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Valorization of Watermelon *(Citrullus lanatus)* into Bioethanol Using Several Yeast Strains of *Saccharomyces Cerevisiae*

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Abstract: Watermelon is a fruit with very high losses, and its waste can attract pests. It's therefore preferable to look for ways to recycle this waste. This study aims to valorize watermelon waste for the production of bioethanol by fermentation. The performance of six yeast strains of Saccharomyces Cerevisiae namely "Zymaflore X16, Actiflore F5, Zymaflore X5, Actiflore BO213, Zymaflore Delta, Zymaflore RX60 and Saf-Levure" were tested, in the presence and absence of urea as a growth factor. The results show that it is possible to obtain ethanol from watermelon waste. Among the six yeast strains studied, four see their fermentation performance improved in the presence of urea. The best yields are obtained for "X5 and RX60" strains with yields close to 18% (compared to less than 4,5% in the absence of urea for the same yeast strains). Nitrogen compounds are therefore nutrients that improve the fermenting power of yeast. The purity of ethanol obtained after distillation of fermentation must varies between 10 and 40% in the absence of urea and between 18 and 42% in the presence of urea. The watermelon from Togo therefore gives interesting and encouraging results for the production of bioethanol with yeast strains that stand out and whose use can be considered for the production of bioethanol on a larger scale. The results obtained will still require optimizing the conditions.

Keywords: Watermelon, Bioethanol, Yeast Strains, Urea

1. Introduction

The disastrous consequences of global warming have been alarming the world for several years. Thus, research is accelerating to find solutions to minimize the negative impacts of anthropogenic activities on human health and the environment. The main causes of these negative effects are high population growth, the use of non-renewable resources as an energy source and the production of large quantities of waste and effluent that are difficult to treat [1, 2]. In this context, the search for alternative, ecological, profitable, sustainable and renewable energy sources has developed in recent decades [3], with the aim of limiting the use of fossil fuels that tend to disappear and are responsible for global warming [4]. Thus, ethanol made from plant biomass fermentation is viewed as an ecological alternative to fossil fuels [5, 6]. It can be mixed with gasoline (at a certain percentage) to reduce the use of fossil fuels, which reduces greenhouse gas emissions responsible for global warming [7]. Several conventional biomass are used for the production of this bioethanol including sugar cane, sugar beet, corn or sorghum. Fruit and vegetables are among the foods with the highest loss rate in the production chain. This is mainly due

to the classification of fruits and vegetables after harvest, due to the established quality standards. Also, in industrialized regions, mass purchasing losses account for 15-30% of the products thrown away by consumers, which have economic, environmental, and social repercussions [8]. In developing countries, the lack of processing and conservation structures for fruit and vegetables is the main cause of post-harvest loss [9, 10]. This food waste is generating a lot of interest, and the UN has designated 2021 as the International Year of Fruits and Vegetables, in order to draw attention to the problem of food waste and loss and to technological innovations aimed at minimizing environmental problems related to these factors [11]. The conversion of fruit waste into bioethanol is therefore a strategy for the recovery of foods that are no longer suitable for human consumption [12, 13]. It is also an opportunity to reduce food waste that leads to significant environmental problems and can cause the proliferation of pests, vectors of certain diseases. The non-exploitation of these residues results in the loss of renewable energy sources and bioproducts that can be recovered from these biomass [14, 15]. Watermelon (Citrullus lanatus) is one of the fruits with the highest crop losses and full utilization potential that has not yet been explored, with a global harvest of 101620420 tonnes in 2021 [16]. According to estimates, about 20% of the crop is not marketed because of surface imperfections or deformations [17]. In addition, a significant percentage is thrown at the places of commercialization due to excessive ripening, superficial spots, white areas and crumbling. Therefore, the use of watermelon waste as a substrate for bioenergy production can be an alternative to the accumulation of such waste and, at the same time, make it possible to economically recover watermelon waste. In Togo, there is currently no precise statistical data on the quantity of watermelon produced annually. However, this fruit is produced both in the great Lomé and in the region of Kara. This fruit which abounds when it is the period and which finds difficult taker on the market is therefore victim of losses for the producers and the resolvers. This is why we are interested in the valorization of watermelon to produce bioethanol.

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).

2.1. Raw Material

The fruits of watermelon (Citrallus lanatus) were obtained in the period June-July 2023, from resellers of the market of Kara - Togo. Watermelons were washed and disinfected with a solution of sodium hypochlorite 1M (NaClO), stored at 13°C and used within 4 days.

2.2. Physico-Chemical Characterization of Samples

Water content was determined by desiccating 10g of watermelon pulp, placed in a Memert insulated oven at 105°C and atmospheric pressure, until a constant mass of the sample was obtained [18]. Ash was determined by heating 10g of watermelon pulp in a Nabertherm oven at 550°C for 2 hours. The total organic matter content was determined by differentiating between the mass of the sample (10g) after 24 hours of drying at 105°C in the oven and the mass of the sample (10g) after carbonization in the oven at 550°C for 6 h.

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

Titratable acidity was determined by titration with NaOH solution at 0.1M up to pH = 8,1 using 1% bromotimol blue as a coloured indicator. Total sugars were determined using the phenol-sulphuric method [19]. The calibration curve shown in Figure 1 was used to determine the exact total sugar content.

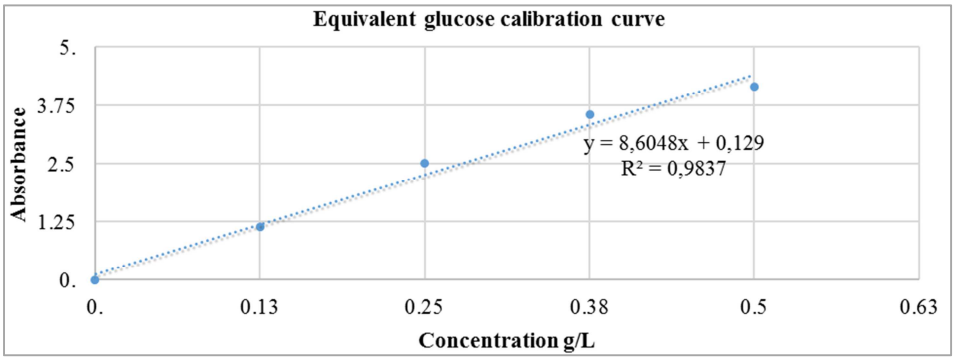


Figure 1. *Calibration curve used to determine total sugar content.*

We used iodometric ascorbic acid assay to determine the content of Vitamin C [20].

2.3. Inoculum Preparation

The ferments used consist of six yeast strains of Saccharomyces cerevisiae for oenological use (Zymaflore X16, Actiflore F5, Zymaflore X5, Actiflore BO213, Zymaflore Delta and Zymaflore RX60) marketed by Laffort Oenologie France and the Saccharomyces Cerevisiae Saf-Levure strain marketed by Lesaffre France company. Two

types of inoculum were prepared: a classical inoculum by introduction of 6,0 g of each of the dry yeast strains (lyophilized) in 100 mL of sugar water; an inoculum with urea was prepared as before with addition of 5% ureaw/w.

2.4. Preparation of the Fermentation Must

The fresh watermelons were cut with a knife. The pulp containing the seeds was ground using an electric mixer. The resulting juice was heated to 90°C for 20 minutes to remove microorganisms and pathogens that may influence fermentation and cooled to room temperature [21, 22]. To achieve the optimal pH zone for watermelon fermentation, soda was added to adjust the pH of the juice to 4,5 [23]. The fermentation must is distributed in sterilized 1 litre glass bottles that serve as fermenters. Controlled fermentation was carried out by adding the inoculum (5%v/v) prepared the day before to the previously obtained watermelon juice.

2.5. Distillation

We extracted bioethanol by fractional distillation of fermented must at the end of the fermentation. The distillation temperature is 79°C at the head of the column [24]. A flame test was performed to verify that the product obtained is ethanol. The density and purity of bioethanol alcohol was determined. The ethanol produced was stored in vials at 4°C.

2.6. Analytical Methods

We used a Schimler pH meter to measure the pH and temperature of the musts. The total soluble solids content (Brix degree) was determined using a Scihemtech manual refractometer. The relative density of the product obtained was determined by the pycnometric method. The purity of the bioethanol produced (% v/v) was determined according to the pycnometric method recommended by AOAC (1984). The ethanol production yield (Rp), which is the ratio of ethanol volumes obtained per volume of fermented must, was also determined.

3. Results and Discussion

Physico-Chemical Characteristics of Watermelon

The results presented in Table 1 below show the physico-chemical characteristics of watermelon studied.

Table 1. *Physical-chemical parameters of citrullus lanatus.*

	Citrullus Lanatus
Water content (%)	93,11 ± 1,27
Ash content (%)	0,92 ± 0,46
Total organic matter content (%)	68,16 ± 0,46
Total sugar content (%)	10,99 ± 2,82
Reducing sugar content (%)	0,46 ± 0,04
Solubility Rate (°Brix)	5
Titrable acidity (g/100g)	0,05
Vitamin C content (mg/100g)	7,13 ± 0,52

Watermelon is a fruit made almost exclusively of water, with a water content of 93%. It is also known for its very thirst-quenching character in case of high heat, because of its high water content [25]. Its content of total sugars (11%) and reducing sugars (0,46) is relatively low. Low levels of total sugar and reducing sugar would be due to sampling period which is in July month. Indeed, July is a month of heavy rains in the Kara región, so that it increases wáter content of watermelon up to 96% of wáter of the simple mass. The Brix degree, with a value of 5, is also low compared to other fruits such as mango, which has a Brix degree around 15 and has already been used as biomass to produce bioethanol.

Evolution of pH During Fermentation

Figure 2 show the variation of the pH during the fermentation process of the must with the different yeast strains in the absence of urea, and Figure 3 in the presence of urea.

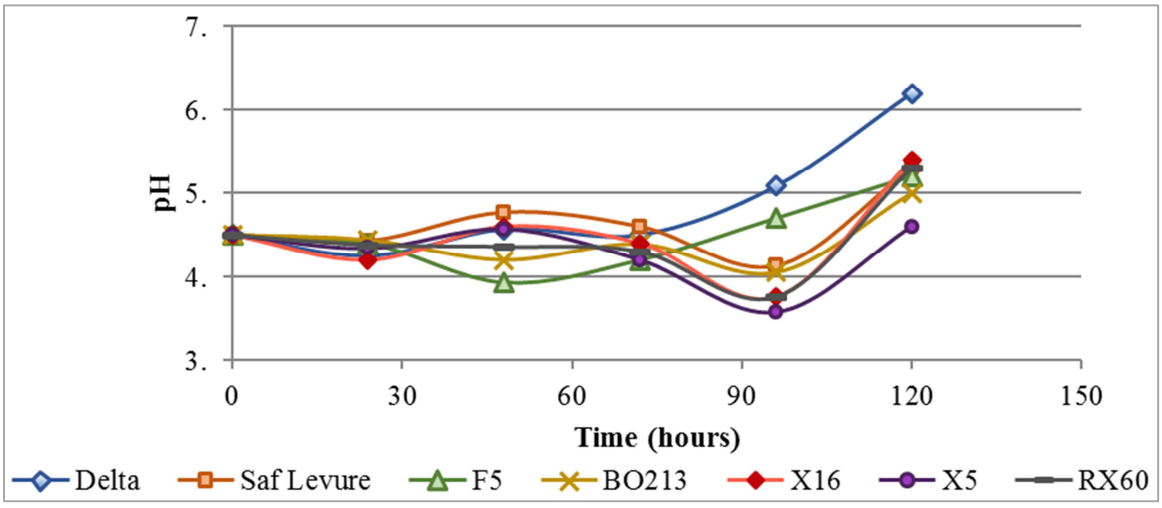


Figure 2. *Evolution of pH in musts during fermentation with urea-free inoculum.*

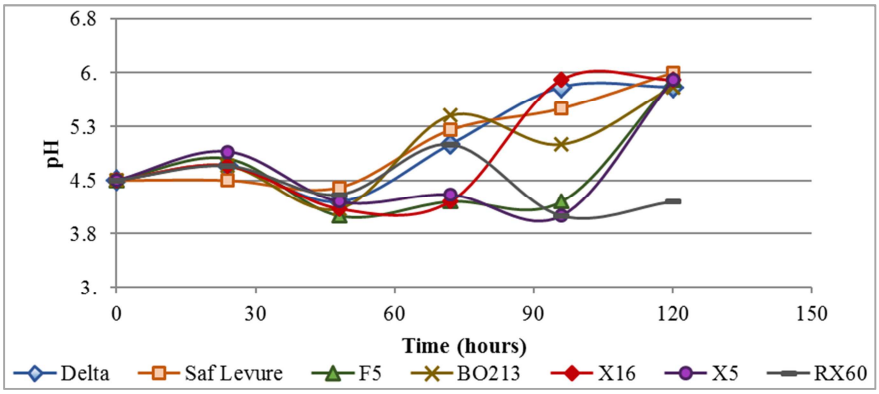


Figure 3. *Evolution of pH in musts during fermentation with urea inoculum.*

Watermelon juice without any treatment is moderately acidic since it has a value of 5,72. Before fermentation, the pH was adjusted to 4,5 by adding sulfuric acid. Regardless of the yeast strain used and with or without urea, a variation in pH is observed during fermentation. With the alcoholic fermentation process, the metabolism of the yeast induces a perpetual change in the medium. Variations in pH are explained by the fact that the production of alcohol from sugars leads to a change in the dissociation of the components of the must and mainly of the organic acids initially present in the must [26]. The consumption of carbon and nitrogen substrates is accompanied by the production of acid or alcohol metabolites. Carbon dioxide or acidic compounds are produced by yeasts which can lead to an increase in acidity. In the presence of ethanol, dissociation is less important and therefore a lower proton concentration and therefore a slight increase in pH. The pH of watermelon must is located within the optimal pH range of bioethanol production which is 4,3 to 5,7 on neem pulp [23], except with Delta yeast with added urea which has a slightly higher pH at the end of fermentation. We can therefore hope to succeed in producing bioethanol from watermelons.

Evolution of the rate of soluble solids during fermentation

Figures 4 and 5 show the evolution of the rate of soluble solids in the must during fermentation according to the yeast strains used, with and without urea.

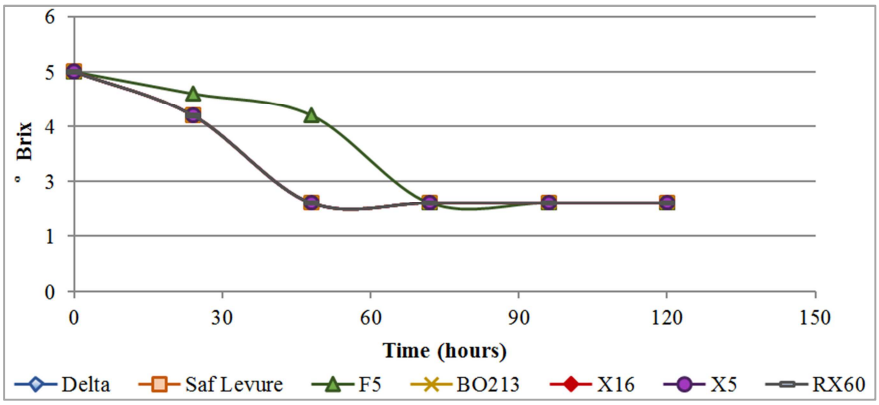


Figure 4. *Brix evolution of watermelon must during the urea-free fermentation process.*

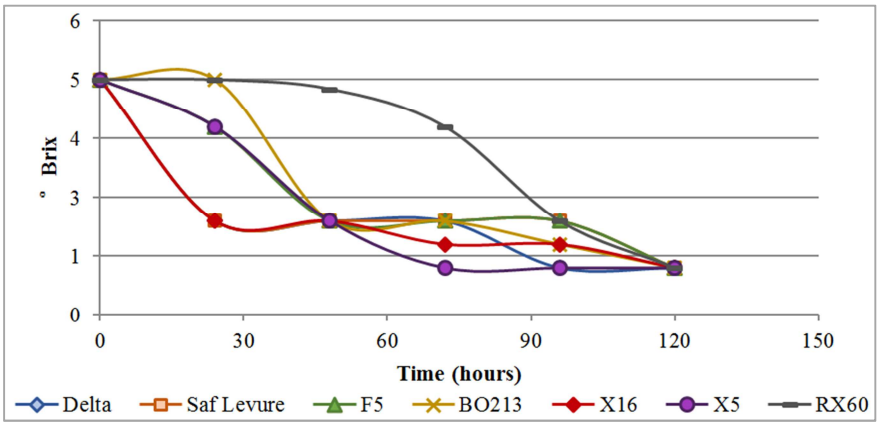


Figure 5. *Brix evolution of watermelon must during fermentation process with urea.*

In the absence of urea, it is noted that all yeasts have the same variation profile of the degree Brix except yeast F5. A decrease is observed during the first 24 hours and stabilizes at 48 h with a degree Brix from 5 to 2. This degree Brix no longer varies during the 96h following during which the musts were left in fermentation. We can assume that the fermentation ended after 48 hours. With urea, the curves have a very different profile: we see that with yeast RX60, a slow decrease is observed and it is after 120 hours of fermentation that the Brix degree reaches its lowest level while with yeasts "X16, Safe Yeast and Delta", the decrease is very rapid and after 24 hours the Brix is 2. At the end of fermentation, all Brix degrees are 1. With these very heterogeneous variations, it is impossible to predict which yeast will produce the best ethanol yield, but it is assumed that there is indeed fermentation in the media.

Yield of bioethanol produced

Figures 6 and 7 present the results of the analysis of bioethanols produced in the absence of urea; those obtained in the presence of urea are presented in Figures 8 and 9.

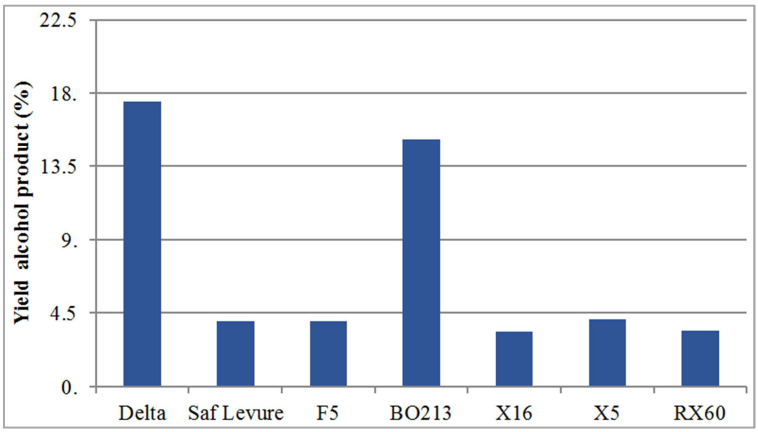


Figure 6. *Yield of bioethanol produced by type of yeast used in the absence of urea.*

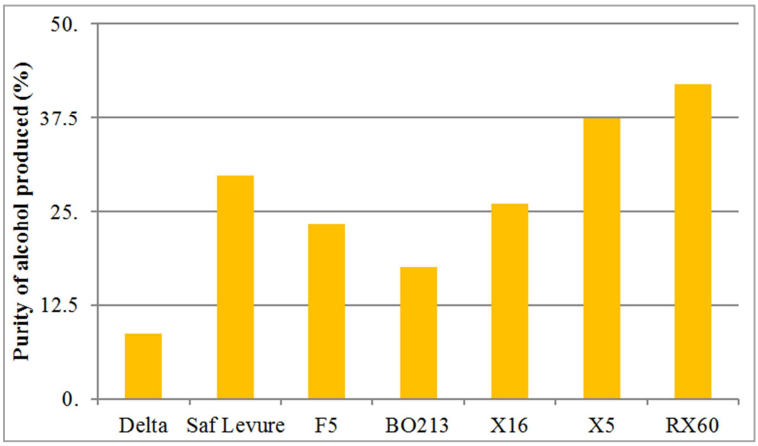


Figure 7. *Purity of bioethanol produced according to the type of yeast used in the absence of urea.*

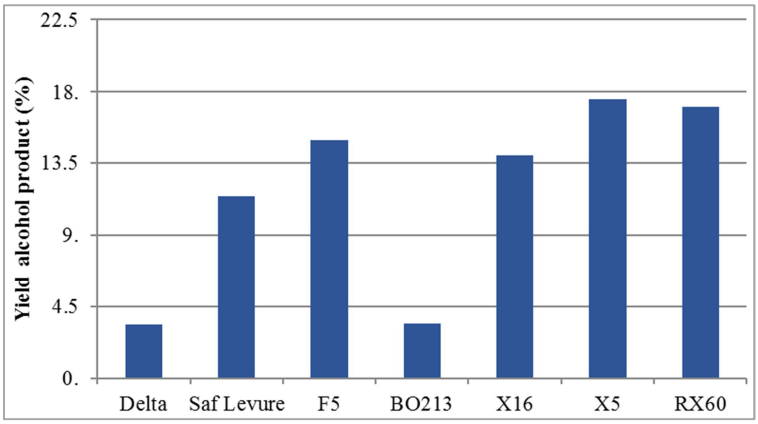


Figure 8. *Yield of bioethanol produced by type of yeast used in presence of urea.*

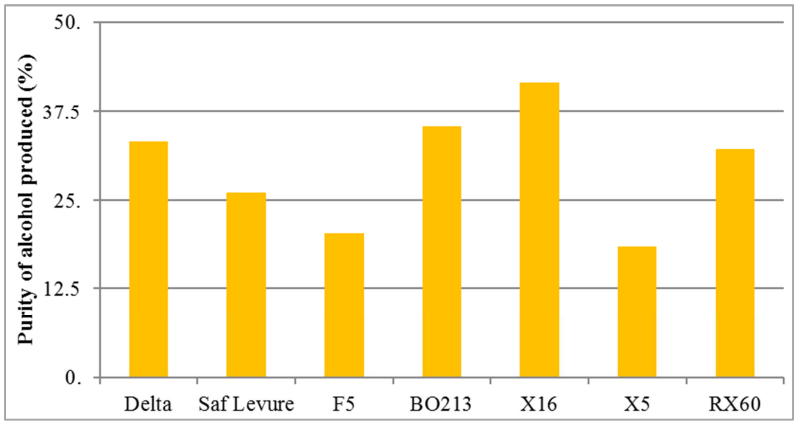


Figure 9. *Purity of bioethanol produced according to the type of yeasts used in presence of urea.*

In the absence of urea, although the variation in the rate of soluble matter was almost identical for all yeast strains, disparities are observed in the production yield of bioethanol. Indeed, it is the "Delta and BO213 yeasts", which give the best yields with a production rate between 15 and 17%. The other yeast strains give low yields not exceeding 4%. The observations are completely opposite in the presence of urea. Indeed, in the presence of urea in the inoculum, it is with "yeasts X5 and RX60" that the highest rate of bioethanol production is observed with a yield of about 17%. Conversely, the lowest yields are obtained for "Delta and BO213" yeasts. Other yeast strains yield between 10 and 15%. It therefore seems that, except for Delta and "BO213", the addition of nitrogen compound improves the fermentation performance of yeasts. For its nutrition the yeast needs to find in the must (fermentation medium) a set of nutrients necessary for its proper development including sugar, nitrogen, mineral salts and vitamins [27]. It should be remembered that nitrogen is a key component of proteins and amino acids, which are necessary for the growth of yeast cells. Thus, the yeasts "X5 and RX60" which recorded the highest production rate of bioethanol in the presence of urea have specific permeases allowing them to transport through the membrane the mineral nitrogen from urea [28]. However, urea may not be the best source of nitrogen for all yeast strains. Indeed, some yeasts prefer forms of organic nitrogen, such as amino acids, peptides.. It is important to note that depending on the yeast strain, the addition of urea in the must can cause an excess of nitrogen ('Crabtree effect') for the yeast thus disrupting the balance of nitrogen compounds in the cell [29]. This can lead to disruptions in metabolic pathways and regulatory problems that affect yeast growth. The latter may result in the lowering of the alcoholic fermentation capacities of yeasts. From the above, it can be understood that the yeasts "BO213 and Delta" see their fermentation performance inhibited in the presence of mineral nitrogen brought by the addition of urea. Regarding the purity of the bioethanol obtained, there is no correlation with the rate of return. It oscillates between 10 and 40% without urea and between 18 and 42% with urea.

4. Conclusion

The present study showed that watermelon is a substrate that is not very rich in total sugars and soluble solids but can be transformed into bioethanol by biotechnological processes. Yeast strains do not react in the same way to this substrate and the presence of urea has an influence on the production yield of bioethanol. During the alcoholic fermentation trials of watermelon, the best production rates of bioethanol were observed in the presence of urea for yeast strains "RX60 and X5". Yields are around 18%. These results are encouraging when we know that watermelon is a fruit whose losses are very important because conservation methods are limited and there are no or few processing plants in the world and even less in Togo. However, these results would seek to be improved by varying the amounts of inoculum introduced into the must, adjusting the pH according to each yeast or optimizing the extraction of sugars from watermelon to optimize the conversion to bioethanol.

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Conflicts of Interest

The authors declare no conflicts of interest.

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