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Bioethanol Production from Mango Waste: Use of Several Oenological Yeasts

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Abstract The surplus mangoes produced, which rot in the environment, become a source of pollution and lead the proliferation of flies, vectors of certain diseases. The aim of this work was to valorize mango waste by producing bioethanol. Several oenological yeast strains were used to determine their influence on kinetics and quantity of bioethanol produced. The results show that each yeast reacts differently with mango substrates. The best mango production rates obtained were 14% with Saf-Levure commercial yeast and 13% with Delta Yeast. Brix levels did not vary after 48h for all yeasts, suggesting that bioethanol production kinetics are two days.

Keywords: *mangifera indica, waste, bioethanol, oenological yeast*

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1. Introduction

With a world population that continues to grow and which approaches the 8 billion inhabitants [1], food and energy requirements and environmental pollution have increased sharply in recent years, posing a serious threat to sustainable development [2]. The world's population is now extremely dependent on fossil fuels such as oil, coal and gas to meet its energy needs. It is currently estimated that about 80 % of the energy consumed comes from fossil fuels [3]. However, the intensive use of these fuels leads to the emission of greenhouse gases such as methane (CH_4), carbon dioxide (CO_2), carbon monoxide (CO), nitrogen oxides (NO_X) and sulphur oxides (SO_X) [4] which are the main cause of global warming. According to projections, to curb global warming, greenhouse gas emissions should be reduced by at least 40 %: this will keep the average increase in Earth's surface temperature at 1.5°C [5]. So we need a change in human activities. In addition, the reserves of non-renewable fossil fuels are running out. It is estimated that by 2050 the oil reserves will be completely exhausted. With the geopolitical context currently very tense, the scarcity of petroleum products is accompanied by a surge in prices, which is hurting several countries in the world. This scenario led to the search for environmentally friendly, cost-effective, sustainable and renewable energy sources [6,7]. Biofuels ranked first among these attributes, strictly meeting the above criterion, and emerged as an attractive choice to meet global fuel needs [8]. Bioethanol is an organic biofuel obtained by alcoholic fermentation of carbohydrates contained in sugary or starchy plants such as sugar cane, sugar beet, corn, sorghum or lignocellulosic biomass and, more recently, microalgae. Bioethanol is low in toxicity, low in volatility and low in photochemical reactivity, reducing ozone and smog formation compared to conventional fuels. It is a versatile fuel that provides a high octane rating, high vaporization heat and other features that allow it to be more efficient than gasoline in compatible and optimized engines. Bioethanol can be mixed with gasoline (at a given percentage) to reduce the use of fossil fuels, provide oxygen and increase the octane rating, promoting more complete combustion, reducing emissions of carbon monoxide, unburned hydrocarbons and greenhouse gases [9-12]. Global bioethanol production has increased sharply since the early 2000s, from 17,25 billion litres in 2000 [13] to over 46 billion litres in 2007 [14]. This is due to the fact that the need for bioethanol continues to increase due to its potential use as a fuel for transport and its great advantage over conventional fuels in various ways, directly or mixed with gasoline, called "gasohol" [15]. At present, it is the northern countries such as the United States and several EU Member States accompanied by Brazil that have the largest biofuel promotion programs in the world [16]. The countries of the South, especially the countries of the African continent, are lagging behind on these issues.

Mango (*Mangifera indica L.*) is a fruit which is mainly grown in tropical and subtropical countries [17]. It belongs to the family Anacardiaceae in the order Sapindales [18]. It is one of the most traded tropical fruits in the world, with global production of about 57,01 million metric tons in 2021 [19] (Food and Agriculture Organization of the United Nations). http://www.fao.org/news/archive/newsby-date/2019/en/). It is a fruit rich in essential nutrients such as carbohydrates, sugar, dietary fiber, pectins, irons, vitamins A, B-6 and C, which is grown naturally in tropical regions. In Togo, mango production reached about 370 thousand tonnes in 2017 [20]. According to the 2015 ECOWAS [21]. Report on the Strategic Orientation for the Mango Value Chain in the Economic Community of West African States (ECOWAS), of the 1,3 million tonnes of mangoes produced per year in ECOWAS, post-harvest losses can reach 50 %. These losses have several reasons : very easy damage to mangoes during harvest and transport, early or late ripening of fruit, attacks of pests such as fruit fly, the difficulty of preserving mangoes and the lack of structures for storing or processing mango. In 2019, according to an FAO study [19] in Togo, of the 370,000 tonnes of mangoes produced, the loss is estimated at 12 to 20 % in the fields (between 45,000 and 74,000 tonnes of mangoes lost). These lost mangoes are sources of environmental pollution, and cause the proliferation of flies, which are the main vectors of certain diseases. A viable alternative for mangoes destined to be lost would be their use in fermentation processes to produce bioethanol due to their high sugar content (16-18 % w/v), sucrose, glucose and fructose being the main sugars contained in ripe mango, with small amounts of cellulose, hemicellulose and pectin [22,23]. For this purpose, the present work aims to use several strains of yeast Saccharomyces cerevisiae to study their influence on the kinetics of producing bioethanol from mangoes in Togo.

2. Materials and Methods

The experimental work was carried out at the Laboratory of Organic Chemistry and Environmental Sciences (LaCOSE) of the University of Kara (Togo).

Raw material

The fruits of the mango tree, (*Mangifera indica L.*) were obtained in the period April-May 2023, as residues in the fields around the city of Kara (Awandjelo), Togo. The mangoes were washed and disinfected with a solution of sodium hypochlorite 1M (NaClO), stored at 13°C and used within 4 days.

Physico-chemical Characterization of Mangoes

The moisture content was determined by desiccating 10 g of fresh mango, placed in an isothermal oven (Memmert, Biotech) at 105°C at atmospheric pressure until a constant mass of the sample was obtained [24].

Ash was determined by incinerating 10 g of mango pulp in a 550°C muffle oven (Nabertherm) for 2 hours.

The rate of total soluble solids was determined by mixing 10 g of mango pulp with 50 mL of distilled water : the mixture was clarified by centrifugation at 6000 tr/ min for 10 min. The rate was measured by direct reading with a refractometer (Scihemtech).

Titratable acidity was determined by titration with NaOH solution at 0,1 M up to pH = 8,1 using bromotimol blue 1 % as a coloured indicator.The rate of total and reducing sugars were determined using the phenol-sulphuric method [25].

Inoculum preparation

The ferments used consist of six yeast strains of *Saccharomyces Cerevisiae* for oenological use (Zymaflore X16, Actiflore F5, Zymaflore X5, BO123, Delta and RX60) marketed by the company Laffort Œnologie France and the strain of *Saccharomyces Cerevisiae* Saf-Yeast marketed by Lesaffre France. Each inoculum was prepared by introducing 6,0 g of each dry yeast strain (freeze-dried) into 100 mL of sugar water.

Preparation of the fermentation must

The fresh mango fruits were peeled and cut with a knife. The collected flesh was ground using an electric mixer (Lucky JL 886). The resulting puree was diluted with water (20 % w/v), pasteurized at 90°C for 20 minutes to remove microorganisms and pathogens that can influence fermentation, and then cooled to room temperature [26,27]. The fermentation must is divided into 1 litre sterilized glass bottles that serve as fermenters. Controlled fermentation was carried out by adding 5 mL of the inoculum prepared the day before to the mango juice previously obtained.

Distillation

At the end of the fermentation, bioethanol was extracted by fractional distillation of fermented musts. The distillation temperature is 79°C at the column head [28]. We performed a flame test to verify that the product obtained is actually ethanol. The density and alcohol content of bioethanol were determined. The ethanol produced was stored in vials at 4°C.

Analytic methods

The pH and temperature of the musts were measured using a pH meter (HANNA). The total soluble solid content (Brix degree) was determined using a Scihemtech hand refractometer. The alcohol content (% v/v) of the bioethanol produced was measured using an alcohol meter that can measure the alcohol purity from 0 to 100% and according to the pycnometric method recommended by AOAC (1984).

3. Results and Discussion

Physical and physico-chemical characteristics of mangoes

Table 1 and Table 2 show respectively the physical and the physico-chemical characteristics of the mangoes that were collected.

Table 1. Physical characteristics of the mangoes studied

	Average weight (g)	Pulp content (%)	Peel content (%)	Core content (%)
Mangifera Indica	425,13	82,89	11,02	6,09

Mango is a very water-rich fruit with almost 77 % moisture content, although this value is lower than that of Boko type mangoes analyzed in Congo and which have a water content of about 82 % [26]. It is also a fruit rich in total sugars and reducing sugars with nearly 24 g/ L of each of these sugars. The solubles solids content is also high at 18°. With such high levels of sugars and total soluble matter, mango pulp appears as an interesting source for bioethanol production.

Table 2. Physico-chemical characteristics of the mangoes studied

	Mangifera Indica
Water content (%)	76,54 ± 2,46
Ash content (%)	23,00 ± 1,74
Total sugars (g/L)	24,14 ± 1,78
Reducing sugars (g/L)	23,22 ± 1,06
Solubles (°Brix)	18
Titratable acidity (%)	0,28

Evolution of pH during fermentation

The variation in pH during the fermentation process of musts with different yeast strains is shown in Figure 1.

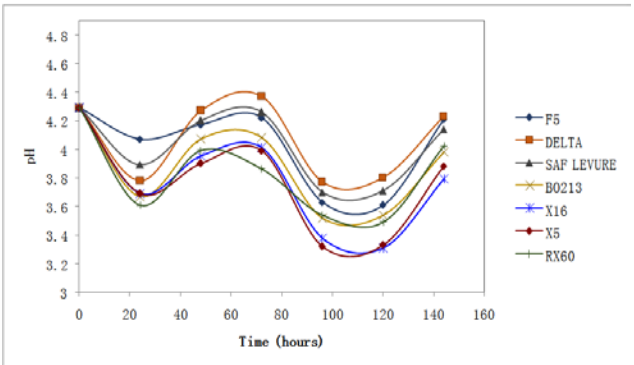

Figure 1. Evolution of pH during the fermentation process of mango musts

The pH of mango must is slightly acidic with a pH of 4,29. During fermentation, we can observe a variation of the pH for all the yeasts used as inoculum. Indeed, for each yeast, a more or less significant decrease is observed during the first 24 hours of the fermentation process : the decrease is greater with yeast RX60 where the pH reaches 3,61 in 24 h and the decrease is smaller with yeast F5 which increases the pH from 4,29 to 4,07 in 24h. In the two days that follow, an increase in pH is observed then, from 72 hours of fermentation, the pH decreases again to reach values between 3,31 and 3,81 after 120 hours of fermentation. Finally, the pH increases slightly in the last hours of fermentation. During alcoholic fermentation, the yeast metabolism induces a perpetual change of the medium. The decrease in pH at the beginning of fermentation may be due to the growth and multiplication of yeast, which results in the production of secondary metabolites. The consumption of carbon and nitrogen substrates is accompanied by the production of acid metabolites or alcohols. Carbon dioxide or acid compounds are produced by yeasts which can lead to an increase in acidity. Variations in pH are explained by the fact that the production of alcohol from sugars leads to a change in the dissociation of the constituents of the must and mainly of the organic acids initially present in the must [29]. In the presence of ethanol, dissociation is less important and it results in a lower proton concentration and therefore a slight increase in pH. The stabilization of the pH at the level of the musts could correspond to the exhaustion of the medium in fermentable sugars or to the saturation of the medium by secondary metabolites likely to inhibit the growth of the yeasts or to slow down their fermentative activity [28]. The pH of mango musts is located in the optimal pH range of bioethanol production which is 4,3 to 5,7 on neem pulp [30]. Thus, mangoes would constitute an interesting biomass with a high production rate of bioethanol.

Evolution of soluble solids during fermentation

Figure 2 shows the evolution of the rate of soluble solids in musts during fermentation according to the yeast strains used and time.

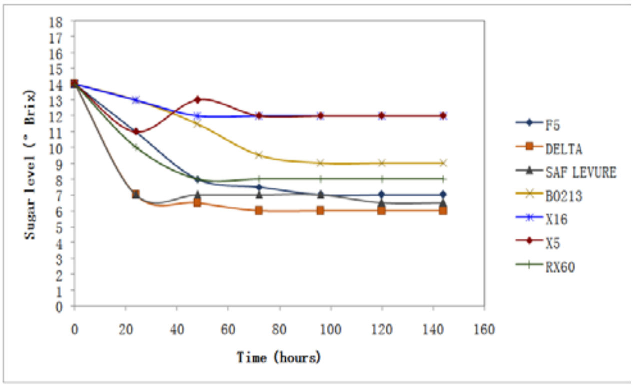

Figure 2. Evolution of Brix degree of mango musts during the fermentation process

For all yeasts, the Brix degree decreases during the first 24 hours of fermentation and then stabilizes from 48 hours of fermentation to remain constant until the sixth day of fermentation. We can therefore assume that with the inoculum, the fermentation process is completed after 48 hours since after that, there is no significant change in the rate of Brix. From a qualitative point of view, the decrease of the rates of soluble matter is the most important with the yeasts DELTA and SAF-LEVURE for which, the rate of Brix goes from 14 to 7. This assumes that the best yields of bioethanol production will be obtained with these two yeast strains. Conversely, the smallest decreases in the rate of soluble matter are observed with yeasts X5 and X16 which both vary degrees Brix from 14 to 12. We can therefore assume that with these two yeasts, the ethanol production rate will be low.

Characteristics of the bioethanol produced

The results of the analysis of the bioethanols produced are presented in Figure 3 and Figure 4.

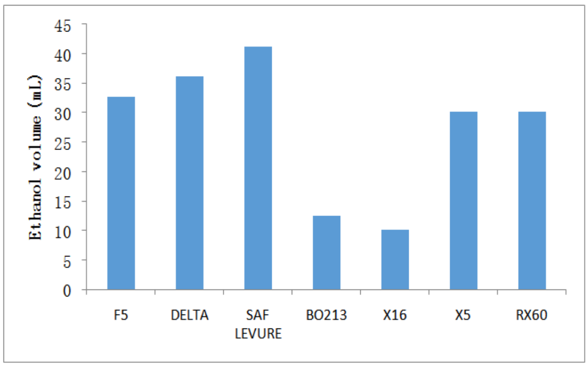

Figure 3. Volume of bioethanol produced by type of yeast used

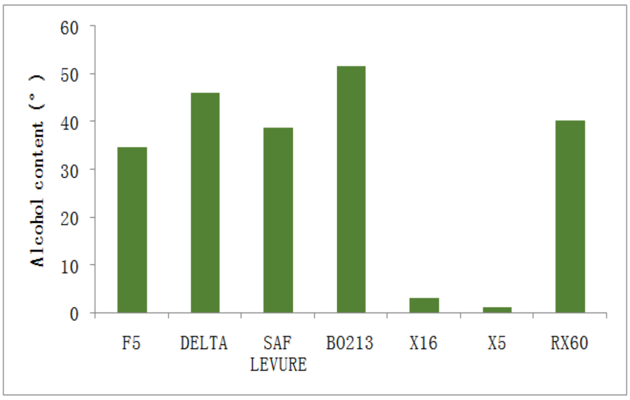

Figure 4. Alcohol content of bioethanols produced by type of yeast used

There is a correlation between the rate of change of the Brix degree and the rate of bioethanol production. Indeed, the best bioethanol production rates are obtained with Saf-Levure yeasts (41 mL) and Delta (36 mL) which are the yeasts for which we observed the greatest decrease in the rate of soluble matter. The production yields of bioethanol are respectively 14 and 12 % with alcohol levels of 46° and 39°. Yeasts X5 and X16, for which the variations of the Brix degree were the lowest, are those which give the lowest yields of production: With yeast X6, a yield of 3 % is obtained; concerning X5 a volume of 30 mL is obtained but with an alcohol rate of about 2 % which shows that the yield is low.

4. Conclusion

The present study showed that mango is a substrate very rich in total sugars and soluble solids, which can be transformed into bioethanol by biotechnological processes. Yeast strains do not react in the same way to this substrate. During the tests of alcoholic fermentation of mango, the best production rates of bioethanol were observed with the commercial yeast Saf-Levure for a yield of 14 % and the oenological yeast Delta for a production yield of 13 %. These results are encouraging when we know that mango is a fruit whose losses are very important because conservation methods are limited and there are no or few processing plants in Togo. However, these results would seek to be improved by varying the amounts of inoculum introduced into the must, by adjusting the pH values according to each yeast or by optimizing the extraction of mango sugars to optimize the conversion to bioethanol. An analysis by GC/MS of hydrolysate and bioethanol would also provide their exact composition.

Conflicts of Interest

The authors of this article declare that there are no conflicts of interest in this publication.

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