REDUCING SILICA CONTENT IN RICE HUSK BY DECANTATION METHODS

Master thesis for Sustainable Biotechnology

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Contents

Abstract

Lignocellulosic biomass is a renewable resource that has an enormous potential to produce heat, power, transportation fuels, and bioproducts. The main benefit of using biomass as an energy carrier is that the net emissions of CO_2 can be neutral or even negative if the biomass comes from sustainable sources like agricultural waste feedstocks.

Rice husk is an important biomass residue found in large quantities. Approximately 680 million tons of rice is grown yearly, producing 120 million tons of rice husk as a byproduct. As a residue from the rice hulling industry, the husk is easy to collect and inexpensive, and its lignocellulose can be used as an energy carrier. However, the high silica content is a barrier to lignocellulose utilization. Thus, huge amounts of rice husk are subjected to wrongful management or disposal, mainly burned in the open.

This thesis developed a method to fractionate rice husk powders by density. An exploratory analysis was implemented to test the hypothesis that density fractionation can separate rice husk residues into low and high silica content fractions. If possible, fractions of low silica content can be used as raw materials for energy generation, while fractions of high silica content can be used in silica-related applications.

The selected method was based on centrifugation. Two different experiments were conducted with milled rice husk samples from Uruguay and Colombia. Variables from those experiments were compiled to recommend a method for future experiments. A decreasing particle size (as determined by grinding time) did not strongly influence the density fractionation, while the time of contact between the rice husk samples and water appears to have a tangible effect on reducing ash content. This latter effect can be attributed to soluble minerals diluting into the water slowly during water-wash. However, there was no evidence that water at room temperature improved the release over water at 50°C.

Small but significant differences in ash content were measured and related to a reduction in silica content using a combination of ATR-FTIR and chemometric tools. Principal component analysis (PCA) and hierarchical cluster analysis (HCA) were able to identify the small differences in ash content. Comparisons between different experiments were made, and the combination proved to be simple, accurate, and reliable.

1 Introduction**Error! Bookmark not defined.**

Decreasing fossil reserves, uncertain prices, concerns over the security of supply, and environmental impacts have led to a global endeavor to enable the use of biomass as low or neutral carbon feedstock (Gunawardena and Fernando, 2013). Agricultural residues composed of lignocellulosic biomass, like for example, rice husk are a renewable energy source that can be used sustainably if the feedstocks (1) do not compete for fertile land because it would increase food prices; (2) do not increase the use of fertilizers and pesticides to create carbon-neutral processes, and (3) protect biodiversity (Anwar et al., 2014).

Approximately 750 million tons of rice is grown yearly (FAOSTAT, 2021), producing 120 million tons of rice husk as a waste residue. Rice is a staple food for more than 3 billion people. Farmers worldwide open burn the byproducts (straw and husk) to eliminate the residues of harvested rice. This practice has negative environmental impacts, especially related to air pollution (El Safty, 2020).

A challenge of using rice husk as an energy source is the high silica content. For example, in combustion applications, silica causes fouling, decreasing boiler performance; when converting rice husk to ethanol, the conversion yields are low (Madu and Agboola, 2018), and the silica is mixed with the lignin-rich distillation residue, limiting the possibility of utilizing the lignin further; the silica will damage engines if used as a biofuel (Cabrera et al., 2016).

The project is an exploratory analysis to test the hypothesis that density fractionation can separate rice husk residues into low and high silica content fractions. If possible, fractions of low silica content can be used as raw materials for energy generation, while fractions of high silica content can be used in silica-related applications.

In the following section, the environmental problems that motivate sustainable biomass use are introduced, followed by a detailed description of rice husk as its potential uses.

1.1 Environmental problems

Surface air temperature has risen considerably at a rate of 0.12° C per decade over the past century (Huang et al., 2017). This temperature change is with a 95% probability caused by human activity (NASA, 2021), such as the production and combustion of fossil fuels, industrial

activities, and agriculture (Kweku et al., 2018). The outcome of all these changes in the atmosphere are not easy to predict, however, on average, it is projected that the earth will become warmer, the glaciers and ice sheets will partially melt, and new patterns of pests and diseases will appear, among others (NASA, 2021).

Under the 2015 Paris Agreement, the global community has agreed to limit the global average temperature increase to below 2°C above pre-industrial levels and pursue efforts to stay below 1.5°C warming (UNFCC, 2017). The Danish Government target a reduction of national GHG emissions by 70% by 2030 (Ministry of Foreign affairs of Denmark, 2020). These national and international agreements call for drastic emission reductions from fossil fuel and agricultural industries to and beyond 2050.

1.1.1 Fossil fuels

Fossil fuels are natural resources formed from fossilized remains of animals and plants that inhabited the earth millions of years ago. Crude oil, coal, natural gas, and other fossil fuels have been used as a primary source for energy generation for centuries (Diercks et al., 2008). During the combustion of fossil fuels, CO_2 is released, which increases global warming. In addition, several studies revealed that fossil fuels are responsible for a considerable amount of air pollution, causing health issues and economic problems (Martins et al., 2018).

Fossil fuels are also used for plastic manufacturing, carbon fibers, lubricants, and other petrochemical industries. These industries are also considered culprits for climate change due to the greenhouse emissions produced in each step of their lifecycle (Hopewell et al., 2009; Rhodes, 2018).

There is a societal need to develop non-fossil, sustainable, renewable carbon molecules to replace fossil fuels.

1.1.2 Agricultural production

Over the last 50 years, agriculture has increased threefold production mainly due to the growing population, the technological contributions to increase productivity, and the expansion of soils for agricultural use. Daily production is around 23.7 million tons of food globally, producing an incredible amount of waste residues (FAO, 2017c). For example, the European Union produces around 1.3 billion tons of residues per year, from which 700 million tons are agricultural byproducts (Babenko, et al., 2018).

This global growth in agriculture has increased environmental pressures, causing negative impacts on the air, soil, and water resources (Duque-Acevedo, et al., 2020). Currently, many of the residues are incinerated, which is harmful to the environment due to greenhouse gas emissions (GHG) (Babenko, et al., 2018; Hýsková et al., 2020). In addition, the expansion of the agricultural frontier is the leading cause of deforestation, and one of the most significant contributors to climate change, with 20 to 35% of the GHG emissions associated with this sector (Clapp et al., 2018). Furthermore, agriculture utilizes around 40% of the earth´s land surface and consumes 70% of freshwater (Braimoh, 2013).

There is a societal need to reduce the environmental impact of agriculture.

1.2 Agricultural waste as a source of renewable carbon molecules

Using agricultural waste to replace fossil raw materials can generate circular biobased economies (FAO and UNEP, 2010). In biorefineries, biomass can be converted into multiple products, such as energy, chemicals, and materials (Attard et al., 2014). Many biorefineries utilize feedstocks that compete with food or feed. Agricultural residues do not compete with food, contributing to the feasibility of biorefineries (Clark and Deswarte, 2015).

Agricultural waste, byproducts, and co-products are defined as unwanted or undesirable residues from plants or animals that are not food or feed, which may cause an environmental and economic load for the primary sector. These byproducts have an enormous potential to produce bioenergy or bio-based products (Gontard et al., 2018).

There are many definitions of biomass. Some define biomass as solar energy converted to organic matter by photosynthesis, or the substance that plants are made of. Biomass can be classified as herbaceous and agricultural biomass, wood and woody biomass, animal and human biomass, aquatic biomass, contaminated biomass, industrial biomass wastes (semi-biomass), and biomass mixtures (blends from the other varieties) (Vassilev et al., 2010). To use biomass as a carbon source, it is important to classify it in terms of its composition (Zhao et al., 2012).

1.2.1 Chemical composition

Biomass is a complex heterogeneous mixture of the main structural organic components, hemicellulose, cellulose, and lignin. Also, organic and inorganic compounds are associated with these lignocellulosic matrices with different origins and content (Vassilev et al., 2012).

Cellulose and hemicellulose are polymers of 6 and 5 carbon polysaccharides. Cellulose consists of a linear chain of several hundred to over ten thousand β (1→4) linked D-glucose units. It makes up to 40% of the secondary cell walls of plant residues. Hemicellulose

polysaccharides are composed of 1, 4-linked β-D-hexosyl residues and may contain pentoses, hexoses, and uronic acids. Lignin is an aromatic polymer that binds the plant cell walls together (Zhao et al., 2012).

The chemical composition of the biomass differs by species, location, harvest, and storage time. Also, each residue has specific moisture and ash contents. The ratio between the components differs for each plant residue. Wood and straw mainly consist of lignocellulose. Green parts, such as foliage and young grass, have a relatively high protein content and are low in lignin (Van Groenestijn et al., 2019).

As mentioned, knowledge of the composition is essential for biobased production and bioenergy conversion processes (Vassilev et al., 2010). The relative ratio of the components for economically important agricultural residues is given in **Error! Not a valid bookmark self-reference.**.

*Table 1.1. Main chemical composition of grass biomass feedstock (adapted from Zhao et al., 2012) (*Rice husk data from Jung et al., 2021; Wu et al., 2018)*

Feedstock	Cellulose (%)	Xylan (%)	Galactan (%)	Araban (%)	Lignin (%)	Mannan (%)	Extractives (%)	Ash (%)
Grass biomass	25-50	20-50	0.5-1.0	1.8-3.0	10-30	0.2-0.6	4-25	2-17
Rice Straw	34.2	24.5	NA	NA	11.9	NA	17.9	16.1
Rice husk*	35.6	19.7	0.8	1.7	23.4	0.4	4.2	15.7
Corn cob	36.4	18.0	1.0	3.0	16.6	0.6	7.3	9.7
Corn stover	40.9	21.5	1.0	1.8	16.7	NA	NA	6.3
Sugarcane bagasse	40.2	21.1	0.5	1.9	25.2	0.3	4.4	4.0
Wheat straw	38.2	21.2	0.7	2.5	23.4	0.3	13.0	10.3
Switchgrass	31.0	20.4	0.9	2.8	17.6	0.3	17.0	5.8

Globally, crop residues such as corn stover or rice straw are produced in great quantities (Table 1.2). Because these resources are byproducts of crop production, their collection and utilization can be coupled with sustainable practices of biomass production (Mohammed et al., 2018).

Table 1.2. Yields (ton/year) of some agricultural byproducts

Feedstock	Production (million tons / year)	Source
Grass biomass		
Rice Straw*	945	Logeswaran et al., 2020
Rice husk	120	Rao et al., 2017
Corn stover**	1148	Murphy and Kendall, 2013; FAOSTATS 2021
Sugarcane bagasse	493	Khattab and Watanabe, 2019

* 1.35 kg of rice straw for each kg of rice produced

** 1 kg of corn stover for each kg of corn produced on dry mass basis

1.2.2. Biomass conversion to bioenergy

Biomass can be used for different purposes (Akhtar et al., 2018). Different processes convert the energy present in biomass to useful heat energy (Gunawardena and Fernando, 2013). Figure 1-1 summarize the methods.
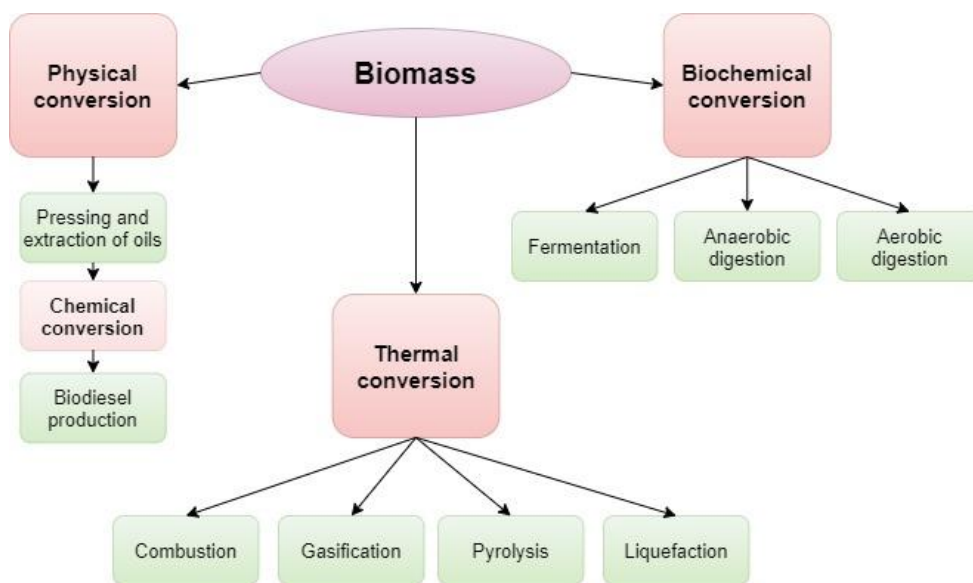

Figure 1-1. Different categories of biomass conversion to energy. (Adapted from Gunawardena and Fernando, 2013).

Physical conversion involves pressing the plants to produce triglyceride oils. Triglycerides cannot be used directly as fuels and need to be processed further. Thus, to produce biodiesel, the triglycerides are transformed through transesterification in the presence of alcohol into fatty acid alkyl (Gunawardena and Fernando, 2013).

Biochemical conversion involves using microorganisms or enzymes to break down biomass into simpler sugars (Gunawardena and Fernando, 2013). The fuel ethanol industry using enzymes is the most mature biofuel industry, with a global production of 110 billion liters in

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2018 (Koehler et al., 2019). Biogas is produced by the anaerobic biological breakdown of organic matter (Sindhu et al., 2019). Biogas production has been largely studied, and although the manufacturing technologies need to be improved due to low conversion yields, the industry is growing (Gontard et al., 2018).

The **thermochemical conversion** activates chemical transformations using heat. Biomass combustion in existing coal-fired boilers is arguably carbon dioxide (CO_2) neutral (Marangwanda et al., 2020) if the biomass comes from sustainable sources. In countries where biomass is available in large quantities, combustion for heat and electricity production and other conversion routes is increasing. Globally, plants for biomass combustion are growing to reach emission reduction targets and end fossil fuel dependency (Cruz et al., 2019).

It is estimated that the demand for bioenergy in Europe is 10.0 EJ/year, and it is increasing (Bentsen and Felby, 2012). Bioenergy is the main renewable energy source in Europe, with around 60% of the share; about 75% of all bioenergy is used in the heating and cooling sector; most biomass demand is met domestically. (Scarlat et al., 2019).

Other thermochemical conversion techniques can be categorized into three main processes: gasification, pyrolysis, and liquefaction (Gunawardena and Fernando, 2013).

Gasification is a type of thermal decomposition where the biomass is heated in an environment poor in oxygen (Balat, 2009). The biomass is converted into a mixture of gases called synthesis gas (or syngas), composed of hydrogen and carbon monoxide. Synthesis gas can be converted into liquid fuels (using Fisher-Tropshc synthesis), hydrogen, and chemicals or directly used in internal combustion engines (Balat, 2009; Gunawardena and Fernando, 2013).

Pyrolysis and **liquefaction** are related routes to produce liquids – called bio-oil or bio-crude. Pyrolysis, unlike gasification, takes place in an oxygen-free atmosphere. The temperature at which the thermal decomposition of biomass takes place is around 450–550°C. The bio-oil portion is initially in the vapor phase and obtained by quenching the volatile output. The yields of the condensable fraction are reported to be as high as 70–80% (Gunawardena and Fernando, 2013).

Biomass pyrolysis has become a renewable and sustainable fuel source and petrochemical substitute (Fahmy et al., 2018). The pyrolysis process can be divided into three subclasses: slow, fast, and flash pyrolysis, depending on the temperature and residence time of the feedstock (Hu and Gholizadeh, 2019).

1.3 Rice husk as a bioenergy source

Rice is considered the most important staple food. Approximately half of the world´s population depends on rice as a source of food, calories, and protein (Chen et al., 2020). It is produced in approximately 100 countries around the world. The biggest producers are China, India, and Indonesia, with 90% global production and consumption (Maraseni et al., 2018). The total annual production is around 750 million tons (**Error! Reference source not found.**) (FAOSTATS, 2021), and it is expected that production increases due to technological developments (Logeswaran et al., 2020).

Approximately 20-22% of the weight of rice paddy is collected as husk (Zerbino et al., 2011; Nagrale et al., 2012), while 50% of the gross weight of the plant is straw (Logeswaran et al., 2020). Around 120 million tons are harvested as rice husk (Rao et al., 2017), and around 1012 million tons as straw (Logeswaran et al., 2020).
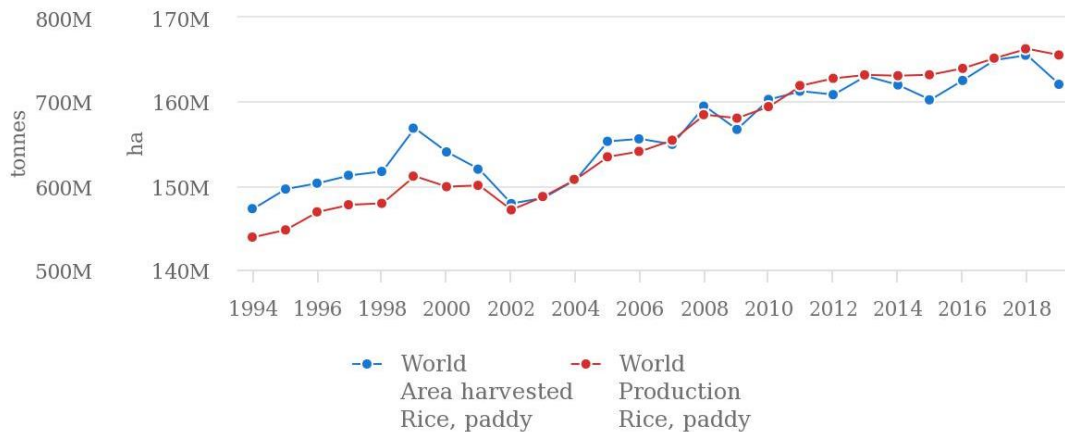

Figure 1-2 Global Production/Yield quantities of rice, paddy from 1994 to 2019 (FAOSTAT, May 2021)

Rice husk is a lignocellulose material that contains around 28-35% of cellulose, 12-29% of hemicellulose, and 15-20% of lignin (Zamani et al., 2019), and approximately 15-20% silica (SiO_2 in amorphous form) and other minerals (Phonphuak and Chindaprasirt, 2015; Ndazi et al., 2008). The rice husk ashes contain more than 90% of silica, which can be used in different industries. The physical properties of the rice husk are shown in Table 1.3

Table 1.3 Physical properties of a rice husk sample (Adapted from Gutiérrez et al., 2020; Jung et al., 2021)

Characteristics	Values
Ash content (%)	15.17
Bulk density (g/mL)	0.73
Moisture content (%)	6.62
Particle size (mesh)	200-16
Solid density (g/mL)	1.5
Surface area (m2/g)	272.5
Surface acidity (meq/gm)	0.1

1.4 Uses of rice husk ashes

As mentioned, the main component of the rice husk ashes is silica. Rice husk ashes can be used as cement and concrete additives and silica gel production, among other industries where silica is the important component (Pode, 2016). Due to its characteristics, rice husk ashes can be used as an additive in different construction materials as insulation panels or refractory bricks, but the properties can vary depending on the firing temperature and time (Phonphuak and Chindaprasirt, 2008). Zerbino et al., 2011, tested the addition of rice husk ashes in cement-based materials, showing a replacement up to 25% of the cement weight for ground ashes. Also, the rice husk ashes are considered a natural pozzolan, and in combination with lime, acquire cementation properties (Pode, 2016).

In certain incineration processes under controlled conditions, it is possible to obtain almost pure silica. However, the ashes also contain some metallic impurities such as iron (Fe), calcium (Ca), sodium (Na), among others, which may affect the purity of the silica and even the color (Bakar et al., 2016). However, the silica obtained is more cost-effective than silica produced from quartz (Todkar et al. 2016). It is important to note that 80% of the residue is burned to use the silica in these processes. Kalapathy et al., 2000, showed that it is possible to obtain pure silica xerogels from rice husk ashes with a 93% silica content, applying low energy chemical method.

Rice husk residues far exceed the demand for silica residues, and the extraction implies technological development that is not always economically competitive (Pode, 2016).

For bioenergy production, rice husk is used as fuel in boilers or power generators for the rice mills. There is potential to use rice husk lignocellulose in more advanced biofuels such as second-generation ethanol, as it can be obtained in massive amounts at one location from the

rice hulling industry with little or no value (Zamani et al., 2019), and most of the time, it is left in the fields or burnt in the open air, causing pollution issues (Prasara and Grant, 2011).

Finding technologies to extract rice husk lignocellulosic residues in techno-economically viable processes is an important challenge for the biobased economy. Efforts have been directed to extract ethanol from the residue (Madu and Agboola, 2018). However, the saccharifiability is lower in comparison with rice straw, it produces higher levels of fermentation inhibitors, and it yields much less ethanol. Factors affecting the recalcitrance included higher lignin content and the larger quantities of enzyme inhibitors (Wu et al., 2018). As discussed by Wu et al. (2018), silica content can also affect saccharifiability.

Several studies have shown that it is possible to extract the lignin and other structural barriers to enzymes using harsh chemicals (Wood et al., 2016). For example, by pretreatment of the rice with hydrochloric acid (Ang et al., 2013) or alkaline peroxide (Wu et al., 2018). Lignin can be removed with an organosolv pretreatment before removing silica, but the process is not cost-effective (Wu et al., 2018). It is possible that harsh chemicals also affect the silica chemistry in the residues.

1.5   Aim of study

This exploratory project aimed to gather scientific evidence to the hypothesis that density fractionation can be used to separate rice husk residues into low and high silica content fractions If possible, fractions of low silica content can be used as renewable carbon molecules, for example, for bioethanol production, while fractions of higher silica content can be used for silica extraction with higher economic incentives as the currently used techniques. Another goal was to develop chemometric approachs to differentiate the fractions based on ATR-FTIR spectroscopy.

Two different experiments were set up, as follows:

1) **Preliminary trials:** Using a sample of rice husk from Uruguay, a methodology for density fractionation was developed. Practical aspects of the experiments were investigated, such as container shape (beakers vs. centrifuge tubes vs. separatory funnels), decantation method (centrifugation vs. no centrifugation), and sample size. For practical reasons, a method using 50 ml Falcon centrifugation tubes, 40 mL of solution, and 2 g of homogeneous material was selected. Then, the variables grinding time and water temperature were evaluated using total ash content and ATR-FTIR spectra patterns as responses.

2) **Density variation**: Using a sample of rice husk from Colombia, the hypothesis that density-fractionation using a saline solution results in different fractionation than density-fractionation using demineralized water was tested.

2 Materials and methods

2.1 Rice husk

The rice husk from Uruguay was a stored sample donated to the Bioresource Chemistry and Technology Laboratory of the University of Copenhagen in 2018. The sample was stored in vacuum-sealed plastic bags. Another sample was imported from the Guaviare region of Colombia. Both samples were previously dried and had 95% dry matter content measured with a Sartorius MA 30 moisture analyzer at 105 °C in triplicates. As mentioned, experiment 1 was performed with the Uruguay material, and experiment 2 with the Colombian sample.

2.2 Particle size distribution

In experiment 1, the grinding time was optimized. The milling process was performed in a mixing mill Retsch mm 400 with a frequency of 30 rpm. Grinding times tested were 2-, 4-, 6- and 8-minutes. The husk was introduced in 2 grinding jars of 25 ml with 3 iron balls of 1 cm in diameter. Approximately 2.5 g of rice husk was added to each jar. The experiment was repeated 3 times for each grinding time. After the grinding, the batches were sieved separately, using the sieve shaker Octagon 2000 at a setting amplitude-6 for 5 minutes. Different sieve sizes were chosen (200, 125, 100, and 63 µm), and the weight of each sieved fraction was measured using a precision balance Sartorius Quintix 224-1s.

2.3 Density fractionation

Two grams of ground husk and 40 ml of testing solution were added to 50-mL Falcon tubes.

For experiment 1, the testing solutions were (1) demineralized water (dm-water) at room temperature (density: 0.997 g/mL at 23.7°C, measured in triplicate with a Handheld Density Meter, Densito 30PX, Mettler Toledo), and (2) dm-water at 50°C. Two repetitions were done for each grinding time (2, 4, 6, 8 minutes).

The tubes were placed in a shaker IKA KS 260 Basic at 300 rpm/min for 20 minutes at room temperature to release surface-bound minerals (note that the shaking method did not allow temperature control). Afterward, the samples were centrifuged in a Sigma 3-18 KS (4000 rpm,

2 min, 20° C). The supernatant was discarded, and the remaining samples were dried at 40°
C for 48 h.

For experiment 2, the testing solutions were: a saline solution (80 g of lab-grade NaCl, Sigma-Aldrich) were added to 500 ml of dm-water, reaching a density of 1.107 gr/cm^3 at 21.7° C) and dm-water. Here, five repetitions were done for each treatment: fractions 2-4 minutes combined, and 6-8 minutes combined with and without salt. The 20 sample tubes were rotated at speed 5 in a Labnet Problot hybridization oven overnight at 50°C. Afterward, the samples were subjected to the centrifugation process as in experiment 1, but the speed was reduced from 4000 to 2000 rpm for gentle precipitation. In addition, the drying was increased from 48 h to 72 h since the samples were not dried in 48 h.

2.4   Fourier-transform infrared spectroscopy (FTIR)

The dried samples were manually divided into an upper and lower section. Around 1 g of each fraction was obtained. ATR-FTIR spectra were recorded using a Thermo Fischer Scientific Nicolet 6700 FTIR spectrometer (Thermo Fischer Scientific, Waltham, MA, USA) equipped with a Goldengate ATR accessory (Specac Ltd, Orpington, Kent, UK). Spectra from 4000 to 600 cm^{-1} were obtained with a 4 cm^{-1} resolution, 200 background scans, and 100 scans for each sample spectrum. The average of the three spectra was calculated for each sample.

The ATR-FTIR spectra were analyzed in R (R core team, 2021, Version 4.0.5). The package *hyperspec* was used to perform baseline correction via the rubberband method (Hovde 2010, Beleites 2020). The package *prospectr* was used for spectra derivatization and smoothing (Wentzell and Brown, 2000), via *savitzkyGolay* function (Savitzky and Golay, 1964), the package *factoextra* to perform hierarchical clustering analysis, and the packages *base* and *ropls* to perform principal component analysis.

2.5   Ash quantification

To quantify ash content, the organic material was incinerated in a Heraeus muffle furnace at 550±25° C for 4 h. This process was performed using numbered crucibles with around 1 gr of the samples each. The crucibles were put in a desiccator to cool the samples previous to weighting. The crucibles were weighed before and after incineration.

3   Results and discussion

3.1   Experiment 1 - Preliminary trials

3.1.1   Particle size distribution

The particle size distribution for the different grinding times was determined (Table 3.1, Figure 3-1). As expected, the longer the grinding time, the smaller the resulting particles. 2 and 4 minutes of grinding accumulated the highest quantity of coarse particles (72% and 53%, respectively, of the husk particles, were between half of 100-125 µm, 125-200 µm and >200 µm – the coarser particles). For 6 and 8 minutes, the percentage was exactly 43% for both fractions.

It is striking that after 6 minutes the two finest fractions (100-63 and <63) decrease their percentage. Which suggesting a clumping effect after 6 minutes, perhaps due to a thermal or electrostatic effect since powders tend to become electrically charged.

Table 3.1. Particle distribution in percentages according to the grinding times

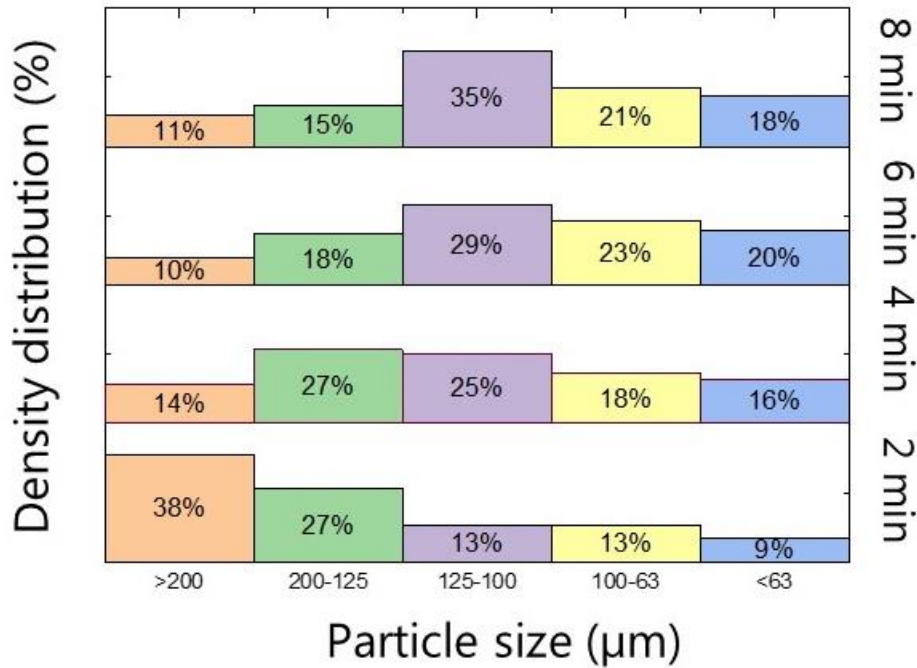| Size (µm)	Particle distribution (%)			
	2 min	4 min	6 min	8 min
>200	38%	14%	10%	11%
200-125	27%	27%	18%	15%
125-100	13%	25%	29%	35%
100-63	13%	18%	23%	21%
<63	9%	16%	20%	18%

Figure 3-1 Particle distribution according to the different grinding time.

3.1.2 Ash quantification

The ash content was determined by incinerating the organic material at 550°C. From literature, it is known that the quantity of silica in rice husk is around 20%, and almost around 90% of the ashes are silica. However, there is great variation between samples. To assess the ash content of the Uruguayan rice husk, eight samples without any treatment were incinerated, giving an average quantity of 17.9%, and a standard deviation of 0.49, which suggest that the method is precise. Results are shown in **Error! Reference source not found.**.

Table 3.2 Ash quantification for a not-treated, Uruguayan rice husk sample

Sample	Initial biomass (gr)	Incinerated biomass (gr)	Ashes (%)
1	1.0331	0.1924	18.62
2	1.0730	0.1879	17.51
3	1.0485	0.1907	18.19
4	1.0170	0.1792	17.62
5	1.0368	0.1926	18.58
6	1.1052	0.1955	17.69
7	1.1249	0.1982	17.62
8	1.0482	0.1822	17.38
		Average	17.90
		Standard deviation	0.49

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The ash content of the samples fractionated with water at room temperature and water at 50°C is presented in Table 3.3. Due to an experimental error, there is no data for the upper fraction at room temperature of 2- and 4-minutes grinding time.

Table 3.3 Ash content for two different experimental set-ups; density fractionation with water at room temperature and water at 50°C. Each result represents the average of two samples

	Samples	Upper Fraction		Lower fraction	
		Sample name	Ash (%)	Sample name	Ash (%)
Room Temperature	2 min A	@Room T-Uruguay 2 min 1	NA	Room T-Uruguay 2 min 1	14.6
	2 min B	@Room T-Uruguay 2 min 2		Room T-Uruguay 2 min 2	
	4 min A	@Room T-Uruguay 4 min 1	NA	Room T-Uruguay 4 min 1	14.35
	4 min B	@Room T-Uruguay 4 min 2		Room T-Uruguay 4 min 2	
	6 min A	@Room T-Uruguay 6 min 1	15.30	Room T-Uruguay 6 min 1	15.61
	6 min B	@Room T-Uruguay 6 min 2		Room T-Uruguay 6 min 2	
	8 min A	@Room T-Uruguay 8 min 1	14.71	Room T-Uruguay 8 min 1	12.72
	8 min B	@Room T-Uruguay 8 min 2		Room T-Uruguay 8 min 2	
50 C	2 min A	@50C-Uruguay 2 min 1	11.99	50C-Uruguay 2 min 1	15.36
	2 min B	@50C-Uruguay 2 min 2		50C-Uruguay 2 min 2	
	4 min A	@50C-Uruguay 4 min 1	11.98	50C-Uruguay 4 min 1	13.82
	4 min B	@50C-Uruguay 4 min 2		50C-Uruguay 4 min 2	
	6 min A	@50C-Uruguay 6 min 1	11.46	50C-Uruguay 6 min 1	11.11
	6 min B	@50C-Uruguay 6 min 2		50C-Uruguay 6 min 2	
	8 min A	@50C-Uruguay 8 min 1	10.00	50C-Uruguay 8 min 1	12.76
	8 min B	@50C-Uruguay 8 min 2		50C-Uruguay 8 min 2	

The results show that all the samples have a lower ash content than the original material, i.e., less than 17.9%. However, there are no apparent differences or any trend in ash content between the upper and lower fractions, suggesting that the decantation method do not separate fractions for their ash content, but the lower ash content was due to water wash.

3.1.3 Exploratory data analysis

Many qualitative and quantitative analysis methods have been developed to quantify silica in biomass (Nakadi et al., 2017). Unfortunately, the most precise characterization methods, such as ICP, are costly, require manual work and time, and use considerable amounts of organic solvents. It prevents the analysis of high volumes of samples.

Spectroscopic techniques, such as ATR-FTIR, can be used to investigate the chemical structure of the biomass samples. These techniques allow the recognition of the presence or absence of silica in the chemical structure of organic compounds and the relative quantification. Statistical and mathematical methods need to be used to maximize the extraction of information from ATR-FTIR spectra: a discipline known as chemometrics (Gredilla et al., 2016).

Three ATR-FTIR spectra of each sample were saved in CVS files and imported into R (V. 4.05) to analyze them with chemometric methods. First, the mean of each triplicate was calculated, and the baseline of each mean spectrum was estimated. Next, the spectra were analyzed from 1700 to 400 cm^{-1} where absorption bands for silicon and lignin are located (Figure 3-2).

The greater variation in the spectra lies in the absorption range for silicon bands (in green in Figure 3-2). These bands correspond to bending and stretching modes of the siloxane bonds Si–O–Si around 455, 799, and 1045 cm^{-1} (Zemnukhova et al., 2015). The lack of absorption bands around 958 cm^{-1}, characteristic of silanol Si-OH bonds (Zemnukhova et al., 2014), indicates low water content.

Figure 3-2 show in blue lignin absorption bands from 1700 to 700 cm^{-1} (Heitner et al., 2010), including:

- Around 1645 cm^{-1} for ring conjugated C=C stretch of coniferaldehyde or sinapaldehyde
- Close to 1515 cm^{-1} for the asymmetric stretching modes of guaiacyl and syringyl aryl rings
- Aromatic skeletal vibration combined with C-H in plane deformation at 1455 cm^{-1} for guaiacyl groups and 1415 cm^{-1} for syringyl groups.
- Around 1370 cm^{-1} for O-CH$_3$ C-H symmetric deformation in both guaiacyl and syringyl groups Around 1330 cm^{-1} for aryl ring breathing with C-O stretch (Collier et al., 1997).
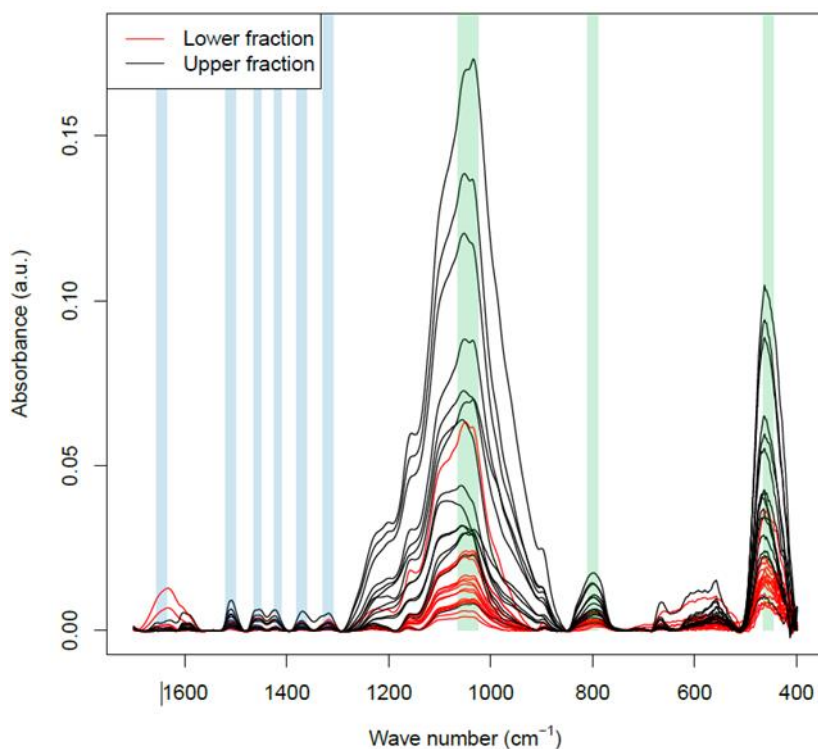
18

Figure 3-2 FTIR spectra for the rice husk treated in experiment 1 in the spectral range of 1700 to 400 cm^{-1}. Absorption bands for silicon are marked in green and absorption bands for lignin in blue.

The spectra in red, which correspond to the lower fractions, tended to have less intensity than the upper fractions. The intensity of absorption bands is related to the concentrations of the absorbing chemical species, through the Beer-Lambert law (Udvardi et al, 2014). Thus, the spectra suggest that the upper fractions have higher silica content than the lower fractions. However, other mathematical tools are needed to subtract instrumental noise.

Variation due to instrumental noise was subtracted from the spectra using baseline correction via the Savitzky-Golay filter function (Wentzell and Brown, 2000). The data are presented in Figure 3-3. This correction is necessary to statistically differentiate the data using Principal Component Analysis (PCA) and hierarchical cluster analysis (HCA).
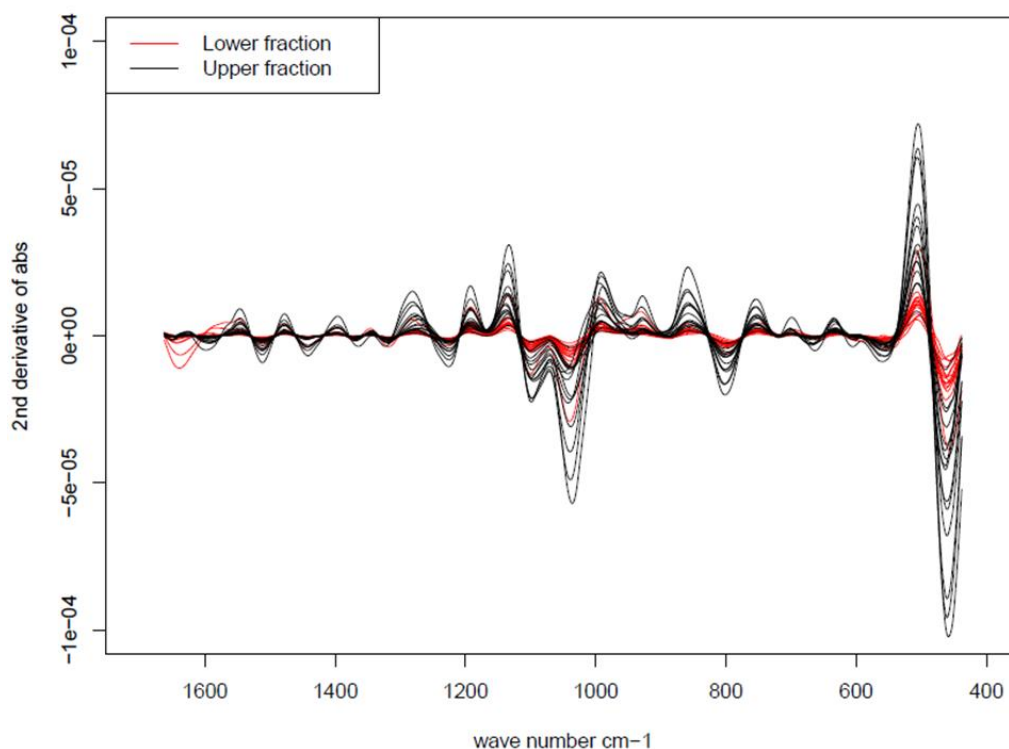
Figure 3-3 Derivated FTIR spectra for the rice husk treated in experiment 1. Red spectra corresponds to lower fraction and black spectra to the upper fraction.

The PCA was used to differentiate the two fractions (Figure 3-4). Each density fraction occupied a different region of the space. 95% confidence ellipses suggest that the chemical species (most probably due to silica content) are different for upper and lower fractions, and the differences were statistically significant and were not instrumental noise.
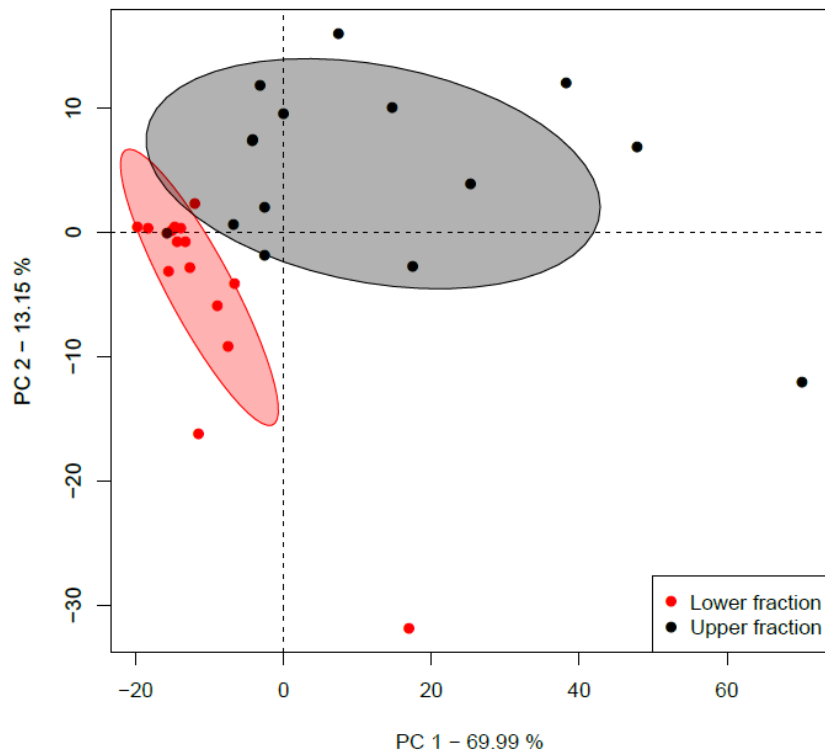
Figure 3-4 Score plot from PCA model calculated on the derivated FT-IR spectra. 1.

3.1.4 Hierarchical clustering analysis (HCA)

Cluster analysis identifies groups within the samples when no information about group membership is available. In other words, cluster analysis tries to find groups containing samples with the most similar ATR-FTIR spectra (Everitt, 1974; Massart and Kaufmann, 1983; Kaufmann and Rousseeuw, 1990; Ripley, 1996; Gordon, 1999)

In hierarchical methods, samples are arranged in a hierarchy of similarity, represented in a tree-like structure or a dendrogram. It allows to determine the optimal number of clusters based on a measure of distance as an inverse of similarity between samples and to see hierarchical relationships between different clusters of samples. (Varmuza and Filzmoser, 2008)

K-means is an algorithm used for partitioning samples into groups of objects in a multidimensional space (Hartigan, 1975). It uses pairwise distances between the objects and requires the input of previously known k clusters. Drawbacks of the random initializations can be overcome by using the hierarchical clustering groups as initial clusters.

Hierarchical clustering analysis of the ATR-FTIR spectra was performed using the hkmeans function available in the factoextra R package.
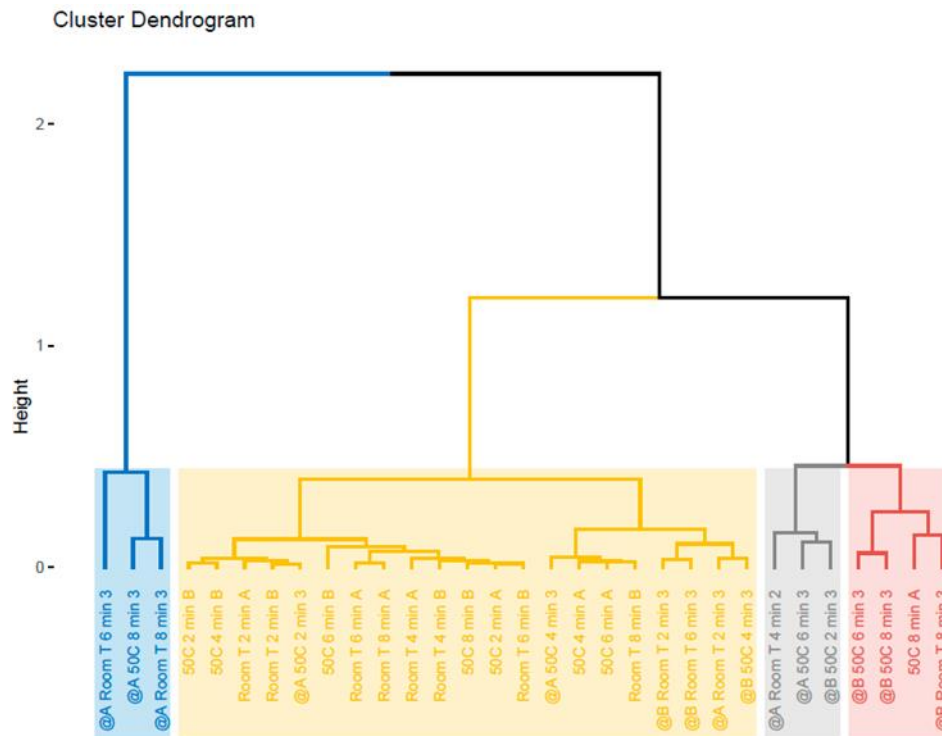
Figure 3-5 Hierarchical relationships between different clusters of rice husk treated with density fractionation. Samples with @represent the upper fraction. Room T stands for room temperature.

The dendrogram in Figure 3-8 suggests that samples from the upper fraction have more similarity between themselves than with the lower fraction samples, as three clear cluster of upper-fractions are formed in the figure in blue, grey, and red. The yellow cluster also distinguishes the upper fraction from the lower fraction. The dendrogram indicates that it is possible to differentiate between these two groups of different density fractions by simply measuring distances in the multidimensional spectral space.

Conclusions for Experiment 1:

- There were no differences in grinding time between 2-4 and 6-8 minutes. After 6 minutes, the grinding effect disappeared.

- There was a reduction in the ash content of all fractions from 17.% to below 15%. However, there was no difference in ash content between upper and lower fractions, which indicate that the reduction could be due to surface-bond minerals removed during the water wash. For the next experiment, the ash content of the controls has to be determined after washing.

- Density fractionation holds the potential to differentiate low and high silica fractions as seen in the PCA analysis and hierarchical analysis; however, different grinding times did not influence the separation.

- The ash content did not correlate with the findings in the FTIR-chemometric analysis. This suggests that the difference in silica content is a very small difference or that the

experimental error during sample preparation or in the ash content determination was high. Since the ash content determination method was proven to be accurate, the error could have been introduced during sample preparation. Probably, the short time that the samples were kept in water before the analysis prevented the complete dissolution of other minerals with low solubility.

3.2 Experiment 2 - Density variation

The goal of this experiment was to compare the effect of liquid density on the protocol developed in Experiment 1. Two densities were compared: dm-water (density: 0.997 g/mL at 23.7°C) and a saline solution (density of 1.107 gr/cm^3 at 21.7°C).

From lessons learned in experiment 1, the grinding fractions 2+4 min and 6+8 min were combined, and the number of repetitions per treatment increased to five. The rice husk samples were kept in the water solutions for a longer time (overnight instead of 2 hours), and the temperature was held at 50°C to ensure an efficient water wash.

Demineralized water

After washing the Colombian rice husk to account for better control than in Experiment 1, the ash determination was performed in triplicate, and the result was 12.28% (s.d. = 0.2).

The ash content of the fractions after decantation with dm-water at 50°C can be seen in Table 3.4.

Table 3.4 Ash content of rice husk samples. The samples were ground for different times: A. a combination of samples ground for 2 and 4 minutes. B. a combination of samples ground for 6 and 8 minutes.

	Samples	Upper Fraction		Lower fraction	
		Sample name	Ash (%)	Sample name	Ash (%)
	1	@50C-Colombia 2-4 min 1	12.63	50C-Colombia 2-4 min 1	10.96
	2	@50C-Colombia 2-4 min 2	12.36	50C-Colombia 2-4 min 2	11.11
	3	@50C-Colombia 2-4 min 3	13.87	50C-Colombia 2-4 min 3	10.86
A (2 + 4 min)	4	@50C-Colombia 2-4 min 4	13.62	50C-Colombia 2-4 min 4	10.48
	5	@50C-Colombia 2-4 min 5	13.79	50C-Colombia 2-4 min 5	10.66
		Average	13.25	Average	10.81
		Standard deviation	0.70	Standar deviation	0.25
	1	@50C-Colombia 6-8 min 1	14.01	50C-Colombia 6-8 min 1	10.38
	2	@50C-Colombia 6-8 min 2	13.81	50C-Colombia 6-8 min 2	9.83
B (6 + 8 min)	5	@50C-Colombia 6-8 min 3	13.33	50C-Colombia 6-8 min 3	9.79
	4	@50C-Colombia 6-8 min 4	14.09	50C-Colombia 6-8 min 4	10.67
	5	@50C-Colombia 6-8 min 5	12.65	50C-Colombia 6-8 min 5	10.38
		Average	13.58	Average	10.21
		Standard deviation	0.60	Standar deviation	0.38

The results in Table 3.4 suggest that a small proportion of ashes was accumulated in the upper fraction. The control sample had an ash content of 12.3%, and all the upper fractions have ash contents between 12.6% and 14.9%, while the lower fraction between 9.8% and 11.1%.

Although the differences are small, they are statistically significant, as evidenced in the t-test (α=0.05), performed in R with the following statistics:

2+4 min: t = 3.55; df = 7.77; **p-value = 0.007796**. 95% confidence interval: 0.712 - 3.380.

6+8 min: t = 10.61; df = 6.82; **p-value = 1.723e-05**. 95% confidence interval: 2.613 -4.122.

The t-test results showed that the ash content in the lower fractions is significantly different from the ash content in the upper fraction. The 95% confidence intervals (C.I) for the difference of means suggest that the 6-8 min differences (C.I.: 2.6 to 4.1) are higher than for the 2+4 min (C.I. 0.7 to 3.4). However, this difference was not statistically significant in a t-test (α=0.05). (statistics: t = 2.06; df = 6.12; **p-value = 0.08351**; 95% C.I.: -0.1969749 2.3969749).

Saline solution

The last trial corresponds to the samples decanted with the saline solution. The results showed a much higher ash content than the sample before due to the presence of salts. (**Error! Reference source not found.**)

Table 3.5 Ash content for the decantation process using the saline solution at 50° C.

	Samples	Upper Fraction		Lower fraction	
		Sample name	Ash (%)	Sample name	Ash (%)
A (2 + 4 min)	1	@50C-Colombia 2-4 min SS 1	46.87	50C-Colombia 2-4 min SS 1	25.87
	2	@50C-Colombia 2-4 min SS 2	50.66	50C-Colombia 2-4 min SS 2	26.44
	3	@50C-Colombia 2-4 min SS 3	46.47	50C-Colombia 2-4 min SS 3	25.56
	4	@50C-Colombia 2-4 min SS 4	50.22	50C-Colombia 2-4 min SS 4	26.41
	5	@50C-Colombia 2-4 min SS 5	47.58	50C-Colombia 2-4 min SS 5	25.78
		Average	48.36	Average	26.01
		Standard deviation	1.95	Standar deviation	0.39
B (6 + 8 min)	1	@50C-Colombia 6-8 min SS 1	48.64	50C-Colombia 6-8 min SS 1	26.05
	2	@50C-Colombia 6-8 min SS 2	49.78	50C-Colombia 6-8 min SS 2	24.45
	5	@50C-Colombia 6-8 min SS 3	49.63	50C-Colombia 6-8 min SS 3	28.59
	4	@50C-Colombia 6-8 min SS 4	51.89	50C-Colombia 6-8 min SS 4	28.93
	5	@50C-Colombia 6-8 min SS 5	48.48	50C-Colombia 6-8 min SS 5	26.80
		Average	49.68	Average	26.97
		Standard deviation	1.36	Standar deviation	1.85

The numeric quantification is meaningless as the rice husk residues absorbed the saline solution. It is likely that during drying, the salt accumulated on the surface of the solid; this can be the reason that the upper layer appears with higher ash content than the lower fraction.

Fortunately, in the ATR-FTIR, the ions in the saline solution are transparent, so the analysis continues as in Experiment 1.

3.2.1   Exploratory data analysis

The analysis of ATR-FTIR spectra was performed as the previous experiment. Three spectra of each sample were obtained and saved in CVS files and imported into R (V. 4.05) to further analysis with chemometric methods. The mean of each triplicate and thr baseline of each mean spectrum were determined.

The spectra were analyzed from 1700 to 750 cm^{-1} where absorption bands for silicon and lignin are located, in Figure 3-6. In this case, the spectral range from 400 to 749 cm^{-1} was removed because of the high noise signal. In Figure 8, these bands are in green and blue, respectively. There is a higher variation in the absorption range for lignin bands in these spectra compared with Experiment 1. However, most of the variation is in the absorption of the silica bands.
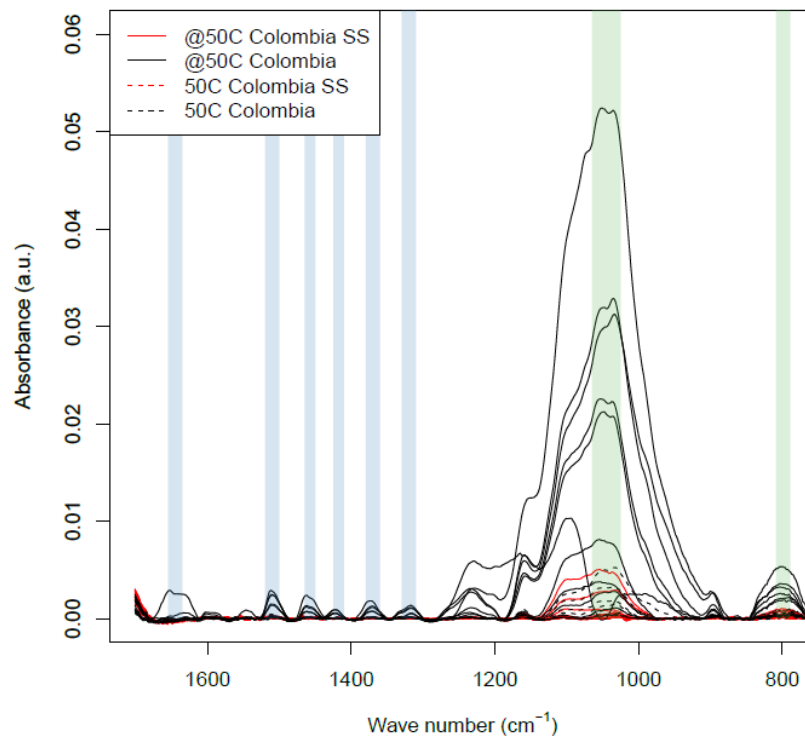

Figure 3-6 FTIR spectra for the rice husk treated in experiment 2 in the spectral range of 1700 to 400 cm^{-1}. SS stands for saline solution; @ represents the spectra of the upper fraction.

As mentioned, the intensity of absorption bands is related to the concentrations of the absorbing chemical species (Udvardi et al, 2014). Thus, all the spectra with saline solution and the spectra of the lower fraction of dm-water appear to had lower silica content than the upper fraction of the dm water. It should be noticed that the dried sodium chloride in the samples weakens the absorption of FTIR spectra, as there is a dissolution effect. Therefore, all samples done in the saline solution experiments appear to be similar to the lower part of the experiments with dm-water.

The spectra were baseline corrected, as in the previous experiment. Figure 3-7 shows the second derivated spectra for the upper and low fractions for the saline and non-saline samples.
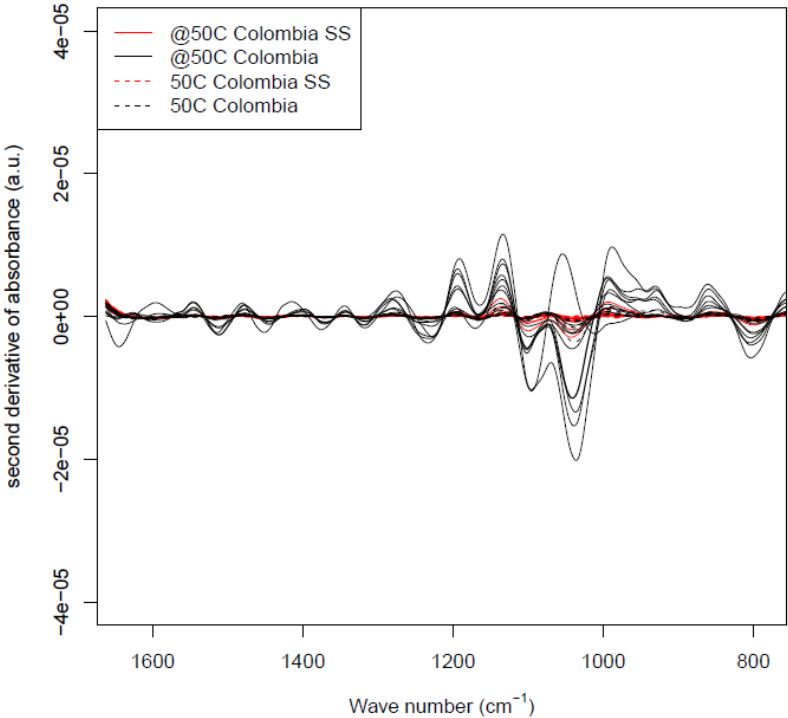

Figure 3-7 FTIR spectra for the rice husk treated in experiment 2 after the experimental noise has been removed.
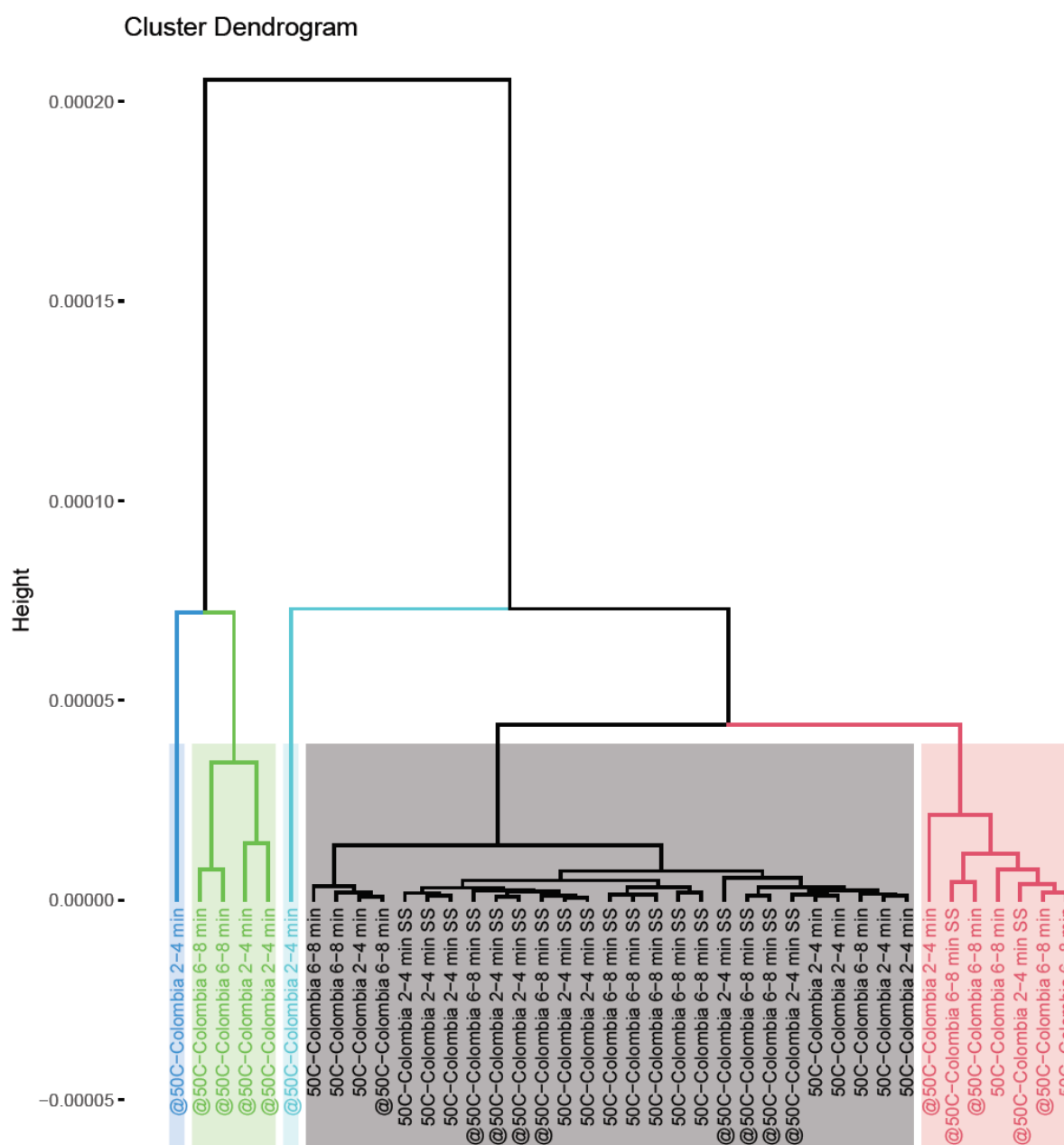
3.2.2 Hierarchical clustering analysis

Figure 3-8 Hierarchical relationships between different clusters of rice husk treated with density fractionation. Samples with @represent the upper fraction. Samples with SS refers to saline solution

Hierarchical clustering analysis (HCA) of the ATR-FTIR derivated spectra of experiment 2 was performed using the hkmeans function as in the experiment 1. In that case, the data is structured in five different clusters (Figure 3-8). It can be seen that saline samples from the upper and lower fraction are almost grouped in the same cluster (grey) together with the lower fraction of non-saline samples. In this experiment, the upper fractions of the non-saline samples are less similar between them.

A different data analysis strategy was applied in this experiment. The HCA results have been used as initial information for the PCA analysis, i.e, HCA was performed before PCA. In the PCA score plot, black color corresponds to the upper and lower fractions from the saline solution and the lower fraction of the non-saline samples. Green, red, blue samples in Figure 3-9 are mostly related to the upper fraction of non-saline samples. There is a difference between the upper fraction of non-saline samples and the rest. This suggests that saline samples, can have reduced the silica content from both upper and lower fractions. However, as the results from the ash determination are inconclusive, further experimentation needs to be done to confirm the effect of the saline solution.
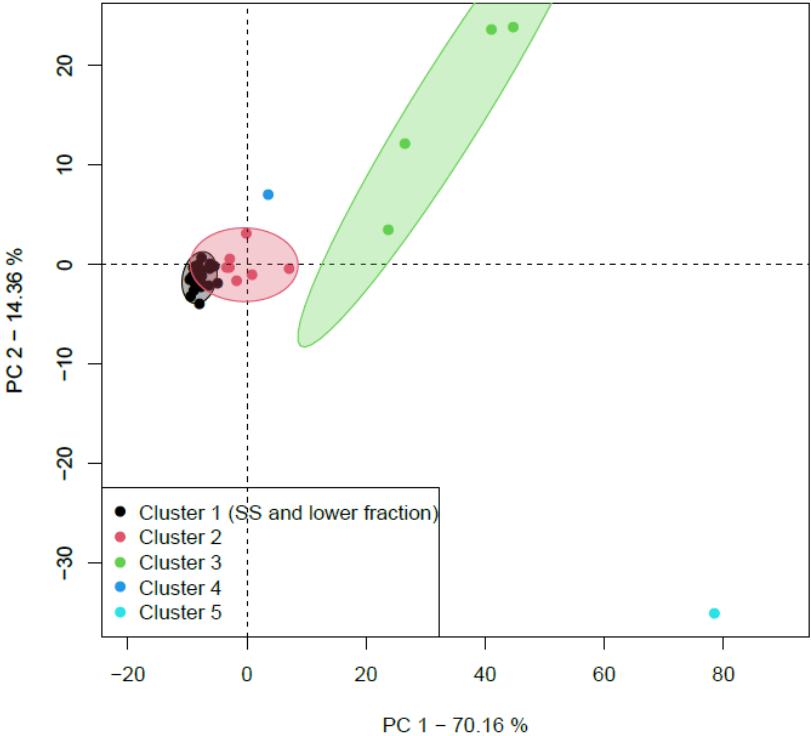

Figure 3-9. Score plot from PCA model calculated on the clusters solved in the HCA analysis .

Conclusions for Experiment 2

- The experiments with DM water showed much better results than those found in Experiment 1, meaning that the method's variations were useful and showed that it is possible to partially concentrate the silica in the upper fraction of the samples.

- In the experiments with saline solution, the evaporation of the saline solution during the drying process caused it to concentrate at the top, where it was in contact with the hot air from the oven. As a result, salt crystals were also observed with the naked eye at the top of the fractions.

- The salt is invisible in the ATR-FTIR, but it has a dilution effect on the rice husk sample, and as a consequence, the bands have lower absorption in the infrared. However, the chemometric tools could still distinguish the upper and lower fractions of samples treated with dm-water.

- Between the first and the second experiments, the time the samples were in contact with the water changed from 2 to 12 hours. This could explain why in the first experiment, almost no difference was seen in the ash determination. Soluble minerals such as sodium or potassium are trapped inside cells and cell walls. Perhaps it takes time for those minerals to dissolve, and a hydration time of 12 hours resulted in better dilution.

4 Conclusions and future work

- Rice husk lignocellulose is an abundant feedstock that has the potential to be utilized sustainably in future biorefineries. However, the high silica content limits this potential.

- In this work, a method to fractionate rice husk powders by density to obtain fractions with low and high silica content was developed. Small but significant differences in ash content were observed and related to a reduction in silica content using a combination of ATR-FTIR and chemometric tools. The main recommendations for future experiments and method optimization are: 1) the implementation of a methodology to wash ions from saline solution, 2) to separate the fractions when they are still wet, and 3) further optimization of hydration time.

- Due to the huge amount of biomass available and its composition qualitative and quantitative analysis methods have been developed to quantify silica in biomass. Unfortunately, the most precise characterization methods are costly, require manual work and time, and use considerable amounts of organic solvents. This prevents the analysis of high volumes of samples in exploratory studies. Spectroscopic techniques, such as ATR-FTIR, offer an alternative to these expensive methods and

can be used to investigate the chemical structure of the biomass samples. These techniques allow the recognition of the presence or absence of silica in the chemical structure of organic compounds and the relative quantification.

- In this work, ATR-FTIR, in combination with the chemometric tools PCA and HCA, was able to identify the small differences in ash content of the samples, and so it can be used to guide future studies. A validation of the results with wet-chemistry, high sensitive silica detection method is recommended.

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