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Comparison of Bioethanol Production of Starches from Different Andean Tubers

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Bioethanol from starchy biomass represents 60% of the global generation. In Ecuador, there is an annual production of potato, cassava and sweet potato of over 600 thousand tons. However, there are no comparative studies that analyze the effects of starch source. Thus, the present work aims to compare the potential for bioethanol production from starches of potato (*Solanum tuberosum*), cassava (*Manihot esculenta*), and sweet potato (*Ipomea batatas*). Starches were subjected to liquefaction with α-amylase. For bioethanol production, two different processes were assessed 1) sequential (SeqSF) saccharification, with gluco-amylase, and fermentation at 30°C for 72h, with previously activated yeast (*S. cerevisiae*, a wine strain), and 2) simultaneous saccharification-fermentation (SSF), where glucoamylase and yeast were added at the same time, at fermentation conditions. Total reducing sugars, after enzymatic digestion and during fermentation, were quantified through 3,5 dinitrosalicylic acid (DNS), and ethanol was quantified through GC. After liquefaction, potato and cassava starches resulted in approx. 44 g/L of sugars, while SP resulted in 33 g/L. For the SeqSF process, after saccharification, potato and cassava presented similar values of sugar concentration, close to 90 g/L (58% conversion), while sweet potato yielded a lower value of 66 g/L (52% conversion). During fermentation, in the case of cassava and potato, over 90% of the sugars were consumed in the first 24h, and less than 1% remained after 48h. For sweet potato, sugar consumption was lower, with about 18% remaining after 72h. In SSF, sugar consumption was slower. For potato and cassava, around 80% of the sugar had been metabolized by 24h, and for SP, it was 68%. However, after 72h, all fermentations presented less than 4% sugars. Ethanol production in general was below 10% v/v, and it was affected by sugar consumption kinetics. Bioethanol production was greater in the SSF process, when compared to the SeqSF.

1. Introduction

Biothanol is considered one of the most important biofuels, and it can be obtained from different biomasses (Honma et al., 2019; Lopez-Diago et al., 2018). However, the most widely used raw materials for worldwide ethanol production are sugar cane and beetroot, with a 40% of global production, and biomass with high content of starch, such as potato, sweet potato and cassava (Bušić et al., 2018). Even though there are many organic raw materials suitable for this purpose, starch is one of the most abundant and cheapest polymers for its production. Particularly, in Ecuador 27.000 to 30.000 hectares of cassava are grown. This amount represents an annual production of 114.000 tons of this tuber, as reported by the Ecuadorian Ministry of Agriculture and Livestock for the year 2017. Nevertheless, this production is not used entirely, nor is it completely destined for human consumption. For example, only in the Metropolitan District of Quito, it is estimated that 36.500 tons of food are wasted each year. Of these, 65% are represented by tubers and vegetables (Dubbeling et al., 2016), making this waste suitable to produce alternative fuels, such as bioethanol due to the starch they contain. In order to generate ethanol from starch, a hydrolysis process is needed, and it can be chemical, enzymatic, or combined. The present study includes the use of an enzymatic hydrolysis to convert the polysaccharides of the starch, amylose and amylopectin, into smaller glycosidic chains. Enzymatic digestions are liquefaction, carried out with α-amylase, and saccharification, through

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glucoamylase (Nielsen et al., 2012). Due to the high quantity and availability of these tubers, their use for the generation of bioethanol is feasible. Particularly in the case of Ecuador, there is a project aimed to the partial addition of biofuels in oil cuts. Currently, it focuses on light duty gasoline “Gasolina Extra” using a 5% of bioethanol in its composition. The studies related to bioethanol synthesis from starches is a widely explored area; however, extensive comparative studies of bioethanol obtained from cassava, potato and sweet potato have not been carried out. Moreover, the literature does not report the use of existing varieties of these tubers (cassava, potato, sweet potato) in Ecuador, and it is known that climate and geographic location can affect starch composition (Zhu, 2015). This is why a characterization of the starches must be done. Similarly, the optimal concentration of α-amylase on the liquefaction process and glucoamylase concentration in the saccharification process have not been studied or compared between different starches in previous works. Thus, the present study aims to compare the potential for bioethanol production from starches of three different tubers produced in Ecuador: potato (Solanum tuberosum), cassava (Manihot esculenta), and sweet potato (Ipomea batatas).

2. Experimental

2.1 Starch extraction and characterization

Starches were mechanically extracted from fresh tubers and characterized. Moisture and ash contents were calculated following methods AOAC 934.01 and 932.01 (Thiex, 2009), respectively. Lipid quantification was performed by a solid-liquid extraction using AOAC 2003.05 and 2003.06 (Thiex et al., 2003) standards. Protein content was obtained by the Kjeldahl method with boric acid 4%, as shown in AOAC 960.52 (Lynch and Barbano, 1999).

2.2 Enzymatic digestions

For liquefaction, 30 g of starch were used in a digestion volume of 150 mL (20% w/v). The digestion was carried out with 500 TAU/g of α-amylase (Gamalpha Spezial AB Enzymes), for 3 hours at 80 °C, under reflux and constant stirring. After liquefaction, the digested solution was diluted in order to obtain equivalent starch concentrations of 20, 15, 10, 6 and 3% w/v. In conical tubes, 15 mL of each starch concentration were added with 1425 GAU/g glucoamylase (Gammadex Cal AB Enzymes). The saccharification was carried out in a HYSC SI-64 150 shaking incubator for 2 hours at 60 °C, with shaking at 50 rpm. Reducing sugars, after liquefaction and saccharification, were estimated by DNS method (Bernfeld, 1955) and measured in a Hach DR 800 colorimeter at 550 nm. The 10% starch concentration was selected as the best option for sequential and simultaneous saccharification/fermentation, as it will be explained later.

2.3 Fermentation

Fermentations were done for 72 hours at 30 °C in a shaking incubator at 50 rpm. Conical tubes were used as fermenter vessels, with cotton/gauze caps and PVC hoses directed to a 2M sodium hydroxide as CO2 trap. All the mentioned materials were sterilized in a steam autoclave at 150 °C for 15 minutes under a 0.17 MPa pressure. After this, 30 mL of enzymatic hydrolysate were added into the vessels, along with 0.24 g (Shanavas et al., 2011) of wine brewing *Saccharomyces cerevisiae* (Lalvin Bourgorouge), which was activated for 30 minutes at 30 °C in the hydrolysate. For simultaneous saccharification/fermentation (SSF), 1425 GAU/g of glucoamylase were added along with the yeast after liquefaction. For sequential saccharification/fermentation (SeqSF) only yeast was added after the saccharification process. Every 24 hours, fermentation samples were taken in order to analyze reducing sugars and quantify ethanol. The latter was measured in a Shimadzu GC-QP2010 Gas Chromatographer, using isopropanol as internal standard.
All experiments were carried out in triplicates (n=3). Results are reported as the average ± standard deviation. Data analysis and significant differences were determined using ANOVA with Tukey-HSD pairwise-comparisons, with a 95% confidence level.

3. Results and discussion

As previously mentioned, this study aimed to compare ethanol production using enzymatically hydrolyzed cassava, potato and sweet potato starches in order to determine the most appropriate Andean starch for sequential and simultaneous fermentation.

3.1 Starch characterization

The proximate compositions of cassava, potato and sweet potato starches are shown on Table 1. The three starches showed a high moisture content, which could be due to water absorption from the surroundings during storage. Protein, ashes and lipid contents were low, below 1%, as reported in previous research

(Horstmann et al., 2017; Moorthy, 2002; Zheng et al., 2016). A small amount of reducing sugars was obtained, being cassava the starch with the highest content of free sugars, which could remain from the extraction process.

Table 1: Proximate composition of cassava, potato and sweet potato starches.

Parameter	Cassava Starch	Potato Starch	Sweet Potato Starch
Protein [%]	0.840 ± 0.002	1.095 ±0.008	0.2098 ±0.002
Lipid [%]	0.020 ±0.0003	0.0321 ±0.001	0.0263 ±0.001
Moisture [%]	94.690 ±0.004	94.254 ±0.001	89.241 ±0.001
Ash [%]	0.371 ±0.001	0.644 ±0.001	0.094 ±0.001
R. Sugars [g/L]	8.027 ± 0.122	3.640 ± 0.106	3.493 ± 0.061

3.2 Enzymatic digestions

As shown in Figure 1 (a), after saccharification, the amount of sugars increased at higher starch concentrations. However, a smaller increase in sugar concentration is observed for 15% and 20% of starch, gradually reaching a reducing sugar production plateau when increasing starch concentration. Statistical analysis showed no significant difference between these two concentrations ($p > 0.05$).
Meanwhile, 3% and 6% starch concentrations provided a low reducing sugar conversion, so the 10% concentration of starch was selected for the fermentation processes. Additionally, 10% digestion showed a lower viscosity than the ones obtained from 15% and 20%, especially for sweet potato starch, which were difficult to handle and analyze due to their high viscosity and suspended solid content.
According to the statistical analyses done for this section, reducing sugar concentration only depended on the starch concentration used, but no differences were found between the starches of different tubers ($p > 0.05$). Similar to reducing sugar concentration, Figure 1 (b) indicates that 3% and 6% starch had the highest sugar yield after saccharification, while 20% and 15% resulted in the lowest; each pair was not significantly different ($p > 0.05$). This analysis corroborates the decision to choose 10% as the most appropriate concentration for fermentation.

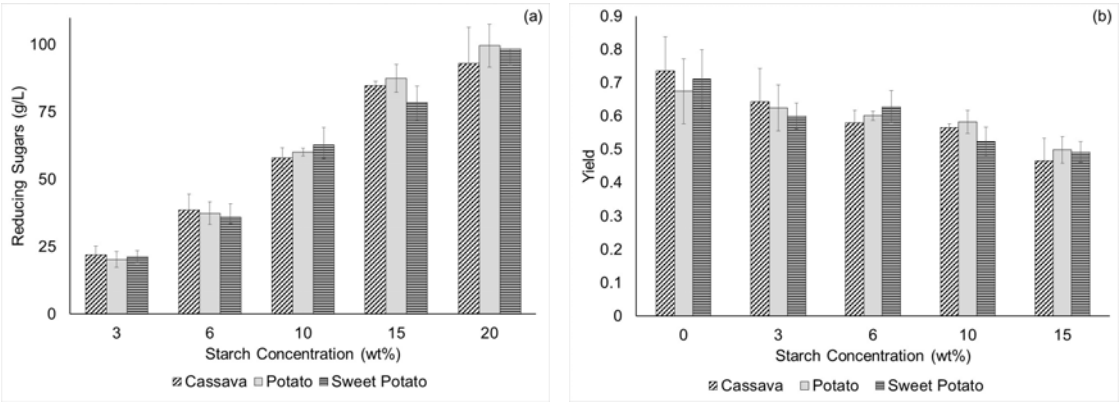

Figure 1. Reducing sugars (a) and yield (b) for different starch concentrations after saccharification.

3.3 Fermentation

Quantification of sugar consumption served two purposes. The first one was to assess how the substrate was being used by the yeast, so ethanol production results can be compared, and the second one was to use it as an indirect method of yeast growth. Sugar consumption during the 72 hours of SSF and SeqSF are displayed in Figures 2 (a) and (b), respectively. For the two methods, a considerable amount of sugar was used within the first 24 h, with up to 94% for cassava and potato starches.
Sweet potato starch shows an interesting behavior because, unlike cassava and potato starches which are quickly consumed by the yeast in both fermentation processes, this one has a slow sugar consumption during SeqSF, with 65% sugars available after the first day. This could happen because the solution showed a high content of dissolved solids and considerable amount of reducing sugar on it, and thereby, a high viscosity.

These characteristics are present in very high gravity (VHG) fermentations, although the fermentations held in this research were not performed at the mentioned conditions.
When working at VHG conditions, the high content of solids and dissolved sugars can rise the osmotic pressure of the solution providing stress to the yeast, and creating a feedback inhibition for glucoamylase (Lareo et al., 2013; Zhang et al., 2010). As a lower concentration of initial sugars is used in SSF, the inhibition factors explained before disappear due to a more balanced production/consumption rate of the reducing sugars, favoring yeast growth rather than the enzyme activity because of the operating conditions (Shanavas et al., 2011). Furthermore, the statistical analysis showed that sugar consumption in SSF is significantly slower than in SeqSF, and that sugar measurements at 48 and 72 hours showed no differences, indicating that most of the sugar has been consumed and fermentation is over after the first 24 hours. This means that the exponential growth phase of yeast occurs within the first day of fermentation, so most of the ethanol will be produced in this stage, as it is a primary metabolite (Henderson et al., 2013). In order to extend exponential cellular growth phase to 48 and 72 hours, a higher amount of sugar could be used at the beginning of the fermentations.

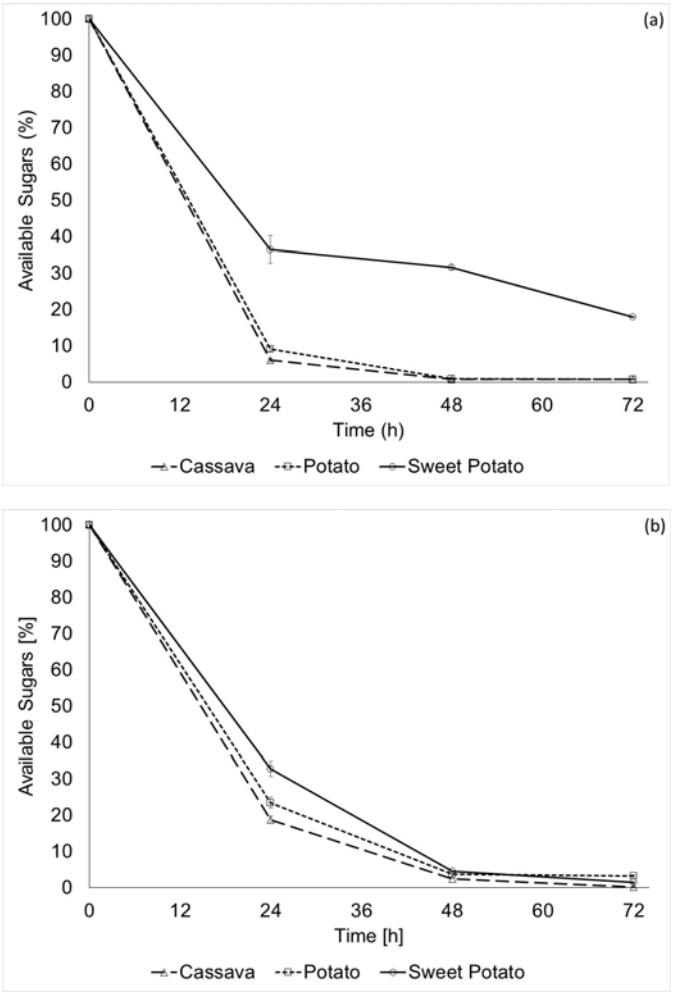

Figure 2. Kinetics of reducing sugar consumption during three-day SeqSF (a) and SSF (b)

Ethanol production from each fermentation process is shown in terms of productivity, defined as grams of ethanol per kilograms of starch, in Figure 3 (a). The highest content of ethanol seems to be obtained from cassava starch in SeqSF and from sweet potato starch in SSF; however, there were no significant differences between starches $(p > 0.05)$. In Figure 3 (b), a considerable raise of ethanol yield is achieved by using SSF, being sweet potato starch fermentations the ones with a larger increment, in comparison to SeqSF, achieving a very high percentage, but again, no significant differences were found between starches.

Statistical analysis of the results presented on the figures of this section, proved that the simultaneous fermentation is a more suitable process overall for obtaining a higher ethanol yield, productivity and efficiency regardless of the type of starch used for the different methods of fermentation.
The obtained values of ethanol productivity are lower than the ones reported in the literature, but it should be taken into account that, in this work, VHG conditions were not applied (Shanavas et al., 2011; Zhang et al., 2010).
As shown in previous studies, SSF is a process that guarantees a greater productivity due to: a reduction on end-product inhibition issues with enzymes, a better overall reaction time and reduced investment and operation costs (Olofsson et al., 2008; Pinaki et al., 2015); thus, in terms of large scale bioethanol production, SSF is a more suitable process for obtaining this biofuel. Given the fact that there is no difference in the use of starches from different sources in order to produce more ethanol, large scale production could be performed from mixed starchy biomass wastes from the tubers herein used.

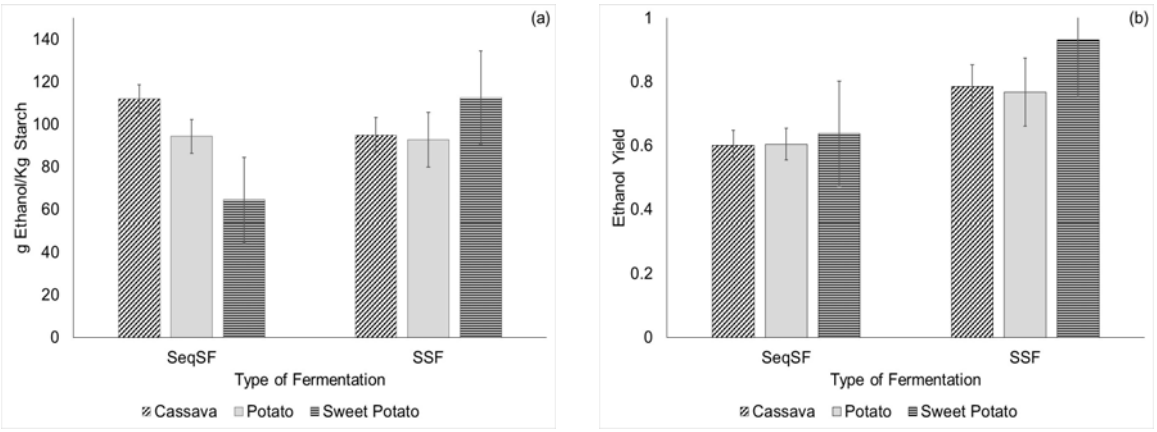

Figure 3. Ethanol productivity (a) and yield (b) for SeqSF and SFF.

4. Conclusions

This comparative study revealed that the starch source is not a factor that significantly affects the amount of reducing sugars after the digestion processes, and, therefore, bioethanol production during fermentation. This fact implies that the use of any agriwaste from these tubers could be equally effective and suitable for the production of the biofuel.
Simultaneous saccharification/fermentation (SSF) process proved to be a better overall process in terms of ethanol productivity, yield and efficiency (data not shown) compared to sequential saccharificaiton and fermentation (SeqSF), regardless of the type of starch used. Additionally, a higher consumption of reducing sugars was observed in SSF proving that this process provides more appropriate conditions for cell growth. As sugars were consumed rapidly on the first 24 hours, it is believed that fermentations could be carried out in shorter periods, and a more frequent sample taking should be done within this period of time in order to observe a more detailed behavior of the yeast growth and ethanol production in the fermentations. Moreover, the peels and wastes of these tubers could be an interesting biomass option for further studies of bioethanol production using SSF.

Acknowledgments

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