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High-Yield Bioethanol Production from Unfit Oranges for Consumption

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Abstract

With their very high fermentable sugar content, rotten oranges stand out as one of the raw materials of choice to maximize bioethanol production. This work was deliberately aimed to evaluate the efficiency of the technology for producing bioethanol from musts prepared from rotten oranges. To achieve this goal, four types of musts (16 ˚Brix, 20 ˚Brix, 24 ˚Brix, and 28 ˚Brix) were prepared from raw juice (9.5 ˚Brix) extracted by mechanical pressing of the oranges. Sucrose was used as the reference fermentable sugar. Sodium glutamate, added to the musts, played the role of increasing yeast cells to effectively boost their enzymatic catalysis. Ethanol fermentation in batch mode and fed-batch mode was adopted. The ethanol fermentation reaction was monitored by refractometric measurement, while the alcoholic strength of the fermented musts was determined by the pycnometric method. The results revealed that the ethanol content (% vol.) produced from the must of 28 ˚Brix from rotten oranges, fermented in batch mode, reached a maximum value of 16.51 ± 0.21 with the addition of sodium glutamate SG (2 g/L), whereas with the same must, only a content (% vol.) of 14.72 ± 0.05 was obtained in semi-continuous mode. However, without SG, an ethanol content (% vol.) of 4.93 ± 0.33 was only produced from the raw juice (9.5 ˚Brix), by mechanical pressing of rotten oranges. In contrast to the results obtained with sucrose, the musts of rotten oranges without SG supplementation produced higher ethanol contents than those supplemented with SG (2 g/L). Finally, batch fermentation of rotten orange musts was more efficient than fed-batch fermentation. However, the addition of SG (2 g/L) in the rotten orange musts fermented by fed-batch mode enhanced the ethanol content, while in batch mode, this addition was only beneficial for the rotten orange musts of 28 ˚Brix and 9.5 ˚Brix. The valorization of rotten oranges into bioethanol therefore fits into local circular economy approaches to promote the sustainable use of natural resources.

Keywords

Inedible Oranges, Valorization, Green Energy, Environmental Protection, Circular Economy

1. Introduction

For a number of decades, energy and environmental concerns have taken center stage in global discourse. Indeed, the rise in fossil fuel prices, the depletion of non-renewable energy reserves, and the intensification of greenhouse gas (GHG) emissions have prompted the scientific community to explore sustainable energy solutions [1]. These solutions share the common goal of reducing dependence on polluting energy sources while limiting their environmental footprint [2]. In this context, biofuels, as an inexhaustible source of green energy, stand out as one of the most outstanding substitutes for petroleum-based fuels [3]. Moreover, the exploitation of new raw material sources, such as algae, offers significant opportunities to reduce environmental impacts and integrate sustainable fuels into the global energy cycle [4].

Biofuels, such as bioethanol, are gaining popularity as alternative solutions to fossil fuels, especially in the transport sector, thanks to their almost low environmental impacts. Indeed, derived from organic materials, these fuels provide a response to energy challenges while mitigating their impact on the environment [5] [6]. However, their large-scale production remains a major challenge, particularly with regard to the preservation of global food security [7] [8]. Accordingly, the use of food crops to produce first-generation bioethanol has sparked strong criticism concerning the competition between energy and food needs [9]. To address the challenge of food competition in bioethanol production, scientists are exploring sustainable alternative solutions. Using non-edible substrates, including agricultural leftovers, especially fruit waste, is one of the attractive solutions that is now generating debate. These agro-industrial by-products, which are naturally rich in fermentable sugars, constitute an underutilized resource for producing bioethanol through fermentation processes with optimal profitability [10] [11]. Consequently, these tactics have a significant positive impact on the environment, particularly in terms of decreasing food waste and satisfying the rising need for renewable energy [12]. Unlike food crops, the exploitation of agro-industrial residues rich in fermentable sugars ensures attractive bioethanol yields while minimizing impacts on food security [13]. In addition, these residues contribute to a circular economy by reintegrating plant-based waste into energy value chains [14]. This raises the question of which method would be the most effective for producing bioethanol with optimal profitability.

The most fermentable carbohydrates for the yeast *Saccharomyces cerevisiae* are glucose, fructose, and sucrose. Glucose is the preferred carbon source for yeast, followed by fructose, then sucrose, which must be hydrolyzed into glucose and fructose to be used [15]. The work carried out by [16] highlighted that the major sugars contained in orange (*Citrus sinensis* L. Osbeck) juice were sucrose, glucose, and fructose, with respective contents (g/L) of 46.40 ± 1.41, 30.99 ± 1.84, and 33.05 ± 1.13, equivalent to a major sugar content of 110.44 ± 1.53 g/L.

In response to this question, bioethanol production from fruit waste undoubtedly requires the optimization of various parameters, including: must sugar concentration, the applied fermentation mode, and the use of nutritional additives to enhance the efficiency of enzymatic catalysis in the bioconversion of fermentable sugars by yeast of the genus *Saccharomyces cerevisiae*. Previous studies have demonstrated that certain nitrogen-based nutrients, such as sodium glutamate, can effectively stimulate yeast growth, and as a result, improve ethanol yields [17] [18]. Furthermore, the adoption of alternative fermentation modes, such as fed-batch fermentation, extends the active fermentation phase, which in turn increases ethanol production yield [19].

The current study approach consists of exploring cost-effective bioethanol production with optimal profitability from oranges discarded in nature due to spoilage. Accordingly, the objective of the present study was to optimize bioethanol production parameters from rotten oranges. To achieve this goal, two fermentation modes were comparatively adopted, namely batch fermentation and fed-batch fermentation. In parallel with these two fermentation modes, the influence of sodium glutamate on the efficiency of bioethanol production was also assessed.

2. Materials and Methods

2.1. Materials Used for Agroethanol Production

Rotten oranges (**Figure 1**), by-products from agri-food distribution chains in Lomé-Togo, served as a raw material rich in fermentable sugars, offering significant

Figure 1. Photo of rotten oranges used as raw material.

potential for bioethanol production. To valorize this waste in this green energy, their juice was mechanically extracted with a juicer after peeling and manual cutting. The resulting must was supplemented with sodium glutamate (SG) to stimulate yeast growth and improve fermentation kinetics. The conversion of sugars into ethanol was carried out by the yeast *Saccharomyces cerevisiae* (Saf-Levure S.I. Lesaffre), usually applied in the form of active dry yeast.

2.2. Experimental Procedure for the Bioconversion of Rotten Oranges into Bioethanol

Prior to its utilization as a raw material, rotten oranges were carefully washed to remove all impurities, including plant debris, plastics, sand, and microorganisms. They were then peeled, cut, and pressed to extract the raw juice. After filtration, the collected filtrate was concentrated by evaporation through heating without reflux in a glass flask to obtain four types of musts with concentrations of 16 ˚Brix, 20 ˚Brix, 24 ˚Brix, and 28 ˚Brix. The ethanol fermentation of the prepared musts was carried out with the genus *Saccharomyces cerevisiae*, used as fermentative microorganisms. Once ethanol fermentation of the musts was complete, the produced bioethanol was recovered by fractional distillation through mass transfer using a simple distillation column in order to determine the ethanol content in the musts at the end of fermentation.

2.3. Physicochemical Characterization of the Raw Material

2.3.1. Determination of Moisture and Volatile Matter Content

The principle adopted for this characterization was based on oven-drying slices of degraded orange, with an initially known mass, placed in a Petri dish at a temperature of about 103˚C ± 2˚C until complete removal of water and volatile matter. The moisture and volatile matter content (MC) was then calculated using Equation (1) [20].

$$\text{MC} = \frac{\text{MH} - \text{MS}}{\text{MH} - \text{MB}} \times 100 \tag{1}$$

where:

MH = mass of the fresh rotten orange + Petri dish before oven-drying;
MS = mass of the dried rotten orange + Petri dish after oven-drying;
MB = mass of the empty Petri dish.

2.3.2. Measurement of Dry Matter Content

Dry matter content (Si), which is the mass percentage (%) of dry matter contained in an organic substance, was expressed relative to its total mass (wet matter). In practice, this percentage is theoretically calculated using the value of the moisture and volatile matter content (MC), according to Equation (2) [20].

$$\text{Si} + \text{MC} = 100\% \tag{2}$$

2.3.3. Evaluation of Ash Content

The experimental protocol used consisted of calcining oven-dried slices of rotten orange for two hours in a crucible placed in a muffle furnace, with the maximum temperature set at 550˚C. This operation was carried out with a heating rate of 10˚C/min from the beginning to the end of the experiment. After the complete destruction and elimination of the organic matter in the form of gases escaping from the crucible, the ash content (AC) was evaluated using Equation (3) [20].

$$\text{AC} = \frac{M_1 - M_0}{M_2 - M_0} \times 100 \qquad (3)$$

where:

AC = Ash content of the rotten oranges;

M_0 = Mass of the empty crucible;

M_2 = Total mass of the crucible and the sample before calcination;

M_1 = Total mass of the crucible and the ashes after calcination.

2.4. Raw Juice Extraction from Rotten Oranges

In this study, the extraction method applied for recovering raw juice from rotten oranges was mechanical pressing, using a juicer, commonly employed by juice vendors in Lomé-Togo for preparing citrus juices. The raw juice obtained through mechanical pressing was heated to 80˚C for 60 minutes, then cooled to room temperature before being stored in a freezer at −23˚C for later use.

2.5. Preparation of Musts for Ethanol Fermentation

To minimize substrate inhibition, the effects of feeding yeast with different sugar concentrations on the ethanol production by batch and fed-batch cultures [21] in a 1-L fermentor were investigated. To achieve this purpose, the raw juice taken from the freezer was first thawed, and the resulting liquid was concentrated by heating without reflux to obtain four types of concentrated musts (16 ˚Brix, 20 ˚Brix, 24 ˚Brix, and 28 ˚Brix). These intentionally prepared musts were stored in a freezer (−23˚C) for later operations. To make a comparison, sucrose musts were also prepared to carry out fermentation in fed-batch mode. Ethanol fermentation of the sucrose musts was used as a reference for that of the rotten orange musts in order to optimize bioethanol production in this study. Indeed, the sucrose musts used as a model substrate to carry out the ethanolic fermentation in order to transpose the method to the case of orange juice because sucrose, glucose, and fructose are the sugars predominantly present in orange juice, as referenced in the work done by [16]. Moreover, the hydrolysis of sucrose leads to the formation of glucose and fructose, which are the monosaccharides whose ethanolic fermentation based on *Saccharomyces cerevisiae* yields the best bioethanol production rates [16].

2.6. Ethanol Pre-Fermentation

Pre-fermentation is the step that precedes ethanol fermentation itself. At this stage, an inoculum was prepared with 1/10 of the volume of each rotten orange must, with the addition of baker's yeast of the genus *Saccharomyces cerevisiae* (1 g/L) and SG in a concentration range from 0 to 8 g/L. The resulting ethanol fermentation broths were left to ferment under anaerobic conditions at room temperature (30˚C

- 32˚C) for 24 hours, allowing the fermentative microorganisms to adapt to their culture medium.

2.7. Initiation of Ethanol Fermentation

At this stage, each pre-fermented must was mixed with the remaining 9/10 of the must to initiate ethanol fermentation properly [22]. After stirring, the mixture was left to ferment at room temperature (30˚C - 32˚C) under two ethanol fermentation modes, namely batch fermentation and fed-batch fermentation.

2.8. Monitoring of Ethanol Fermentation and Measurement of Ethanol Content

The ethanol fermentation reaction was periodically monitored every 24 hours from the start until the exhaustion of each must by measuring the total soluble solids (TSS), expressed in degrees Brix. An Abbe/Azzota refractometer, model AR-2 [20], was used for this measurement. At the end of fermentation, the limit attenuation (LA) of each fermented must was determined using Equation (4).

$$\text{LA} = [(\text{Initial Brix} - \text{Final Brix})/\text{Initial Brix}] \times 100\% \quad (4)$$

The alcoholic strength, or ethanol content produced from the musts at the end of fermentation, was determined using the pycnometric method according to the recommendations of AOAC (Association of Official Analytical Chemists), method 982.10.

2.9. Applied Ethanol Fermentation Modes

Ethanol fermentation in batch mode and fed-batch mode were comparatively applied with the aim of maximizing ethanol yield.

3. Results

3.1. Physicochemical Characteristics of the Rotten Oranges

The physicochemical properties of rotten oranges, namely moisture and volatile matter content (MC), dry matter content (Si), mineral fraction or ash (AC), as well as the proportion of organic matter (OM), are illustrated in the form of a two-dimensional pie chart (**Figure 2**).

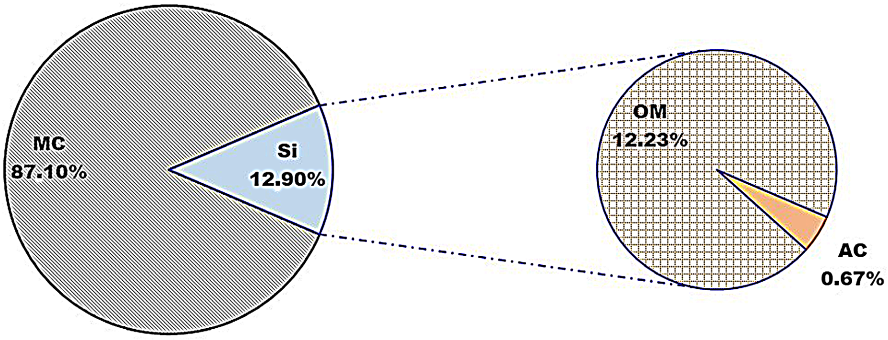

Figure 2. Physicochemical characteristics of rotten oranges.

The analysis of the data from this representation indicates that the decomposed oranges used in this study have a moisture and volatile matter content of 87.10% ± 0.50%. In addition, the dry fraction corresponding to a dry matter content (Si) of 12.90% ± 0.50% is divided into an ash content (AC) of 0.67% ± 0.01% and an organic matter content (OM) of 12.23 ± 0.49%.

3.2. Influence of SG on Sucrose Must Fermentation

3.2.1. Influence of SG on the Variation of Total Soluble Solids in Sucrose Musts during Batch Ethanol Fermentation

The variation of TSS as a function of time during batch fermentation of sucrose musts with normal density (20 ˚Brix) and very high density (28 ˚Brix), supplemented with SG at different concentrations, produced the curves illustrated in Figure 3(A) and Figure 3(B).

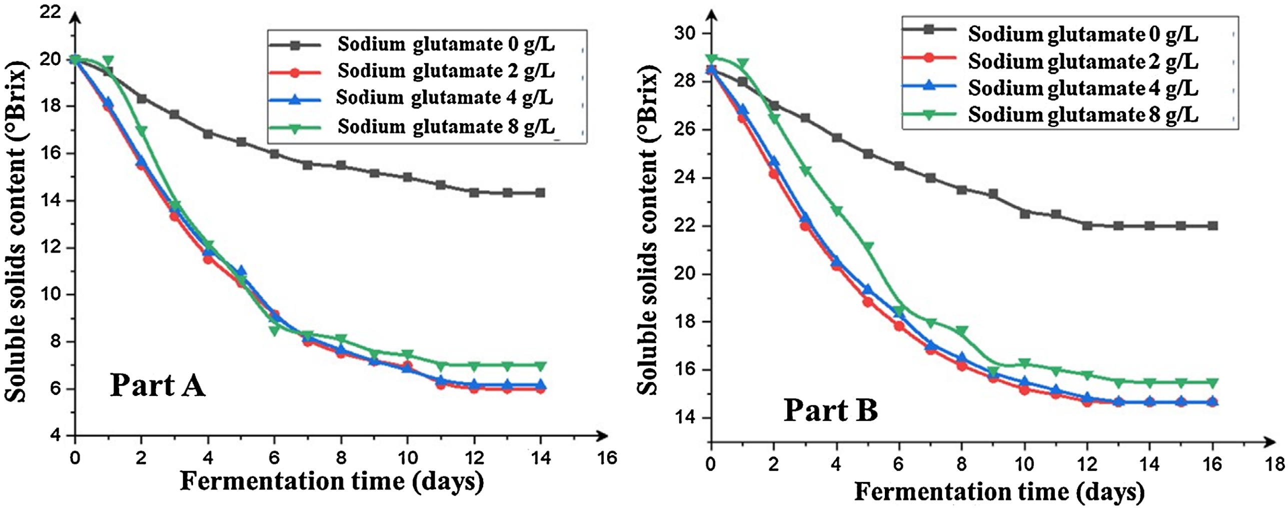

Figure 3. Variation of TSS over time in sucrose musts NG (Part A) and VHG (Part B), supplemented with SG during batch fermentation.

A decrease in TSS over time was observed in each must. However, this decrease in TSS was noticeably more pronounced in the sucrose musts supplemented with SG.

3.2.2. Influence of SG on Ethanol Content Produced with Sucrose Musts in Batch Mode

The ethanol contents (EC) obtained through batch fermentation of sucrose musts NG (20 ˚Brix) and VHG (28 ˚Brix), with or without SG supplementation, are shown in Figure 4.

From the results presented in Figure 4, it can be observed that, regardless of the batch fermentation method adopted or the sucrose must concentration, whether NG (20 ˚Brix) or VHG (28 ˚Brix), the use of SG as a growth factor led to significantly higher ethanol contents compared to the control musts.

3.2.3. Influence of SG on the Experimental Yield of Bioethanol Production in Batch Mode from Sucrose Musts

The results shown in **Figure 5** present the experimental yields of ethanol production (EYP) in batch mode with sucrose musts NG (20 ˚Brix) and VHG (28 ˚Brix).

The ethanol EYPs in this study are relatively close to the theoretical Gay-Lussac yield of 100%. They range from 35.46% ± 0.67% to 87.92% ± 0.65% for NG sucrose musts and from 31.40% ± 0.11% to 66.85% ± 0.33% for VHG sucrose musts.

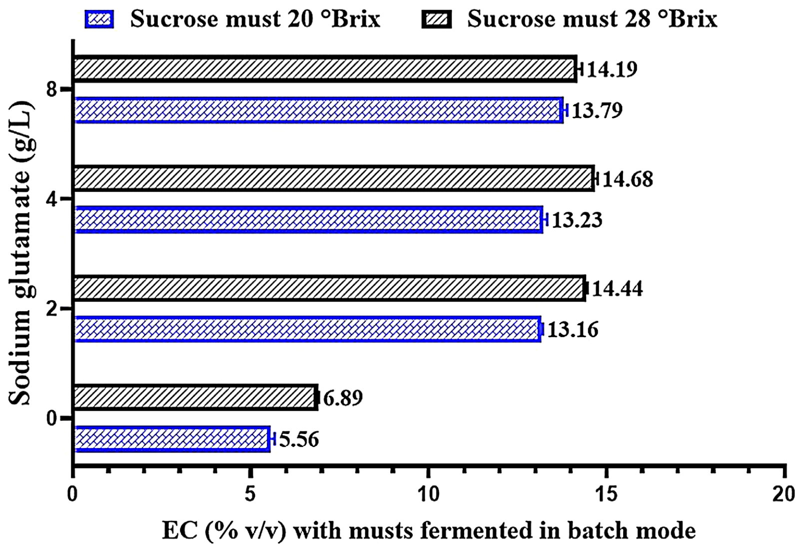

Figure 4. Influence of SG on EC through batch fermentation of sucrose musts NG (20 ˚Brix) and VHG (28 ˚Brix).

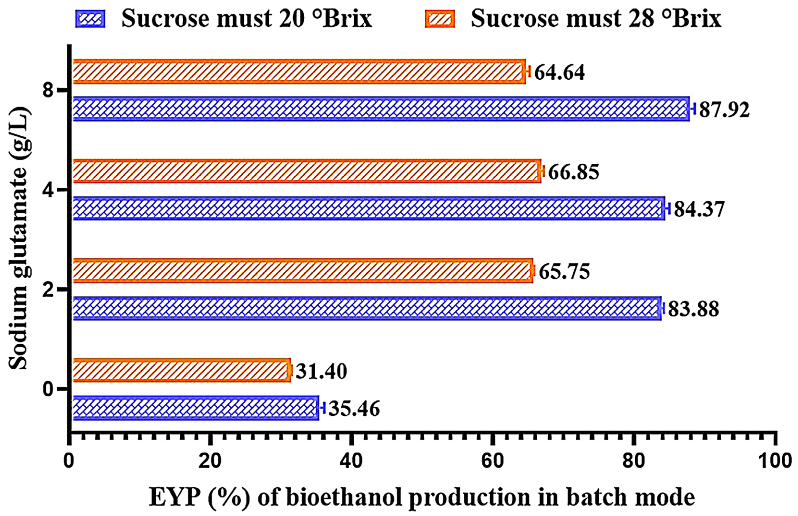

Figure 5. Influence of SG on the EYP (%) of ethanol through batch fermentation of sucrose musts NG (20 ˚Brix) and VHG (28 ˚Brix).

3.2.4. Influence of SG on the Evolution of Sucrose Must Concentration during Fed-Batch Fermentation

The curves in **Figure 6** illustrate the variation of TSS over time in VHG (28 ˚Brix) sucrose musts under semi-batch fermentation.

It can be seen in this figure that the decrease in TSS is not continuous due to the periodic additions of sugars to the fermentation broths, which resulted in

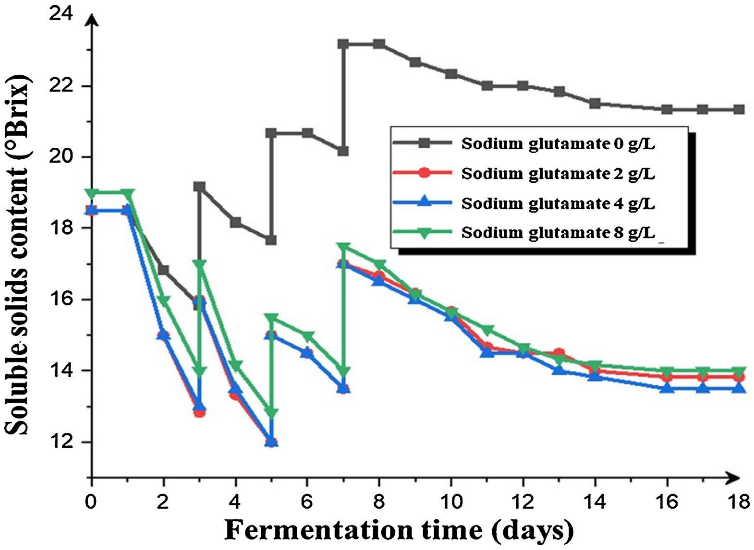

Figure 6. Variation of TSS in VHG sucrose musts during fed-batch fermentation.

cascading increases in TSS in the musts over time. This practice is particularly intended to reduce the stress that fermentative microorganisms would undergo if they were exposed to a must with an initially high sugar concentration (Crabtree effect). Thus, to lower the osmotic pressure across the yeast cell membrane surface during ethanol fermentation, it has been wisely recommended not to introduce the total amount of fermentable sugars into the culture medium at once, but rather progressively.

The analysis of these curves shows that, at the end of semi-batch fermentation, the SG concentration of 4 g/L appeared to produce the most significant decrease in TSS, as indicated by the corresponding curve reaching the lowest level at the end of fermentation. However, this decrease in TSS is practically similar to that obtained with SG concentrations of 2 g/L and 8 g/L. In contrast, in the neutral sucrose must, the decrease in TSS was comparatively much lower.

A more detailed analysis of the results presented in this section shows that the addition of SG had a very significant positive effect on sucrose consumption during fed-batch fermentation.

3.2.5. Influence of SG on EC through Fed-Batch Fermentation of Sucrose Musts

Table 1 records the ethanol contents (EC) obtained from fed-batch fermentation of sucrose musts (28 ˚Brix) as a function of SG concentrations.

From the results presented in Table 1, it can be observed that the use of SG as a growth factor led to significantly higher EC values compared to the neutral must.

In this study, the increase in EC resulting from the use of SG is consistent with the results illustrated in Figure 6.

3.2.6. Influence of SG on the Experimental Yield of Bioethanol Production through Fed-Batch Fermentation of Sucrose Musts

The experimental yields of production (EYP) of bioethanol from fed-batch fermentation of sucrose musts (28 ˚Brix), as a function of SG concentrations, are presented in **Table 2**. The EYPs from fermentation of sucrose musts (28 ˚Brix) ranged between 67.34% ± 0.77% and 69.66% ± 0.42% for sucrose musts supplemented with SG, whereas that of the neutral sucrose must was only 32.99% ± 0.18%.

Table 1. EC in VHG sucrose musts fermented in fed-batch mode.

SG (g/L)	EC (% v/v)/VHG/Fed-batch mode
0.00	07.60 ± 0.30
2.00	14.79 ± 0.17
4.00	15.12 ± 0.04
8.00	15.29 ± 0.09

Table 2. EYP (%) of bioethanol from fed-batch ethanol fermentation of sucrose must (28 ˚Brix).

SG (g/L)	EYP (%)/VHG/Fed-batch mode
0.00	32.99 ± 0.18
2.00	67.34 ± 0.77
4.00	68.85 ± 0.18
8.00	69.66 ± 0.42

The supplementation of sucrose musts (28 ˚Brix) with SG improved the experimental yield of bioethanol production in fed-batch mode, with values ranging from 104.12% to 111.15% compared to the neutral sucrose must.

3.2.7. Comparison of Limit Attenuation Values for the Three Methods of Bioethanol Production with Sucrose

The results presented in **Table 3** indicate that the limit attenuation (LA) values of ethanol fermentation in NG sucrose musts (20 ˚Brix) were higher than those of VHG sucrose musts (28 ˚Brix). The presence of SG in the musts had a beneficial effect on LA values. Moreover, fed-batch fermentation yielded higher LA values than batch fermentation for the high-density sucrose must (28 ˚Brix).

Table 3. Limit attenuation (LA) of fermented sucrose musts.

SG (g/L)	LA (%)		
	NG/Batch mode	*VHG/Batch mode*	*VHG/Fed-batch mode*
0.00	28.33 ± 0.83	19.64 ± 0.00	23.81 ± 0.60
2.00	70.00 ± 0.00	42.26 ± 0.60	50.60 ± 0.60
4.00	70.00 ± 0.00.	47.62 ± 1.19	51.79 ± 0.00
8.00	64.17 ±0.83	46.77 ± 0.00	50.00 ± 0.00

However, it was observed that the LA values of NG sucrose must (20 ˚Brix) fermented in batch mode were significantly higher than those corresponding to the fermentation of VHG sucrose must (28 ˚Brix).

3.3. Influence of SG on Batch Fermentation of Orange Musts

3.3.1. Influence of SG on the Decrease of TSS in Orange Musts during Batch Fermentation

Figures 7-11 present the influence of SG on the decrease of TSS in rotten orange musts during ethanol fermentation in batch mode, with different initial must concentrations in ˚Brix: 9.5, 16.0, 20.0, 24.0, and 28.0.

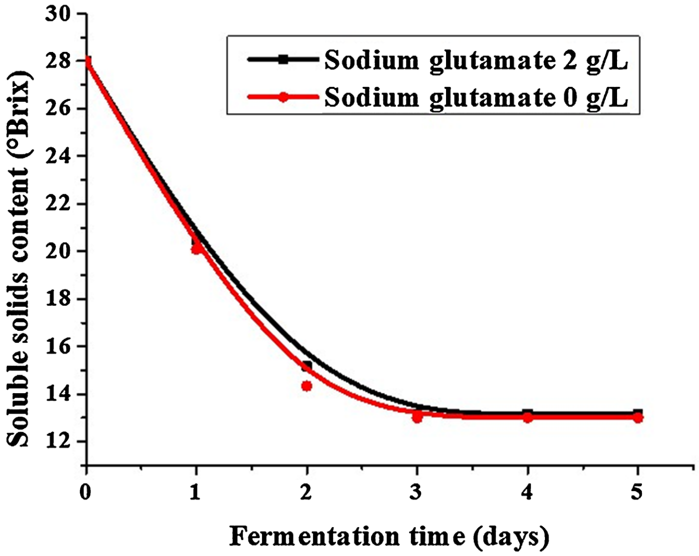

Figure 7. Influence of SG on the decrease of TSS during batch fermentation of rotten orange must (28 ˚Brix) over time.

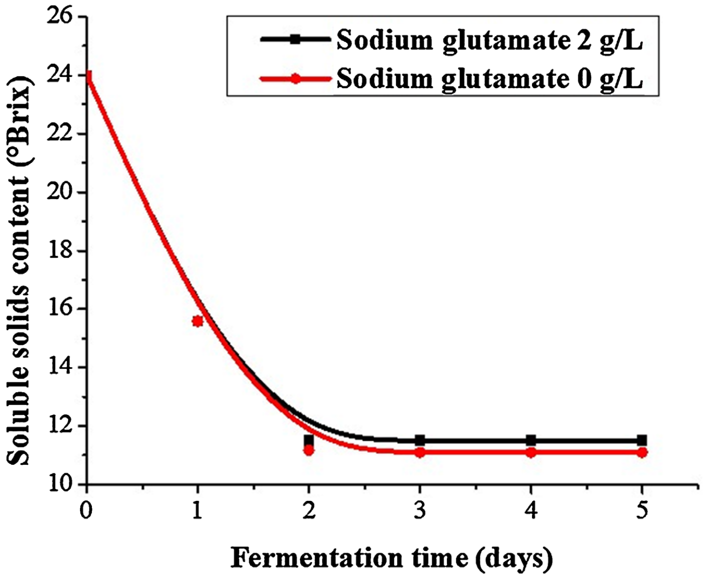

Figure 8. Influence of SG on the decrease of TSS during batch fermentation of rotten orange must (24 ˚Brix) over time.

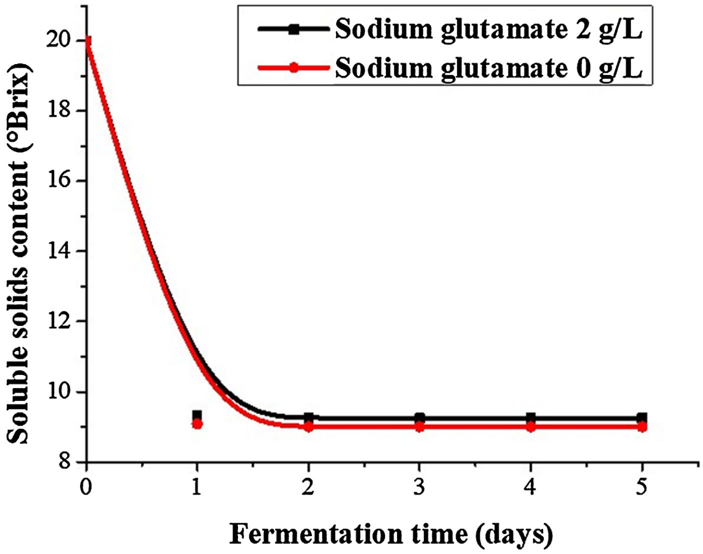

Figure 9. Influence of SG on the decrease of TSS during batch fermentation of rotten orange must (20 ˚Brix) over time.

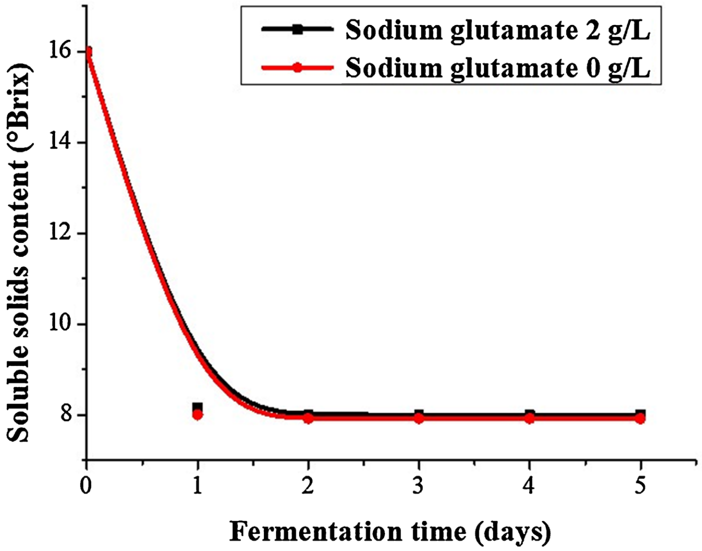

Figure 10. Influence of SG on the decrease of TSS during batch fermentation of rotten orange must (16 ˚Brix) over time.

The shape of these curves reveals that there are no significant differences between the pure must and the musts supplemented with SG (2 g/L). In particular, in Figure 11 a markedly less pronounced decrease in TSS is observed in the must supplemented with SG (2 g/L) compared to the neutral must. This suggests that the presence of SG in rotten orange musts slows down the consumption of fermentable sugars.

3.3.2. Influence of SG on EC through Batch Fermentation of Rotten Orange Musts

The ethanol contents (EC) obtained during batch fermentation of rotten orange musts with initial concentrations ranging from 9.5 ˚Brix to 28.0 ˚Brix are shown in the histograms of Figure 12.

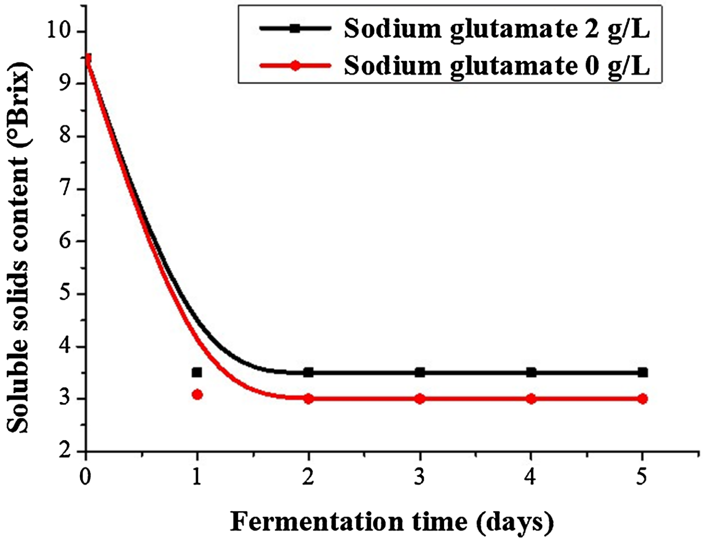

Figure 11. Influence of SG on the decrease of TSS during batch fermentation of rotten orange must (9.5 ˚Brix) over time.

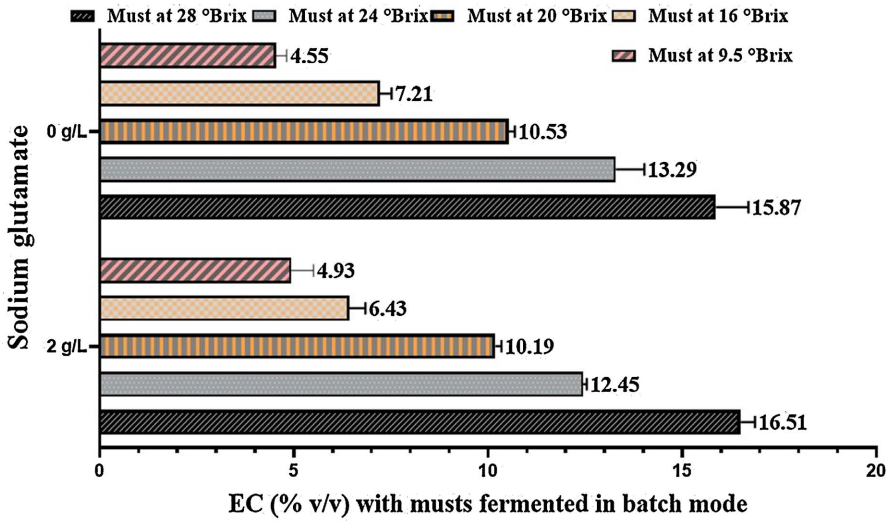

Figure 12. Influence of SG on EC through batch fermentation of sucrose musts fluence of SG on the experimental yields of bioethanol production through batch fermentation of rotten orange musts.

The results indicate that ethanol production increased progressively with the initial concentration of the rotten orange musts. Thus, in the present study, fermentation of rotten orange musts produced ethanol contents (% v/v) ranging from 4.55% ± 0.15% to 16.51% ± 0.21% for initial concentrations from 9.5 to 28.0 ˚Brix. The highest ethanol content (16.51% ± 0.21% v/v) was obtained with the must having the highest initial concentration, 28.0 ˚Brix, and supplemented with SG (2 g/L), while the lowest ethanol content was recorded in the neutral must with the lowest initial concentration, 9.5 ˚Brix.

The experimental yields of production (EYP) of bioethanol from rotten orange musts (28 ˚Brix) through batch fermentation are presented in **Table 4**. The highest ethanol EYP, 75.15% ± 0.96%, was obtained from the rotten orange must with an initial concentration of 28 ˚Brix supplemented with SG (2 g/L).

Table 4. Influence of SG on the EYP of ethanol through fed-batch fermentation of rotten orange musts [9.5 ˚Brix - 28 ˚Brix].

SG (g/L)	**EYP (%) of ethanol through batch ethanol fermentation of rotten orange musts**				
	9.5 ˚Brix	**16.0 ˚Brix**	**20.0 ˚Brix**	**24.0 ˚Brix**	**28.0 ˚Brix**
0.00	61.15 ± 2.08	57.44 ± 1.42	67.09 ± 0.61	70.58 ± 2.25	72.23 ± 2.19
2.00	66.30 ± 4.44	51.21 ± 1.90	64.95 ± 0.56	66.12 ± 0.28	75.15 ± 0.96

The lowest EYP, 51.21% ± 1.90%, was recorded for the must with an initial concentration of 16 ˚Brix supplemented with SG (2 g/L). Overall, it can be observed that, with the exception of musts having initial concentrations of 28 ˚Brix and 9.5 ˚Brix, all other musts showed higher ethanol EYPs when supplemented with SG (2 g/L).

3.3.3. Comparison of the Five Methods of Bioethanol Production through Batch Fermentation from Rotten Orange Musts

The results presented in **Table 5** indicate the final capacities of consumption of fermentable sugars contained in rotten orange musts, expressed as limit attenuation LA (%), through batch fermentation using *Saccharomyces cerevisiae*.

Table 5. LA (%) of rotten orange musts fermented in batch mode.

SG (g/L)	LA (%) of rotten orange musts				
	9.5 ˚Brix	16.0 ˚Brix	20.0 ˚Brix	24.0 ˚Brix	28.0 ˚Brix
0.00	68.42 ± 0.00	50.00 ± 0.00	55.00 ± 0.00	53.47 ± 0.35	53.57 ± 0.00
2.00	63.16 ± 0.00	49.48 ± 0.52	53.75 ± 0.00	52.08 ± 0.00	52.98 ± 0.60

3.4. Influence of SG on the Fermentation of Rotten Orange Musts in Fed-Batch Mode

3.4.1. Influence of SG on TSS

The curves presented in **Figure 13** show the evolution of total TSS in rotten orange musts (28 ˚Brix) over time through ethanol fermentation in fed-batch mode.

The shape of these curves shows that there is no positive improvement between the neutral must and the musts supplemented with SG.

3.4.2. Influence of SG on EC through Fed-Batch Fermentation of Rotten Orange Musts

Among the EC values (**Table 6**) obtained through fed-batch fermentation of very high-density rotten orange musts (28 ˚Brix), the highest, 14.72% ± 0.12% (v/v), was achieved with supplementation of the must with SG (2 g/L).

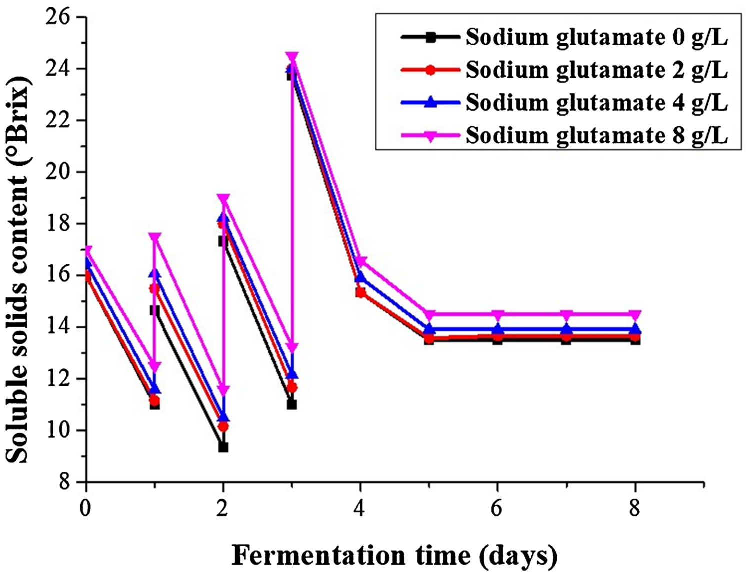

Figure 13. Influence of SG on total TSS over time through fed-batch fermentation of rotten orange musts.

Table 6. Influence of SG on EC through fed-batch fermentation of rotten orange musts (28 ˚Brix).

SG (g/L)	EC (% v/v) from rotten orange musts (28 ˚Brix)
0.00	14.00 ± 0.05
2.00	14.72 ± 0.12
4.00	14.33 ± 0.14
8.00	14.69 ± 0.05

3.4.3. Influence of SG on the EYP of Bioethanol through Fed-Batch Fermentation of Rotten Orange Musts

The ethanol EYPs from fed-batch fermentation of rotten orange musts at 28 ˚Brix are recorded in Table 7.

Table 7. Influence of SG on the EYP of bioethanol through fed-batch fermentation of rotten orange must (28 ˚Brix).

SG (g/L)	EYP of bioethanol (%) through fed-batch fermentation of rotten orange musts (28 ˚Brix)
0.00	63.72 ± 0.25
2.00	66.98 ± 0.54
4.00	65.22 ± 0.65
8.00	66.86 ± 0.21

These results indicate that supplementation of the must with SG (2 g/L) produced the highest yield, with a value of 66.98% ± 0.54%, compared to 63.72% ± 0.25% for the neutral must, which gave the lowest yield.

3.4.4. Comparison of the Influence of SG on Bioethanol Production through Fermentation of Sucrose and Rotten Orange Musts (28 ˚Brix) in Fed-Batch Mode

The results presented in Table 8 show that ethanol fermentation of neutral VHG rotten orange musts yielded higher LA values than neutral VHG sucrose musts, i.e., 51.79% ± 0.00% versus 23.81% ± 0.60%. While supplementation of sucrose musts with SG had a beneficial effect on LA, which generally increased with SG concentration, supplementation of rotten orange musts, on the other hand, had a negative effect on LA, which worsened as SG concentration increased.

Table 8. LA (%) for sucrose and rotten orange musts (28 ˚Brix) fermented in fed-batch mode.

SG (g/L)	LA (%) through fed-batch fermentation	
	Sucrose musts (28 ˚Brix)	Rotten orange musts (28 ˚Brix)
0.00	23.81 ± 0.60	51.79 ± 0.00
2.00	50.60 ± 0.60	51.19 ± 0.60
4.00	51.79 ± 0.00	50.30 ± 0.30
8.00	50.00 ± 0.00	48.21 ± 0.00

3.4.5. Comparison of the Efficiency of the Two Fermentation Modes of Rotten Orange Musts

When the two fermentation modes of rotten orange musts are compared (Table 9), it is found that batch mode achieved higher LA values.

Table 9. LA (%) of rotten orange musts (28 ˚Brix) fermented in batch and fed-batch modes.

SG (g/L)	LA (%) of rotten orange must fermentation (28 ˚Brix)	
	Batch mode	Fed-batch mode
0.00	53.57 ± 0.00	51.79 ± 0.00
2.00	52.98 ± 0.60	51.19 ± 0.60

4. Discussion

Supplementation with SG optimized ethanol production in sucrose musts studied at different concentrations, regardless of the ethanol fermentation mode applied. This nutritional additive promoted an increase in sucrose consumption, which was reflected in a rise in limit attenuation. The optimal SG concentration is estimated at 2 g/L. Thus, in batch mode, the LA at the end of fermentation reached 70% for NG sucrose must (20 ˚Brix) supplemented with SG (2 g/L), while for VHG sucrose must (28 ˚Brix), the value obtained was 46.43%. In fed-batch mode, the LA reached a value of 51.79% for the VHG must supplemented with SG (4 g/L), whereas the neutral VHG must yielded only 23.83%. The increase in LA due to supplementation of sucrose musts with SG is consistent with the findings of [23], who highlighted those nitrogenous substances tend to enhance yeast cell growth necessary for continuous ethanol production, resulting in more intense sugar consumption. Similarly, [24] demonstrated that nitrogen supplementation promotes yeast productivity, while [25] showed that sodium, by passively diffusing through the cell membrane, stimulates sugar transport. This increase in LA is always accompanied by a rise in the EYP of bioethanol. With batch fermentation of NG sucrose musts, significant improvements in yields were observed, with rates ranging between 136.55% and 147.94%, thus confirming the efficiency of sucrose bioconversion into bioethanol only in the presence of a low SG concentration. However, VHG sucrose musts fermented in both batch and fed-batch modes showed moderate improvements, with values ranging from 105.86% to 112.90% and from 104.12% to 111.15%, respectively. Although batch fermentation yielded the highest EYP of bioethanol for NG sucrose musts, it was the fed-batch fermentation of VHG sucrose musts that provided the most notable performance in terms of EC at the end of fermentation. This finding is consistent with the work of [21]. The advantage of a higher EC at the end of fermentation lies in the reduction of energy required during the distillation step. Indeed, a high EC allows for an estimated energy saving of about 29% per liter of pure ethanol when the ethanol content in the musts reaches 15% v/v compared to those containing 10% v/v, according to [26].

In batch mode, fermentation of the VHG sucrose must generated relatively higher EC values than those obtained with the NG sucrose must. However, these EC values from VHG musts remained below the theoretical value, estimated at 21.96%.

Another advantage highlighted in this study is the adoption of fed-batch fermentation, which plays a role in reducing yeast osmotic stress. Indeed, through the progressive addition of fermentable sugar into the fermentation broth, the fed-batch mode helps better manage high-sugar conditions that create both elevated osmotic pressure and increasing ethanol concentrations. The combined mechanism of these two factors is likely to hinder yeast growth and fermentative activity, given that maintaining cell integrity under osmotic variations is crucial for achieving optimal yield. Thus, osmotically stable conditions enhance yeast tolerance and longevity. This strategy optimizes fermentation performance while limiting metabolic constraints, consistent with the conclusions of [27] and [28], who emphasized the importance of maintaining yeast cell membrane integrity to maximize ethanol production. To make the bioconversion of rotten orange musts more cost-effective, the techniques previously developed for the bioconversion of sucrose musts were applied. For this purpose, the raw juice (9.50 ˚Brix) from rotten oranges was first concentrated into four different types of musts with concentrations of 16 ˚Brix, 20 ˚Brix, 24 ˚Brix, and 28 ˚Brix. These carefully prepared musts were then supplemented with SG (2 g/L). Finally, two fermentation modes were tested. These three strategies developed in the present work aim to optimize bioethanol production from cheaper raw materials.

In this study, fermentation of rotten orange musts yielded EC values ranging from 4.55% ± 0.15% to 16.51% ± 0.21% (v/v). The highest ethanol content (16.51% ± 0.21% v/v) was obtained with high-density musts (28 ˚Brix) enriched with 2 g/L of SG, while the lowest was found in low-density musts (9.5 ˚Brix), also enriched with 2 g/L of SG. Overall, the rotten orange must (28 ˚Brix) supplemented with SG achieved an EC of 16.51% ± 0.21% (v/v), compared to 15.87% ± 0.48% (v/v) for the neutral must.

Only a maximum ethanol content of 8.1% (v/v) was obtained in the orange wine under optimized conditions by [30], demonstrating that the current study's orange juice maximum ethanol production rate is extremely high, around double that obtained by [29].

This was achieved by the supplementation of sucrose musts with SG improved the EYP of bioethanol, whereas supplementation of rotten orange musts instead led to sluggish ethanol fermentation. Indeed, the presence of SG as a nitrogenous substance in sucrose musts increased the yeast population, which in turn stimulated sugar consumption and enhanced the EYP of bioethanol [30] [31]. On the other hand, since rotten orange musts originally contain other organic and mineral substances, this may have resulted in an inhibitory effect of SG [32]. These findings allow us to deduce that the optimal conditions for the ethanolic fermentation of a pure sugar substrate like sucrose may not transfer directly to a fruit must because of the complexity of this type of must in additional substances that can act favorably or against the bioethanol production.

It was also observed that the fermentation time of rotten orange musts was shorter (about 3 days) than that of sucrose musts (about 12 days for a must with a concentration of 28 ˚Brix). This difference could partly be explained by the availability of other favorable factors in orange musts, particularly the nature of the fermentable sugars. Indeed, fermentable sugars such as fructose and glucose are already present in rotten orange musts and are directly assimilable by yeast. This is not the case for sucrose, which is a disaccharide that must first undergo hydrolysis to yield glucose and fructose. Only after this inversion step do the yeast begin to assimilate the two monosaccharides formed, leading to ethanol production.

Moreover, orange juice naturally contains nutritional factors such as vitamins (B1, B6, B12), minerals (calcium, magnesium, potassium), as well as essential amino acids, all of which are indispensable for the growth and enhancement of the fermentative capacity of *Saccharomyces cerevisiae*. The presence of these naturally occurring nutritional factors largely explains the disparity observed in the fermentation kinetics of sucrose musts compared to rotten orange musts. However, another limiting factor that may act against ethanol fermentation of rotten orange musts is the presence of natural inhibitory compounds such as essential oils (e.g., limonene), phenols, and flavonoids, which can hinder the growth of *Saccharomyces cerevisiae* [33]-[35]. These compounds alter the membrane permeability of yeast cells and inhibit certain key metabolic enzymes involved in fermentation, thereby slowing down their activity [36]. In addition to these potential inhibitors, the ethanol produced by the yeast itself also acts as an inhibitor when its concentration rises significantly in the fermentation broth. In fed-batch mode, these inhibitors of ethanol fermentation accumulate progressively with each substrate addition, since the medium is not renewed. This prolonged accumulation creates suboptimal conditions for the yeast, thereby reducing the fermentation rate. However, in batch mode, even though these inhibitors are present from the start of fermentation, the rapid kinetics of rotten orange must fermentation allow the process to be completed before the inhibitors reach toxic levels [37]. In contrast, in fed-batch mode, the successive additions of substrate prolong yeast exposure to inhibitors and accumulated ethanol, which limits its fermentation performance. These inhibitors would be more detrimental in fed-batch mode compared to batch mode, because the fed-batch mode is seriously vulnerable to bacterial contamination, which is very detrimental to bioethanol production, leading to a decrease in bioethanol yield. Bacteria can consume sugar and ethanol in a fermentation broth, producing undesirable by-products and inhibiting yeast activity [38].

Based on the various criteria previously mentioned, it appears that supplementation of rotten orange musts with SG is not necessary to improve the EYP of bioethanol. Regarding the two fermentation modes applied, it was noted that fed-batch mode yielded ethanol contents of 14.00% ± 0.05% (v/v), corresponding to an ethanol EYP of 63.72% ± 0.25% for neutral sucrose musts, compared to an EC of 14.72% ± 0.12% (v/v) for sucrose must supplemented with SG (2 g/L), corresponding to an ethanol EYP of 66.98% ± 0.54%. As for batch mode, fermentation of rotten orange musts yielded EC values of 15.87% ± 0.15% (v/v) and 16.51% ± 0.12% (v/v), respectively for the neutral must and the must supplemented with SG (2 g/L), with corresponding ethanol EYPs of 72.23% ± 2.19% and 75.15% ± 0.96%. These results show that batch fermentation of rotten orange musts produced more bioethanol than fed-batch fermentation. On the other hand, for sucrose musts, it was fed-batch fermentation that resulted in a higher ethanol content.

5. Conclusions and Perspectives

The valorization of rotten oranges into bioethanol proved particularly successful in the present study, as it enabled the production of bioethanol with a maximum ethanol content of about 16.51% ± 0.12% (v/v), corresponding to a maximum concentration of 130.26 ± 0.95 g/L of ethanol. Large-scale application of the technology developed in this work could generate additional benefits for the various stakeholders involved in orange production and sales in Togo, such as a reduction of wastes for fruit vendors, a decrease of the reliance on fossil fuels in local transport, or a creation of a value-added product from agricultural surplus. In addition to these all-socio-economic outcomes, there would also be positive impacts on environmental protection.

For future work, it is important to identify other, more effective growth factors for the bioconversion of rotten oranges into bioethanol.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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