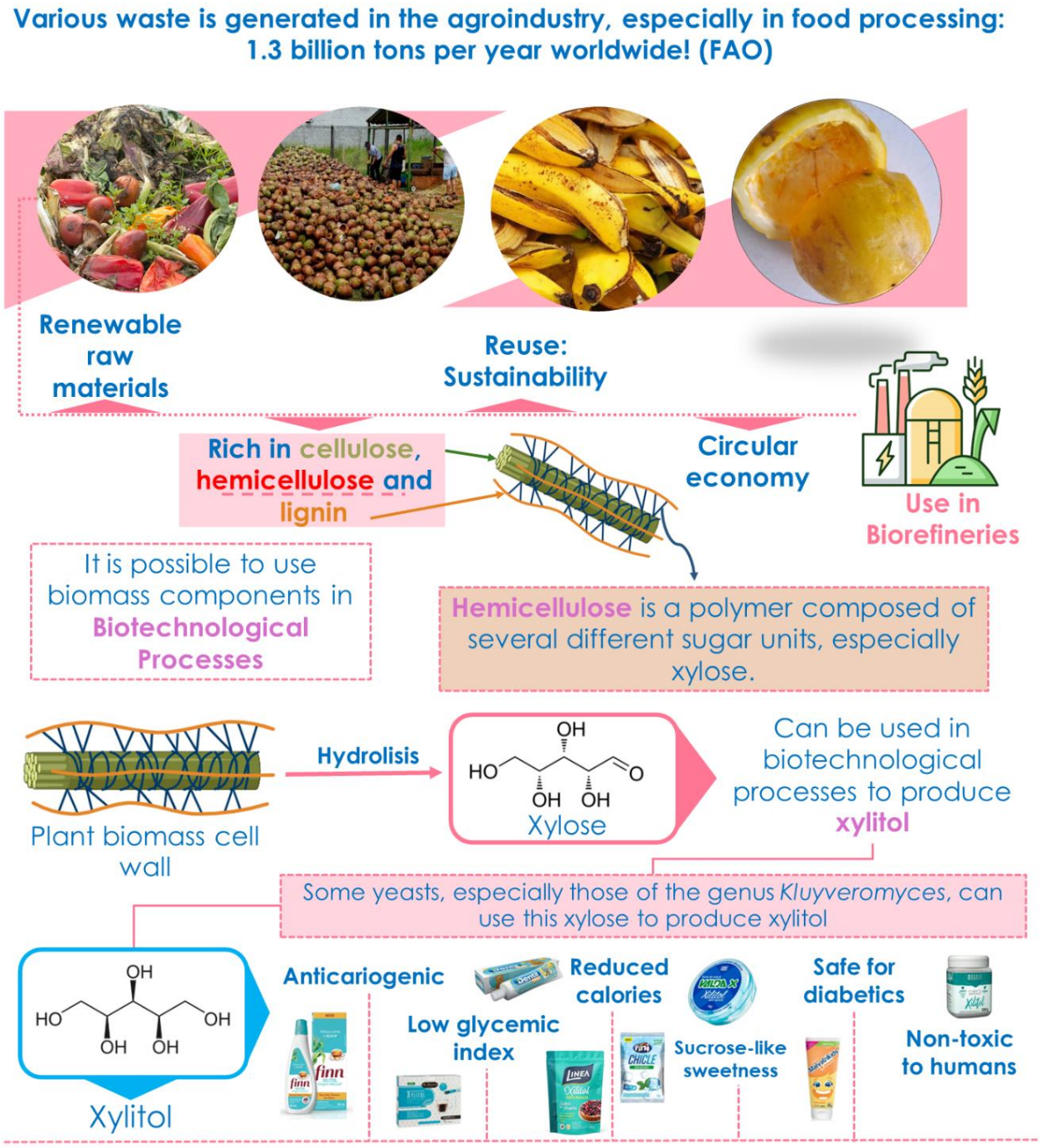
One strategy to mitigate the environmental impacts resulting from the large volume of agro-industrial waste is the valorization of these biomasses. By employing technologies that utilize these wastes as raw materials for producing high-value compounds, it is possible to effectively utilize them integrally on an industrial scale, aligning with the principles of green economy, sustainability, and total biomass utilization. The use of lignocellulosic waste for the production of higher-value compounds, such as polyol carbohydrates, substitutes for sugars, and conventional sweeteners, is prominent in the scientific realm, as exemplified by the utilization of cashew bagasse (de Albuquerque *et al.*, 2023), sugarcane bagasse (Narisetty *et al.*, 2021) and corn cobs (Du *et al.*, 2020) can be utilized for the generation of fermentable sugars. Corn cob is primarily composed of pectin, cellulose, hemicellulose, and lignin (Santos *et al.*, 2023), which can be utilized to produce sugars such as glucose and xylose. These sugars, in turn, can be converted into industrially relevant compounds (de Albuquerque *et al.*, 2023).

Xylitol stands out among the compounds produced from these sugars as a substitute for sucrose due to its low caloric content, insulin-independent metabolism, and potential for caries prevention. (Albuquerque *et al.*, 2014; de Albuquerque *et al.*, 2023; Mäkinen, 2000). This polyol is produced on an industrial scale through the chemical hydrogenation of xylose. However, this method entails high operational costs due to the high temperatures and pressures involved and the required purification steps (de Albuquerque *et al.*, 2023; Santos *et al.*, 2008).

In this context, new technologies have been seeking to produce xylitol through microbial pathways, utilizing yeast capable of converting xylose into this biocompound (de Albuquerque *et al.*, 2023; Du *et al.*, 2020; Narisetty *et al.*, 2021). Therefore, this study aims to investigate the possibility of developing

a fermentative process aimed at producing xylitol from the hemicellulosic hydrolysate of passion fruit peel. The Figure 1 displays the conceptual diagram of this work's objective, aiming to contribute to the promotion of scientific and technological processes for the efficient utilization of this agro-industrial waste, thereby advancing the circular economy and striving to balance environmental and economic aspects, through the production of high value products.

Figure 1: Conceptual diagram illustrating the potential of utilizing lignocellulosic waste as raw material for xylitol production.



2 MATERIAL AND METHODS

2.1 Materials

Glucose and $(NH_4)SO_2$ were obtained from *Neon Comercial* (Suzano, SP); D-xylose, peptone, activated charcoal, and yeast extract were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO); H_2SO_4 was obtained from *Exodo Científica* (Sumaré, SP). H_3PO_4 was obtained from *Dinâmica Química Contemporânea* LTDA (Indaiatuba, SP). The Passion fruit (*Passiflora edulis*) peel (PFP) was obtained from the local juice production market in the city of Fortaleza, Ceará, Brazil.

2.2 Characterization of passion fruit peel (PFP)

The passion fruit peels (PFP) were washed three times, dried at 60 °C, crushed to obtain passion fruit peel in powder (PFPP), and standardized between 0.17 mm and 0.84 mm (20 and 80 meshes). The PFPP was stored at 25 ºC for use in subsequent experiments. The characterization of the concentration of cellulose, hemicellulose, lignin, extractives, and ash in the material was carried out using a methodology (Gouveia *et al.*, 2009).

2.3 Obtaining fermentable sugars.

Fermentable sugars (glucose, xylose, and arabinose) were obtained through hydrolysis of the hemicellulose in PFPP (de Albuquerque *et al.*, 2023). Thus, an H_2SO_4 (0.7 M) heat treatment was carried out for 22 minutes at 121 °C, previously optimized conditions. The proportion of PFPP biomass was kept constant at 10% (w/v). After hydrolysis, the liquid fraction was separated by filtration, neutralized with $Ca(OH)_2$ until pH 6.5, and analyzed to determine the final sugar concentration.

2.4 Microorganism and inoculum production

The *Kluyveromyces marxianus* ATCC 36907 strain, obtained by the Biotechnological Processes Research and Development Group (GPBio), was used in this research for xylitol production. The yeast was stored in Petri dishes containing YEPD medium (10 g/L yeast extract, 20 g/L peptone, 20 g/L dextrose, and 20 g/L agar). Then, three colonies were transferred to 125 mL Erlenmeyer flasks containing 50 mL of YEPD broth of the same composition for inoculum production, in orbital shaker for 24 h at 30 ºC and 200 rpm.

2.5 Xylitol Production by *K. Marxianus*

Xylitol production from PFPP was carried out in 125 mL Erlenmeyer flasks containing 50 mL of neutralized PFPP hydrolyzate and maintained at 30 °C and 200 rpm for up to 120 h, using an initial cell concentration of 1.0 g/L transferred from the previously produced inoculum. As a control, the synthetic media MX (D-xylose, 40 g L^{-1}, yeast extract, 10 g L^{-1} and $(NH_4)SO_2$, 5 g L^{-1}) and MXG (D-xylose, 40 g L^{-1}, D-glucose, 20 g L^{-1}, yeast extract, 10 g L^{-1} and $(NH_4)SO_2$, 5 g.L^{-1}) (de Albuquerque *et al.*, 2015).

2.6 Evaluation of PFPP hydrolyzate detoxification treatment

The neutralized hydrolyzate was detoxified with activated charcoal (1% w/v) for 1h, at 30 ºC and 200 rpm, to reduce the concentration of inhibitors present in the culture medium, according to a methodology adapted from Albuquerque et al. 2023.

2.7 Analytical Methods

2.7.1 Cell growth

Cell biomass growth was assessed by measuring the samples' optical density using a UV-visible spectrophotometer at 600 nm. The biomass concentration, expressed in g/L, was then determined using a calibration curve correlating the dry weight (g/L) with the optical density at 600 nm.

2.7.2 Determination of carbohydrate concentration

To determine the concentration of cellulose and hemicellulose components, high-performance liquid chromatography (HPLC) was performed on a Waters system (Waters, Milford, MA, USA), using Aminex HPX-87H (Bio-Rad, Hercules, CA, USA), and the eluent consisted of 5 mmol/L H_2SO_4, with a flow rate of 0.5 mL/min at 65 °C. The concentration of glucose, xylose, arabinose, ethanol, and xylitol was carried out by high-performance liquid chromatography (HPLC) using a Thermo HPLC system (Waters, Waltham, MA, USA) equipped with a Supelco 610-H column with H_3PO_4 mobile phase. 0.1% (v/v), under a flow of 0.5 mL/min at 30 ºC. Samples were identified by comparing retention times with those of previously analyzed standards.

2.7.3 PFPP morphological assessment

Structural and morphological changes were evaluated by scanning electron microscopy (SEM, FEF Quanta 450) with energy dispersive X-ray spectroscopy (EDS). Samples were pre-conditioned with carbon tape and covered with silver using a Quorum QT150ES metalizer. The SEM chamber was

subjected to a pressure of 10 Pa, with an electronic incidence radius of 20 kV.

3 RESULTS AND DISCUSSION

3.1 PFPP characterization and obtaining fermentable sugars

The characterization obtained from the material analyzed indicated a concentration of extractables lower than that reported in previous works for other biomass (Santos et al. 2023) (Table 1), which may indicate the lower concentration of fats and organic acids in passion fruit. The total portion of lignin corresponds to 12.29% of the total mass, which represents the potential of this residue for use in the production of lignin-based compounds. A low ash content was also observed, indicating the low presence of inorganic compounds and salts (da Costa Correia *et al.*, 2022; Santos *et al.*, 2023). The material had 68.83% hemicellulose content, represented by the sum of the percentage of cellulose (48.03%) and hemicellulose (20.8%), both carbohydrates that can be hydrolyzed to produce fermentable sugars (glucose and xylose).

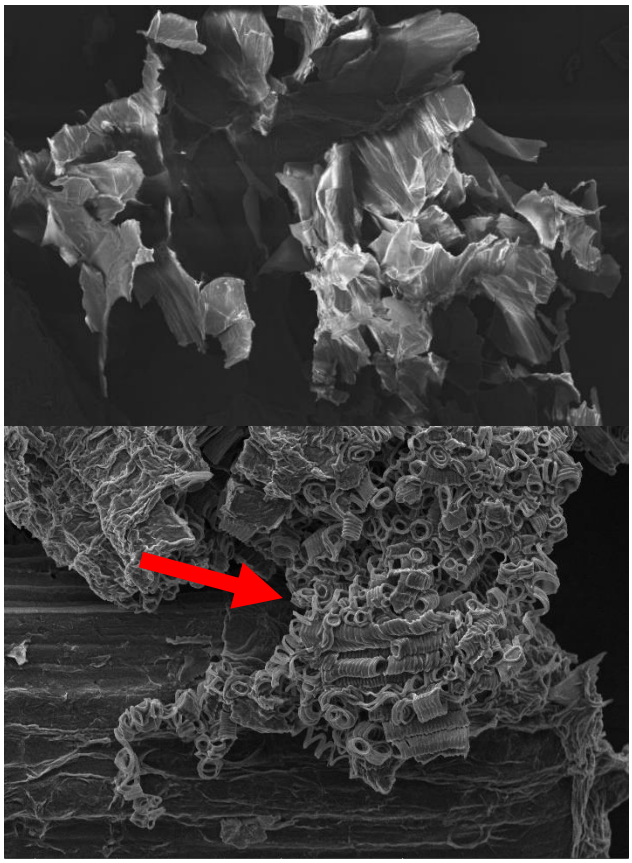
Table 1: Characterization of dried yellow passion fruit peel

Compound	Fraction (%)
Cellulose	**48.03 ± 0.5**
Cellobiose	*3.96* ± 0.5
Glucose	*25.03*± 0.1
Formic acid	*15.26*± 0.2
HMF	*0.4* ± 0.1
Hemicellulose	**20.8 ± 0.9**
Xylose	*1.73* ± 0.03
Arabinose	*1.0* ± 0.03
Galactose	*10.9* ± 0.05
Furfural	*0.00* ± 0.0
Acetic acid	*7.17* ± 0.1
Extractable	**17.47 ± 0.7**
Soluble lignin	**8.68 ± 0.25**
Insoluble lignin	**3.61 ± 0.19**
Ash	**1.75 ± 0.58**
Total	**100.34**

From the data obtained, it is also possible to determine that PFP has the potential to release sugars to be used in the production of xylitol, in particular, xylose (de Albuquerque *et al.*, 2023). Thus, after the acid hydrolysis of hemicellulose, carried out with PFPP, a high xylose concentration was obtained (19 g/L), which can produce xylitol.

After the acid hydrolysis of PFP, fermentable sugars can be released, and morphological changes in the material's structure can also be noticed after treatment. In Figure 2A, the morphological structure of passion fruit flour can be seen before hydrolysis. In Figure 2B, after hydrolysis, the exposure of cellulose and the loss of its crystalline structure is notable, causing the release of sugars, which also reinforces the efficiency of the process in releasing xylose from this residue, highlighting its potential use to produce xylitol.

Figure 2: Morphological analysis of the PFP (A) before and (B) after acid hydrolysis with H_2SO_4 (0.7 M) for 22 min at 121 °C.



3.2 Xylitol production

After hydrolysis of PFPP to obtain xylose, sugars were fermented to produce xylitol (Table 2). The bioprocess took place in a shaker at 200 rpm at 30 ºC for 120h.

Table 2: Glucose, xylose, and arabinose remaining in the culture medium and production of xylitol, acetic acid, and ethanol by *K. marxianus* 36907 for 120 h at 30 ºC and 200 rpm

Culture medium	Glucose (g/L)	Xylose (g/L)	Arabinose (g/L)	Xylitol (g/L)	Acetic acid (g/L)	Ethanol (g/L)
MXG	0.00 ± 0.00[a]	21.82 ± 1.36[a]	0.00 ± 0.00[a]	0.80 ± 0.24[a]	0.06 ± 0.02[a]	0.42±0.12[a]
MX	0.00 ± 0.00 [a]	10.27 ± 2.34[b]	0.00 ± 0.00[a]	4.94 ± 0.14[b]	0.12 ± 0.05[b]	0.43±0.15[a]
PFPP hydrolyzed and neutralized	0.18 ± 0.02[b]	2.44 ± 0.08[c]	1.15 ± 0.07[b]	0.69 ± 0.12[c]	0.01 ± 0.01[c]	0.40±0.03[a]
PFPP hydrolyzed, neutralized, and detoxified	0.18 ± 0.06 [b]	0.20 ± 0.01[d]	0.86 ± 0.04[c]	1.30 ± 0.10[d]	0.20 ± 0.01[d]	0.63±0.04[c]

It can be observed that glucose is consumed in all culture media, and arabinose is consumed almost entirely, except in media produced from the hydrolysis of PFPP. In the MXG medium, it is observed that xylose remains in high concentrations after 120 h, which results in a lower production of xylitol in the MXG medium (0.80 ± 0.24 g/L) compared to the MX medium (4.94 ± 0.14 g/L), which is an indication that in the presence of high concentrations of glucose, the microorganism tends towards metabolic routes that favor the consumption of glucose, disfavoring the production of xylitol (de Albuquerque *et al.*, 2015).

Xylitol production was more significant in media containing a higher xylose concentration without glucose (MX). In media obtained from acid hydrolysis of PFPP, lower production was obtained than that observed in the synthetic medium (MX), indicating the presence of inhibitors that cause production deviation. This can be observed by the 53.1% increase in the production of this polyol after detoxification of the culture medium with activated charcoal. This technique has already been used in previous reports to increase xylitol production due to its adsorptive potential that allows the removal of inhibitory compounds such as phenolics and other volatiles (de Albuquerque *et al.*, 2015).

High glucose concentrations in culture media tend to direct microbial metabolism towards routes that prioritize glucose consumption, inhibiting xylitol production. This phenomenon, known as the Crabtree effect, is evidenced by the lower production of xylitol in the MXG medium compared to the MX medium (Lee *et al.*, 2021). Furthermore, inhibitors originating from the acid hydrolysis of PFPP contribute to metabolic deviation, resulting in a lower production of xylitol. The application of detoxification techniques, such as activated charcoal, effectively removes these inhibitors, significantly increasing xylitol production. (de Albuquerque et al., 2023)

It was also observed that the production of acetic acid was better in media where a more outstanding production of xylitol was obtained, MX medium (4.94 g/L) and detoxified hydrolyzed medium (MHD) (1.30 g/L), which may be an indication that this organic acid is a co-product of the production of this polyol. This results in a decrease in the pH of the medium after production, which may explain the end of production before the total consumption of sugars, such as xylose, in the MX medium (0.12 g/L) and the MHD medium (0.20 g/L). Ethanol production remained constant in almost all assays evaluated, showing no significant difference in the analysis of variance (ANOVA) at 95% confidence, indicating that ethanol production probably does not affect xylitol production under the conditions studied however increase was observed in the medium MHD, which presented a higher ethanol production in comparison to other media.

Thus, it was possible to increase the production of xylitol in the MHD medium, obtaining a final xylitol concentration of 1.30 g/L. The presence of glucose in the culture medium resulting from the hydrolysis of cellulose probably resulted in metabolic deviations that reduced the yield of the synthetic medium; however, optimizing techniques, such as detoxification, is effective in increasing this production.

4 CONCLUSIONS

Thus, this study demonstrated that the production of xylitol from hydrolyzed passion fruit peel flour (PFP) is possible. After hydrolysis, high concentrations of xylose (19 g/L) were obtained, which were converted into xylitol (1.30 g/L). The use of detoxification techniques was effective in increasing this production, generating a 53.1% higher result. Therefore, new technologies can be applied to further increase the viability of this synthesis. Thus, looking for new ways of using waste, following the contemporary models of green chemistry and circular economy, which seeks to use agro-industrial waste that is still little used.

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REFERENCES

ALBUQUERQUE, Tiago Lima de *et al.* Biotechnological production of xylitol from lignocellulosic wastes: A review. **Process Biochemistry**, [*s. l.*], v. 49, n. 11, p. 1779–1789, 2014.

DA COSTA CORREIA, Jéssyca Aline *et al.* Different design configurations of simultaneous saccharification and fermentation to enhance ethanol production from cashew apple bagasse pretreated with alkaline hydrogen peroxide applying the biorefinery concept. **Biomass Conversion and Biorefinery**, [*s. l.*], v. 12, n. 7, p. 2767–2780, 2022.

DE ALBUQUERQUE, Tiago Lima *et al.* Xylitol production by different yeasts: Kinetic study and biosynthesis from cashew apple bagasse hydrolysate. **Canadian Journal of Chemical Engineering**, [*s. l.*], v. 101, n. 7, p. 3668–3679, 2023.

DE ALBUQUERQUE, Tiago Lima *et al.* Xylitol production from cashew apple bagasse by Kluyveromyces marxianus CCA510. **Catalysis Today**, [*s. l.*], v. 255, p. 33–40, 2015.

DU, Cong *et al.* Production of bioethanol and xylitol from non-detoxified corn cob via a two-stage fermentation strategy. **Bioresource Technology**, [*s. l.*], v. 310, n. March, p. 123427, 2020.

FERREIRA, C. C. *et al.* Agronomic Performance of Passion Fruit Genotypes in the Federal District, Brazil. **Journal of Agricultural Science**, [*s. l.*], v. 14, n. 5, p. 116, 2022.

FRANCISCHINI, Danielle Da Silva *et al.* Development and application of green and sustainable analytical methods for flavonoid extraction from Passiflora waste. **BMC Chemistry**, [*s. l.*], v. 14, n. 56, p. 1–11, 2020.

GOUVEIA, Ester Ribeiro *et al.* Validação de metodologia para a caracterização química de bagaço de cana-de-açúcar. **Química Nova**, [*s. l.*], v. 32, n. 6, p. 1500–1503, 2009.

IBGE - INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA. Sistema IBGE de Recuperação Automática – SIDRA. Produção Agropecuária, 2023. Available in: https://www.ibge.gov.br/explica/producao-agropecuaria/maracuja/br. Acesso em: April 07 of 2024.

LEE, Sae-Byuk *et al.* Crabtree/Warburg-like aerobic xylose fermentation by engineered Saccharomyces cerevisiae. **Metabolic Engineering**, [*s. l.*], v. 68, p. 119–130, 2021.

MÄKINEN, K. K. Can the pentitol-hexitol theory explain the clinical observations made with xylitol?. **Medical Hypotheses**, [*s. l.*], v. 54, n. 4, p. 603–613, 2000.

NARISETTY, Vivek *et al.* High level xylitol production by Pichia fermentans using non-detoxified xylose-rich sugarcane bagasse and olive pits hydrolysates. **Bioresource Technology**, [*s. l.*], v. 342, p. 126005, 2021.

SANTOS, Jéssyka R. *et al.* Effect of different pretreatments of Passiflora edulis peel biomass on the conversion process into bioproducts for biorefineries. **Sustainable Chemistry for the Environment**, [*s. l.*], v. 2, p. 100013, 2023.

SANTOS, Diego T. *et al.* Use of sugarcane bagasse as biomaterial for cell immobilization for xylitol production. **Journal of Food Engineering**, [*s. l.*], v. 86, n. 4, p. 542–548, 2008.