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THE PROXIMATE COMPOSITION AND METABOLITE PROFILING OF SUGARCANE (*Saccharum officinarum*) MOLASSES

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ABSTRACT

The transformation of organic by-products into valuable materials has become a necessity and a common practice in the food industry. By-products oftentimes offer low economic value and conferred numerous health benefits. Sugarcane molasses is a waste from the sugar manufacturing process with many valuable compounds. It is known to be rich in nutrients, and the various processing stages might be the cause of the complex nature of sugarcane molasses. Therefore, this study aimed to analyze the proximate composition of sugarcane molasses and to ascertain the sugars and amino acid profile. The result of this study showed that sugarcane molasses is composed of high carbohydrates (75.10±0.7%) and has high sucrose content (38.10±2.4 g). Besides, it comprises several amino acids, namely tyrosine, glysine, proline, glutamic acid, and valine. As the large scale of proximate composition revealed plenty of metabolites present in sugarcane molasses, it can be concluded that this by-product has great nutritional benefits.

Key words: Amino acid, proximate, sugarcane molasses, sugar, sucrose

INTRODUCTION

The food industry produces an enormous amount of waste or by-products annually around the world through the transformation of raw materials into new products. These industrial by-products are exploitable sources of nutraceuticals, bioactive, functional ingredients, minerals, and phytochemicals that are inherently functional and good for human health (Martins *et al*., 2017). Since the essential nutrient is a human necessity, food industries are looking for valuable by-products for potential functional food ingredients (Helkar *et al*., 2016).

Sugar is one of the age-old commodities globally (Dotaniya *et al*., 2016). The product has become an essential item for human consumption daily as it provides satisfaction and pleasure. In the past decades, the graph of sugar production increased by 2% every year, from 2009 to 2015 (Sahu, 2018). In 2020, the production of sugar accounted for 193.2 metric million tons (IMARC, 2021). According to Li & Yang (2015), about 90% of sugar production is from sugarcane extract. Sugarcane is a tropical crop that has a high contribution to generating carbon fixation process. The process led to the production of carbohydrates, thus making the plant the best source to produce sugar extensively (Santchurn *et al*., 2012). The production of sugar from sugarcane generated various essential by-products including pressmud (3-5%), bagasses (25-30%), and molasses (3.5-5%) (Singh *et al*., 2015; Sahu, 2018). It has been reported that 1 kg of sugarcane can produce approximately 0.3 kg of molasses (Sahu, 2018).

Many valuable compounds are present in sugarcane molasses, mainly sugar molecules, dominated by sucrose, fructose and glucose, and minerals (Soukoulis & Tzia, 2018). Due to its high nutritional value, molasses is widely used as a raw material for acetic and lactic acid distilleries, fuel alcohol, livestock and poultry feed, functional food fermentation, and many pharmaceutical products (Hashizume *et al*., 1966; Takara *et al*., 2007). Even though numerous studies reported sugarcane by-products such as juice and bagasse-derived sugar molecules, amino acids, and minerals, there are very limited study was carried out on molasses. Besides, different molasses treatments used may change the results of their composition. In this study, the research was subjected

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to analyzing the composition of unsulphured sugarcane molasses using proximate analysis and to determine the profile of sugars and amino acids.

MATERIALS AND METHODS

Sample preparation

The sugarcane molasses (900 g) was purchased from Matahari Sdn. Bhd., Selangor, Malaysia and packaged in glass. The molasses had been extracted three times by repeated boiling from matured organic sugarcane without sulphur treatment (Jamir *et al*., 2021).

Proximate analysis

Proximate analysis comprising moisture, ash, crude fat, crude proteins, and carbohydrates contents were determined by their respective formulation of raw material using standard AOAC procedures (2000) as described below;

Moisture content

The moisture content of sugarcane molasses was analyzed using the oven drying method (method 926.5). Five grams of sugarcane molasses sample were dried in a conventional oven (Thermo Fisher Scientific, U.S) at 105 °C for 24 h. Then, the sample was cooled in a desiccator for 20 min and weighed. The moisture content was determined as the percentage weight loss of the sample using Equation 1:

$$\text{Moisture (\%)} = \frac{\text{(Weight of sample used-Dry weight of sample)}}{\text{Weight of sample used}} \times 100$$

Ash content

The ash content of sugarcane molasses was determined using the dry ashing method (method 930.22). Five grams of sugarcane molasses sample were placed into the Muffle furnace (Carbolite, England) at 550 °C for approximately 12 h until it turned to ash. The ash content was determined using Equation 2:

$$\text{Ash (\%)} = \frac{\text{(weight of ash+weight of crucibles)-(weight of crucible)}}{\text{weight of sample}} \times 100$$

Crude fat

The analysis of crude fat in sugarcane molasses was carried out with the Soxhlet method (method 935.38). Five grams of the sample were transferred into a Soxhlet extraction flask (FatExtractore E-500). Next, 200 mL of petroleum ether (Merck, Germany) were poured into the boiling flask attached to the Soxhlet extraction flask. The apparatus was heated in the boiling water bath to reflux the solvent for 8 hr. Then, the sample was cooled down by rotary evaporation. The flask was then placed in the conventional oven for 15 min at 105 °C. Lastly, a desiccator was used to cool down again the flask containing the sample and weighed. The crude fat was measured using Equation 3:

$$\text{Fat (\%)} = \frac{\text{Weight of oil}}{\text{Weight of sample}} \times 100$$

Crude proteins

For crude protein content, the micro-Kjeldahl method (method 950.36) was used. 0.15 g of sugarcane molasses shifted in a boiling tube. Then, 0.8 g of mixed catalysts and 2.5 mL of concentrated sulphuric acid (H_2SO_4) (Merck, Germany) were added to the tube and heated. Next, 10 mL of 45% sodium hydroxide (NaOH) solution (Merck, Germany) were added slowly to the distillation tube to separate the two layers of the solution. The conical flask containing 2% of boric acid (Merck, Germany) was placed at the distillate platform, and distillation of ammonia was allowed to take place. The ammonium borate in the distillate was titrated with 0.05 N H_2SO_4 until the endpoint was reached and the amount of titrating was recorded. The percentage of proteins was calculated using Equation 4:

$$\text{Protein (\%)} = \frac{\text{(The volume of titrating sample-Volume of titrating blank)} \times 0.05\text{N} \times 1.4}{\text{Weight of sample}} \times 6.25$$

Carbohydrates

The total carbohydrates content of sugarcane molasses was determined by difference using Equation 5:

$$\text{\% total carbohydrate} = 100 - (\text{\% moisture} + \text{\% ash} + \text{\% crude fat} + \text{\% crude proteins})$$

Sugars profiling

To determine the sugar monomers, approximately 100 g of sugarcane molasses were mixed with 400 mL of boiling distilled water and blended homogeneously for 5 min. Then, the sample was shaken on an automatic shaker for 1 h to dissolve the sugar. The sample was subjected to filtration through filter paper Whatman No. 2 and dried in vacuum condition in a rotary evaporator (Buchi Rotavapor R200). After that, the monosaccharide contents were determined using gas chromatography (GS-MS Agilent 6890, GC Plus, US). The column used was a 30 m long DB-5 fused silica capillary column with 1.0 µm thickness. The glucose, fructose, and sucrose were quantified after the injection of a 2.0 µL sample.

Amino acids profiling

To qualify and quantify the amino acids in sugarcane molasses, three phases were necessary; the release of amino acids from sugarcane molasses, the separation, and the quantification of amino acids. Approximately 2 g of sugarcane molasses were weighed and mixed into 15 mL of 0.1 M hydrochloric acid (HCl) (Merck, Germany). The sample was homogenized in a stomacher for 4 min. Then, the sample was transferred into a micro test tube and centrifuged at 2000 × *g* for 15 min at 4 °C. The supernatant was stored at -80 °C until further

analysis. To separate the amino acids individually, 100 µL of the sample were deproteinized with 250 µL of acetonitrile (ACN) (Merck, Germany) in a micro test tube. Then, the mixture was centrifuged at 2000 × *g* for 3 min. 100 µL of supernatant were placed into a heat-resistant tube and 100 µL of internal standard nor leucine solution (Merck, Germany) were added.

The test tube was then dried in nitrogen. After that, the residual water was removed and 50 µL of dichloromethane was added and dried again in nitrogen. Finally, 50 µL of acetonitrile and 50 µL of N-tert-butyldimethylsilyl-N-methyltrifluoroacetamide (MTBSTFA) (Merck, Germany) were added to the tube and placed in a shaking incubator at 100 °C for 60 min to induce the derivatization reaction. Next, the tube was refrigerated and analyzed by GC-MS within the next 24 h. The quantification was carried out with GC equipment 5890 series II coupled to a mass selective detector (MSD) electron impact (EI), model 5973. The column used was a 50 m × 0.32 mm i.d., 1.05 μm, HP-5 (Hewlett-Packard), and the column head pressure was 12.8 psi. The flow rate was 1.2 mL/min at 280 °C. Firstly, the temperature was set at 170 °C for 5 min, then, gradually increased from 4 °C per min to 200 °C. After that, the temperature was set to increase at 4 °C per min until 290 °C. Next, the temperature was gradually increased at 20 °C per min until 325 °C was reached. The transfer line to the mass spectrometer program was set as follows: temperature set at 280 °C for 35 min and gradually increased 10 °C per min until 320 °C. The quantification was carried out in the selected ion monitoring (SIM) mode.

RESULTS AND DISCUSSION

Proximate Composition of Sugarcane Molasses

Proximate analysis is used to assess the nutritional value of the macronutrients in food samples including moisture, ash, fat, proteins, and carbohydrates contents (Thangaraj, 2016). The results of the proximate composition of sugarcane molasses were shown in Table 1.

Table 1. Proximate composition of sugarcane molasses

Composition	Values (%)
Moisture	17.55±0.2
Ash	5.35±0.1
Fat	-
Proteins	2.00±0.0
Carbohydrates	75.10±0.7

*-: no detection

The main composition of sugarcane molasses is carbohydrates. The carbohydrate content determined in this by-product was 75.10±0.71%. This result was supported by Ogunwole *et al*. (2020) who reported that carbohydrates content in sugarcane molasses was 71.84% and is predominantly made up of sucrose, glucose, and fructose.

The moisture content in sugarcane molasses was noted as the second-highest value with 17.55±0.2%. The high moisture content of sugarcane molasses originated from the sugarcane itself as the freshly harvested sugarcane and also by the processing of sugarcane molasses, mainly sugar extraction was reported to have high water content (Hess *et al*., 2016). The crop is known to require a large amount of water and irrigation throughout its cultivation period is essential. A previous study stated that the water content present in sugarcane molasses was about 20% which agrees with this finding (Olbrich, 2006).

Subsequently, sugarcane molasses showed a moderate ash content of 5.35±0.1%. Ash content is referring to the inorganic residue that remained after water and organic matter have been removed by heat through the combustion process (Afify *et al*., 2017). According to Jaffe (2015), sugarcane molasses constitutes several essential mineral elements such as calcium, potassium, phosphorus, magnesium, manganese, and chromium. However, the concentration of the minerals present is low. These trace elements are recognized to be necessary for the growth, development, and physiology of crops (Bowen, 1986).

Interestingly, the study found that sugarcane molasses has 0% crude fat. Previously, Perez (1995) has reported that molasses does not contribute to the fat content of a food product. Since the high-fat content in food is often associated with chronic diseases, consumption of fat-free food products with sugarcane molasses as the substitute for commercial sugar is highly suggested to individuals that suffered from coronary heart disease, diabetes, obesity, and metabolic disorder (Delas, 2011).

While analyzing the crude protein content, the result showed that sugarcane molasses had a low value presented at only 2.00±0.0%. is the protein content of sugarcane molasses found by this study seems to be the lowest when compared to the previous studies by Ogunwole *et al*. (2020) and Sureshkumar *et al*. (2016), which documented the value of crude proteins of 4.38% and 3%, respectively. According to Curtin (1983), molasses contains a low amount of nitrogen which consists of non-protein nitrogen compounds including amides, albuminoids, and amino acids.

Sugars profile

Sugar is a basic diet throughout the globe as it provides sweetness and energy for humans (Zaitoun & Harphoush, 2018). It is a building block of carbohydrates and is naturally found in various foods. As the sugarcane molasses is rich in carbohydrates with a valuable content of 75.10±0.71% this by-product largely consists of sugar components. Through the sugars profiling analysis, the monosaccharides in sugarcane molasses are presented in Table 2.

Table 2. Sugars profile of sugarcane molasses

Sugar	Value (g/100 g)
Sucrose	38.10±2.4
Fructose	8.45±0.5
Glucose	7.8±0.1
Maltose	ND
Lactose	ND

*ND: no detection

The sucrose content was found to be the highest with a value of 38.10±2.4 g/100 g of samples investigated. It was also observed that the amount of fructose was appreciable at 8.45±0.5 g/100 g, followed by glucose at 7.8±0.1 g/100 g. However, the two disaccharides namely lactose and maltose were not detected. Sugarcane molasses is known to contain a high amount of solids which 50% of it are predominantly simple sugars such as sucrose, fructose, and glucose (Xu *et al*., 2014). Given that sugarcane is in demand globally for sucrose production, it is undeniable that the sucrose content in the by-product is significantly the highest. However, the moderate concentration of fructose and glucose might be due to the invertase enzyme activity that hydrolyzed a slight portion of sucrose during the boiling process of sugarcane molasses (Olbrich, 1963; Nadeem *et al*., 2019).

Amino acids profile

Protein is a building block of amino acids that contributes to various biosynthesis processes in the human body. Sugarcane molasses is a by-product that is comprised of a considerable nitrogenous non-sugar component such as betaine and other amino acids (Olbrich, 1963; Varaee *et al*., 2019). Amino acids were obtained during sugar extraction in the form of a solution. Alkaline hydrolysis of sugar during the boiling stage of sugarcane molasses caused protein degradation yielding amino acids (Ali *et al*., 2019). In the present assessment, amino acid composition exposed that tyrosine is the major amino acid with 0.87%, followed by glysine, proline, glutamic acid, and valine with 0.56%, 0.46%, 0.27%, and 0.10%, respectively (Table 3).

However, the sugarcane molasses recorded no trace of other amino acids namely arginine, aspartic acid, alanine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, serine, and threonine. This study contradicts Varaee *et al*. (2019) who reported that aspartic acid, glutamic acid, alanine, and lysine are the amino acids present in the sugarcane molasses. However, this study is by Olbrich (1963) as the author revealed that sugarcane molasses is comprised of tyrosine, glysine, proline, glutamic acid, and valine.

Table 3. Amino acids profile of sugarcane molasses

Amino Acid	Value (%w/w)
Arginine+	-
Aspartic Acid+	-
Alanine+	-
Glutamic Acid+	0.27
Glycine+	0.56
Histidine+	-
Isoleucine+	-
Leucine+	-
Lysine+	-
Methionine+	-
Phenylalanine+	-
Proline+	0.46
Serine+	-
Tyrosine+	0.87
Threonine+	-
Valine+	0.10

*-: no detection

CONCLUSION

In summary, the results drawn from this study revealed that sugarcane molasses without sulphur treatment is characterized by high carbohydrate content. A large-scale metabolite profiling particularly sugars and amino acids analysis was performed thoroughly. The study also reported unsulphered sugarcane molasses rich in sucrose contents. As the sugars are used to preserve food and provide human satisfaction, the identified sugars enhance the beneficial and nourishing value of the sugarcane molasses. The results show sugarcane molasses could be an important by-product in the food industry as they would contribute a great nutritional benefit.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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