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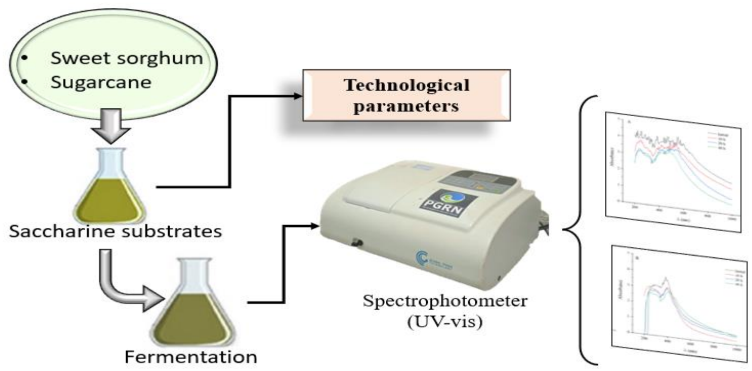
# The Use of UV-vis Spectroscopy Technique for Investigation of Organic Compounds in Saccharine Substrates

Maria do Socorro Mascarenhas [a], Margareth Batistote [a], and Claudia Andrea Cardoso* [a]

Sugar substrates are rich in organic compounds. However, its concentration may vary depending on the culture. Thus, the objective of this work was to evaluate the composition of sugarcane and sorghum juices using the UV-vis technique. An evaluation of the composition of the substrates was carried out and a pre-inoculum with YPSAC 5% medium, where 0.10 grams of FT858 yeast was inoculated and kept incubated at 30°C for 24h at 250rpm. The cells were recovered by centrifugation, resulting in a concentration of 10 mg mL$^{-1}$ of wet mass that was inoculated into 50 mL of the previously prepared fermentation medium with a concentration of 22°Brix and pH 5.0. Aliquots were taken for evaluation of molecular absorption spectra using a spectrophotometer (Global Trade Technology). These substrates are composed of water and fermentable sugars. The evaluation of the molecular absorption spectra showed a difference in the profile of the substrates as well as in the subsequent hours of fermentation, with a decrease in the intensity of the peaks being observed that may be related to the absorption of nutrients by the yeast. vis shows promise for this type of analysis.

## Graphical abstract




## Keywords

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## 1. Introduction

The growing need to expand the use of renewable energy sources in a sustainable way has driven research on the production of biofuels. However, these alternative sources must have attributes that allow meeting the demand for energy with a view to solidifying global energy security and mitigating the effects of global warming [1]. In fact, the use of renewable natural resources, biomass, as sources of energy can result in economic, social and environmental benefits.

Biomass are crops that have high energy potential and can

[a] Programa de Pós-Graduação em Recursos Naturais PGRN da Universidade Estadual de Mato Grosso do Sul/UEMS. Rodovia Dourados/Itahum Km 12 -Cidade Universitária, zip code 79804-970, Dourados Mato Grosso do Sul, Brazil. *Corresponding author. E-mail: **claudia@uems.com**

be used as raw material for various products such as biofuels and other biocommodities [2]. The global situation shows that there is an eminent need for changes, especially in relation to the use of renewable energy sources compared to fossil fuels, in this sense, biomasses are promising sources that have characteristics that make them an option to meet this demand and that depend only on the transformation technologies [3].

In this scenario, Brazil has been standing out, as it has a climate and soil conducive to the cultivation of biomass, as well as technology for the processing and transformation of these raw materials [4]. An example of success is the production of bioethanol, which was boosted by the National Alcohol Program (Proálcool), which encouraged both the expansion of distilleries and subsidized the development of ethanol vehicles, besides the mixing of ethanol with gasoline and research and development of technologies applied to ethanol production, which resulted in the technological advancement of this important agribusiness sector [5].

Brazil has established itself as the second largest producer and important world exporter of ethanol with the raw material, sugarcane, considered the most profitable in terms of production/conversion into a final product and economically profitable [6]. Brazilian ethanol is the most efficient in terms of mitigating greenhouse gas (GHG) emissions through the carbon balance, when compared to ethanol obtained from other raw materials such as wheat, sugar beet and corn [7].

A crop similar to sugarcane that has been attracting interest for the production of ethanol is sweet sorghum [*Sorghum bicolor* (L.) Moench], which is being recognized as a versatile energy crop to be used in the production of ethanol, as it has a high biomass productivity with a low production cost. It is a tolerant crop to water stress that can be cultivated in different soils and provides grains and stems, that can be used in different production processes [8]. Sweet sorghum is easy to grow, planted by seeds and with the possibility of obtaining multiple crops per year [9], which can collaborate to expand the production capacity of processing units and provide greater flexibility to the harvest.

The Brazilian sugar-alcohol sector, despite having a consolidated process [10], new technologies can still be introduced that provide improvements to the biotransformation process, as highlighted Lopes et al. [11], in addition, some stages of the process must be monitored more frequently, such as juice extraction and fermentation, which still need improvement. Another essential element is related to the nutritional conditions of the substrate to be fermented, as it must contain nutrients to ensure the good metabolism of the yeasts.

Saccharomyces cerevisiae are excellent ethanol producers, however they need a substrate composed of carbon and nitrogen sources that can be metabolized by these microorganisms in order to also ensure their physiological integrity, therefore, the fermentation medium must contain amounts of essential nutrients for a good yeast performance, which will result in better ethanol production [12].

In fact, these substrates are formed by organic compounds and have a characteristic color. The color precursors in sugarcane juice are reducing sugars and amino acids, that when heated produces colored polymers of high molecular weight [13] and which can be analyzed by different techniques. In this context, UV-vis spectrometry stands out, as it can be used to evaluate changes in the profile of samples. According to Valderrama et al. [14], it is a simple technique, but it can provide important information such as the possible presence of organic compounds, although it is not very selective. Therefore, this study aimed to evaluate the profile of sugarcane juice and sorghum-based saccharine substrates using the UV-vis technique.

## 2. Results and Discussion

Three The analysis of the composition of sugarcane juice and sweet sorghum carried out by Rochón et al. [15], shows that there is a similarity in composition, but that they differ in concentration. It is noted that these substrates are composed of water and sucrose, being 76% sucrose and 40% water for sugarcane, whereas saccharine sorghum has 75% sucrose and 32% water. Sugarcane juice contained 2% glucose and 2% fructose, while sorghum contained 5% glucose and 3% fructose (Figure 1A and 1B). These are promising substrates to be used in biotechnological processes such as ethanol production, as they present high concentrations of fermentable carbohydrates, which favor the fermentation process.

Studies developed by Silva et al. [16], analyzing the composition of sweet sorghum and sugarcane broths, observed that the sorghum broth contained a greater abundance of nutrients and minerals, mainly phosphorus, potassium and magnesium. According to Santos et al. [17], the nutritional composition of the saccharine substrates acts on the fermentation performance of the yeast during the fermentation process, directly influencing the final ethanol yield. For Dar et al. [9] and Silva et al. [16], sweet sorghum is considered a substrate with economic advantages for the production of ethanol because it has a high content of fermentable sugars and mineral elements that are essential to the process.

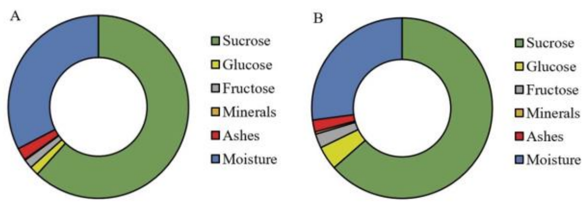


**Fig. 1.** Composition of saccharine substrates based on sugarcane juice (A) and sorghum (B). Source: Adapted [15].

The evaluation of the molecular absorption spectra showed a difference in the spectrum profile of the substrates (Figures 2A and 2B). The spectra suggest the presence of organic compounds, including amino acids and proteins (240 – 310 nm) and carbohydrates (289 – 423 nm). They can also indicate the presence of compounds with functional groups that absorbs in these regions. The present study is in agreement with Skoog et al. [18], who infer that organic compounds have functional groups with specific characteristics in the range of radiation absorption, being carbonyl in the range (186 – 280 nm), amine (214 nm). The presence of the band between 300 – 460 and at 720 nm may be related to both color and the presence of chlorophyll, and may also be responsible for the turbidity of the sample [19, 20]. In the subsequent hours of fermentation, there was a decrease in the intensity of the peaks, this fact can be related to the metabolism of the yeast, since it absorbs nutrients present in the substrate, to maintain its fermentative capacity.

During the fermentation, the yeasts assimilate the sugars and amino acids that are present in the substrate to maintain its functional metabolism and, in return, release ethanol and $CO_2$ to the medium. So, the composition of the fermentation medium and the availability of nutrients directly interfere in the final production of ethanol [21] and in the permanence of viable cells during the process [22].

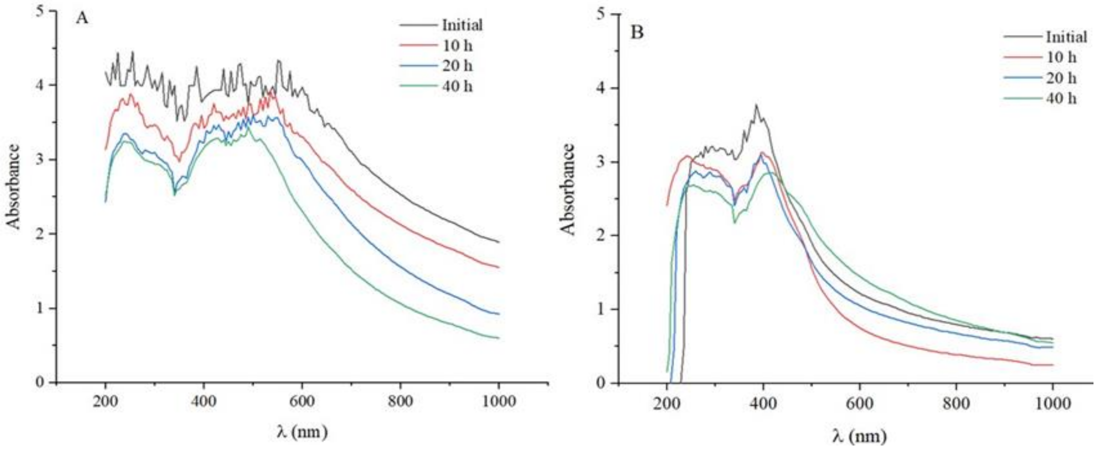


**Fig. 2.** Evaluation of the molecular absorption profile of the saccharine substrates sugarcane juice (A) and sorghum (B), during the fermentation process using the FT858 yeast at different cultivation times at 30 ºC.

Thus, the nutrient composition and the presence of sugars in the substrates are important parameters to be investigated in the fermentation medium, since they are fundamental for the good performance of the yeast. Mueller et al. [23], in their studies comparing the composition of sugarcane and sweet sorghum juices, observed that the sorghum juice contained a higher concentration of amino acids to the functional metabolism of the yeast, these being serine, arginine, alanine and tryptophan, that in this substrate the highest rates of ethanol production occurred.

The spectra obtained in this study suggest the presence of the main sugars that make up sugarcane and sweet sorghum juices, which are sucrose, glucose and fructose. In line with the literature that indicate the presence of carbohydrates in the wavelength between the spectrum range from 315 to 480 nm [24, 25].

In this study, spectrum bands were observed between (240 – 310 nm), which suggests the presence of amino acids in the analyzed substrates. This result is in agreement with the literature, since the amino acids phenylalanine, tyrosine and tryptophan interact with light at a length between 200 and 280 nm [26, 27].

The Table 1 presents studies related to the use of the UV-vis technique to evaluate the composition of samples. UV-vis spectrometry, although considered simple, shows the composition profile of the samples [28].

**Table 1**. Studies related to the use of the UV-Vis technique in samples of sugarcane juice and sorghum.

| Composition | Wavelength (nm) | Reference |
|---|---|---|
| Sucrose | 315 – 340 | [24,25] |
| Glucose | 490 | [24,25] |
| Fructose | 480 | [24,25] |
| Dextrins and starch | 315 | [25] |
| Waxes | 230 – 270 | [29] |
| Pphenolic compounds | 240 – 280[1]; 270 – 350[2] | [30[1];31[2]] |
| Amino acids and proteins | 240 – 310[1]; 240 – 295[2] | [26[1]; 27[2]] |
| Chlorophyll | 430 – 665[1]; 450[2] –710[2] | [19[1];20[2]] |

Source: Prepared by the authors.

The color of the substrates can also be observed in the spectra, considering that the absorbance of the colors changes from violet to red, and the maximum absorption peaks occur approximately between 500 nm, where the green color of the electromagnetic spectrum is transmitted. In this study, bands between 300 – 460 and up to 720 nm occurred, indicating the presence of chlorophyll. For Samide and Tutunaru [19] and Porto et al. [20], in their studies related the presence of chlorophyll in the spectrum range from 430 to 710 nm.

## 3. Material and Methods

### 3.1 Study development location

The study was developed at the Laboratory of Biotechnology, Biochemistry and Biotransformation of the Center for Studies in Natural Resources – CERNA of the State University of Mato Grosso do Sul- UEMS/Dourados-MS.

### 3.2 The composition of the technological parameters of saccharine substrates

The evaluation of the compounds present in the saccharine substrates was carried out in an exploratory qualitative way through a comparative bibliographic study.

### 3.3 Obtaining saccharine substrates

The sugarcane juice was obtained from a factory in the Grande Dourados region, packed in sterilized flasks and transported at low temperature (4 ºC) to the laboratory. The material was filtered through filter paper in order to remove impurities. The sorghum juice was obtained from Embrapa Agropecuária Oeste/Dourados, transported to the laboratory and prepared under the same conditions as the sugarcane juice. Both substrates had their sugars concentrated at 22 °Brix by evaporation on a hot plate accompanied by a portable scale refractometer (0 - 32) with pH adjusted to 5.0 using hydrochloric acid (1 mol $L^{-1}$). The broth after preparation was promptly used in the fermentation assays.

### 3.4 Pre-inoculum and fermentation condition

For the pre-inoculum, the liquid culture medium YPSAC 5%

was used, containing 1.0% (w $v^{-1}$) of yeast extract; 5.0% (w $v^{-1}$) peptone and 5.0% (w $v^{-1}$) sucrose, sterilized in an autoclave at 120 ºC for 20 minutes and 0.10 grams of lyophilized yeast FT858 was inoculated. The flasks, containing the inoculum, were incubated at 30 °C in a shaker model CT-712R, at 250 rpm for 24h. After growth, cells were collected by centrifugation (800 rpm, 20 min), resuspended and washed three consecutive times in sterile saline solution (0.85%), resulting in a concentration of 10 mg $mL^{-1}$ of wet mass. Fermentation experiments were carried out in 125 mL Erlenmeyer, containing 50 mL of sorghum and sugarcane broth sterilized at a concentration of 22 °Brix, and incubated at 30 ºC, at 250 rpm. Aliquots were taken for analysis at initial, 10, 20 and 40 hours.

### 3.5 Data analysis

Samples of saccharine substrates were evaluated at different fermentation times by molecular absorption spectrum at a wavelength between 200 and 1000 nm with intervals of 5nm. A quartz cuvette was used to perform the scans and an optical path of 1 cm. The equipment used was a spectrophotometer (Global Trade Technology).

### 3.6 Data analysis

The data were analyzed using Excel 16 and Origin 8 software. All experiments were performed in triplicate.

## 4. Conclusions

Craft The saccharine substrates have high levels of sugars, amino acids and minerals, essential elements for the fermentation process, such substrates are important for the production of ethanol, which adds economic advantages to this important biotechnological product.

The application of the UV-vis spectrometry technique showed that it can be applied in the analysis of complex mixtures such as saccharine substrates, showing the potential of this technique in the field of investigation in fermentation processes.

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## Author Contributions

M. S. M. = Conceptualization; Investigation; Methodology; Writing original draft; M. B.= Investigation; Writin original draft; C. A. L. C. = Supervision; Writing review & editing.

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