Biobased Chemistry and Technology

BSc Thesis Biobased Chemistry and Technology

Improving the Value of Spent Coffee Grounds by Converting Carbohydrates into Fermentable Sugars

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Abstract

Spent coffee grounds is a waste stream after coffee is brewed. To improve the value of spent coffee grounds in a sustainable way, research is done on how to convert carbohydrates into fermentable sugars which can be used to make value added products. To accomplish this various acid hydrolysis pre-treatments with sulphuric, acetic and lactic acid are done. After this an enzyme hydrolysis is performed followed by a fermentation to obtain ethanol. In addition to that, the influence of oil is also taken into account on the production of glucose. Because these oils can easily be extracted and made, for example, into biodiesel. The highest glucose concentration, 63% of the maximum glucose content, is obtained when spent coffee grounds with oil is pre-treated with 1 M sulphuric acid. The solution of spent coffee grounds with acids and enzymes is fermentable and inhibitory compounds are not formed in significant amounts. It was also discovered that when oil is in the spent coffee grounds and pre-treated with sulphuric acid, more glucose is released in contrast to when it is pre-treated with acetic or lactic acid. Oil has no observable influence on the fermentation.

Contents

1. Introduction

Coffee is the most consumed drink in the world and the average person in the Netherlands drinks around 3 cups a day (Bontridder, 2017). The coffee bean has to be roasted and ground before use. When a cup of coffee is made, the grounds, in other words spent coffee grounds (SCG) remain. Around eight million tonnes of SCG is produced every year worldwide (Burrows, 2015). Around half of that SCG is produced in the factory as a waste stream during the production of instant coffee. This is convenient because all this SCG is produced in one place reducing transportation, if there is a sustainable process for conversion to a useful product. Instant coffee is gaining popularity, especially in Asia and the Middle East, so the production of instant coffee will likely increase (Lacsamana, 2016). That is why it is even more important that a sustainable process to make use of the SCG is developed and carried out.

SCG is composed of a variety of organic compounds, with carbohydrates being the most abundant (45%, see figure 1). SCG is used nowadays for low value applications such as composting, fertiliser, bioenergy production, mushroom growth or fuel (Campos-Vega *et al*., 2015). As mentioned before, the volume of SCG is increasing so it is attractive that a process with a higher value end product is being developed. Some have tried to improve the value of SCG by carrying out biorefinery processes to produce oil or make the carbohydrates available for fermentation (Campos-Vega *et al*., 2015). However, it has been shown that the carbohydrates cannot be fermented efficiently because of the lignin content (Faaij, 2007). Therefore, this thesis will focus on the hydrolysis of SCG to make fermentable sugars.

The coffee companies do not make full use of the compounds in SCG. For example, lipids can easily be extracted and made into biodiesel. In this way the companies could be more self-sufficient and reduce their CO_2 footprint. After extraction of the oil the defatted SCG still has valuable compounds in it. There are still carbohydrates in the defatted SCG, these can be used for fermetation by yeast to produce bioethanol for example.

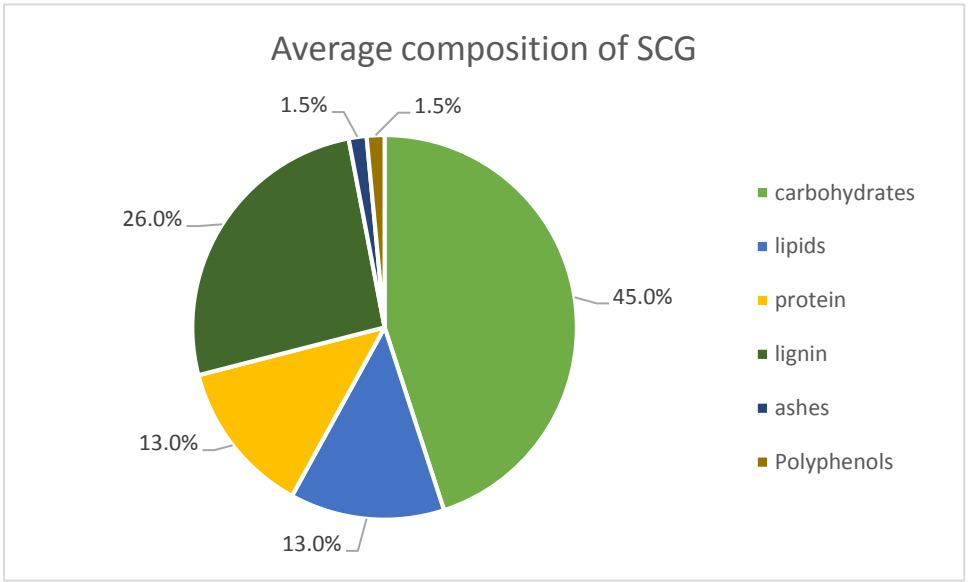


Figure 1: Average composition of spent coffee grounds according to (Mussatto et al., 2011) (Mussatto et al., 2017) (Campos-Vega et al., 2015)

1.1 Research objectives

This thesis will focus on improving the value of spent coffee grounds by converting carbohydrates into fermentable sugars. This will be achieved by answering the following research questions:

- How can carbohydrates be converted to a fermentable medium?
- What acid and enzyme hydrolysis and under which conditions (concentration, temperature and time) should be carried out to make the carbohydrates best available for fermentation?
- Does the lipid content influence the hydrolysis or fermentation of SCG?

Acid and enzyme hydrolysis of SCG to make fermentable sugars is the main focus. At a later stage of research, it will be important to look into what kind of fermentation is possible. However, due to the limited amount of time, only an alcohol fermentation will be performed to test the fermentability. The influence of lipids will be checked because these lipids can be used to make biodiesel and could have an influence on the hydrolysis or fermentation (Kwon *et al.*, 2013).

1.2 Approach

With the experimental setup shown in figure 2, the research questions can be answered. Dry SCG is used for an oil extraction. To measure how much oil is extracted from the coffee, rotory evaporation is used to evaporate the solvent of the oil extraction. Then 2 hydrolysis steps are performed. First an acid hydrolysis which is a pre-treatment step, then an enzyme hydrolysis is performed. Finally, a fermentation with yeast is done to obtain ethanol.

Both the hydrolysis and fermentation products will be analysed by high-performance liquid chromatography (HPLC). The influence of oil on the hydrolysis and fermentation will be investigated by eliminating the oil extraction step and using dry SCG for the hydrolysis directly. This means that the direct and indirect effect of the oil and solvent in measured.

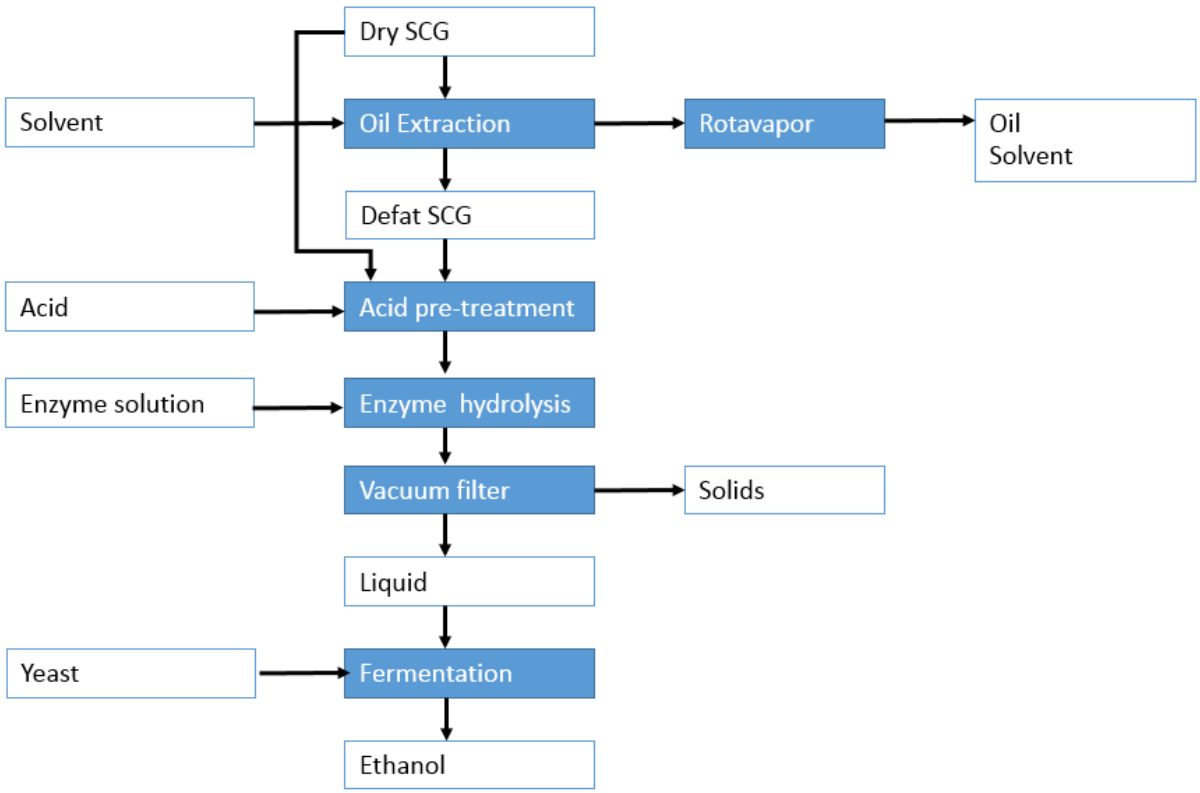


Figure 2: Experimental setup

2. Background

In this chapter information about the coffee bean processing process is given. Then the composition of the SCG is discussed and applications, were these compounds can be used, are suggested. Finally, some information about lignocellulose and its fermentation is given.

2.1 From bean to coffee

Coffee beans are the seeds of the coffee cherry that grows on the *Coffea* plant. As shown in figure 3 on the right side, there are two coffee beans in one cherry. Two main coffee species are cultivated today; Arabica coffee (*Coffea arabica*) accounts for 75-80% and Robusta coffee (*Coffea canephora*) accounts for around 20% of the world's production (Coffee Research Institute, 2006). On the left side of figure 3 a centre cut of a cherry is shown. The cherry is covered with multiple layers, consisting of outer skin, pulp, pectin layer and parchment. Around the bean itself is a layer of silver skin.

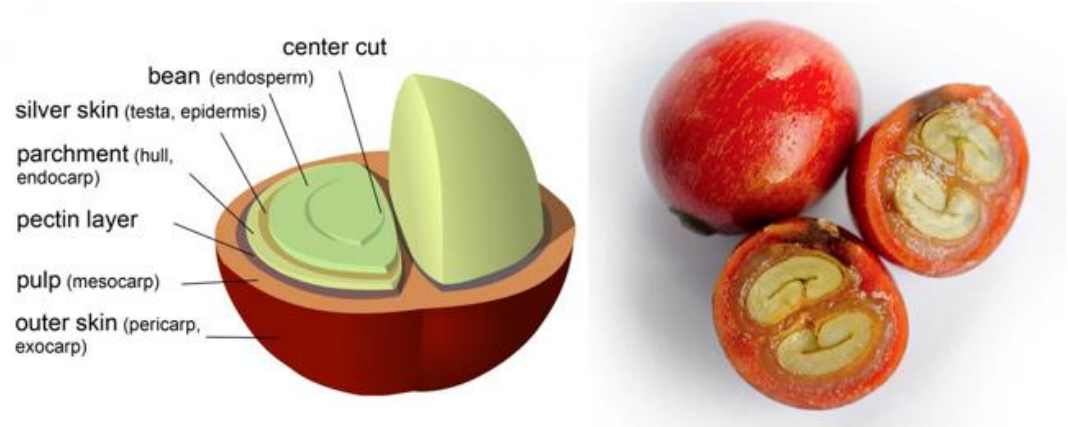


Figure 3: Left; Centre cut of a coffee cherry (National Coffee Association). Right; Ripe coffee cherry (Gotts, 2015).

The process from cherries to coffee is shown in figure 4. The cherries are harvested when they are ripe. Then the cherries are sorted and pulped to get rid of the outer layer and debris. The beans are fermented and then dried in the sun to prevent spoiling. Now the coffee can be stored for several months or years depending on the temperature and humidity. The bean is milled to get rid of the silver skin around the bean. Then professional coffee graders classify the coffee bean according to quality. The fermented coffee beans are distributed all over the world. Then the coffee beans are roasted at 550°C and grinded (Beller, 2001)(National Coffee Association). Finally, a cup of coffee can be made and SCG are produced as a by-product.

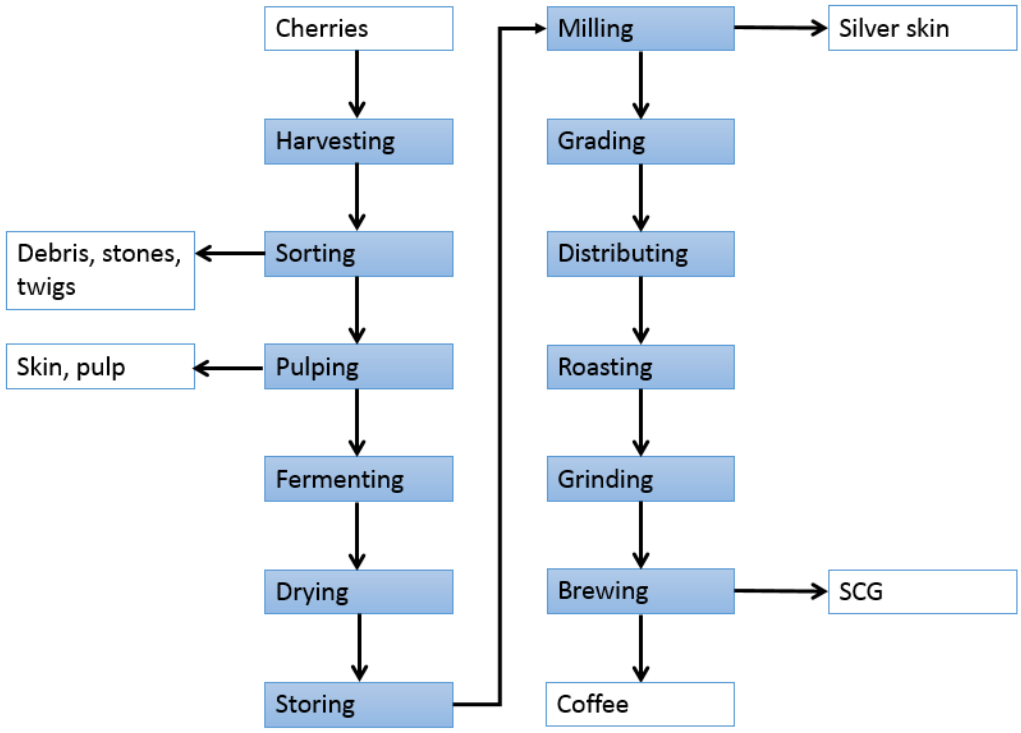


Figure 4: Process from cherries to coffee.

2.2 Composition and applications of SCG

The reported composition of SCG is has a lot of variation. Therefore, an average composition table is made out of all the data obtained from literature, see table 5. As seen from the error bars, the lipid and lignin content has a greater variation compared to the carbohydrates and protein content. This is because of the different species of coffee beans and conditions where the beans were grown. This thesis is focussed on making the carbohydrates available for fermentation. It is also interesting to know out of which sugars these carbohydrates consist, see figure 6. If the carbohydrates are fully broken down, the following monomers are released: mannose, galactose, arabinose and glucose.

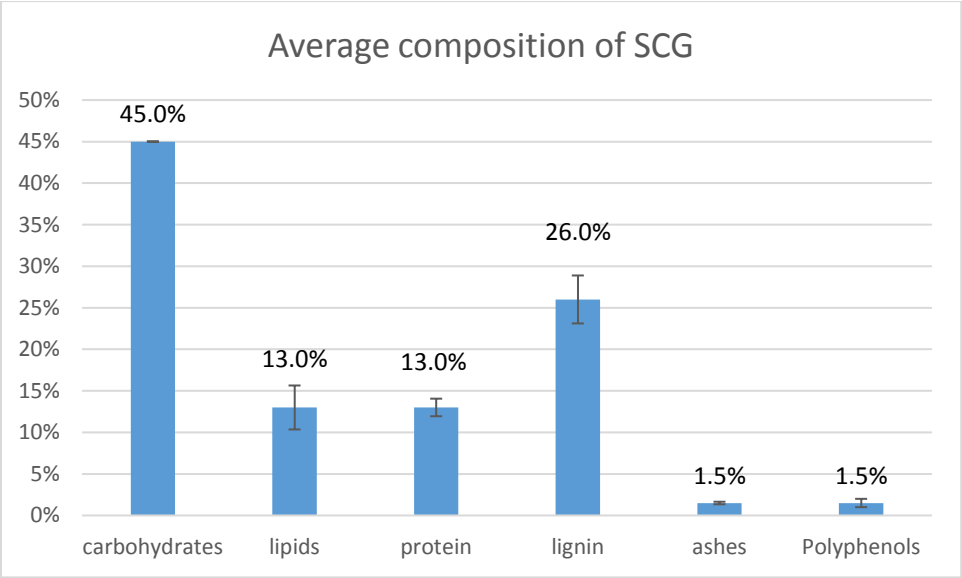


Figure 5: Average composition of SCG according to three sources. Error bars are the range of differnt values. (Campos-Vega et al., 2015)(Mussatto et al., 2011)(Mussatto et al., 2017)

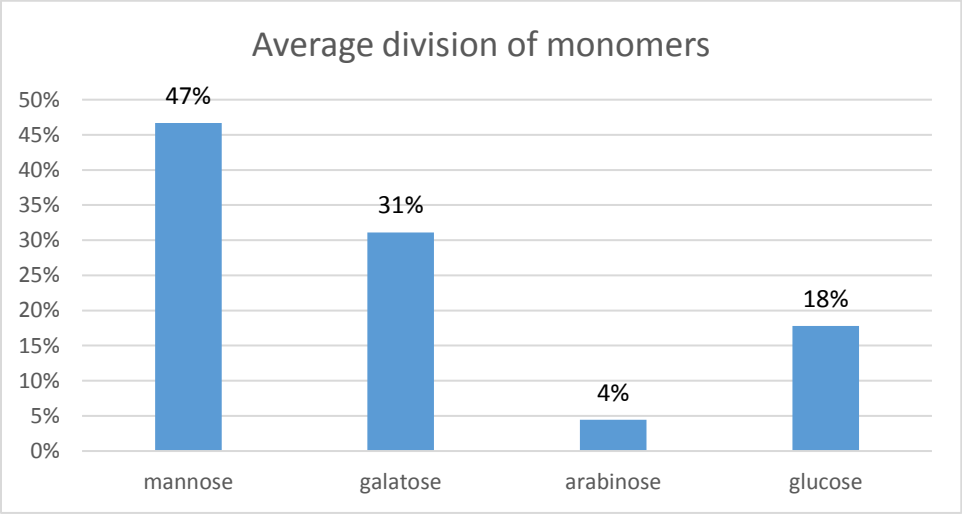


Figure 6: Average division of monomers of SCG according to (Campos-Vega et al., 2015)(Mussatto et al., 2011)(Mussatto et al., 2017)

Although all these compounds can be used, nowadays industries do not fully exploit the potential of SCG. When in fact SCG has been thoroughly studied for all of it uses, see figure 7. Companies now burn the SCG for heating or it is used as compost or as soil for mushroom cultivation. If the coffee producing companies do not use the SCG they send it to land fill.

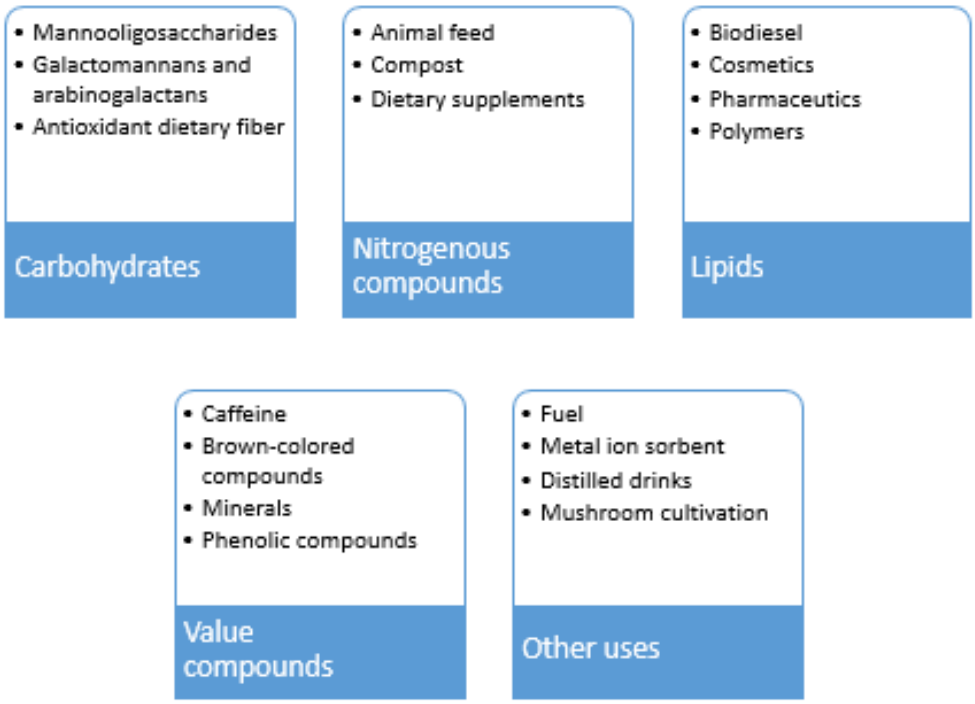


Figure 7: Applications of SCG according to (Campos-Vega et al., 2015)

2.3 Lignocellulose

The coffee bean also has a lignocellulosic structure. Lignocellulose consists mainly of three polymers: cellulose, hemicellulose and lignin, see figure 8. The composition of the lignocellulose varies per plant species, the state of development of the plant and under which conditions it grows. To break down lignocellulose a harsh treatment is necessary to release the monomers (Faaij, 2007). Hemicellulose is relatively easy to break down, in contrast to cellulose, which is relatively hard to breakdown. Therefore, besides the acid hydrolysis an enzyme hydrolysis is preformed to release the glucose.

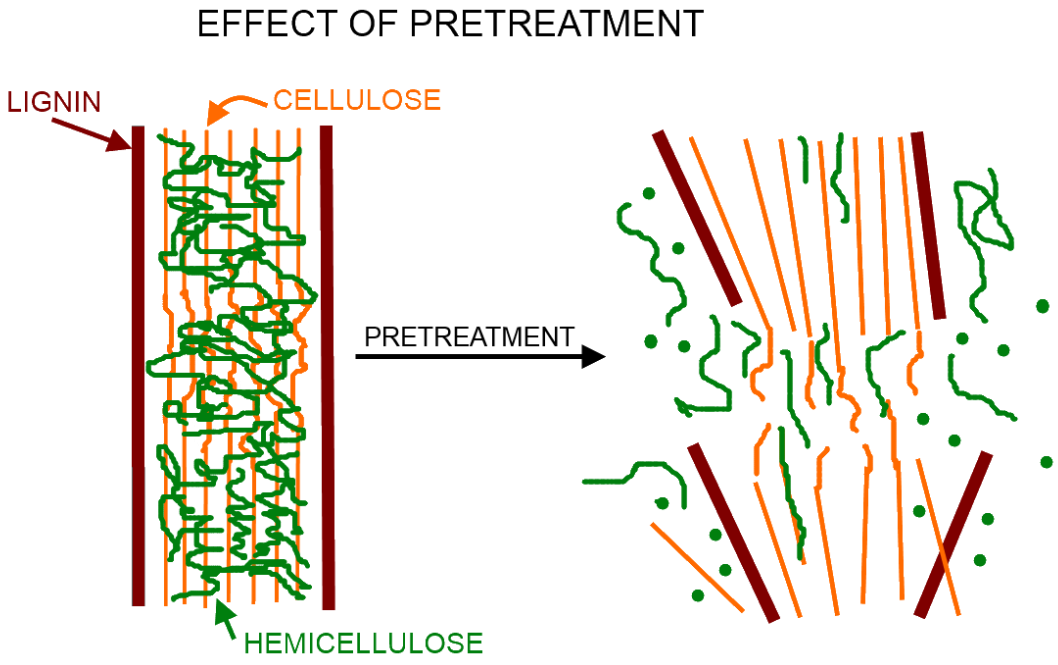


Figure 8: Schematic lignocellulose structure before and after pre-treatment (Blanca-Ocreto, 2013)

Cellulose is a polysaccharide, consisting of a linear chain of β (1→4) linked D-glucose units. The chains are linked together by hydrogen bonds and van der Waals forces. This causes the cellulose to form micro fibrils. The hemicellulose and lignin cover the micro fibrils. Hemicellulose is not build up out of one structure as cellulose is, but out of different monosaccharides. In SCG those are mannose, galactose and arabinose (Faaij, 2007). Generally, hemicellulose is built out of pentoses, hexoses and uronic acids in different compositions. Lignin is a complex and large molecule (around M_w 20.000, Wang, 2015) with a structure containing cross-linked polymers of phenolic monomers which comes in different forms. Lignin acts like the glue in lignocellulose, it binds the different components together, thus making it insoluble in water (Faaij, 2007).

Through the acid and enzymatic hydrolysis steps and the processing steps for the coffee, the lignocelluloses will break open and release the other components. Depending on the hydrolysis steps that are preformed different monomers are released.

2.4 Fermentation

Fermentation is a process that occurs in yeast and bacteria, and other micro-organisms. A lot of different fermentation products can be produce depending on which strain of organism is used, see figure 9. The first step of the glycolysis produces NADH, which is then used to reduce pyruvic acid into further products (Kim, 2010). For this research baker's yeast, *Saccharomyces cervisiae*, will be used. *S. cervisiae* produces CO_2 and ethanol.

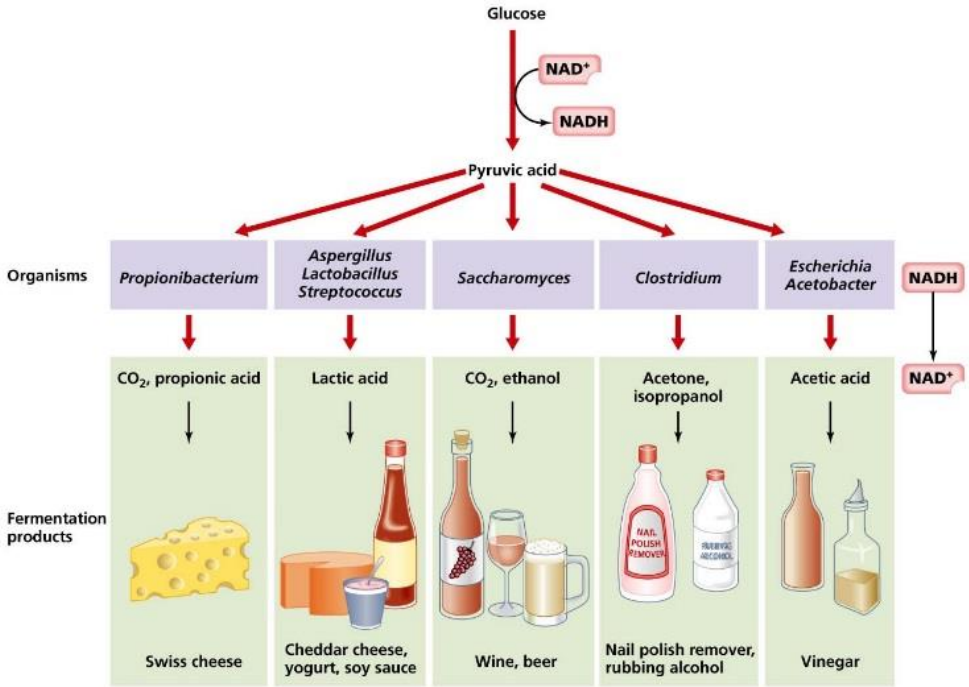


Figure 9: Overview of different fermentation products (Kim, 2010)

3. Materials and methods

An overview of the research is given in chapter 1, figure 2. In this chapter, all the processing steps and how they are performed, are explained. A table of all the chemicals that are used in the process steps is given, see table 1. In the paragraphs only the compound name is used.

Table 1 Chemicals used

Compound	Purity	Supplier	Application
Hexane	97%	SIGMA-ALDRICH	Oil extraction
Sulphuric acid	98%	SIGMA-ALDRICH	Acid hydrolysis
Lactic acid	90%	SIGMA-ALDRICH	Acid hydrolysis
Acetic acid	99-100%	SIGMA-ALDRICH	Acid hydrolysis

3.1 Drying

The purpose of this thesis is generating more value out of SCG. Therefore, the starting material for the experiments is SCG. A drying step is added because the presence of water could result in mould growth and the weight could vary because of the water content. Wet SCG is obtained from the coffee machine in Biobased Chemistry and Technology (BCT) department of Wageningen University and Research. The SCG is dried for 4 to 6 days in an oven at 60°C. From this point on the dried SCG is referred to as 'dry SCG'.

3.2 Oil content

To investigate the influence of oil on the reactions, the oil is extracted from the dry SCG with a Soxhlet apparatus, see figure 10. A 300 ml Soxhlet apparatus is used with 225 ml hexane as solvent. The thimble is filled with dry SCG. Timing is started when the temperature reaches 95°C and after 4 hours the extraction is stopped. The experiment is done in duplo.

Figure 10: Soxhlet apparatus setup

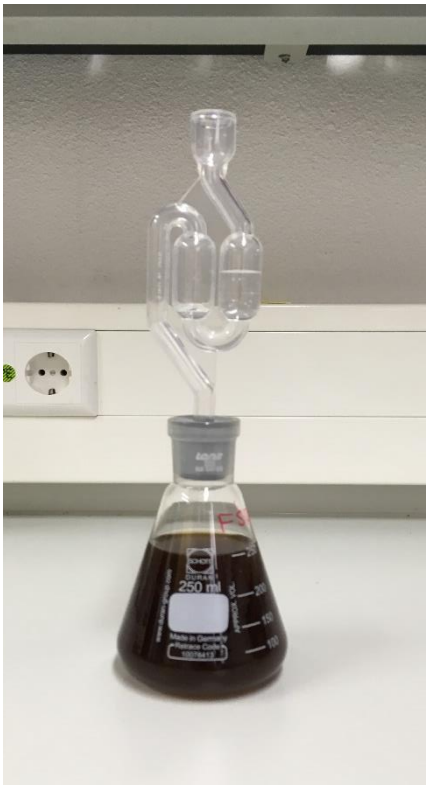


Figure 11: Erlenmeyer with water lock

3.3 Acid hydrolysis

To make the carbohydrates available for fermentation an acid hydrolysis is performed. Dry SCG and an acidic solution are stirred at 100 rpm for 60 minutes at 55°C. The different acids are prepared as a stock solution and then added (table 2). 10 g of dry SCG or defatted SCG is used as sample material and 200 ml of stock solution is added to obtain a ratio of 1 g to 20 ml. This ratio is chosen in order to obtain a SCG solution with a fluent consistency that can be easily stirred. The starting time is when the solution with the SCG is at 55°C. The end time is 60 minutes, samples for HPLC analysis are taken from the Erlenmeyers with the acid hydrolysis. This is done in duplo. For the control, water is used as liquid and the same amount of SCG or defatted SCG is added.

Table 2: Acids used for acid hydrolysis

Acid	Molecule formula	Concentration (M)	pKa
Sulphuric acid	H_2SO_4	0.5, 1.0	-3.0
Acetic acid	$C_2H_4O_2$	0.5	4.76
Lactic acid	$C_3H_6O_3$	0.5	3.86

3.4 Enzyme hydrolysis

To further convert carbohydrates to free sugars for fermentation, an enzyme hydrolysis is performed. Enzymes that are used is Multifect®GC Extra cellulase, which is a customer ready cellulase enzyme cocktail. The enzymes have an activity of 6200 IU/ml (minimum). The whole mixture from the acid hydrolysis experiment is used for the enzyme hydrolysis. First the pH is adjusted to 5.0 as this pH is optimal for the enzyme activity (MULTIFECT GC Extra, 2013). To measure the pH a pH-meter is used. Base solution of 1 M and 10 M NaOH and an acid solution of 1 M HCl was used to adjust the pH. 0.2 ml of enzyme cocktail is added and samples are taken after 0, 1, 2, 4, 24, 48, 72, 96, 168, 192 hours of the reaction time. Untill 24 hours this is done in duplo, after 24 hours only one reaction is continued. The control is the acid hydrolysis control with enzymes added.

3.5 Fermentation

To check if there are fermentable sugars in the samples that are investigated, an alcohol fermentation is used. Baker's yeast, *Saccharomyces cervisiae*, is used as it can perform an alcohol fermentation even if the conditions are suboptimal. Alcohol fermentation by *S. cervisiae* produces ethanol, see equation 3.1. However, alcohol is toxic to yeasts in large amounts, therefore samples will be diluted appropriately in order to keep the concentration below the maximum concentration for *S. cervisiae* (Held, 2012).

$$C_6H_{12}O_6 \rightarrow 2 * C_2H_5OH + 2 * CO_2 \quad (3.1)$$

The glucose that is released during the hydrolysis steps can subsequently be converted to ethanol. First, the samples of the enzyme hydrolysis are separated with a vacuum filter. The solid is dried and weighted to calculate how much of the compounds of the SCG are released in the liquid. Water is added to the liquid sample to obtain a volume of 250 ml. 0.7 g of dry yeast, *S. cervisiae*, is added to the liquid sample. 0.7 g of dry yeast is made from 2.5 g of fresh yeast. This results in a ratio of 1 gram fresh yeast per 100 ml sample. Then, a water lock is placed on top of the Erlenmeyer flask and filled with water, see figure 11. The water lock is necessary because ethanol is volatile and the water lock prevents the ethanol from leaving the Erlenmeyer flask. Samples will be incubated at 30°C and stirred at 100 rpm for 2 days. Samples will be taken before yeast is added, after 2 days and from the water in the water lock.

3.6 HPLC analysis

Products that are analysed in the samples are: glucose, galactose, arabinose, mannose, fructose, sorbitol, furfural, 5-(hydroxymethyl)furfural and ethanol. The samples are analysed with Dionex UltiMate 3000 RS auto-sampler HPLC on an Aminex HPX-87, 300x7.8 mm (Bio-Rad 125-0140) column at 50°C. For glucose, galactose, arabinose, mannose, fructose and sorbitol calibration curves with concentration of 2.0, 1.0, 0.5, 0.1 g/l are made. For furfural and 5-(hydroxymethyl)furfural calibration curves with concentration of 0.2, 0.1, 0.05, 0.01 g/l are made. These are lower because furfural and 5-(hydroxymethyl)furfural are detected with UV at 280 nm and this is more sensitive. For ethanol, a calibration curve with concentrations of 10, 5 and 1 g/l is made because the refractive index detector, RI, is less sensitive for ethanol. Only higher concentration of ethanol, 0.5 g/l or higher, are detected.

4 Results and Discussion

The results are discussed step by step in the process. First of all, information is given about the HPLC analysis. Secondly, the compounds found in SCG are discussed.

Samples from the acid pre-treatment, enzyme hydrolysis and fermentation are taken for HPLC analysis. All these samples are analysed for glucose, galactose, arabinose, mannose, fructose, sorbitol, furfural and 5-(hydroxymethyl)furfural (5HMF) content. The samples that are taken from the fermentation are also analysed for ethanol. These compounds are measured for different reasons. Glucose is measured because this sugar is used by *S. cerevisiae* the produce ethanol. Glucose levels are expected to increase in the hydrolysis steps and decrease in the fermentation step because it is converted to ethanol. Galactose, arabinose and mannose are measured because these are the monomers that are released from hemicellulose when completely degraded. These are the only monomers that are found in SCG. In the hydrolysis steps these are expected to increase and in the fermentation step to remain the same, because *S. cerevisiae* only uses glucose as substrate. Fructose is measured because in an acidic environment it can be formed from glucose. In the acid hydrolysis fructose levels are expected to increase because of the acidic environment. In the enzyme hydrolysis and the fermentation steps fructose concentration should remain the same if there is production in the acid hydrolysis. Sorbitol is measured after it was found in a sample. It was not expected that sorbitol is measured as is it not meantioned in figure 6. Furfural and 5HMF are measured because these are degradation products of sugars. Furfural is formed from pentoses; five carbon sugars. 5HMF is formed from hexoses; six carbon sugars. Concentrations higher than 30ppm of furfural or 5HMF, could inhibit the fermentation process (Faaij, 2007). In all the steps furfural or 5HMF are unwanted products. Ethanol is measured because this is a fermentation product. In both the hydrolysis steps ethanol is not expected to be formed. In the fermentation step ethanol is expected as it is the fermentation product of *S. cervisiae.*

In the RI the peaks of galactose, fructose and mannose overlap. Different settings and columns have been tried but avoiding overlapping peaks was deemed impossible. When these compounds are discussed their results will be combined because there is no distinction between the peaks. As mentioned before, sorbitol was found in the samples when measured. In the first HPLC samples of the sulphuric acid hydrolysis, an unknown peak was measured in the spectrum, which was later proven to be sorbitol by LC-MS, see appendix figure A and B. Sorbitol can be formed from glucose when hydrogen is present. In the process from bean to coffee this could be produces. Another possible reason why sorbitol is present, could be due to the reaction of glucose with myo-inositol in the wet processing of the coffee (Joët *et al*,. 2010). During wet processing, the fruit covering the beans is removed before drying. When 10 g of coffee is stirred with 250 ml of water. On average 11 mg of sorbitol is detected.

4.1 Oil extraction

The average oil content can be seen in table 3. The results show an oil content of 14%, which is in the range of lipids content average determined from literature in figure 5. The average lipid content in the literature was 13%. This oil extraction step is very efficient. As oil extraction is a technique that is widely used in industry and the focus of this thesis is not on oil extraction, no further attempts were made to optimize oil extraction.

Table 3: Oil content

Product	Dry mass (g)	Percentage
SCG	51.80	100%
Defatted SCG	44.12	85%
Oil	7.15	14%
Loss	0.53	1%

4.2 Acid pre-treatment

The main goal of the acid hydrolysis is to increase the enzymatic digestibility of the lignocellulosic material. In order to study the influence of concentration of acid, sulphuric acid is being used at concentrations of 0.5 M and 1 M. To study the influence of the different kinds of acids, sulphuric-, acetic- and lactic acid, in the same concentration is used. To limit influence of other parameters the same temperature, time and stirring speed are used.

Sulphuric acid is used because this acid is frequently used in literature. Acetic and lactic acid is used because these are organic acids. This is an advantage because the SCG can then be used as feed for animals after the carbohydrates are obtained.

4.2.1 Sulphuric acid in different concentrations.

When the acid pre-treatment with sulphuric acid was done, arabinose was released. There were no other compounds released that were not already present in the SCG. Acid hydrolysis is a pre-treatment step, therefore it is not strange that no other compounds are released. However, it is expected that the lignocellulose is degraded to a smaller degree. This has to do with the structure of the cellulose and the hemicellulose.

As seen in figure 12, arabinose production is displayed. When 0.5 M of sulphuric acid is used around 30 mg of arabinose is produced. When 1.0 M of sulphuric acid is used around 160 mg is produced. There is no a significant difference between SCG or defatted SCG.

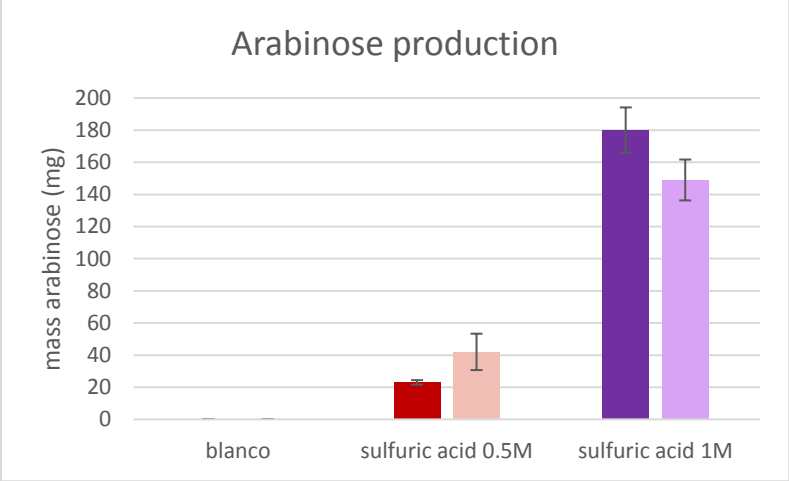


Figure 12: Arabinose production by acid hydrolysis with sulphuric acid in different concentration at 55C for 60 minutes. Dark colour bar: SCG sample. Light colour bar: defatted SCG sample

4.2.2 Effect of acid type in concentration of 0.5 M.

When the acid pre-treatment is done with sulphuric, acetic and lactic acid with a concentration of 0.5M. There were no other compounds released that were not already present in the SCG, expect arabinose with the sulphuric acid pre-treatment. Acid hydrolysis is a pre-treatment step, therefore is not strange that no compounds are released. However, it is expected that the lignocellulose is degraded to a smaller degree. This has to do with the structure of the cellulose and the hemicellulose.

As seen in figure 13, arabinose is produced. Only with the 0.5M sulphuric acid arabinose is produced while for 0.5M acetic acid and 0.5M lactic acid no arabinose is produced. A reason that arabinose only is released when sulphuric acid is used for the hydrolysis is that the [H^+] strength of sulphuric acid is higher than the [H^+] strength of acetic acid and lactic acid. More about the [H^+] strength is explained in paragraph 4.6.

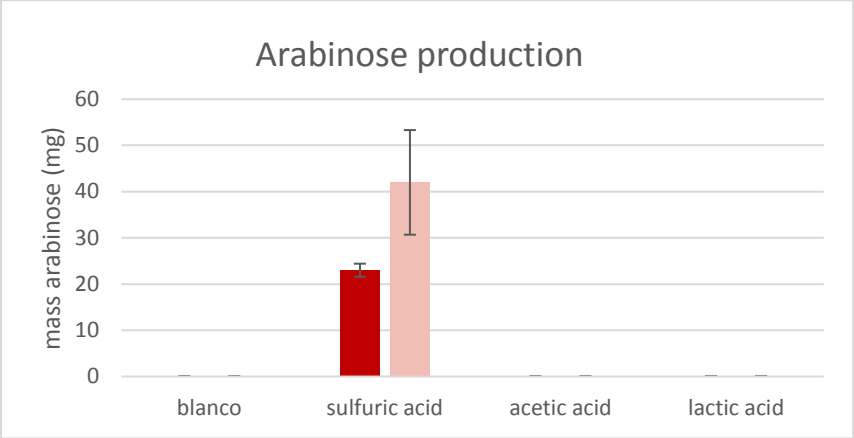


Figure 13: Arabinose production by acid hydrolysis with 0.5M different acid at 55°C for 60 minutes. Dark colour bar: SCG sample. Light colour bar: defatted SCG sample.

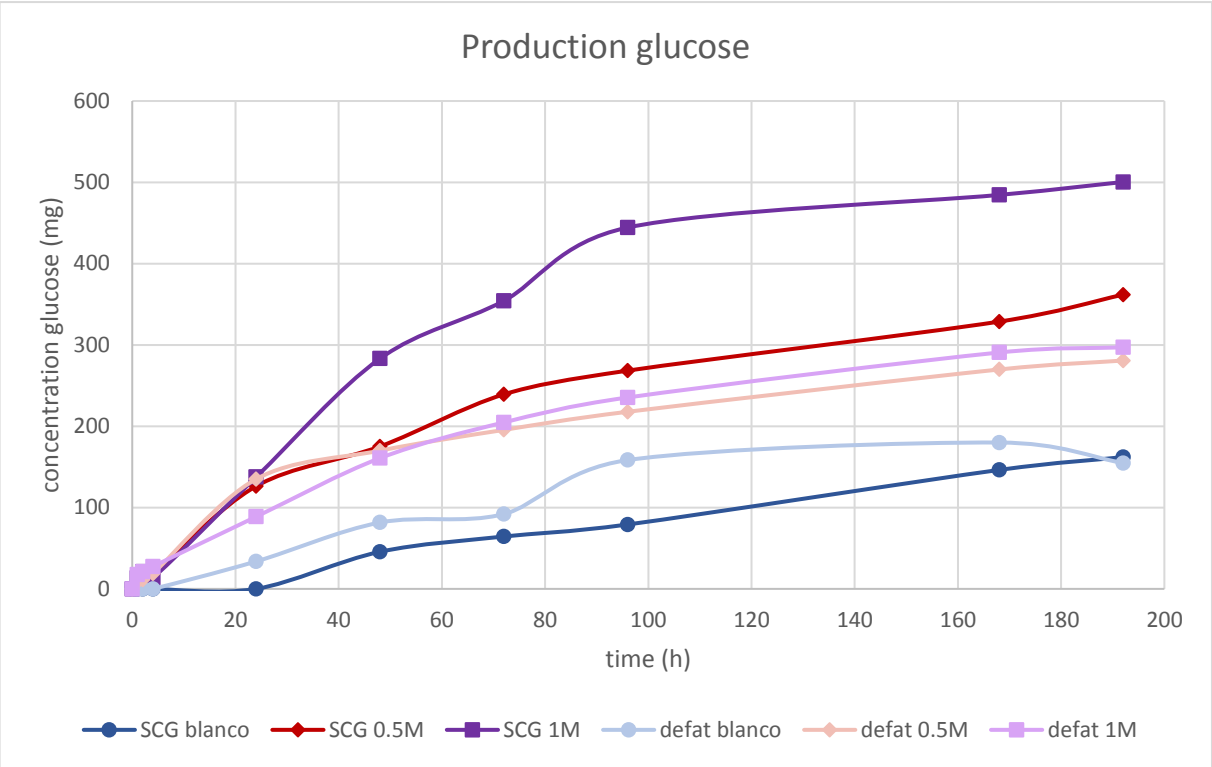
4.3 Enzyme hydrolysis

The main goal of the enzyme hydrolysis is to digest the lignocellulose to fermentable glucose. In order to study the influence of the acid hydrolysis pre-treatment these are taken as sample material. Before performing the enzyme hydrolysis, a 24 hour enzyme hydrolysis seemed best suited for the process. After 24 hours of enzyme hydrolysis it turned out that the enzyme had not finished converting the carbohydrates to monomers. This was done in duplo. Therefore, one of every 24 hour enzyme hydrolysis sample was taken out of the fridge and put back in the oven to perform the reaction for 8 days in total. The enzyme hydrolysis samples pre-treated with sulphuric acid, with concentrations of 0.5 M and 1 M, were stored in the fridge for 13 days and the enzyme hydrolysis samples pre-treated with acetic and lactic acid, were stored in the fridge for 7 days. When a sample for HPLC analysis was taken at the first enzyme hydrolysis at 24 hours and after the destined time in the fridge there were small difference in concentration of the compounds in the sample. The enzyme cocktail should be inactive at a temperature below 20°C, however, the acid can influence the composition of the sample. For the results, an average of the 24 hours measuring point is taken.

In addition to the different composition after the samples had been in the fridge, the liquid in the sample evaporated in the oven at 55°C. The parafilm that covered the Erlenmeyer flasks ripped due to gas formation. Every time a sample for HPLC analysis was taken, new parafilm was placed but this did not prevent that the enzyme hydrolysis sample evaporated. Since the HPLC measured sample in concentration; g/l, a conversion table was made for calculating the approximate amount of compound volume; l. There is made use of the formula: $Weight\ in\ mg = Concentration\ in\ \frac{g}{l} * volume\ in\ l * 1000$. The conversion table is based on the average volume left in the Erlenmeyer flasks, see appendix table A.

4.3.1 Enzyme hydrolysis with sulphuric acid in different concentrations as pre-treatment

Glucose production over time is describe because the enzymes are releasing it from the cellulose. As seen in graph 1, glucose is produced in time. One can observe that both sulphuric acid pre-treatments samples have significantly more glucose production than the control. The control had no pre-treatment with acid. The highest concentration of glucose is produced when the sample is pre-treated with 1.0 M sulphuric acid. Both samples with a SCG as starting material had a higher glucose production compared to defatted SCG.



Graph 1: Glucose production in time with pre-treated samples with different concentrations of sulphuric acid at 55°C for 196hour. Dark colour line: SCG sample. Light colour line: defatted SCG sample.

Arabinose remains in the solution when the enzyme hydrolysis is performed. The bars are an average of all the concentration measured in time. This explains the large error bars. As can be seen in figure 14, arabinose is produced during the acid hydrolysis and in the enzyme hydrolysis nothing is happening to the produced arabinose.

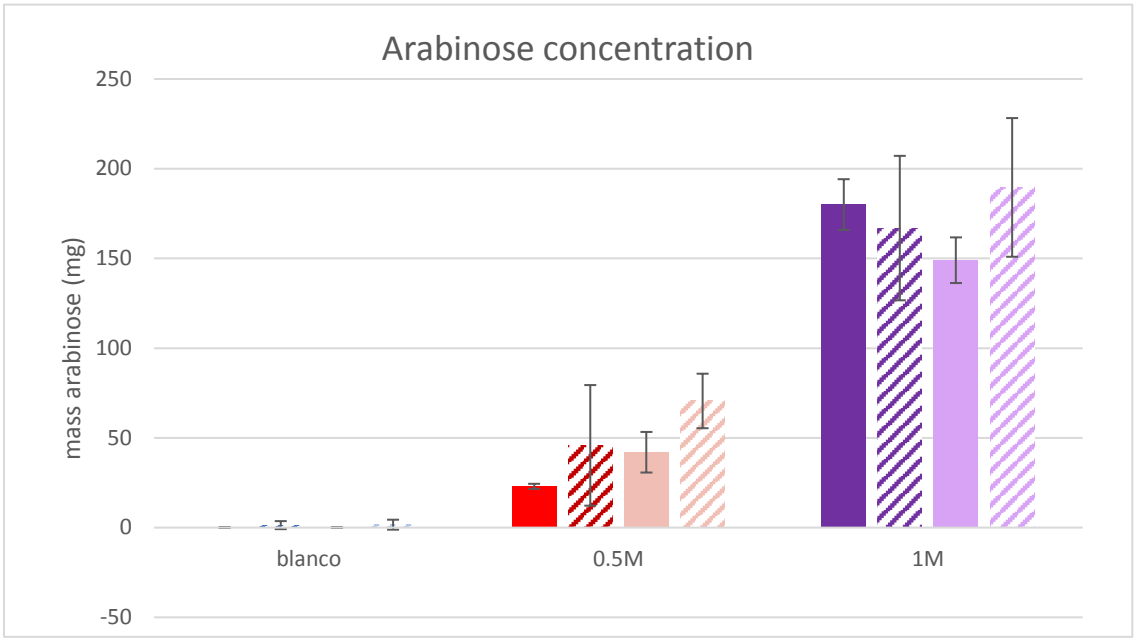
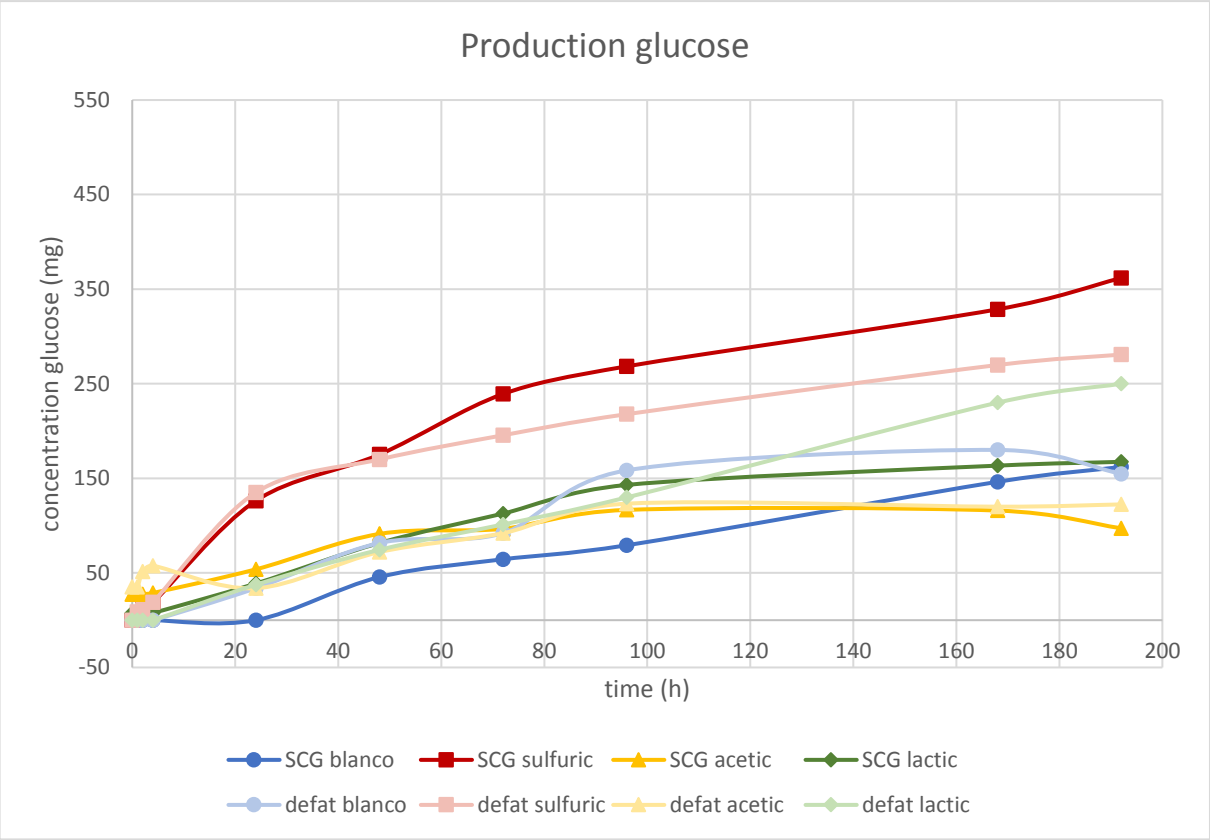


Figure 14: Arabinose production. Solid: by acid hydrolysis with sulphuric acid in different concentration at 55C for 60 minutes. Striped: enzyme hydrolysis with pre-treated samples with different concentrations of sulphuric acid at 55°C for 192hour. Dark colour bar: SCG sample. Light colour bar: defatted SCG sample.

The other compounds that were measured, were released in tiny amounts or not at all. During the enzyme hydrolysis it was expected that more monomers would be released. At the same time, there is no production of the inhibitor compounds, furfural or 5HMF.

4.3.2 Enzyme hydrolysis with sulphuric, acetic and lactic acid in concentration of 0.5 M as pre-treatment

Glucose production over time is describe because the enzymes are releasing it from the cellulose. As seen in graph 2, glucose is produced over time. Pre-treatment with sulphuric acid is most effective for the glucose production. Additionally, the pre-treatment with acetic or lactic acid does not differs from the control in glucose production.



Graph 2: Glucose production in time with pre-treated samples with 0.5 M different acids at 55°C for 192hour. Dark colour line: SCG sample. Light colour line: defatted SCG sample

4.4 Fermentation

The main goal of the fermentation is to convert all the glucose to ethanol. All data points are in single. The concentration of compounds is slightly changed between the last measuring point of the 192 hours enzyme hydrolysis and the first measurement of the fermentation. There are 21 days between the enzyme hydrolysis and the fermentation because the material required was not available during this period. As said in the enzyme hydrolysis, the acid can still influence the composition of the sample. It is unlikely that the enzymes in the sample can influence it, as they should be inactive at temperature below 20°C.

As seen in figure 15, there is a change in glucose concentration, mostly negative, in the time between enzymatic hydrolysis (EH196) and start of the fermentation (F). There is not a clear trend in what the time is doing to the glucose or if it depends on which acid is used for the pre-treatment. This might be because the data used is single. What is clear is that all the glucose in the sample is converted to ethanol by the *S. cerevisiae*. Arabinose is degrading over time when fermented in contrast to sorbitol which remains constant in the fridge and in the fermentation process.

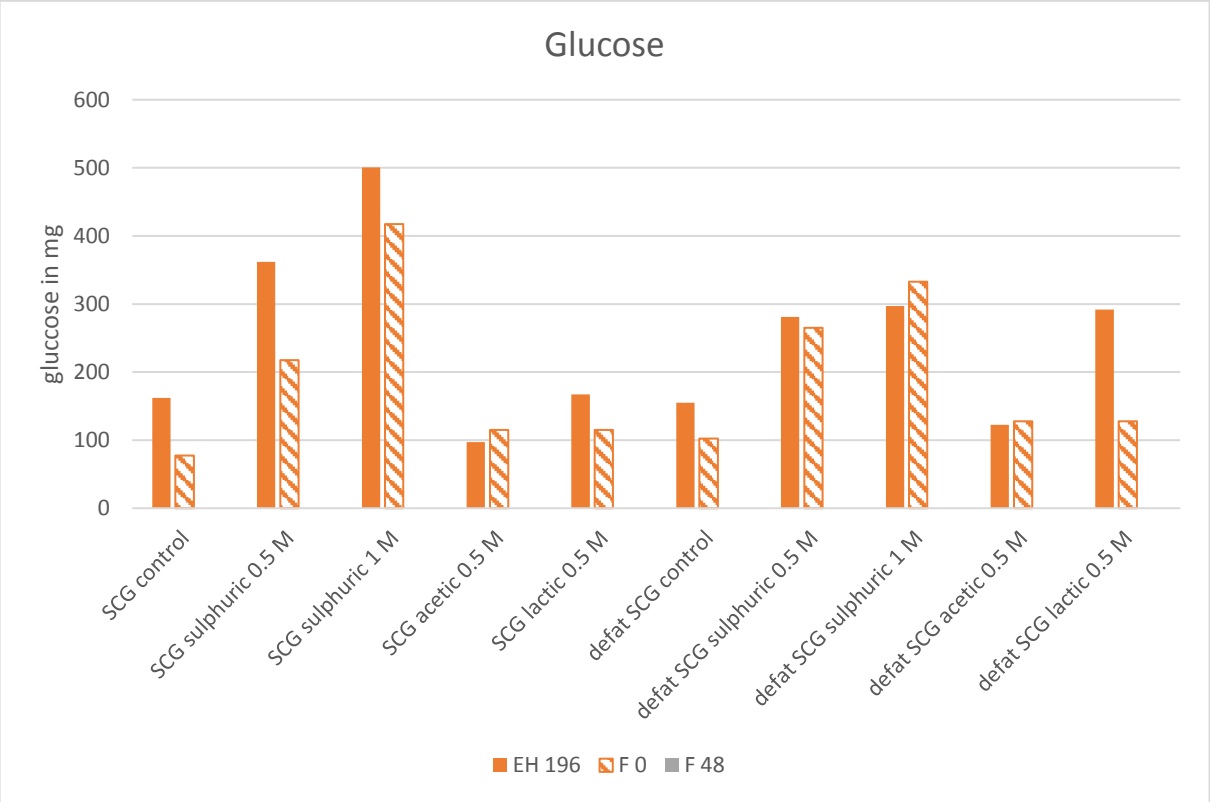


Figure 15: Glucose weight. EH196: at t= 192 hour with enzyme hydrolysis, F0 and F48 are respectively for t= 0 hour and t= 48 hour with fermentation.

As explained in chapter 3, every glucose molecule is converted by *S. cerevisiae* into two molecules of ethanol and two molecules of CO_2., see equation 3.1. One mole of glucose gives two moles of ethanol and two moles of CO_2. If 100mg of glucose was in the fermentation sample at t= 0h, then the expected weight of the produced ethanol is 51mg, calculated by equation 4.1.

$$weight_{ethanol} = \frac{100mg\ glucose * (2 * 46\ M_{w\ ethanol})}{180\ M_{w\ glucose}} = 51mg \quad (4.1)$$

In figure 16, glucose is displayed at the t= 0 and ethanol is displayed at t= 48 h of the fermentation. Ethanol was only measured when the fermentation was done. As calculated, around half of the glucose weight should be converted to ethanol. In every sample ethanol weight is higher than half the glucose weight. A possible reason could be that *S. cerevisiae* uses other sugar molecules, for example sugar molecules that are not measured with HPLC, as substrate instead of glucose. Another reason could be, that HPLC is not precise with measuring ethanol, so the real amount of ethanol could be different.

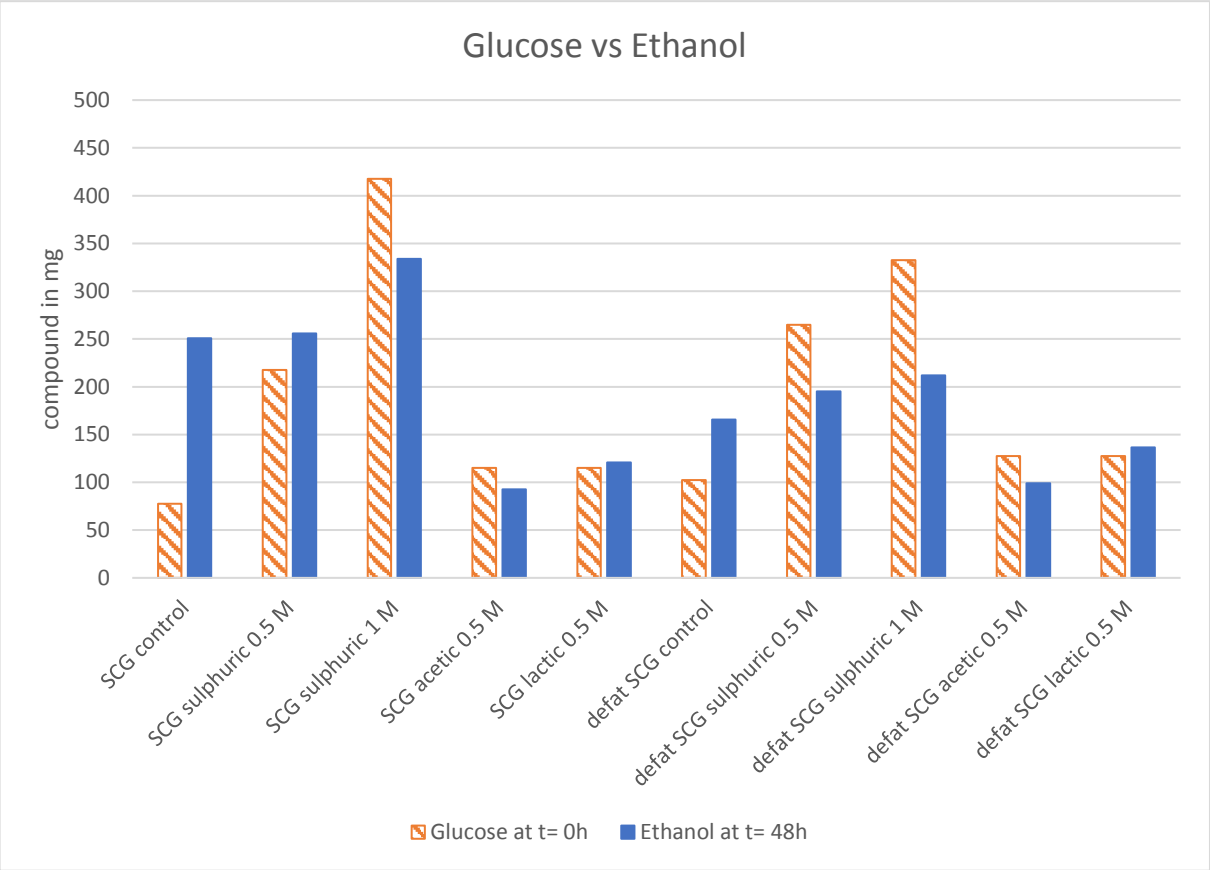


Figure 16: Glucose weight at t=0 hour with fermentation, and ethanol weight with fermentation at t= 48 hour.

Galactose, mannose and fructose were measured in amounts lower than 50 mg, 5HMF were even measured in amounts lower than 1 mg. Furfural was not measured at all after the fermentation.

4.5 Mass balances

To get more insight into the process, all data acquired is used to set up a mass balance. The mass balance gives an overview of all the in- and outlet by every step. The process starts with 10.0 g (100%) SCG, when an oil extraction step is proceed a part of the SCG which consist out of oil is removed. The hexane can be recycled after rotor evaporating. A small amount is lost because SCG sticks in the thimble from the Soxlet apparatus or oil and hexane mixture stick to the Soxhlet apparatus bulb when transferring in the rotor evaporating bulb. Then the samples are prepared from the acid pre-treatment and enzyme hydrolysis, both process step are done in the same Erlenmeyer flask, so nothing should be lost. After enzyme hydrolysis, the solution is filtered with a vacuum filter to determine the amount of SCG in the liquid fraction. The liquid fraction is used for fermentation so that inhibitory compounds in the solid fraction cannot interfere with the fermentation reaction.

If the SCG mass balance (figure 17) and defatted SCG mass balance (figure 18) are compared with both an acid pre-treatment of sulphuric acid 0.5 M. There is a big difference in percentage of the liquid fractions. The lower percentage of water in the defatted SCG sample can be due to the oil extraction step. This would mean that the difference between the two samples is the oil content.

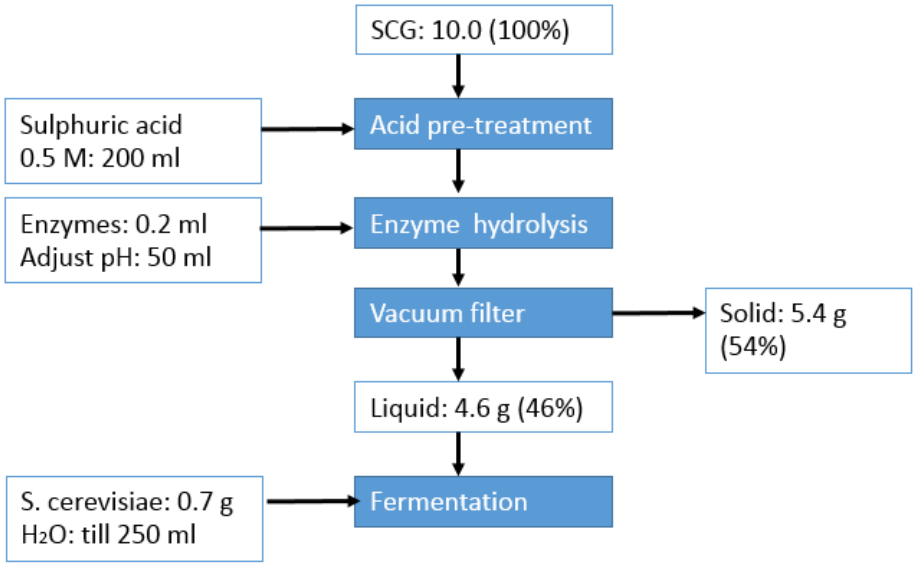


Figure 17: Mass balance for SCG with acid hydrolysis with 0.5 M sulphuric acid.

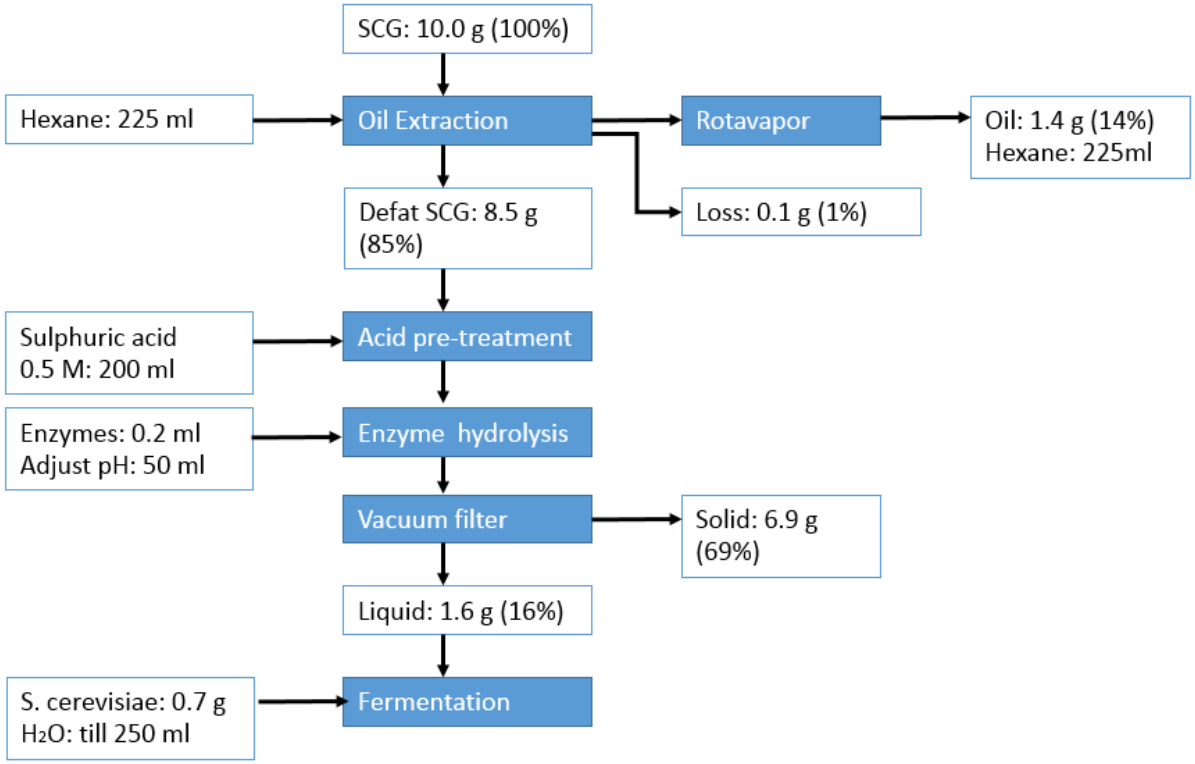


Figure 18: Mass balance for defatted SCG with acid hydrolysis with 0.5M sulphuric acid.

The yield of glucose is calculated to give an insight on how effective glucose is released which each acid pre-treatment. The maximum concentration of glucose is calculated with literature data (figure 6), there is around 0.8 g of glucose and 0.9 g of glucose in 10 g of SCG or defatted SCG respectively. The yields of the maximum mass of glucose is given in figure 19.

As seen in figure 19, when there is a sulphuric acid pre-treatment on normal SCG, compared to defatted SCG, it has a yield increase of 14% and 30% with 0.5 M and 1 M acid respectively. The other way around, this is valid for acid pre-treatment with lactic acid 0.5 M. Here, defatted SCG has 11% more glucose released than when the starting material for the acid pre-treatment is normal SCG. When pre-treatment is done with acetic acid 0.5 M the kind of SCG does not influence the amount of glucose released. The same can be seen for the control but it has a higher glucose yield than a pre-treatment with acetic acid.

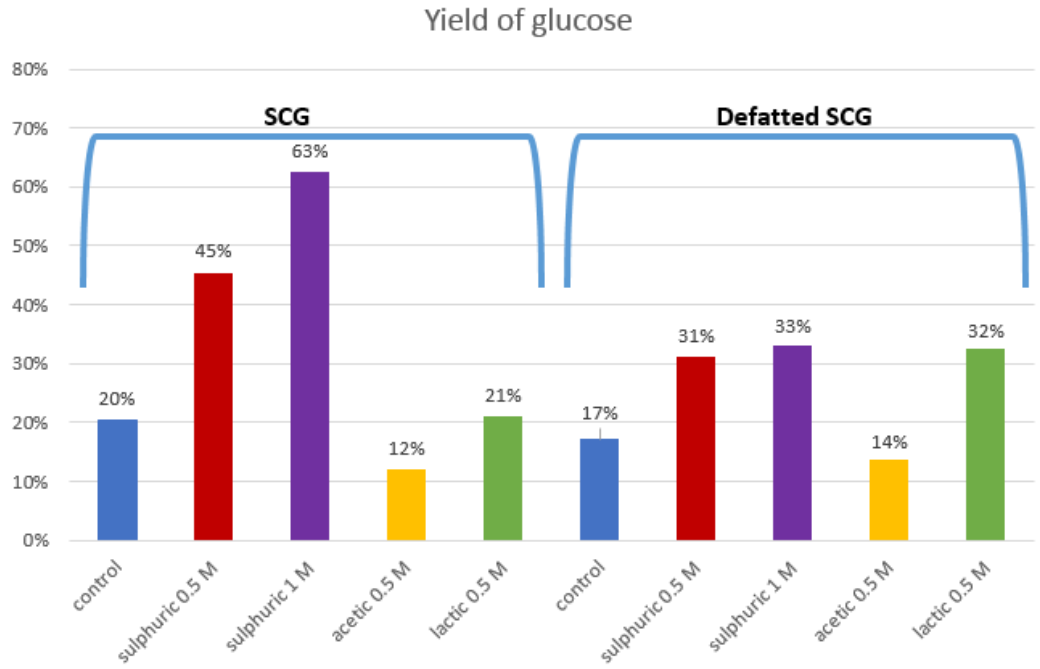


Figure 19: yield of glucose for SCG and defatted SCG.

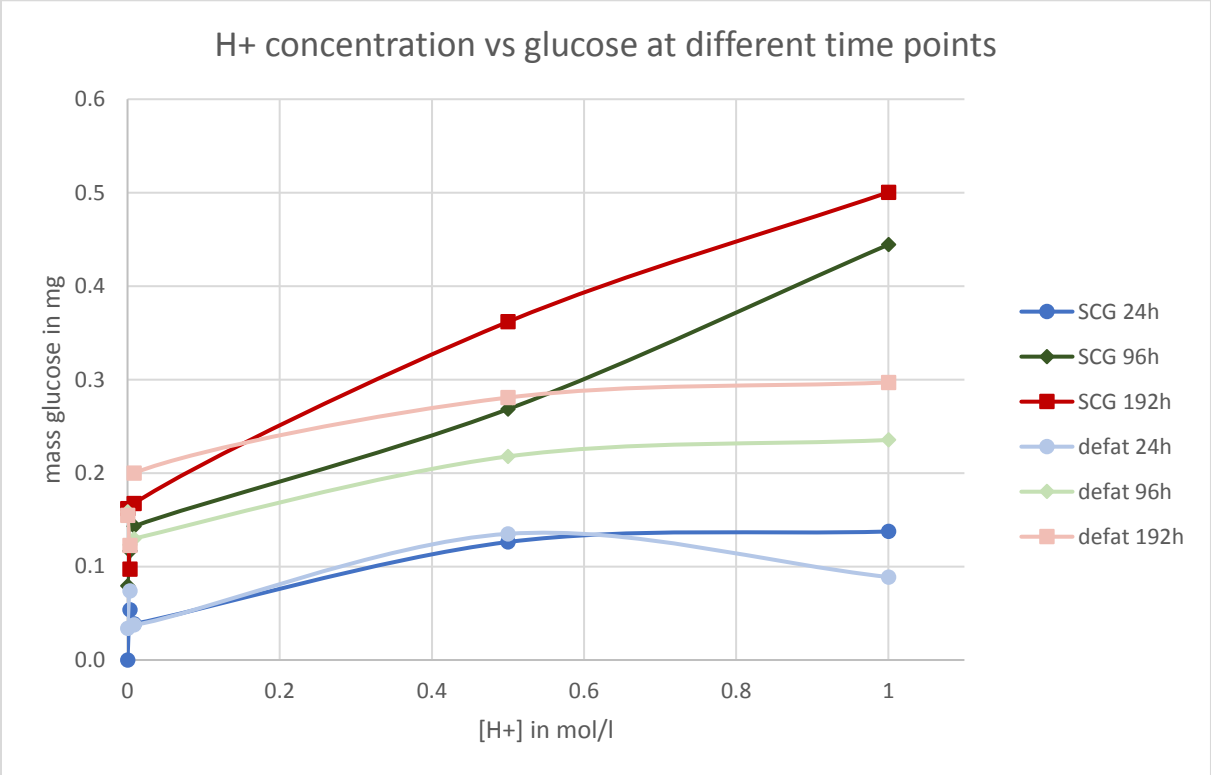
4.6 [H^+] strength

This graph is made to connect the H^+ concentration of the acid that are used for the acid pre-treatment to the maximum amount of glucose released in the enzyme hydrolysis. The H^+ concentration is interesting because different acid with different H^+ concentrations are used as pre-treatment. The concentration H^+ is calculated with the K_a value for water, acetic acid and lactic acid. These [H^+] values of the water, acetic acid and lactic acid are close to zero, therefore not really noticeable in graph 3. Sulphuric acid is a strong acid, therefore has been assumed that sulphuric acid is completely dissociated in water to form H^+.

The H^+ concentration is plotted against the amount of glucose produced at a specific point in time (24,h, 96h and 192h). The glucose production can be seen in graph 1 and 2. The 24h time point is chosen because a small amount of glucose had been produced by this time. The 96h time point is chosen because it is in the middle of the other two time points. The 192h time point is chosen because it is the last point measured.

In graph 3, when looking at SCG (darker coloured lines), especially the 96h and 192h, [H^+] has a clear effect on the glucose that is produced in the enzyme hydrolysis. If the graph is extended, a higher [H^+] would increase the glucose production.

When looking at the lighter coloured lines, with defatted SCG, [H^+] does not influence the production of glucose as much as it does with the SCG. The absent of oil could have a negative effect on glucose production.



Graph 3: Concentration H^+ against weight of glucose at different times. T= 24 hour, t= 96 hour and t= 192 hour in the enzyme hydrolysis. Darker colour: SCG, lighter colour: defatted SCG.

5 Conclusion and Recommendations

It has been proven that products with a potential larger value can be made of spent coffee grounds. Carbohydrates can be converted into fermentable sugars. Although, the process has to be improved. 63% of the glucose available in SCG is extracted under the best condition used in this research, which was 1M sulphuric acid with SCG. If a greater percentage of glucose is extracted, more glucose can be converted to ethanol and the value increases.

To answer the research question, carbohydrates can be converted into a fermentable media. Sulphuric acid is the best acid for an acid hydrolysis at a concentration of 1 M. But using such a strong acid has a negative effect. When the pH has to be adjusted for the enzymes a lot of basic solution is necessary and the solid waste cannot be used as animal feed. The lipid content has an influence on the hydrolysis and fermentation. If the oil is in the SCG and pre-treated with sulphuric acid more glucose is released in contrast to when it is pre-treated with acetic acid or lactic acid. However, the question remains whether it is the oil itself that plays a role or whether the extraction with hexane influences the yields. Oil has no observable influence on the fermentation.

Arabinose is only released when acid hydrolysis is done with sulphuric acid. At higher concentration of sulphuric acid more arabinose is released. Arabinose is converted slowly when fermented.

When the enzyme hydrolysis after 24 hours is compared with the enzyme hydrolysis after 192 hours, the control with water needed more time to release glucose than the samples that were pre-treated with acid. At 192 hours only the samples which had a pre-treatment with sulphuric acid released more glucose than the control, except for the defatted SCG with a lactic acid 0.5 M pre-treatment. After 192 hours of enzyme hydrolysis there was still an increasing trend in the amount of glucose, as seen in the graphs 1 and 2. Therefore a longer enzyme hydrolysis is recommended to obtain more glucose.

The acid hydrolysis should be investigated further. A more effective acid hydrolysis can also improve the reaction of the enzyme hydrolysis, which might shorter the reaction time. Different acids can be used. In addition to investigating other acids, a different reaction time, temperature or concentration could also be of great influence on the results. Especially, the temperature of the acid hydrolysis should be investigated because it is thought that great improvements can be made here, because the temperature that is used is relatively low for an acid hydrolysis. A temperature of 100°C will work better and even higher temperatures will increase the efficiency even further but this is more difficult because the experiments would have to be done at elevated pressures.

To increase the accuracy of the measurements, all experiments should be done at least in duplo, preferably however with even more repetitions. When doing the HPLC analysis, write down the volume or fill till a certain volume, to get the exact amount of component in the sample. Before starting, make sure everything that is needed in the experiments is there, so that the samples do not have to be in the fridge for too long. Because this influences the composition of the sample.

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Appendix

Table A: Conversion table for enzyme hydrolysis from the average volume left in the Erlenmeyer flask

Time in hours	**0**	**1**	**2**	**4**	**24**	**48**	**72**	**96**	**168**	**192**
Volume in ml	250	250	250	250	250	240	230	220	190	180

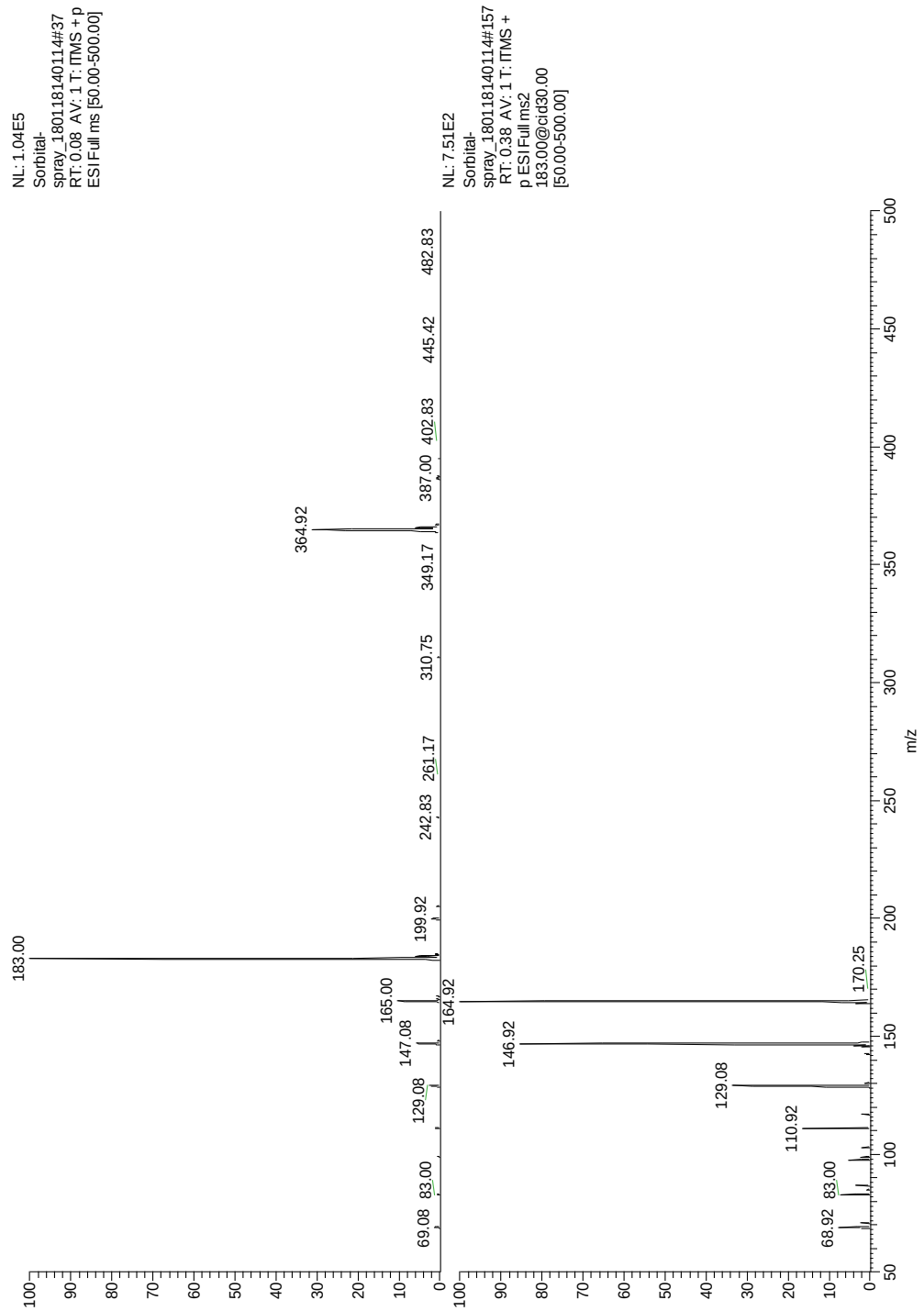

Figure A: LC-MS spectrum of sorbitol 10g/l

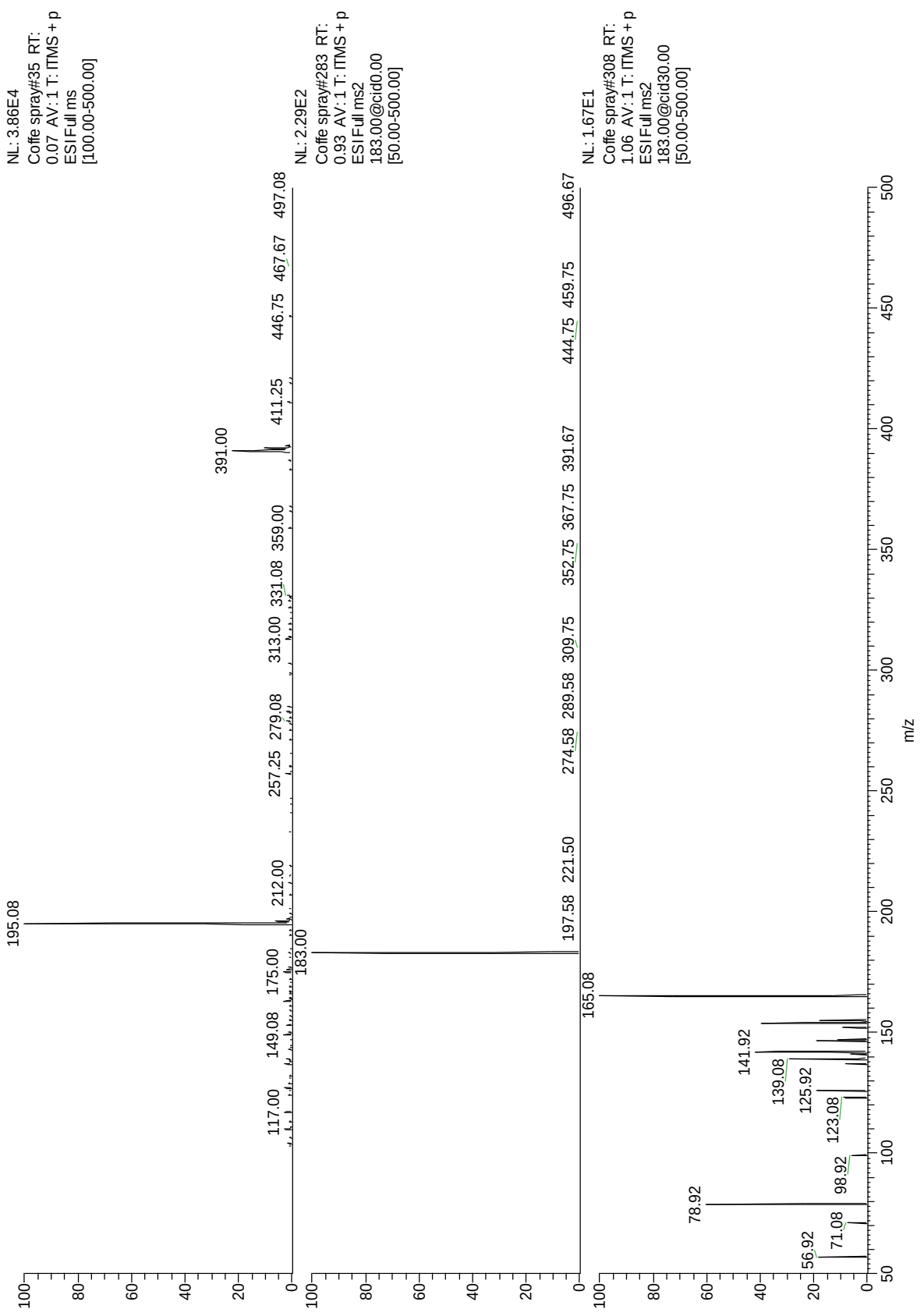

Figure B: LC-MS spectrum of spent coffee grounds 10gram solved in 200ml water. First spectrum: overall sample, second spectrum: only ms of 183, third spectrum: only ms of 183 with 30 energ.