ACTA SCIENTIFIC MICROBIOLOGY (ISSN: 2581-3226)

Volume 2 Issue 9 September 2019

Research Article

The Potential of Using Millet Cobs for the Production of Alcohol and Organic Acids

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Received: July 25, 2019; **Published:** August 09, 2019

DOI: 10.31080/ASMI.2019.02.0331

Abstract

Different species of *Aspergillus, Rhizopus, Bacillus, Saccharomyces* and *Candida* are able to convert several alternative carbon sources to different organic acids and alcohols. The study investigated the amount of organic acids and alcohols in the millet cob sample and evaluated the sugar content of the millet cobs. Thus, from the untreated degraded millet cob sample, it was observed that tartaric acid has the highest content on Day 8 of the degradation period among the four organic acids (tartaric acid, malic acid, citric acid and acetic acid) reaching a value of 1.69 ± 0.00 mg/L, while acetic acid recorded the lowest amount during the degradation period of the millet cob sample, with the lowest value of 0.01 ± 0.00 mg/L, registered on the Day 12. The alcohol content ranges between 0.023 ± 0.000% v/v and 2.430 ± 0.030% v/v. Lactose content was highest among the assayed sugars (glucose, fructose and lactose), with a value of 33.68 ± 0.11 mg/g recorded on Day 20 of the degradation period while fructose content was lowest with a value of 0.99 ± 0.05 mg/g recorded in the control sample. Therefore, millet cobs that are indiscriminately discarded and burnt could serve as an alternative source for organic acids and alcohol production.

Keywords: Organic Acids; Alcohols; Sugars; Millet Cobs

Introduction

Alcohols have a long history of array uses. Alcohol are used for the production of formaldehyde, fuel additive, solvent, alcoholic beverage, placticizers and detergents [9]. Examples of organic acids include acetic acid, malic acid, tartaric acid, citric acid, benzoic acid, lactic acid, formic acid, etc. Organic acids are used in myriad of industries such as pharmaceutical, agrochemicals, paints, beverage, food and detergent.

There is significant interest among industrial and academic researchers around the world in replacing feedstocks for fuel and chemical production with sustainable biomass resources to supply the increasing population while using cutting-edge technologies to counteract environmental problems such as global warming [16]. The increasing concerns of environmental pollution and protection have forced us to seek a new generation of cleaner industrial production to maximize productivity and simultaneously minimize contamination [13]. Lignocellulosic materials are a potential resource that is not used for the production of not only alcohol but also organic acids of industrial importance.

Millets are vital crops in semi-arid tropics of Asia and Africa with 97% of its production in developing nations whereas in the developed world millets are not significant [6]. Short growing season under dry, high-temperature conditions favours its production. Millets are important staple food in tropical Africa where the colossal number of both wild and cultivated millet are used as food. Whole grains may as well be directly dry-milled to give a range of products: broken or cracked grains, grits, coarse meal and fine flour. The flour thus obtained is used in the preparation of an extensive variety of simple to complex food products. In India, millet flours are fermented and used to make a wide range of pancakes [17]. Such traditional fermented breads as injera, kisra, dosa, massa and galettes do exist. Malted millet grains have been widely used in Africa for centuries to make alcoholic and nonalcoholic fermented beverages [17]. Despite these highlighted usefulness of millet, its cobs constitute nuisance to the environment creating unfriendly site to the general public. The purpose of this study was to investigate amount of organic acids and alcohols in the millet cob sample and also, to evaluate the sugar content of the millet cobs.

Materials and Methods

Preparation of millet cobs

The millet cobs (pearl millet) collected from Gusau, Zamfara State, Nigeria were sun-dried and grinded using a blender. The powdered millet cobs were separated into two portion "A" and "B". The "A" served as the control sample which was not degraded, while "B" served as the degraded sample [2].

Submerged substrate degradation

Ten [10] grams of the portion "B" of the substrate was soaked in 100.0 ml of sterile distilled water. The submerged degradation was carried out for 20 days in which analyses were carried out on both the degraded sample and control sample. Sugar, alcohol and organic acids were evaluated at 4 day interval.

Determination of sugars of samples

The standard methods of Sadasivam and Manickam [25] were used to evaluate glucose, fructose and lactose content of millet cobs samples.

Determination of Alcohol content

The alcohol content of the millet cobs sample was determined by distillation using the techniques of AOAC [1].

Determination of organic acids

Tartaric, malic, citric and acetic acids contents were estimated by using the standard method of Bogusław., *et al.* [3] done in four stages such as extraction, filtration, distillation and titration.

Results

Sugar content of samples

The sugar content, expressed in mg/g, is shown in the table 1. The value of glucose content of the millet cob samples ranges from 2.14 ± 0.10 to 10.54 ± 0.00. The highest value of fructose content is 18.25 ± 0.01 while the lowest value is 0.99 ± 0.05. The value of fructose content ranges from 0.44 ± 0.00 to 33.68 ± 0.11.

Sugar Content	Undegraded sample	Day 4	Day 8	Day 12	Day 16	Day 20
Glucose (mg/g)	6.31 ± 0.01^{e}	3.62 ± 0.05^{b}	4.10 ± 0.04^{c}	10.54 ± 0.00^{f}	4.48 ± 0.11^{d}	2.14 ± 0.10^{a}
Fructose (mg/g)	0.99 ± 0.05^{a}	4.30 ± 0.03^{b}	5.28 ± 0.03^{c}	18.32 ± 0.03^{e}	18.25 ± 0.01^{e}	18.10 ± 0.01^{d}
Lactose (mg/g)	0.44 ± 0.00^{a}	1.40 ± 0.14^{b}	2.60 ± 0.08^{c}	15.69 ± 0.14^{d}	26.81 ± 0.01^{e}	33.68 ± 0.11^{f}

Table 1: Sugar content of millet cobs samples during degradation.

Organic acids content of samples

Figure 1 shows the result of organic acids content of millet cob samples. The values of organic acids content ranges from 0.08 ± 0.00 to 1.17 ± 0.00, 0.08 ± 0.00 to 1.22 ± 0.00, 0.03 ± 0.00 to 0.42 ± 0.00 for tartaric, malic, citric and acetic acids respectively.

Alcohol content of samples

Alcohol content of millet cobs samples ranges from 0.023 ± 0.000% v/v to 2.430 ± 0.030% v/v. The alcohol production from millet cob sample with was highest on Day 8 of the biodegradation period and lowest on Day 1 (undegraded sample) which is shown in figure 2.

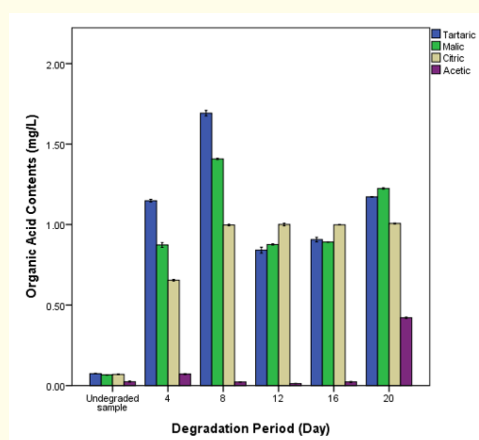

Figure 1: Organic acid contents of millet cobs during degradation. biosynthesized by *A. Niger*. Scale bar = 100 nm using TEM.

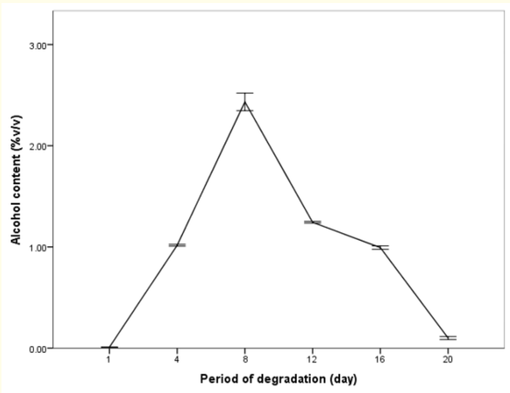

Figure 2: Alcohol content of millet cobs samples during degradation.

Discussion

The decrease in sugar concentrations could be attributed largely to the activities of Lactic Acid Bacteria such as *Lactococcus lactis* and *Lactobacillus lactis* which may be present in the millet cobs which metabolized and converted sugars into organic acids during maize fermentation [27]. Fungi and Lactic Acid Bacteria can metabolise sugars within the millet cobs which is converted to organic acids [27]. Continued saccharolytic activities of *Bacillus subtilis, Bacillus cereus* and *Bacillus licheniformis* which can be present in the millet cobs may lead to the release of sugars particularly on Day 8 which agrees with the findings of Arogunjo and Arotupin (2) when *Bacillus cereus, Bacillus subtilis, Bacillus licheniformis, Corynebacterium fasciens, Flavobacteriun ferrugineum, Lactobacillus lactis, Lactococcus lactis, Candida albicans, Candida krusei, Saccharomyces cerevisiae, Zygosaccharomyces rouxii, Geotrichum albidum, Aspergillus fumigatus, Aspergillus flavus, candidus, Rhizopus stolonifer, Fusarium poae* and *Scopulariopsis brevicaulis* were isolated from millet cobs during degradation.

Acetic acid content in millet cobs sample during degradation could be produced by methanol carbonylation [4]. It could be synthesized by oxidation of acetaldehyde [10]. Costa., *et al*. [5] reported that acetic acid is a bye-product of fermentative lactic acid production, hence, the presence of lactic acid bacteria could be responsible for its production. *Clostridium acetobutylicum* and *Acetobacter* such as *Acetobacter oboediens, A. pomorum, A. intermedius, A. cerevisiae, A. malorum and A. oenietal*., and other known acetic acid bacteria have been identified as microorganisms that can synthesize acetic acid [21], thus, the absence of all these microorganisms in millet cobs during degradation may be responsible for the low concentration of acetic acid when compared to the other organic acids.

The presence of tartaric acid in the undegraded sample agrees with the report of Duarte., *et al*. [8] that tartaric acid can be found naturally in fruits, most notably citrus (particularly grapes), but also bananas and tamarinds. Hronská., *et al*. [11] documented that tartaric acid is present in plants, tartaric acid is partly free and partly present as potassium, calcium or magnesium salts. Lisa and Paolo [12] reported that tartaric acid can be obtained from lees, a solid byproduct of fermentations and dead yeast or residual yeast and other particles that precipitate. The author also documented that tartaric acid could be produced as a result of multistep reaction from maleic acid. One of the process or the combination of the two processes could lead to tartaric production particularly from Days 4 through 20. All these factors could be responsible for its maximum production among the assessed organic acids.

The availability of malic acid in the undegraded sample agrees with the report of Duarte., *et al*. [8] that malic acid occurs naturally in fruits, such as apricot, blackberries, blueberries, cherries, grapes, mirabelles, peaches, pears, plums and quince and it is also present in lower concentrations in citrus, therefore, malic acid may occur naturally in millet cobs. The probable presence of fumaric acid in the millet cobs could be responsible for the formation of malic acid, this concurred with the reports of Roa-Engel [23] and Martin-Dominguez [14] that malic acid can be obtained by the fermentation of fumaric acid. Any or combination of the isolated bacteria could be responsible for the production for the transformation of fumaric acid into malic acid which aligned with the report of Hronská., *et al*. [11] that globally dominant production of malic acid through the conversion of fumaric acid is carried out by bacterial producers immobilized in kcarrageenan. The presence of *C. fascians and L. lactis, S. cerevisae* could be responsible for malic acid production [11,22].

The progressive increase in citric acid concentration maybe due to the presence of *A. flavus* which is known producer of citric acid. Also, the presence of *A. fumigatus* and *A. candidus* may be responsible for the production of citric acid. Papagianni [20] reported that *A. niger, A. awamori, A. nidulans, A. fonsecaeus, A. luchensis, A. phoenicus, A. wentii, A. saitoi, A. flavus, Absidia sp., Acremonium sp., Botrytis sp., Eupenicillium sp., Mucor piriformis, Penicillium janthinellum, P. restrictum, Talaromyces sp., Trichoderma viride* and *Ustulina vulgaris* have been found to accumulate citric acid. Besides mould, it is known that several yeasts produce citric acid [15]. The presence of *Candida* sp [28], *S. cerevisiae* and *Zygosaccharomyces rouxii* [18] could be responsible for the production of citric acid. The pH may also be responsible for increased production in citric acid. Nielsen and Arneborg [18] found that *S. cerevisae* and *Z. bailii* exhibited similar tolerances to citric acid at pH values of 3.0, 4.0, and 4.5. Temperature is another factor that maybe responsible for the production of citric acid. Max., *et al*. [15] documented that spores germinate at 32°C in a prefermenter, using a molasses solutions containing 150 g/l of sugar in the presence of cyanide ions to induce the formation of mycelium in granular form. The accumulation of citric acid is strongly influenced by the composition of the medium, especially in submerged fermentation processes [15]. The high yield of citric acid on Day 20 maybe due to the increase in sucrose content of the millet cobs samples which has been adjudged to be a good substrate for the production of citric acid [27]. Progressive reduction in nitrogen level within the millet cobs can also be responsible for the steady increase in the citric acid composition [15]. The presence of minerals within the millet cobs could affect the rate of production of citric acid. Max., *et al*. [15] stated that the presence of zinc, manganese, iron, copper and heavy metals should be in limiting concentrations.

The decrease in glucose, fructose and lactose content and increase in alcohol content on Day 8 agrees with the findings of Roberta., *et al*. [24] when fermentation process for the production of apple-based kefir vinegar was carried out. This was also observed by Domizio., *et al*. [7] during the fermentation of grape must under control temperature condition. *Candida tropicalis* have been demonstrated to produce ethanol from a mixed-sugar stream [19]. Co-culturing of *C. tropicalis* and *S. cerevisiae* may increase alcohol production [26].The pH could be factor that contribute to alcohol production, this correlate with the findings of Roberta., *et al*. [24] which states that the appropriate pH values required by fermenters for ethanol production is 3.08.

Conclusion

The potentials of millet cobs for alcohol and organic acids production has been ascertained. Hence, millet cobs that are disposed indiscriminately which could cause environmental and aesthetic hazard could be exploited for the purpose of alcohol and organic acids production. Advance studies should be carried out on improving the lignocellulosic structure of the millet cobs in order to get high yield of alcohol and organic acids.

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