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Recovering Apple Agro-Industrial Waste for Bioethanol and Vinasse Joint Production: Screening the Potential of Chile

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Abstract: Bioethanol production has increased in demand as a replacement for conventional fuels. This work studies the use of apple pomace, which corresponds to 45% (*w*/*w*) of dehydrated apple production, as a reliable and inexpensive source for bioethanol production. Additionally, the vinasse obtained from the process as a byproduct is analyzed. Apple pomace has important properties for energy purposes, with high soluble sugar (6%–8%), organic compounds and low protein content. The carbohydrates were consumed in 99.3% in 144 h at a temperature of 30 °C and in a yeast *Saccharomyces cerevisiae* (YSC) concentration of 0.10 g/L. The bioethanol purity produced, 99.5% (*v*/*v*), was quantified by gas chromatography and calorific value (23.21 MJ/kg). This high purity, which fulfills the EN 15376, ASTM D 4806 Standard, allows its use as a fuel and oil additive. Moreover, it can be stated that vinasse obtained from alcohol distillation is a compound that has physicochemical values like other vinasses. Finally, Chile, as the most important exporting country of dehydrated apples in the world, has great potential to take advantage of the use of this raw material for bioethanol and vinasse production.

Keywords: biofuel; fermentation; apple pomace; lignocellulosic wastes; *Saccharomyces cerevisiae*

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1. Introduction

The use of renewable lignocellulosic biomass sources for biofuel production has been proposed as a suitable alternative to address fossil fuel depletion and the mitigation of climate change. First-generation biofuels refer to those produced using specific cultivation areas from seeds, grains and starch-based feedstock. However, it is expected that this type of biofuel does not represent a long-term viable fuel source, since its production requires cultivable land that, in turn, generates conflicts with food/feed use of feedstock [1]. On the other hand, second-generation biofuels produced from lignocellulosic biomass, such as crop residues or woody crops (rice straw, corn cob, wheat straw, sugarcane bagasse and cotton stalk) are advantageous alternatives in terms of output/input energy ratio, lower costs and high availability [2–4]. Bioethanol can be mixed in different ratios with gasoline or used as pure bioethanol in specially conditioned motors. All the factors mentioned above allow taking advantage of its high concentration of cetanes and its vaporization heat level [5]. Furthermore, it is an excellent fuel for future hybrid vehicles since they have a cleaner combustion than fossil fuels [6]. It is also an oxygenated and biodegradable fuel

(35% O_2) with reduced particulate matter and NOx content [6–8]. In the world market, bioethanol is predicted to achieve 140 billion liters in 2022 [4].

Several studies propose alternatives to produce bioethanol from different types of lignocellulosic raw materials such as straw, wood waste, fruit waste, sawdust, among others [9–13]. Moreover, techno-economic analyses were carried out to verify the opportunity to produce biofuels from wheat and rice straw [14,15], tomatoes, potatoes, oranges and olives [16], wood and grass species, bagasse and crop residues [17]. Apple pomace is a biomass corresponding to 45% (*w*/*w*) of dehydrated apple production. Globally, Chile stands out in the sale of dehydrated apples as the largest exporting country [18]. Nowadays, apple pomace is deposited in agricultural land without taking advantage of its rich properties in cellulose, hemicellulose, soluble sugars and soluble fibers. Earlier studies described the potential use of apple pomace to produce ethanol, representing 20% of energy recovery from the total energy present in pomace [19–21]. Pathania et al. [19] studied a solid-state fermentation system for production of ethanol from an apple pomace fermentation system using the Montrachet strain of *Saccharomyces cerevisiae*. Ngadi et al. [22] evaluated the effects of mixing speed and initial moisture of apple pomace on the kinetics of solid-state ethanol fermentation. Patle et al. [23] reviewed the usability of the mixed culture of *Zymomonas mobilis* and *Candida tropicalis*. Parmar et al. [11], used apple pomace to produce fermentable sugars, ethanol and acetic acid. Finally, Evcan & Tari [2] used apple pomace hydrolysate with cocultures of *Trichoderma harzianum*, *Aspergillus sojae* and *Saccharomyces cerevisiae*. However, these studies did consider vinasse production, a residue generated after the fermentation and distillation processes. This residue is very harmful to effluents when settled without any previous treatment, causing environmental issues such as: polluting soil and groundwater, adverse effects on micro-organisms, plants at disposal sites, or reducing sunlight penetration in rivers and lakes, decreasing photosynthetic activity [24–27]. On the other hand, studies show that the most used micro-organisms for bioethanol production are *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Zymomonas mobilis* bacteria, *Porum fusariumoxys* fungus, *Pachysolen tannophylus* yeast-type fungus, and *Thermophilic* bacteria in sugarcane residues (bagasse and waste) and bagasse [28,29]. Among them, *Saccharomyces cerevisiae* and *S. Pombe* are the most used at the industrial level due to their high performance [13].

Currently, Chile and its agro-industrial sector requires advances in the reduction of greenhouse gases, as well as the implementation of adaptation actions to stop climate change and reach the environmental targets proposed in the Chilean Nationally Determined Contributions [30]. Therefore, this sector needs to apply strategies to avoid water and energy scarcity and, most importantly, the recovery of organic waste (except for composting and animal feed) to produce new bioproducts or clean energy in a circular economy approach following the proposed national roadmap [31].

The aim of this paper is to evaluate the recovery of agro-industrial apple pomace waste (i.e., shell, core and discarded apples) to obtain two products: ethanol and vinasse. To do this, pomace will be transformed into bioethanol using yeast *Saccharomyces cerevisiae* UCLM S 377 to generate renewable and low-cost alternative feedstock for fossil fuel production. Moreover, this research carries out a characterization of the vinasse obtained as a byproduct, thus performing a characterization analysis of its properties. Finally, it is expected that this work can lead decision-makers in the agro-industrial sector to consider a new alternative to assess their residues. The above is a crucial variable where the self-generation of bioethanol is possible and valuable for energy integration. Additionally, bioethanol generation produces a reduction of agro-industrial solid wastes.

2. Materials and Methods

2.1. Agro-Industrial Waste and Microorganisms

Chile is the largest exporter of dehydrated apples in the world, reaching a production level above 4800 tons in 2016. The companies responsible for dehydrated apple production are few, with only three of them handling approximately 99% of the exports [18]. The apple

pomace was obtained directly from one of these companies, which is located in Romeral, Maule Region, Chile. Currently, the residual apple pomace obtained from the dehydrated production is not valued by the company. In this sense, this research seeks to propose a new alternative to this subproduct in order to avoid its final disposition and obtain energy to close the loop of this subproduct, making the process more sustainable and avoiding energy consumption based on fossil resources (Figure 1).

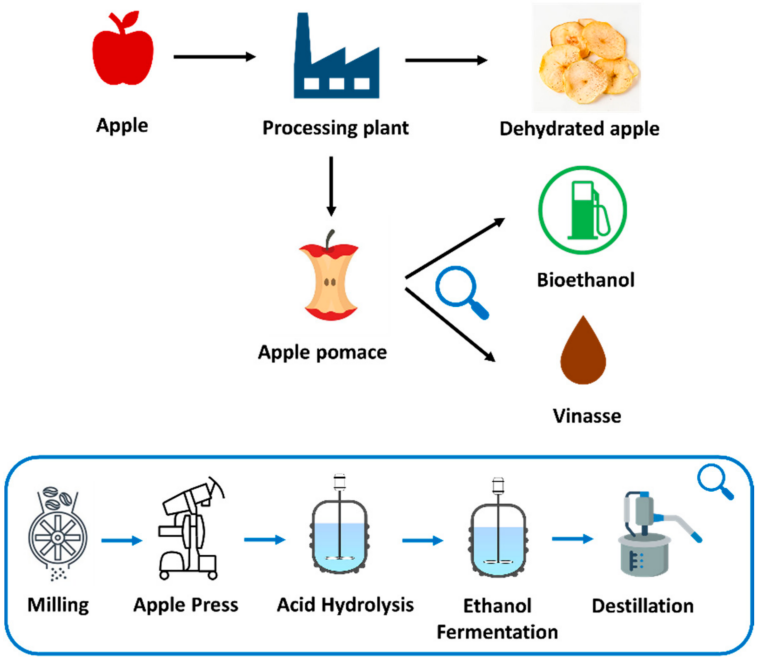

Figure 1. Apple pomace ethanol and vinasse production overview.

The residual pomace was packed in 10 L sealed containers and transported directly to the laboratory, nine kilometers away from the company. Later, it was milled in an industrial blender (Calvac, gastronomic equipment, San Bernardo, Chile) and pressed (manual hydraulic press OL463, Manfredi, San Secondo di Pinerolo, Italy), extracting the free liquid phase corresponding to 58.5% (v/v). The liquid mentioned above has been organoleptically characterized, showing a light brown color, a soft texture, and a sweet–sour taste. Subsequently, the waste was sterilized and distributed in glass flasks to be subjected to a fermentation process. The latter used YSC with different concentrations, temperatures and fermentation times. All samples were diluted with sterilized water at 10% (v/v). YSC was used as a fermenter since it has been reported as the best yeast for transforming carbohydrates to bioethanol in samples of waste in sugarcane, bagasse, coconut milk, pineapple juice and tuna juice [13,19,28,29]. The solid phase, 41.5% (w/w), obtained from the pressing step corresponds to the remaining waste, which can be used for composting [32–34].

2.2. Sample and Yeast Inoculation Preparations

2.2.1. YSC Initial Solution

20 mL of water at 30 °C and 2 g of yeast were added to a sterilized 250 mL flask. The solution was homogenized for 20 min at a speed of 80 rpm in a temperature-controlled bath (YCW-010 Gemmy, Gemmy Industrial Corporation, Taipei, Taiwan). Next, the second solution of 20 mL of Fermaid O fermentative nutrient solution was added to a 0.4 g/L concentration together with 60 mL of sterilized water at 30 °C. The new solution was again stirred in a temperature-controlled bath for 20 min at 80 rpm. Finally, the solution was refrigerated at 1 °C for one h.

2.2.2. Control Samples

Three 250 mL control samples of the initial solution were prepared in 500 mL flasks at a yeast concentration of 0.1 g/L, as previously specified in Section 2.2.1. The culture was composed of 15 g/L glucose, 5 g/L peptone, and 2 g/L malt extract to compare the results with samples of the fermented pomace. The above mixture of nutrients is usually applied to grow the YSC strain [13].

2.2.3. Fermented Samples

In sterilized 500 mL flasks, three 250 mL samples of pomace juice were prepared at concentrations of 0.02 g/L, 0.05 g/L, 0.10 g/L, 0.15 g/L, and 0.20 g/L from the initial YSC strain solution. These samples were tested at different temperatures (25–35 °C), times (1–160 h), and stirring speeds (80–120 rpm) to quantify the bioethanol concentration produced from carbohydrates present in the apple pomace. The flasks were closed with perforated rubber stoppers and a curved capillary tube (2 mm diameter) is used to allow oxygenation and the release of carbon dioxide (CO_2).

2.3. Fermentation Kinetics

The fermentation process was carried out in triplicate in 500 mL round-bottomed flasks. Each flask contained volumes of 250 mL. Each sample was processed at different fermentation times, from 0 h to 200 h, and different YSC concentrations were previously prepared using the temperature-controlled bath with a stirring speed of 100 rpm (Gemmy YCW-010). The cell growth speed and bioethanol production (depending on the number of carbohydrates in samples and their reduction), were measured during the process. Other parameters, such as pH measurement (Hanna pH 211 model, Woonsocket, RI, USA) and degrees Brix (Hanna HI96800 model, Woonsocket, RI, USA), were considered in the fermented pomace samples.

2.4. Analysis Methods

2.4.1. Growth Determination

A microscopic cell count was performed to quantify yeast growth. The procedure was carried out using a Neubauer chamber with a 1 mL sample as described by Marković et al. [35] This procedure was performed every eight hours for each sample analyzed.

2.4.2. Sugar Determination

Following the procedure implemented by Domínguez-Bocanegra et al. [13] to measure reduced sugars in agro-industrial wastes, the 3.5-dinitrosalicylic acid (DNS) method by Miller [36] was applied. For the above, 1 mL was extracted from the liquid phase obtained from the pressing process (Section 2.1), and it was added to a 100 mL flask with 5 mL of HCl 1:1 (v/v). The resulting solution is heated at 65 °C in a water medium for 10 min to perform complete sugar hydrolysis. The solution was cooled at room temperature and neutralized with NaOH (10% w/v). Subsequently, it was made up to the mark with distilled water and is stirred for 5 min. at 300 rpm. Later, in a 20 mL test tube, 1 mL was extracted from the top of the solution and mixed with 1 mL of DNS reagent. Finally, this blend was heated to 65 °C for 5 min and quickly cooled on ice to room temperature in a period of 15 ± 5 min. Next, the sample was diluted by adding 5 mL of distilled water and stirred again for 5 min in an Agitator Vortex AX681/5 (Hamburg, Germany). Subsequently, the sample was read at a 575 nm wavelength by a spectrophotometer (Optizen-alpha Mecasys model, Yuseong-gu, Daejeon, Korea) using the reference calibration curve with Merck's, Darmstadt, Germany, glucose standards.

2.4.3. Quantification of Purity of Bioethanol

After the fermentation process, three samples were separated to be measured in triplicate. Samples were distilled by means of a fractionated column, and the concentration of produced bioethanol was determined by gas chromatography (GC) (thermo Fisher

Scientific Trace 1300, Milan, Italy) with a capillary column Rtx-5MS w/integra-guard (Supelco Inc., 30 m × 0.25 mmID × 0.25 un df, North Harrison Road, MI, USA). As a carrier gas, helium was used with a purity of 99.9%. The heating value determination was performed with 1 mL of sample and using a calorimetric bomb (Parr model 1341, Moline, IL, USA) and DIN 51900 standard. Degrees Brix were analyzed in all samples before and after fermentation at 20 °C with a refractometer (Maselli model LR-02, Parma, Italy). In all fermented pomace juice samples, pH was measured before and during the fermentation process at 20 °C using a pH meter (Hanna pH 211).

2.4.4. Statistical Analysis

All tests were performed in triplicate. A triple factorial variance analysis was applied to YSC cellular density values. and Covariance statistical analysis post hoc comparisons were carried out using the Newman–Keuls (p = 0.05) test [37–39]. A similar statistical analysis was used for bioethanol content, and the differences between treatments applied were also determined by the Newman–Keuls (p = 0.05) post hoc test. XLSTatistics software (New York, NY, USA) was used to perform statistical analysis.

2.4.5. Bioethanol Waste: Vinasse

After the fermentation, selective distillation and subsequent condensation processes of each sample, a residue called vinasse was generated. This organic liquid contained natural impurities such as ash and other organic byproducts derived from the juice extraction and fermentation process. Vinasse corresponds to 93.5% (v/v). The vinasse was analyzed through several physicochemical methods to quantify its properties and estimate the possible final use for this waste. After its characterization, laboratory tests were performed to determine the capacity of the vinasse to improve the compost processes of agro-industrial organic matter.

3. Results and Discussion

3.1. Chemical Composition of Pomace Waste

Table 1 shows the results of the physicochemical characterization of pomace, where it can be observed that there is an important contribution of soluble carbohydrates and soluble solids. The latter shows evidence of the presence of sucrose, which can be fermented entirely by YSC, as reported by Domínguez-Bocanegra et al. [13]. Further, the percentages of soluble solids (juice) found in this work were 11.78%. In this sense, the value reported here is higher than the range of soluble solids found by Evcan & Tari [2] for apple pomace at 2.23%. Moreover, the value of carbohydrates found in this work is similar to the value reported by Shim [40].

3.2. YSC Growth

Figure 2 shows YSC growth at different concentrations considering a temperature of 30 °C for each sample in triplicate, using 250 mL of extracted pomace juice as a culture medium. Results show that the highest growth (5.0×10^7 cell number/mL) was for samples with a concentration of 0.10 g/L. As a result, a control sample of 250 mL was prepared at the optimal concentration to observe yeast growth. The results obtained showed a growth value of 8.0×10^7 cells/mL in this sample, which facilitated a comparison of the results and demonstrated YSC growth at that concentration.

Figure 3 shows YSC growth at different times (in hours) for all concentrations prepared in triplicate using 250 mL of extracted pomace juice as a culture medium. Results show that the highest cellular growth (5.0×10^7 cells/mL equivalent to a concentration of 0.52 g/L) was obtained after 144 h from the fermentation process. Afterwards, no significant growth was observed. It is important to state that, in the previous stage of the fermentation process above, the growth gradually increased as the fermentation time also increased, only observing that the highest growth of the control sample occurred at 48 h with a value of 8.0×10^7 cells/mL (equivalent to a concentration of 0.8 g/L), which decreased over time.

This fact is caused by the purity of the samples, since pomace juice has other nutrients such as fats, proteins and fibers, which may produce a delay in the yeast action.

Table 1. Physicochemical characterization of apple pomace obtained.

Properties	Unit	Value	Determination Method
Moisture content (wb) [a]	Mass %	86.90 ± 0.1	Oven method—AOAC 945.15 [41]
Proteins (db) [b]	Mass %	1.35 ± 0.2	Kjeldahl method—AOAC 979.09 [41]
Fats (db) [b]	Mass %	0.71 ± 0.1	Soxhlet method—AOAC 963.15 [41]
Carbohydrates (db) [b]	Mass %	10.36 ± 0.3	Miller method [32]
Fiber (db) [b]	Mass %	$2.7 \times 10^{-2} \pm 0.2$	Gravimetric method—AOAC 920.169 [41]
Ashes (db) [b]	Mass %	0.65 ± 0.2	Muffle method—AOAC 940.26 [41]
Calorific value (db) [b]	kcal/kg	2232.33 ± 0.2	DIN Serie 51.900 Standard [42]
Density, 20 °C (wb) [a]	kg m^{-3}	1043.17 ± 0.3	ASHRAE R08 2006 [43]
Thermal conductivity, 20 °C (wb) [a]	W/(m·K)	0.57 ± 0.4	ASHRAE R08 2006 [43]
Thermal diffusivity, 20 °C (wb) [a]	m^2 s^{-1}	$1.42 \times 10^{-4} \pm 0.1$	ASHRAE R08 2006 [43]
Specific heat, 20 °C (wb) [a]	kJ/(kg·K)	3.84 ± 0.5	ASHRAE R08 2006 [43]
Soluble solids (juice) (wb) [a]	° Brix	11.78 ± 0.2	Refractometer method—AOAC 932.12 [41]
pH (juice) (wb) [a]	Dimensionless	3.80 ± 0.1	

[a]: wet basis (wb), [b]: dry basis (db).

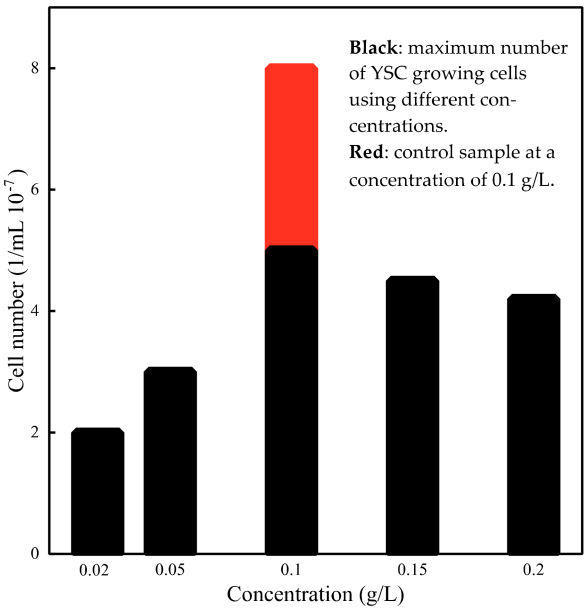

Figure 2. Concentration ratio (g/L) of pomace juice, with respect to the number of YSC growth cells at 100 rpm and 30 °C.

As a result, it can be observed that in the case of pomace juice samples, carbohydrates were consumed by 99.3% in 144 h at a temperature of 30 °C and YSC concentration of 0.10 g/L. This shows that the highest cellular increase occurred during the fermentation time, temperature, and initial yeast concentration used herein. Subsequently, the fermentation process reached a stationary stage, where no significant growth was observed. This scenario indicates that pomace juice has a rich composition of nutrients capable of producing bioethanol, making it comparatively similar to the results reported in other studies using YSC [8,13,44].

Results showed that the highest YSC growth was at a concentration of 0.10 g/L, and the sample reached its lowest growth at a concentration of 0.02 g/L ($p < 0.001$). The comparison between the control sample and the pomace juice sample at a concentration of 0.10 g/L of YSC showed no significant growth differences ($p > 0.05$).

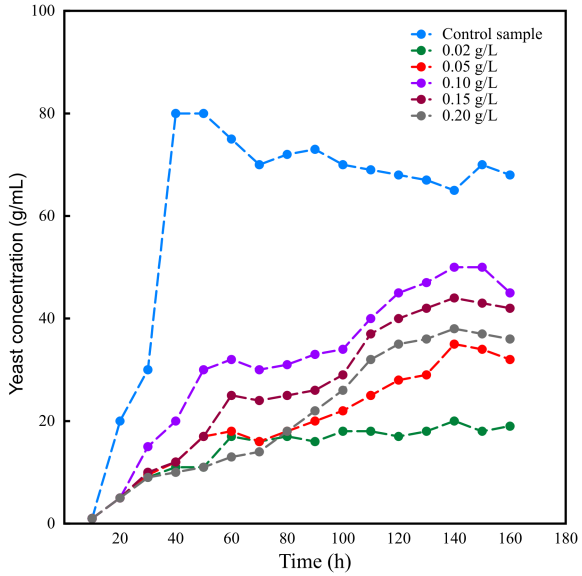

Figure 3. YSC concentration at different times using the control sample and extracted juice from pomace as a culture medium at different concentrations with a stirring speed of 100 rpm in a thermo-regulated bath and at a temperature of 30 °C.

3.3. Bioethanol Production

The graph displayed in Figure 4 shows bioethanol production of 6.85% from pomace juice at a YSC concentration of 0.1 g/L over a time of 144 h of fermentation at a temperature of 30 °C. This occurs because, during the bioethanol fermentation, yeasts transform most of the glucose and fructose into bioethanol and CO_2. For the control sample, 9.3% bioethanol was measured for 48 h at a temperature of 30 °C and at YSC concentration of 0.1 g/L. The latter assumes that approximately 100% of the conversion of sugars into bioethanol was achieved at the above fermentation time. For apple pomace juice, samples at a concentration of 0.10 g/L, a bioethanol generation of 65.69% of its maximum production value at a fermentation time of 48 h could be observed. This is because YSC is a glucophilic yeast; hence the kinetic fermentation of glucose is promoted compared with the fermentation of fructose. Nevertheless, the proportional quantity of fructose increases along the fermentation process [8,13,44].

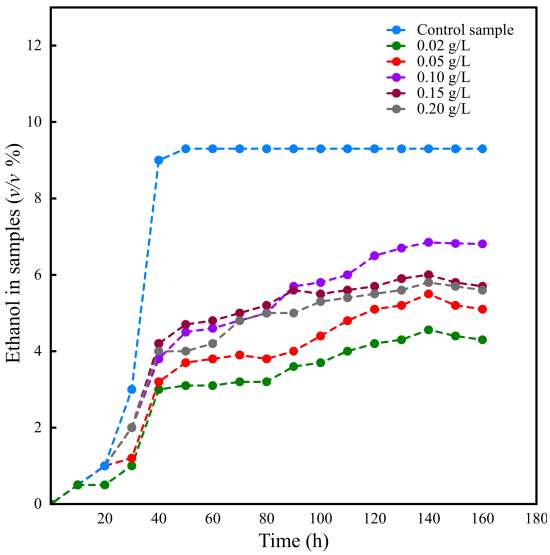

Figure 4. Quantification of bioethanol in the control sample and pomace juice samples, using YSC at a stirring speed of 100 rpm and temperature of 30 °C.

Bioethanol purity, after conventional distillation, was experimentally determined using a density. Results showed a purity of 93% (w/w) $\times 1 = 0.84$, quite close to the azeotropic composition at atmospheric pressure [45]. In addition, the samples were analyzed by gas chromatography showing that no other compounds were present in the sample. The produced bioethanol can be purified again using dehydration, azeotropic destination, cosolvent addition, or other methods [46,47] to fulfill the required concentration of 99.5% (v/v) by the EN 15376, ASTM D 4806 Standard for its use as a fuel and oil additive [48]. Additionally, a calorific value analysis was also conducted, showing an average value of 23.21 MJ/kg, similar to those reported in other experimental measurements [49,50].

3.4. Sugar Concentration

Based on the data obtained, the quantification of the carbohydrate content in the apple pomace juice was 10.36 g/L, whereas the content in the control sample was 17.36 g/L. Figure 5 shows the sugar consumption at different times, demonstrating that after 144 h, for pomace juice samples at a YSC concentration 0.1 g/L, a carbohydrate consumption of 61.38% could be observed between 0 h and 48 h. In contrast, the control sample showed a consumption of 88.47% after the first 48 h and 99.84% after 144 h.

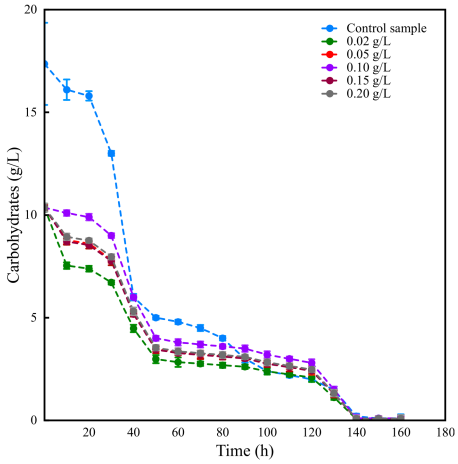

Figure 5. Decrease in the carbohydrate concentration in the control sample and pomace juice at different YSC concentrations.

It is important to note that in Figure 5, there are two significant changes concerning the decrease of carbohydrates, which take place at 50 h and 120 h. The opposite effect occurs in Figure 4 simultaneously, where the percentage of bioethanol generated in the samples increased at a concentration of 0.1 g/L.

Table 2 shows degrees Brix and pH of the samples. In the case of Brix degrees, it can be observed that the minimum value was reached at 144 h, indicating that at this time, there were no carbohydrates in the samples due to the conversion to bioethanol and the low pH level represents a significant fermentation factor regulating yeast growth by controlling its fermentation speed and alcohol production. During fermentation, yeasts extract nitrogen from organic amino acids, despite their amphoteric characteristics, converting them into acids, which causes a pH decrease in the medium [51].

Table 2. Quantification of degrees Brix and pH for samples at different concentrations.

		° Brix					pH				
Time (h)	**Control Sample**	**0.02 g/L**	**0.05 g/L**	**0.10 g/L**	**0.15 g/L**	**0.20 g/L**	**0.02 g/L**	**0.05 g/L**	**0.10 g/L**	**0.15 g/L**	**0.20 g/L**
0	17.00	11.78 ± 0.1	11.78 ± 0.2	11.78 ± 0.2	11.78 ± 0.2	11.78 ± 0.2	3.80 ± 0.2	3.80 ± 0.2	3.80 ± 0.1	3.80 ± 0.3	3.80 ± 0.3
10	16.00	11.00± 0.1	11.20 ± 0.2	11.60 ± 0.4	11.30 ± 0.2	11.10 ± 0.2	3.80 ± 0.2	3.80 ± 0.3	3.80 ± 0.3	3.80 ± 0.3	3.80 ± 0.2
20	15.00	10.00 ± 0.1	11.00 ± 0.4	11.50 ± 0.2	11.00 ± 0.4	11.00 ± 0.1	3.70 ± 0.3	3.70 ± 0.3	3.70 ± 0.4	3.70 ± 0.5	3.70 ± 0.3
30	14.50	10.00 ± 0.3	9.50 ± 0.5	11.40 ± 0.2	9.20 ± 0.3	10.00 ± 0.2	3.70 ± 0.2	3.70 ± 0.4	3.70 ± 0.5	3.70 ± 0.1	3.70 ± 0.4
40	14.00	9.50 ± 0.2	9.17 ± 0.6	11.00 ± 0.2	9.10 ± 0.5	9.00 ± 0.3	3.60 ± 0.1	3.60 ± 0.1	3.60 ± 0.3	3.6 0± 0.2	3.60 ± 0.6
50	5.00	6.15 ± 0.3	6.67 ± 0.3	8.00 ± 0.2	7.00 ± 0.2	6.00 ± 0.4	3.50 ± 0.2	3.50 ± 0.3	3.50 ± 0.2	3.50 ± 0.1	3.50 ± 0.3
60	4.00	5.77 ± 0.4	6.25 ± 0.2	7.50 ± 0.1	6.00 ± 0.1	5.70 ± 0.2	3.40 ± 0.5	3.40 ± 0.3	3.40 ± 0.2	3.40 ± 0.2	3.40 ± 0.2
70	3.00	5.38 ± 0.3	5.83 ± 0.3	7.00 ± 0.2	5.30 ± 0.2	5.30 ± 0.5	3.30 ± 0.2	3.30 ± 0.4	3.30 ± 0.5	3.30 ± 0.3	3.30 ± 0.1
80	2.80	5.00 ± 0.3	5.42 ± 0.2	6.50 ± 0.2	5.20 ± 0.2	5.10 ± 0.2	3.20 ± 0.2	3.20 ± 0.5	3.20 ± 0.3	3.20 ± 0.3	3.20 ± 0.2
90	2.70	4.62 ± 0.3	5.00 ± 0.5	6.00 ± 0.2	5.00 ± 0.1	4.80 ± 0.1	3.20 ± 0.1	3.20 ± 0.3	3.20 ± 0.2	3.20 ± 0.3	3.20 ± 0.3
100	2.50	3.85 ± 0.2	4.17 ± 0.3	5.00 ± 0.1	4.30 ± 0.3	4.00 ± 0.2	3.10 ± 0.2	3.10 ± 0.1	3.10 ± 0.2	3.10 ± 0.2	3.10 ± 0.3
110	2.00	3.08 ± 0.3	3.33 ± 0.1	4.00 ± 0.1	3.80 ± 0.4	3.70 ± 0.3	3.00 ± 0.3	3.00 ± 0.2	3.00 ± 0.3	3.00 ± 0.3	3.00 ± 0.4
120	2.00	2.31 ± 0.3	2.50 ± 0.4	3.00 ± 0.3	3.50 ± 0.2	3.20 ± 0.2	3.00 ± 0.3	3.00 ± 0.2	3.00 ± 0.2	3.00 ± 0.2	3.00 ± 0.3
130	2.00	2.10 ± 0.1	2.08 ± 0.2	2.50 ± 0.4	2.00 ± 0.2	2.30 ± 0.2	2.90 ± 0.4	2.90 ± 0.3	2.90 ± 0.1	2.90 ± 0.1	2.90 ± 0.2
140	2.00	2.00 ± 0.1	2.00 ± 0.1	2.00 ± 0.2	2.00 ± 0.1	2.00 ± 0.1	2.80 ± 0.3	2.80 ± 0.2	2.80 ± 0.2	2.80 ± 0.2	2.80 ± 0.5
150	2.00	2.00 ± 0.3	2.00 ± 0.4	2.00 ± 0.2	2.00 ± 0.1	2.00 ± 0.1	2.80 ± 0.3	2.80 ± 0.3	2.80 ± 0.1	2.80 ± 0.1	2.80 ± 0.3
160	2.00	2.00 ± 0.2	2.00 ± 0.4	2.00 ± 0.3	2.00 ± 0.2	2.00 ± 0.2	2.80 ± 0.1	2.80 ± 0.2	2.80 ± 0.3	2.80 ± 0.2	2.80 ± 0.3

3.5. A Comparison of Bioethanol Performance with Other Studies

Table 3 presents the comparison result obtained in this study with those presented in the scientific literature about bioethanol production based on apple pomace. According to this table, *Saccharomyces cerevisiae* is the most frequently used micro-organism in different varieties, since these micro-organisms present better pH behavior and sugar levels. Another common factor was the temperature level used for the fermentation process with 30 °C most often. This is a result of these micro-organisms presenting better life conditions and reaching higher efficiency levels at this temperature. Regarding stirring speeds and fermentation time, the process applied to obtain the maximum ethanol concentration takes as much time as other reviewed studies to convert sugar into bioethanol.

Table 3. Results comparison with other bioethanol production studies from apple pomace.

Reference	**Microorganism**	**Initial Sugar Content**	**Temperature (°C)**	**Rpm**	**Maximum Ethanol Concentration**	**Fermentation Time (h)**
[21] *	*Saccharomyces cerevisiae* (baker's yeast)	-	40	150	190 g/L	168
[23] *	*Zymomonas mobilis* and *Candida tropicalis*	122 g/L	-	-	50 g/L	-
[2]	*Harzianum harzianum*, *Aspergillus Sojae* and *Saccharomyces cerevisiae* NRRL Y-139	16.16 g/L	30	200	8.75 g/L	100
	Saccharomyces cerevisiae NRRL Y-139				4.46 g/L	-
[52]	*Saccharomyces cerevisiae*	-	30	150	53.6 g/L	72
[53]	*Trichoderma harzianum*, *Aspergillus sojae*, and *Saccharomyces cerevisiae*	-	-	-	8.75 g/L	-
[54]	*Saccharomyces cerevisiae*	116.3 g/L	30	-	53.1 g/L	72
	Kluyveromyces marxianus			-	29.5 g/L	72
The present study	*Saccharomyces cerevisiae* UCLM S 377	108.07 g/L	30	100	31.30 g/L	144

* In these studies, the methods and substrates are different, but they are based on apple pomace, -: Data not found

Initial sugar content and the maximum bioethanol concentration obtained present differences among these studies. This may occur since apples have different origins, quality and chemical composition. The maximum ethanol concentration obtained in this study is higher than others [2,53]. However, it is lower than the concentration obtained by many more [21,23,52,54]. The main difference with the latter studies lies in the enzymatic hydrolysis process applied as apple pretreatment before fermentation, instead of acid hydrolysis, as performed in this study. Additionally, Parmar et al. [21] and Magyar et al. [52] obtained the apple pomace from juice manufacturers. Moreover, both studies present a higher composition of sugars than this study: for example, Parmar et al. [21] present

18.2 g total reducing sugars (glucose and fructose), cellulose 22.2 g, and hemicellulose 5.5 g per 100 g dry mass, while Magyar et al. [52] present glucan (21%), xylan (3.7%), fructose (19.2%) and sucrose (1%). Thus, as the obtained apple pomace evaluated corresponds to a subproduct from dehydrated apple production, it only contains shells, discarded apples (those in poor condition) and cores (seeds), which implies less sugar content. Therefore, it is possible to say that the initial sample characterization is relevant for the analysis.

In order to reach better ethanol yields, it is possible to apply other pretreatment processes like enzymatic hydrolysis; however, this manuscript seeks to make the process simple, without bringing in an expensive and complex pretreatment process to produce sugar degradation products, avoiding increasing the cost of ethanol production. Ethanol production is not the main purpose of the company, and this study represents an alternative to the valorization of the apple pomace produced as subproduct. Hence, acid hydrolysis is presented as a reliable alternative due to its low catalyst cost and it is occurring in shorter time periods than enzymatic hydrolysis [55]. Regarding economic costs associated with ethanol production, the feedstock logistics represent a great challenge since it requires obtaining the apple pomace from multiple farmer locations. However, this is not pertinent to this study as the pomace was obtained directly as a waste stream from the dehydrated apple production. Therefore, the advantage of the system is that bioethanol is obtained as a coproduct and thus one of the major economic barriers, such as the feedstock price affecting the bioethanol production cost is avoided. Consequently, equipment investment represents the main economic issue. Considering the process as a whole, bioethanol product yield from sugar feedstock is higher compared to lignocellulosic sources [17,56]. Moreover, the company processes 2.8 thousand tons of dehydrated apples per annum and generates a total of six thousand tons of residue yearly. Therefore, a future study to perform a deeper tecno-economic analysis of this strategy needs to take place.

3.6. Physiochemical Analysis of Vinasse

After the distillation of all samples, a physicochemical analysis of vinasse was performed, and the results are shown in Table 4. Vinasse corresponds to 93.5% (v/v). The analyzed samples of vinasse show physicochemical properties like those of sugar beet vinasse [57] and sugar cane fields [58], especially with those referring to pH, nitrogen, BOD_5 and COD, among others.

Table 4. Physicochemical analysis of vinasse.

Analysis	Unit of Measurement	0.02 g/L	0.05 g/L	0.10 g/L	0.15 g/L	0.20 g/L	Determination Method
pH	Dimensionless	4.50 ± 0.2	4.50 ± 0.3	4.60 ± 0.2	4.60 ± 0.3	4.60 ± 0.3	
COD	g/L	112.4 ± 0.2	111.9 ± 0.3	113.1 ± 0.3	111.7 ± 0.3	112.5 ± 0.2	Hach TNT822 [59]
BOD_5	g/L	85.3 ± 0.1	84.2 ± 0.2	82.4 ± 0.4	83.4 ± 0.4	83.8 ± 0.1	Hach standard method [60]
Nitrogen	g/L	1.91 ± 0.2	1.94 ± 0.3	1.98 ± 0.2	1.96 ± 0.1	1.95 ± 0.3	Kjeldahl method—AOAC 979.09 [41]
Raw proteins	g/L	8.21 ± 0.3	8.23 ± 0.4	8.23 ± 0.4	8.24 ± 0.1	8.24 ± 0.1	Kjeldahl method—AOAC 979.09 [41]
Ashes	g/L	19.54 ± 0.3	19.54 ± 0.5	19.76 ± 0.2	19.54 ± 0.4	19.78 ± 0.5	Muffle method—AOAC 940.26 [41]
Total solids	%	60.31 ± 0.5	60.32 ± 0.3	60.31 ± 0.6	60.32 ± 0.6	60.32 ± 0.2	Mehod 2540 D—APHA 2005 [61]
Organic matter	g/L	48.43 ± 0.6	48.44 ± 0.5	48.56 ± 0.2	48.54 ± 0.2	48.36 ± 0.1	TNT Hach TOC MR [62]
Electrical conductivity	dS/m	17.31 ± 0.4	17.41 ± 0.5	17.33 ± 0.3	17.53 ± 0.2	17.45 ± 0.1	Method 2510—APHA 2005 [61]
Density	g/mL	0.96 ± 0.4	0.97 ± 0.3	0.98 ± 0.4	0.98 ± 0.5	0.98 ± 0.5	ASHRAE R08 2006 [43]
Potassium (K_2O)	g/L	4.21 ± 0.3	4.32 ± 0.2	4.42 ± 0.3	4.31 ± 0.5	4.20 ± 0.5	The X-ray fluorescence (XRF) technique (Bruker S8 tiger, Ettlingen, Germany)
Phosphorus (P_2O_5)	g/L	0.06 ± 0.6	0.06 ± 0.5	0.07 ± 0.2	0.07 ± 0.2	0.07 ± 0.3	The X-ray fluorescence (XRF) technique (Bruker S8 tiger, Ettlingen, Germany)

Table 4. *Cont.*

Analysis	Unit of Measurement	0.02 g/L	0.05 g/L	0.10 g/L	0.15 g/L	0.20 g/L	Determination Method
Calcium (CaO)	g/L	1.40 ± 0.2	1.41 ± 0.6	1.43 ± 0.2	1.42 ± 0.6	1.43 ± 0.2	The X-ray fluorescence (XRF) technique (Bruker S8 tiger, Ettlingen, Germany)
Manganese (MnO)	g/L	0.21 ± 0.4	0.23 ± 0.3	0.23 ± 0.5	0.25 ± 0.1	0.26 ± 0.4	The X-ray fluorescence (XRF) technique (Bruker S8 tiger, Ettlingen, Germany)

Comparing the physicochemical vinasse results obtained in this study with those of the literature, it is possible to observe that the COD average value of 112.32 g/L is within the range of 27.5–299.25 g/L for sugarcane vinasse [63]. However, other studies present lower average values: for example, for sugarcane vinasse, [64] which obtains 22.1 ± 0.46 and 32.4 ± 10 g/L, [65] reaches 67.3 ± 1.4 g/L, while 13.38 g/L was presented by Christofoletti et al. [66]. Other raw materials analyzed also present lower COD levels: for example, [60] indicates 30.4 g/L for cane juice, 84.9–95 g/L for cane molasses, 26–50.2 g/L for grapes (wine), 55.2–66.3 g/L for agave (tequila), 79.9 g/L for sweet sorghum, and 55.5–91.1 g/L for beet molasses. On the other hand, BOD_5 levels obtained in this study are also higher than those presented by Christofoletti et al. [66] for sugarcane (50.46 g/L), grape (wine) (18.9 g/L), beet (78.3 g/L) and sweet sorghum (0.46 g/L). Again, España-Gamboa et al. [27] indicate BOD_5 values for cane juice (16.7 g/L), cane molasses (39.5 g/L), grapes (wine) (14.54–16.3 g/L), agave (tequila) (20.6 g/L), sweet sorghum (46 g/L) and beet molasses (27.5–44.9 g/L), which are lower than the DBO_5 obtained in this study. Particularly in Chile, vinasse is considered as liquid industrial waste, because its high BOD_5 level makes its use difficult. In this context, Chilean Regulation 1333 [67] on Irrigation Water Quality allows for the application of a maximum amount of stillage in specially authorized and controlled places equivalent to 112 kg BOD_5 ha^{-1} d^{-1}.

Nitrogen values allow vinasse to be used together with other phosphorus-rich materials for compost processing and improvement as well as a soil improver (fertirrigation). In this sense, nitrogen enhances microbial activity and, hence, accelerates the decomposition process, reduces the preparation time of organic manures, and increases potassium, calcium, sulfur and carbon contents. The use of 150 m^3/ha vinasse as a fertilizer in fields is equal to 61 kg/ha of nitrogen, 40 kg/ha of phosphorus, 343 kg/ha of potassium, 108 kg/ha of calcium and 80 kg/ha of sulfur [63]. However, vinasse does not have its own standards for direct use in soil [68] in contrast with some countries such as Brazil [63].

3.7. Production Potential in Chile

Chile is the largest exporting country of fresh apples in the Southern hemisphere, and the first one in the exportation of dehydrated apples globally. In 2016, Chile exported 4852 tons of dehydrated apples [18]. The dehydration industry uses the commercial category apple as raw material, which is the one sold in the domestic market, different from apples used in the juice industry. Therefore, competition between the processing industries is low. Chilean apple production is chiefly dedicated to export (61.8%), with the main source of raw material for the industrial process corresponding mainly to the discards from orchards or process plants. Considering the Chilean apple supply chain, the pomace produced represents 13.5% of the total amount of apple production, equal to more than 164,000 tons of the harvest fruit, considering discarded apples both for export and industrial use [69]. Currently, apple pomace is used mainly by companies for compost, animal feed or it is sent to landfill. In this sense, the non-use of apple pomace in the Chilean dehydrated industry represents an opportunity to produce bioenergy in a circular economy approach, closing the loop both for apple pomace to obtain bioethanol, and for vinasse (bioethanol subproduct) to produce biogas through an anaerobic digestion process [70–73].

However, due to the seasonal nature of apple production, apple pomace can be collected only post-production, which may greatly limit the development of the biorefinery

industry to produce bioethanol, as well as increasing production costs accordingly. Consequently, different feedstocks should support the long-term development of the future biorefinery systems for bioethanol production in Chile. If only apple pomace is considered, Chile may have a theoretical potential production of more than 6.23 million L of bioethanol. Nevertheless, the literature proposes different feedstocks to obtain ethanol, such as olive [74], wheat and rice straw [75,76], corn [77] and rape straw [78], among others. In this sense, Chile has a rich variety of crops that may be useful as raw materials for bioethanol production. The most important crops nationally are wheat, oats, corn, rape and rice, which together represent 75% of the total farming area [69], while the total area planted with fruit trees is led by table grape (14.9%), followed by walnuts (11.5%), apple trees (10.6%), cherry trees (9.4%) and avocado trees (9%). Further, other dehydrated systems are based on cherry, tomato, onion, blueberry and plum production, among others [18]. Likewise, Chile has great potential to replicate the system proposed both in dehydrated and crop production systems in order to avoid dependence on feedstock season and/or increase bioethanol production.

4. Conclusions

This work addresses the viability of producing bioethanol based on apple pomace obtained from dehydrated apple production. The results obtained quantitatively show that an important amount of bioethanol was produced. The purity of the obtained bioethanol was 99.5% (v/v) after distillation, which fulfills the international standards for its use as a fuel and additive. The experimental results demonstrate that the apple pomace waste is an excellent culture medium for YSC UCLM S 377 without the use of any additional nutrient.

It is important to notice that the apple pomace chemical composition found in this study was like any other apple waste and other biological sources in the literature. The YSC growth and bioethanol production showed an optimum value at 0.1 g/L. The carbohydrates were consumed by 99.84% in 144 h at a temperature of 30 °C and YSC concentration of 0.1 g/L. The production of bioethanol reached a planar maximum around 48 h and no significant increase of this variable was observed.

Finally, it can be established that apple pomace is a highly competitive second-generation substrate. Furthermore, this waste has an important capacity to produce bioethanol and reduce environmental pollution, due to its physicochemical properties, availability and low cost. Moreover, it can also be stated that vinasse obtained from alcohol distillation is a compound that has physicochemical values like other vinasses, and it has the potential for energy use due to its high COD and BOD_5 levels.

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