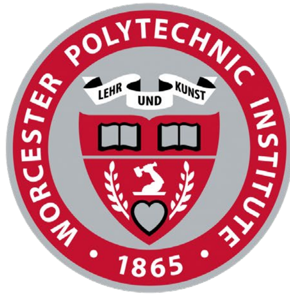

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Valorization of Poultry Feather Protein Residues Using Subcritical Water Hydrolysis

A Major Qualifying Project Report

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Abstract

This experiment analyzed the effect of subcritical water hydrolysis on natural poultry feathers for the isolation of high-value amino acids. Subcritical water hydrolysis is the process by which water in the range of 100 - 374 °C converts and breaks down biomass into micromolecules. Often this process can result in the production of high-value products from poultry feathers. Poultry feathers are approximately 90 percent protein, which suggests that poultry feathers can be used to produce valuable amino acids. A non-toxic, economically viable, and environmentally favorable way to break down these poultry feathers is by subcritical water hydrolysis. In particular, this experiment focused on the effect of temperature on the hydrolysate. The hydrolysis machine was operated at three different temperatures (210, 230, and 250 °C) at a constant flow rate of 10 mL / min. Differences between the hydrolysates were defined by performing Total Nitrogen, Chemical Oxygen Demand, and Nelson-Somogyi method testing. Initial hydrolysate analysis testing suggests that a low operating temperature and an operating time below 10 minutes may assist in getting optimum protein levels. No amino acid testing was conducted. All amino acid related results will be available later.

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Chapter 1: Introduction

The poultry industry is one of the largest industries in Brazil. It currently makes up about 1.5% of the nation's Gross Domestic Product, and employs more than 3.6 million people (ABPA, n.d.). Globally, the poultry industry produces about 1 million tons of poultry feathers (Cheng et al., 2008). Due to the large volume of poultry products, there is a high amount of chicken feathers that are discarded. Poultry feathers have the potential to be used as a cheap alternative to help in producing nutrient-dense animal feed, natural gas, wool, and wastewater treatment (Ramakrishnan et al., 2011). This is due to the extremely high protein content within the feather, which is about 82% crude protein (Tesfaye et al., 2017).

Currently, most biomass is disposed of by two main methods: dumping in landfills or incineration. Both ways lead to major environmental and health problems such as greenhouse gas emissions, high lechates concentration, dioxin release, as well as the release of harmful pathogens. Instead, subcritical water hydrolysis offers a safer method of waste disposal. Subcritical water hydrolysis is a great way to convert organic material into high-value nutrients. This process can otherwise be known as 'valorization,' which describes the recycling of waste such as poultry feathers into high-value products like amino acids. Subcritical hydrolysis is a great way to break down organic matter because of the unique properties water takes on within the subcritical liquid temperature and pressure range of 100 - 374 °C and 1 - 218 atm, respectively (Lachos-Perez et al., 2011). In this range, water can be used as a cleaner medium of chemical reactions.

The objective of this study was focused on the potential yield of amino acids from hydrolyzed poultry feathers because of their many versatile applications. Amino acids are currently being used to aid in agriculture, textile, and pharmaceutical industries. Some of the amino acids in poultry feathers include cysteine, glutamine, proline, and serine, which each have their own unique uses (Saravanan and Dhurai, 2012). The retained amino acids can also be isolated and used as health supplements. As a result of all the benefits associated with amino acids, this experiment set out to find the parameters necessary to produce them.

Properties such as operating temperature, retention time, and operating pressure can play a role in the chemical makeup and amino acid yield from poultry feather hydrolysates. Research suggests that a temperature range of 200 - 290 °C with an operating time between 5 - 30 minutes is better at optimizing amino acid yield (Cheng et al., 2008). This experiment loosely followed that model by running six runs of hydrolysis with natural poultry feathers at 210, 230, and 230 °C at 10 mL/min at 15 MPa. Each run was carried out in duplicate. This experiment focused on one parameter, temperature, in affecting the chemical makeup of hydrolysate. Hydrolysate analysis included Total Nitrogen, Chemical Oxygen Demand (COD), and Nelson-Somogyi testing. Before hydrolysis testing, the natural poultry feathers chemical makeup was characterized by performing Total Solids and Volatile Solids analysis, as well as Total Nitrogen testing.

Chapter 2: Background

2.1. The Poultry Industry in Brazil

The poultry industry is one of the largest industries in Brazil. It currently makes up about 1.5% of the nation's Gross Domestic Product, and employs more than 3.6 million people (ABPA, n.d.). Globally, the poultry industry produces about 1 million tons of poultry feathers (Cheng et al., 2008). After the United States and China, Brazil is the world's third-largest poultry manufacturer, accounting for about a third of the world's poultry. Due to the large volume of poultry products, there is likewise a high amount of chicken feathers that often are unaccounted for. In particular, the removal of chicken feathers is a great source of distress for many chicken farmers. Blood and harmful bacteria usually coat the chicken feathers, which has led to high mortality rates among people who must dispose of the contaminated feathers. The mortality rate for chicken farmers is usually about 0.1% and even reaches 0.25% per day (ABPA, n.d.).

Poultry feathers have the potential to be used as a cheap alternative to help in producing nutrient-dense animal feed, natural gas, wool, and wastewater treatment (Ramakrishnan et al., 2011). This is due to the extremely high protein content within the feather, which is about 82% crude protein (Tesfaye et al., 2017). Poultry feathers are reported to be made up of 35-40 protein residues (Cheng et al., 2008). Feathers are made up of beta-keratin, which is a fibrous protein that is insoluble and chemically inert (Ramakrishnan et al., 2011). In the past, feather degrading bacteria *Bacillus licheniformis*, which produces keratinases, has been used to break down the rigid structure of poultry feathers (Ichida et al., 2001). Once the poultry feathers are broken down then they can be used in many applications. The way in which we have proposed to extract value from poultry feathers is through subcritical water hydrolysis.

2.2. Subcritical Water Hydrolysis Application

Subcritical water hydrolysis is a great way to convert organic material into high-value nutrients. This process can otherwise be known as 'valorization,' which describes the recycling of waste to yield high-value products. The water in subcritical hydrolysis is so unique because of the properties it takes on within the subcritical liquid temperature and pressure range of 100 - 374 ℃ and 1 - 218 atm, respectively (Lachos-Perez et al., 2011). In this range, water behaves like a nonpolar solvent and can thus be used as a cleaner medium of chemical reactions. Water in this state can also be applied in cases of energy and mass transfer due to the fluctuations in density and low dielectric properties. Furthermore, the iconic content of subcritical water is approximately a thousand times higher than that of standard water, which suggests that acidic and alkaline catalysts can be applied to the subcritical fluid.

The current research on subcritical water shows promise in many areas, including the extraction of bio-oils from palm tree fruits, fuel gas from hydrogen and methane, and amino acids from protein-rich biomass (Tu et al., 2016). In this case, we are primarily concerned with

the latter. As aforementioned, poultry feathers have the potential to break down the proteins using subcritical water hydrolysis to produce valuable amino acids.

Currently, most biomass is disposed of by two main methods: dumping in landfills or incineration. Both ways lead to major environmental and health problems such as greenhouse gas emissions, high lechates concentration, dioxin release, as well as the release of harmful pathogens. Instead, subcritical water hydrolysis offers a safer method of waste disposal. At the industrial level, current hydrolysis technology involves chemical or enzymatic hydrolysis. Chemical Hydrolysis requires extreme reaction conductions and produces environmentally harmful by-products, while enzymatic hydrolysis is very expensive and long to carry out (Cheng et al., 2008). One shortcoming that has been cited of subcritical water hydrolysis is the lack of research on how technology changes the nutritional makeup of organic compounds (Tu et al., 2016).

It has been shown that properties like operating temperature, retention time, and operating pressure play a huge role in both the rate of extraction and production of amino acids. One paper that worked with de-oiled peanut meal found the optimal temperature range for increasing amino acid production to be within 140 - 160 °C. The same paper also found that a long retention time of 1.5 - 2 hours also helped in increasing total nitrogen production. However, other research suggests that a higher temperature range of 200 - 290 °C is better at optimizing amino acid yield (Cheng et al., 2008). In this case, researchers were working with poultry feathers in addition to other organic compounds. The same researchers also cited a lower operation time of about 5 - 20 minutes. At a run of 30 minutes an operating temperature of 260 °C yielded optimal amino acid production in the case of chicken feathers. We loosely followed the operating conditions of the second paper by performing hydrolysis at higher temperatures but for shorter periods of time at lower operating pressures. This experiment operated on a semi-continuous hydrolysis machine that can be seen labeled in Figure 1. Shorter reaction times were utilized because of the rapid decomposition of amino acids. Also, high operating pressures can become costly and difficult to operate.

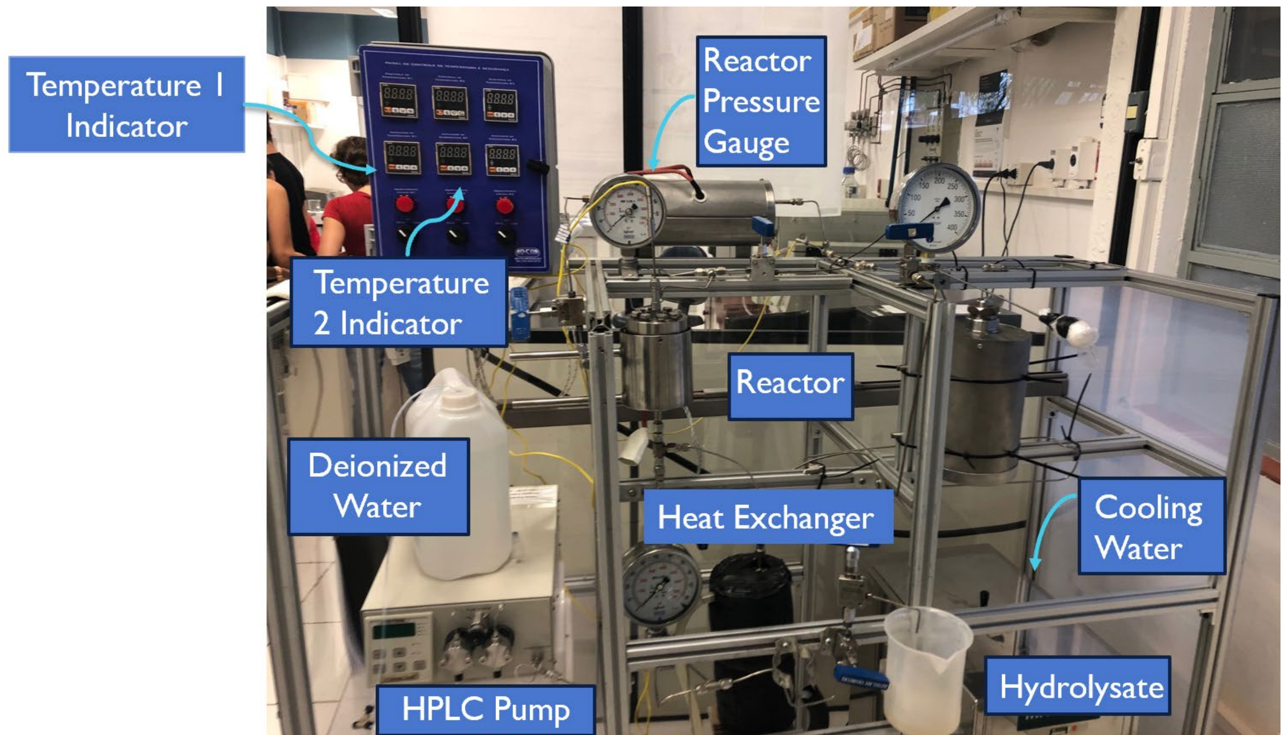

Figure 1. Subcritical water hydrolysis unit used to perform the experiments.

2.3. Extraction of Valuable Amino Acids

As we mentioned earlier, the high keratin protein content of poultry feathers yields a high volume of amino acids that can be used for a variety of applications. An approximate analysis of chicken feathers states that it is 82.36% crude protein, 64.47% carbon, 10.41% nitrogen, 22.34% oxygen, and 2.64% sulphur (Tesfaye et al., 2017). However, the exact percentages of each component vary depending on the type of feather being analyzed. Furthermore, research on the amino acid composition of poultry feathers has found the organic matter particularly high in cysteine, glutamine, proline, and serine (Saravanan and Dhurai, 2012). Poultry feathers are known to be low in nutritionally essential amino acids like methionine, lysine, histidine and tryptophan, which makes it hard for chickens to digest in feed meal (Onifade et al., 1998).

We plan on testing how the temperature of the subcritical hydrolysis technology affects the protein and organic content makeup of the hydrolysate. Research has shown that the temperature, pressure, initial pH, and retention time, among other factors, can affect the amount of amino acids obtained. However, for the purposes of this experiment we only concerned ourselves with temperature.

Amino acids have versatile applications and can be of use in multiple industries including agriculture, textile, and pharmaceuticals. Some of the amino acids in great excess within the chicken feathers can be valuable in aided industry needs. One industry where amino acids can be of value is the meet and farming industry. As of now, 17 trillion kgs of animal feed is used per year at a steep cost of 48 billion dollars (Ritchie and Roser, 2019; Becker, 2008). Amino acids

derived from poultry feathers can help substitute up to 0.42% of current global animal feed. The substitution of amino acids can lead to a savings of 200 million dollars per year, making it a very economically viable option for the farming industry.

Cysteine is responsible for the rigid structure and chemical activity of the high amount of keratin in the poultry feathers (Ramakrishnan et al., 2011). Disulphide bonds are connected between two cysteine molecules and account for the strength and resistance against proteolytic enzymes. Cysteine, in its supplement form, is referred to as N-acetyl cysteine (NAC), which is used by the body to produce antioxidants and vitamins that can help repair cell death (N-Acetyl Cysteine, n.d.). Cysteine supplements retail for about $10.50 (N-Acetyl-L-Cysteine, n.d.). NAC has shown success in treating acetaminophen overdose, bronchitis, liver failure, and neurological damage.

Another amino acid in abundance is glutamine. Glutamine is essential for providing fuel to the body and in supporting gut and immune health. Research has shown promise with taking glutamine to treat sickle cell disease, surgery recovery, bone marrow transplants, HIV/AIDS, and cancer (GLUTAMINE, n.d.). Glutamine is the most abundant amino acid in the human body and as a byproduct of this it has a lot of potential applications. The supplement form of glutamine is referred to as L-glutamine and retails for $12.00 (Tinsley, 2018; L-glutamine, n.d.). Proline is an additional high-value amino acid that can be used in maintaining elastic skin, muscle repair, and skin damage (Proline, n.d.). Proline is a building block of protein that serves as an essential component of collagen and retails for about $10.00 (L-Proline, n.d.). Poultry feathers can be utilized to produce proline that can be taken as a supplement in the form of L-proline that can support joint and tendon function.

Serine, in its supplemental form L-serine, can help in supporting brain function. Serine is an essential component of phosphatidylserine, which helps in maintaining brain cell survival (L-SERINE, 2018). In its supplement form Serine is priced at $22.00 (L-Serine, n.d.). Companies like Merck are also using serine as a dietary supplement and nutritional additive (Koning et al., 2003). Poultry feathers have many amino acids beyond cysteine, glutamine, proline, and serine that can be beneficial in many industries.

2.4 Characterization techniques

2.4.1 Solids Testing

Total solids testing was used in order to characterize the dry matter in the natural and processed feathers. Total solids testing was conducted in order to measure the weight of the poultry feathers without any moisture. The absence of moisture in the poultry feathers allowed us to quantify the number of solids before and after subcritical water hydrolysis. More specifically, how well the hydrolysis technology solubilizes the poultry feathers to produce amino acids. Furthermore, volatile solids testing was done to measure the amount of organic and inorganic matter within the natural and processed chicken feathers. Volatile solids were calculated by

subtracting the total solids from the fixed solids. Fixed solids, the inorganic material, are the remaining solids in the material after igniting it at 550 °C.

2.4.2 Total Nitrogen

Total nitrogen was tested for both the natural and processed feathers using the Kjeldahl Nitrogen method. The total amount of nitrogen is important in knowing the amount of proteins and amino acids within the samples since nitrogen accounts for about 16% of proteins (Allowances, 1989). The Kjeldahl Total Nitrogen measures both organic and inorganic forms of nitrogen such as ammonia nitrogen, however nitrogen coming from azide, azo, hydrozones, nitrile, semicarbazones, and oximes are not detected (Applequist, 2012). enabled us to see the chemical makeup of poultry feathers. Total nitrogen tests were conducted before and after hydrolysis. The second test was run mainly as a means of measuring the extraction efficiency and the effect, or lack thereof, hydrolysis may have on the poultry feathers. In addition, total nitrogen was used as both a characterization test and a hydrolysate test.

2.3 Hydrolysis Analysis

2.3.1 Chemical Oxygen Demand (COD)

The Chemical Oxygen Demand (COD) was measured on each hydrolysate sample to get a better picture of the amount of organic matter. As the amount of organic material decreases in a sample, so does the level of COD in the sample. COD measures the amount of oxidant that can interact with organic matter in a sample (Muenchow, 2019). In this case, we used potassium dichromate ($K_2Cr_2O_7$) as the digestive solution, which resulted in the chromate ion, $Cr_2O_7^-$, serving as the oxidant. As the chromate ion interacts with the organic material in the poultry feathers, it reduces to Cr^{3+}. It is important to note that both organic and inorganic material is able to oxidize, however organic matter is able to oxidize at much higher levels.

2.3.2 Nelson-Somogyi Method

The Nelson-Somogyi method was used in order to characterize the sugar composition of the poultry feathers hydrolysate. It is well-known that poultry feathers are almost entirely protein; however, it is important to note the exact composition before performing amino acid composition analysis. The Nelson-Somogyi method determined the amount of reducing sugars such as glucose, galactose, lactose and maltose (Marais et al., 1966). When alkaline copper tartrate is heated, it reduces to cuprous oxide, which, when treated with arsenomolybdic acid, forms a blue color. Each of the eight samples from each hydrolysis run undergoes this colorimetric reaction and the absorbance of each sample was measured at 540 nm.

Chapter 3: Materials and Methods

3.1 Raw materials and Reagents

Solid residues consisted of natural and processed feathers that were provided by Oriente Comercio Ltda (Parana, Brazil). The feathers were milled using a knife mill (Marconi, model MA 340, Piracicaba, SP, Brazil) and later used for hydrolysis testing. All experiments were done using distilled water. Sulphuric acid, sodium hydroxide, boric acid, bromocresol green/methyl red mixed indicator, and hydrochloric acid were all (Wako Pure Chem. Ind., Ltd., Osaka, Japan) used without purification to determine the composition of nitrogen within the raw material. A catalytic blend with 7g K_2SO_4 and 0.8g $CuSO_4$ was also made for total nitrogen testing. The Somogyi-Nelson method was carried out with alkaline copper tartrate and arsenomolybdic acid, which were both prepared within the laboratory. Chemical Oxygen Demand testing was done with a digestive solution made up of potassium dichromate, $K_2Cr_2O_7$, and a catalytic solution of silver sulfate, Ag_2SO_4. Potassium biftalato, KHP, was used as a standard solution. All COD reagents were made within the laboratory.

3.2 Material Characterization

3.2.1 Total Solids

Both the processed and natural poultry feathers were analyzed for moisture content, organic and inorganic matter, and nitrogen and protein content. Total solids and volatile solids tests were performed and calculated using the analytical methods within NREL Determination of Total Solids and Ash in Algal Biomass (Wychen and Laurens, 2015). About 0.3g were weighed out for the natural feathers and 1g was weighed out for the processed feathers. All experiments were carried out in triplicate. All samples were placed in the oven (Fenem, Model 315 SE) at 105 C for 24 hours, left to cool for an hour, and weighed on lab scale. Volatile solids were carried out at 550 °C and placed in the muffle furnace for two hours and left to cool for an additional two hours. After cooling, the sample plus the crucible was weighed on a standard lab scale. Total solids and volatile solids were calculated using Eq 1 and Eq 2. The calculations were made on Excel and can be found in Appendix E.

$$Total\ Solids\ \% = \frac{Mass_{crucible\ +\ sample} - Mass_{crucible}}{Mass_{before\ heating}} * 100 \qquad \text{Eq (1)}$$

$$Volatile\ Solids\ \% = (1 - (Mass_{crucible+\ burned\ sample} - Mass_{crucible})) * 100 \qquad \text{Eq (2)}$$

3.2.2 Total Nitrogen

Total nitrogen testing was done using the Kjeldahl Nitrogen test outlined in the 20th edition of Standard Methods for the Examination of Water and Wastewater (American Public Health Association, 1915). Samples of 0.2g were taken of the processed and natural feathers and

placed in a kjeldahl tube. Total nitrogen testing was done in triplicate. Half a gram of the catalytic blend was added to each tube along with 5 ml of sulphuric acid (95-98% concentration). All tubes were placed in the digester and heated until the temperature arrived at 400 ℃. The kjeldahl tubes were left until complete digestion was reached, indicated by a green color after approximately seven hours. The samples were cooled and 10 ml of water and 25 ml of 40% m/v sodium hydroxide were added to each tube. The samples were placed into a distiller, where 10 ml of boric acid solution and five drops of 4% w/v protein indicator, were placed in an Erlenmeyer flask. Distillate was stopped after 100 ml of each sample was collected. The 100 ml solution was eventually titrated using 0.1 M of standard hydrochloric acid. Total nitrogen and total protein content were calculated using Eq 2 and Eq 3. The general conversion factor of 6.25 was used in order to convert nitrogen content to protein content. Calculations were made on Excel and can be found in Appendix B.2. Total nitrogen tests were carried out on both the raw and hydrolyzed poultry feathers. All tests were carried out in triplicate.

$$N\,\% = \frac{HCL\ ml * HCL\ M * 0.014}{sample\ weight} * 100 \quad \text{Eq (2)}$$

$$Protein\,\% = 6.25 * N\% \quad \text{Eq (3)}$$

3.3 Subcritical Water Hydrolysis

3.3.1 Subcritical water Hydrolysis

Hydrolysis testing was done on natural and processed poultry feathers at different temperatures and retention times. All tests were made using subcritical water in a semi-continuous reactor made of SS316 (Mayanga-Torres, 2017). Figure 2 shows a schematic of the hydrolysis system. The system has one reactor with an internal volume of 100 ml with maximum working conditions of 230 ℃ and 40 MPa. A liquid pump delivers pressurized water to the reactor, which is controlled by a pressure regulator (High-Pressure Piston-Sensing Back-Pressure Regulators, KHB Series, Lafayette, LA) and two pressure transducers. The reaction temperature was monitored by two thermocouples (type K) and was outside of the reactor, with each thermocouple measuring the top and bottom points within the reactor. The existing reactor effluent was cooled to below 27 ℃ in a stainless-steel shell-and-tube heat exchanger paired with a temperature-controlled recirculating bath (Marconi, model MA-184, Piracicaba, SP, Brazil). The recirculating bath was maintained at −10 ℃ using aqueous ethylene glycol 50% as the heat transfer fluid.

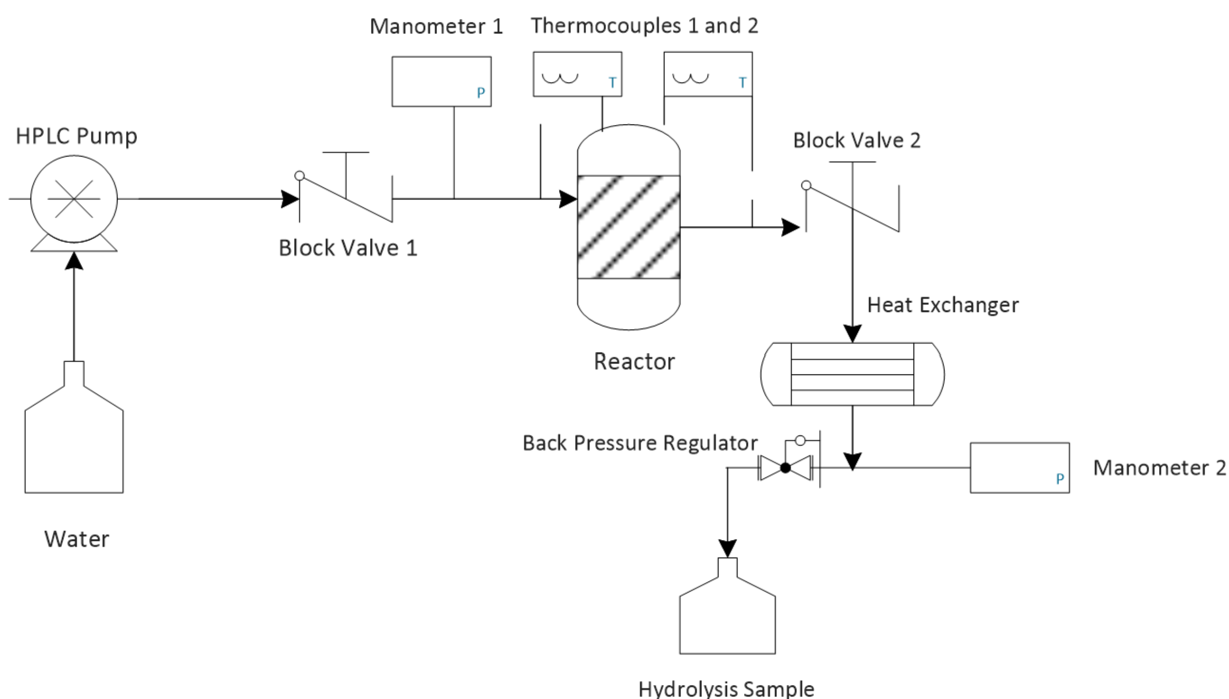

Figure 2. Process Flow Diagram of the subcritical hydrolysis process of poultry feathers.

The feed was placed in the stainless-steel reactor and consisted of 10 g of poultry feathers. The liquid pump was then turned on and pressurized the system as well as filled the system with deionized water. Once the system reached 15 MPa, the pump was turned off. It took approximately 10 minutes for the system to be pressurized. After the system was pressurized, the temperature was set by turning on the heating system. It took approximately 20 minutes for the set point temperature to be reached. After the system was sufficiently heated the system was pumped with water. Samples were collected once 120 ml of water was collected from the system. Hydrolysate samples were collected approximately every four minutes for 32 minutes and stored in plastic Falcon tubes until further analysis. A photo of the collected hydrolysates can be seen in Figure 3. All experiments were performed in duplicate. The independent variable in this study was system temperature.

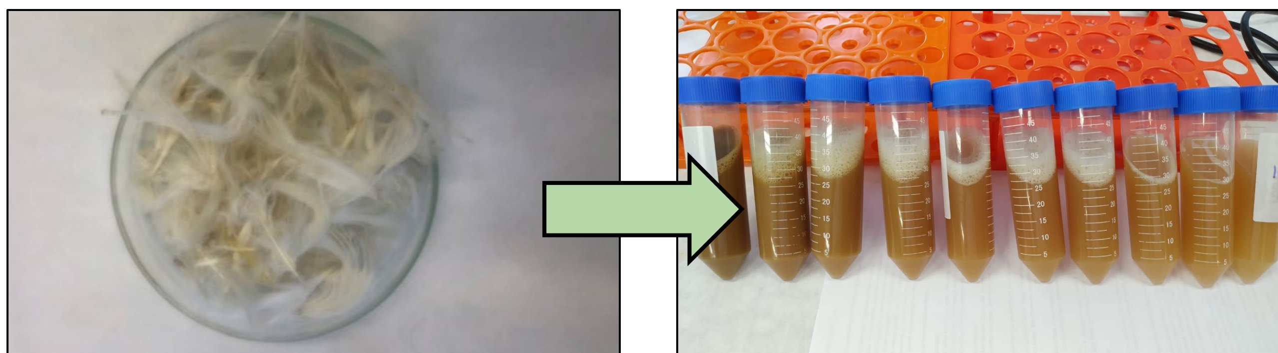

Figure 3. Depiction of the poultry feathers before and after subcritical water hydrolysis.

A total of six runs were made with natural poultry feathers at three different temperatures (210, 230, and 250 ℃) at 10 ml/min at a pressure of 15 MPa. Water flow rate was kept constant throughout each run.

3.4 Analysis of Hydrolysate

3.4.1 Somogyi-Nelson Method for TRS and RS

The Somogyi-Nelson colorimetric method was used to quantify the reducing sugars (RS) and total reducing sugars (TRS). The poultry hydrolysate undergoes acid hydrolysis to break down any polymers into monomers that can be picked up as reducing sugars. About half of the eight poultry hydrolysate samples taken from the hydrolysis machine were diluted to 1:10, while the other half was diluted by 1:5. Each sample was diluted with deionized water so that each readings were within the appropriate calibration range. Poultry hydrolysates that were taken later in the hydrolysis run were not as concentrated as samples taken earlier, which required a lower dilution ratio for these later samples of 1:5 as opposed to 1:10.

A mL of the diluted hydrolysate was added with 2 mL of the Somogyi Nelson I reagent (Alkaline Copper). This mixture was heated and then placed in an ice bath, upon which 2 mL of the Somogyi Nelson II reagent (arsenomolybdic acid) was added. After the addition of the second reagent, all samples were vortexed and left to rest. The sample then was transferred to a volumetric flask and the total volume reached by adding deionized water. Once the colorimetric reaction was done, the absorbance of each sample was measured at 540 nm using a spectrophotometer (Hach, model DR / 4000U, Loveland, CO, USA). Each sample was done in triplicate. RS and TRS values were obtained using a known glucose standard calibration curve based on standard glucose solutions (0.05 - 0.6 $g \cdot L^{-1}$). RS and TRS values were calculated using Excel and can be found in Appendix C.1.

3.4.3 Chemical Oxygen Demand (COD)

A standard solution of potassium dichromate, $K_2Cr_2O_7$, and silver sulfate, Ag_2SO_4, were used as the digestive and catalytic solutions, respectively. Potassium biftalato, also referred to as potassium hydrogen phthalate, was used to make a standard curve for COD testing, which can be

seen in Appendix D. All reaction samples were mixed and heated with a potassium dichromate digestive solution and a silver (II) sulfate/sulfuric acid catalytic solution and read in a Hach spectrophotometer (Hach, model DR / 4000U, Loveland, CO, USA) at 610 nm. The amount of COD was measured using Eq 5.

$$COD = Absorbance * Standard\ Slope \qquad \text{Eq (5)}$$

3.4.4 Amino Acid Composition

The natural and processed feather hydrolysates will be sent to the Technical University of Denmark to Solange I. Mussatto's Biomass Conversion and Bioprocess Technology group to have the composition of amino acids discovered. Here, the amino acid percentages will be discovered using a Phenomenex (USA) EZ: fast amino acid analysis kit. A mass spectrometry detector (Agilent 1100 series, LC/MSD Trap, Agilent Technologies, Denmark) along with liquid chromatography will then be used to identify certain amino acids (Qin et al., 2018). The sum of the amount of all amino acids after hydrolysis will determine the total amino acid content (mg/100 g feather hydrolysate).

Chapter 4: Results and Discussion

4.1 Characterization Tests

Characterization tests such as total nitrogen and total solids were performed on the natural and processed feathers to better understand the composition of the poultry feathers. The moisture content of the natural feathers was slightly lower than that of the processed feathers, however both values were not far from other research conducted on natural feathers by Tesfaye et al. (2017). The only major difference was seen in the nitrogen and protein content. Overall, the numbers suggest that the chicken feathers used are higher in protein than the average feathers. This high protein content is favorable with the ultimate pursuit of the chicken feathers being for high-value amino acids. All composition data for total nitrogen and total solids can be seen in Table 1 with the calculations available in Appendix B.2 and Appendix E, respectively.

Table 1. Raw characterization of poultry feathers

Composition	Natural Feathers [%]	Processed Feathers [%]
Moisture	7.60 ± 6.40 12.33*	8.20 ± 0.97
Ashes	1.20 ± 0.25 1.49*	2.00 ± 0.03
Nitrogen	14.80 ± 0.10 10.41*	13.70 ± 0.07
Protein	92.40 ± 0.60 82.36*	85.70 ± 0.40

Values presented as mean +/- standard deviation
*Values taken from Tesfaye et al., 2017

4.2 Reactor Heat Transfer

The heat transferred through the system reactor for each hydrolysis run was modeled to better understand the system mechanics. Figure 3 shows that the heat transfer did not change much with temperature. The graph was generated using excel and can be seen in Appendix A. Instead, the heat transfer was nearly identical for the first three runs with more heat being lost throughout the reactor for the last three runs. The difference in heat transfer in the last three runs can be attributed to the fact that the reactor no longer worked after running three and had to be repaired. Thus, the subsequent runs were carried out with a slightly different reactor. The uniformity of the reactor's heat transfer profile is ideal because it shows that nothing odd or unexpected is happening in the reactor, and that the reactor is successfully carrying out the hydrolysis of the poultry feathers.

Furthermore, the shape of the curves in Figure 3 help to describe the hydrolysis process. As the temperature of the system is heating up to the set operating temperature, the heat transferred into the reactor increases until it decreases and plateaus at around the 25-minute mark. It is important to note that the actual hydrolysis process doesn't begin until around 35 minutes into the process. Before this, the machine is heated and ran with just water. At the point of hydrolysis, the heat transferred into the reactor is much lower and constant at around 60 kW with some fluctuation. Analyzing the heat transfer within the reactor allowed for the experiment to be clearly demonstrated and explained.

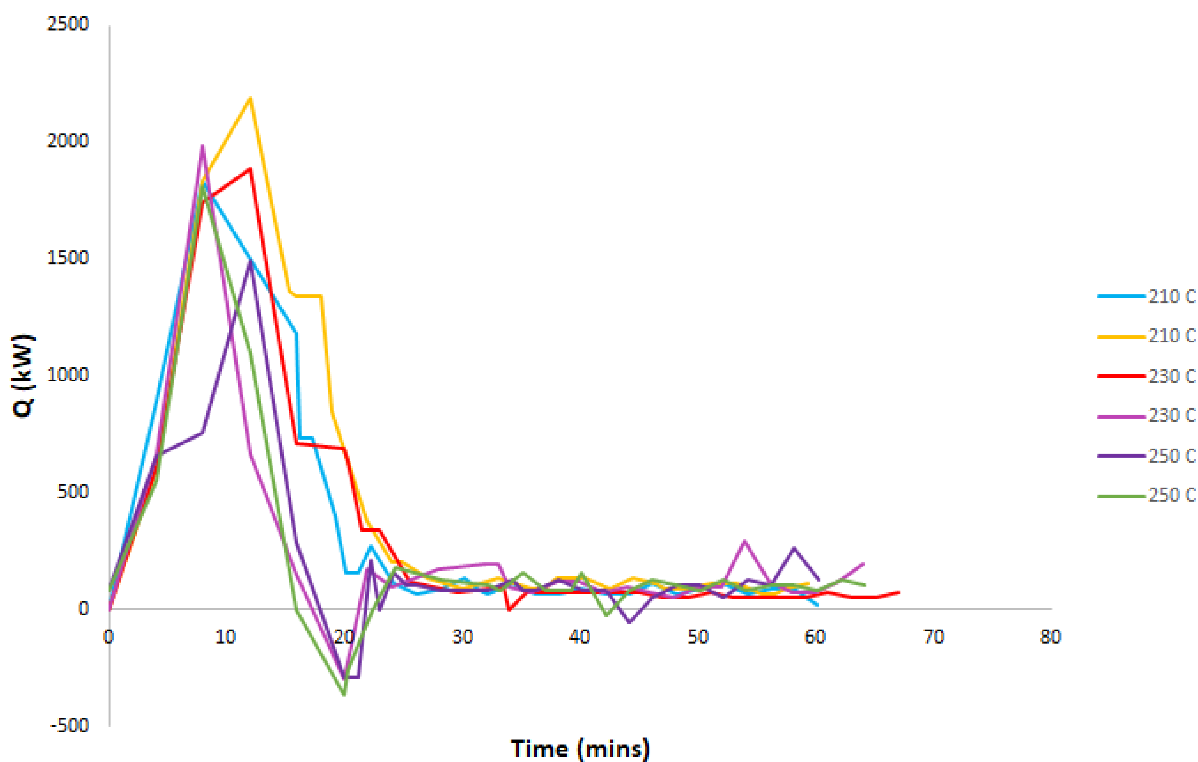

Figure 4. Heat transferred through the reactor at each temperature over the course of the runs.

4.3 Hydrolysate Analysis

4.3.1 Total Nitrogen

Total nitrogen testing was conducted at two different temperatures—230 and 210 °C each at 10 ml/min. Unfortunately, due to the faulty equipment and other external reasons we were unable to obtain additional temperatures and additional flow rates. It can be seen from Figure 4 that the nitrogen recovery was best at the lower temperature of 210 °C. The excel sheet used to generate Figure 4 can be found in Appendix B.1. The raw natural poultry feathers are about 15% nitrogen, which can be seen from Table 1. It is important to note that not all the nitrogen

recovered is from ammonia acids. Since the experiment measures total nitrogen, ammonia nitrogen is also included in the nitrogen amounts seen in Figure 4.

At 210 °C, the nitrogen recovery was the highest earlier on in the hydrolysis process, with the highest nitrogen recovery being about 4.5% at the first collection point. It is important to note that the first collection point was taken about 30 minutes into the entire process, but only about four minutes into the hydrolysis process. The hydrolysis process, at a flow rate of 10 mL/min, takes about 32 minutes, but the full hydrolysis process takes about an hour with the necessary steps. From the first collection point, the amount of nitrogen recovered goes down a percentage until about the fourth collection point. The last four collection points tend to decrease, but not by as much. Furthermore, it can be seen in Figure 4 that the run with an additional 20 °C did not recover as much nitrogen. This may suggest that higher operating temperatures beyond 230 °C may be too high in trying to recover amino acids. Since amino acids consist of nitrogen this inference can be made.

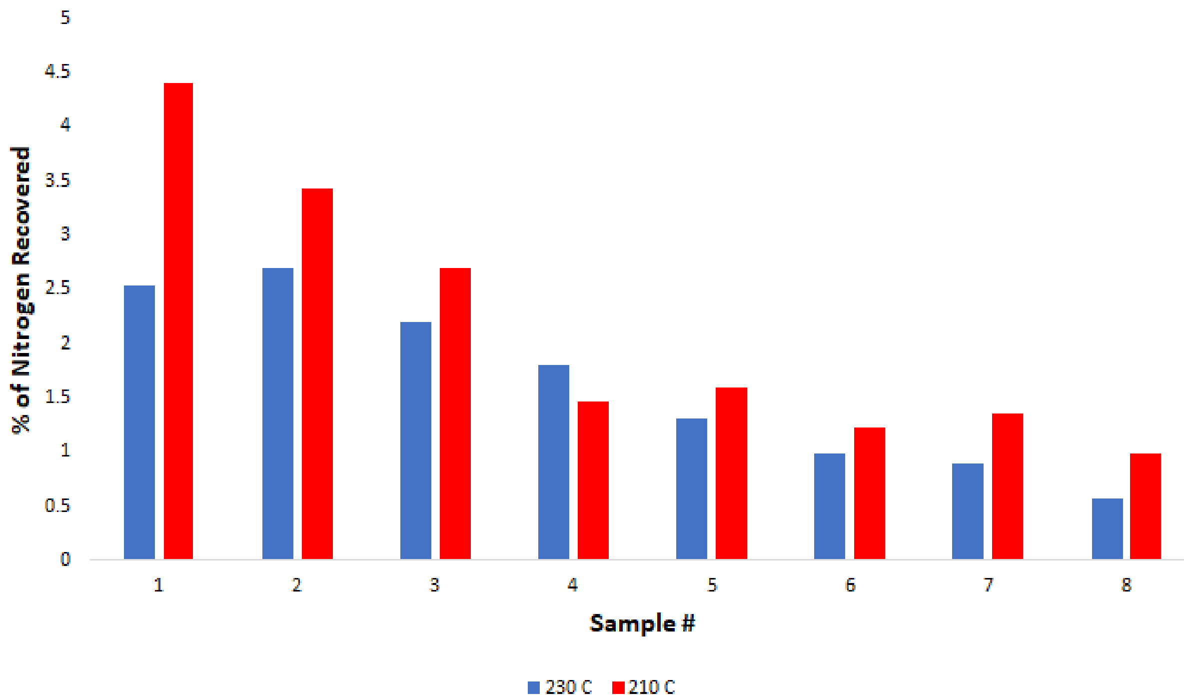

Figure 5. Nitrogen recovered from the raw poultry feathers at 210 °C and 230 °C.

The accumulated nitrogen, or the maximum of nitrogen the machine was able to recover through the hydrolysis process, was also calculated. A depiction of the nitrogen accumulation at 210 °C and 230 °C can be found in Figure 5. It can be seen from the graph that the accumulation follows a logarithmic trend, meaning that the nitrogen content in the hydrolysate decreases by less and less as the process goes on. In other words, the nitrogen content that is recovered from the raw feathers is highest at the onset, and it proceeds to decrease, but this decrease is less and less significant the longer the hydrolysis process continues. This indicates that shorter reaction times, such as only 10 minutes, may be ideal for ultimate nitrogen recovery. Beyond the 10-

minute mark, the accumulated nitrogen content begins to plateau with the decreasing nitrogen content. In addition, the higher amount of nitrogen recovered took place at 210 °C (blue curve). At 210 °C, about 17% of the initial nitrogen content was able to be recovered over the course of the hydrolysis process. This amount is higher relative to the approximately 13% that was recovered at 230 °C.

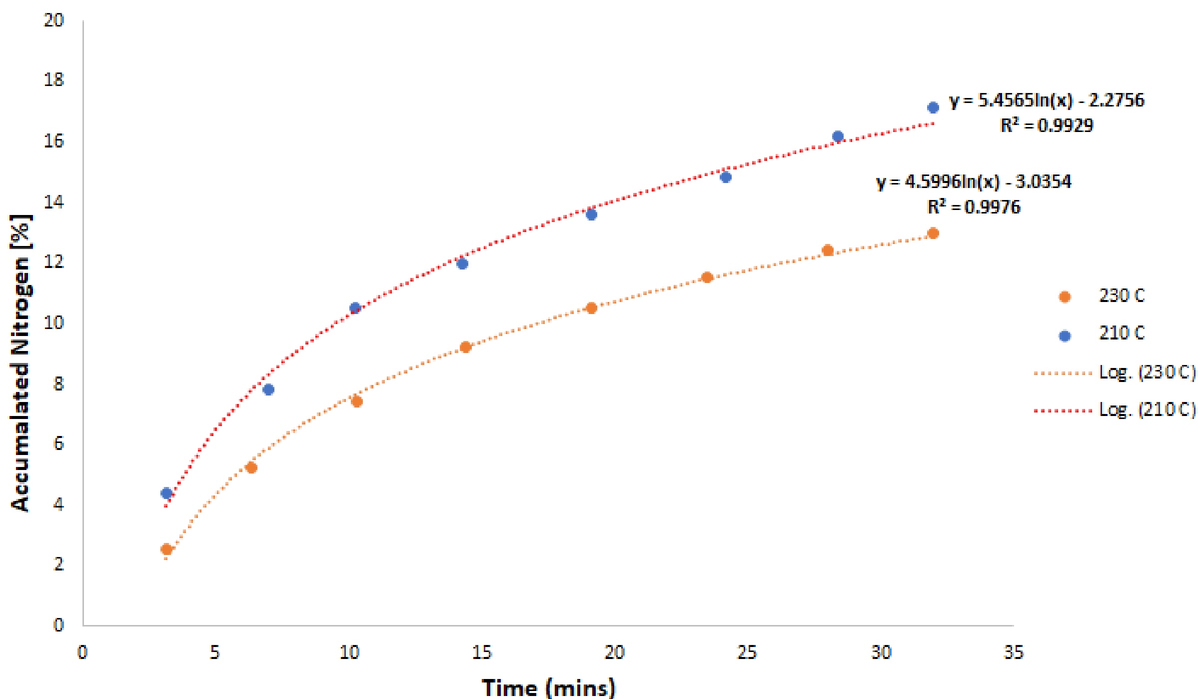

Figure 6. Nitrogen accumulated throughout the hydrolysis process at 210 °C and 230 °C.

4.3.2 Chemical Oxygen Demand (COD)

COD tests were done for three different temperatures—210, 230, and 250 °C at one constant flow rate at 10 ml/min. DQO values measure the oxygen demand in grams of oxygen per liter of solution. From Figure 6, it is evident that the oxygen demand is higher during the first half of hydrolysis than at the latter half. All COD calculations were made on excel and can be seen in Appendix D. As mentioned before, COD is a good indicator of the amount of organic content within a solution because. Thus, the organic matter during the first half, or for the first four collection points, is higher than in the last four collection points. The highest temperature of 250 °C does appear to have, overall, the highest organic matter. However, the difference in DQO values among the three different temperatures decreases with time.

In addition, in Figure 6 the organic matter in the hydrolysates for 230 and 210 °C are very similar, with the only exception being at collection point two. Collection point two has a much lower at 230 °C because of experimental error. A different dilution factor, 1 instead of 5, was used for the 230 °C sample and resulted in a much smaller number. However, we can assume that the actual value would be close to 50 g/L because 230 °C and 210 °C seem to follow the

same trend. Moreover, the lower organic content as the hydrolysis process proceeds suggests that the amount of amino acids at the later collection points might also be lower relative to the earlier points. Another point of discussion is that the temperature doesn't seem to have an effect on the organic matter of the hydrolysate and that after the halfway point all hydrolysates, regardless of temperature, behave very similarly.

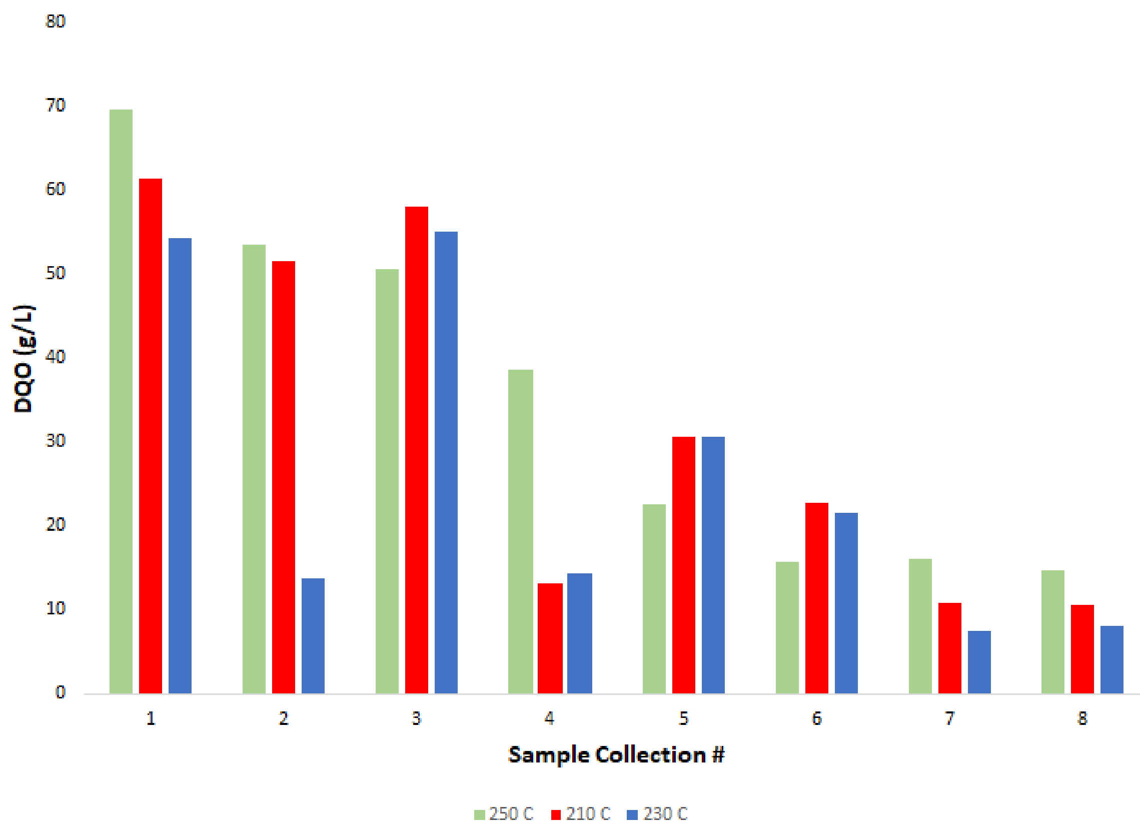

Figure 7. Chemical Oxygen Demand of all hydrolysis runs.

4.3.3 Nelson-Somogyi

Poultry feathers are notably very low in carbohydrates. The Nitrogen Free Content (NFE) of natural chicken feathers is reported to be around 1% of the feathers themselves (Tesfaye et al., 2017). NFE includes compounds like carbohydrates, sugars, starches, and hemicellulose from biomass. Thus, due to the already very low amount of carbohydrates in poultry feathers, we suspected that the Nelson-Somogyi tests were going to yield small values.

Figure 7 shows the sugar formation of the poultry feathers hydrolysates over the roughly 30-minute hydrolysis process at 230 and 210 ℃. At the beginning of hydrolysis, the amount of sugars present is very low at 0.31% at 230 ℃ and 0.15% at 210 ℃. However, as the hydrolysis process continued the amount of sugar present in the liquid hydrolysate increased. The increase in sugar is due to the subcritical water breaking down the carbohydrates of the natural feathers in sugar compounds, much like it does with proteins to form amino acids. Overall, the sugar

formation follows a logarithmic trend, which means that at the onset sugar formation is happening very rapidly, but eventually it levels out at about 1.6% sugar. The logarithmic trend coupled with the low values indicates that there are not many carbohydrates in our poultry feathers to begin with, which is expected due to the fact that the feathers used were found to be almost entirely protein. All calculations were made in excel and can be reviewed in Appendix C.

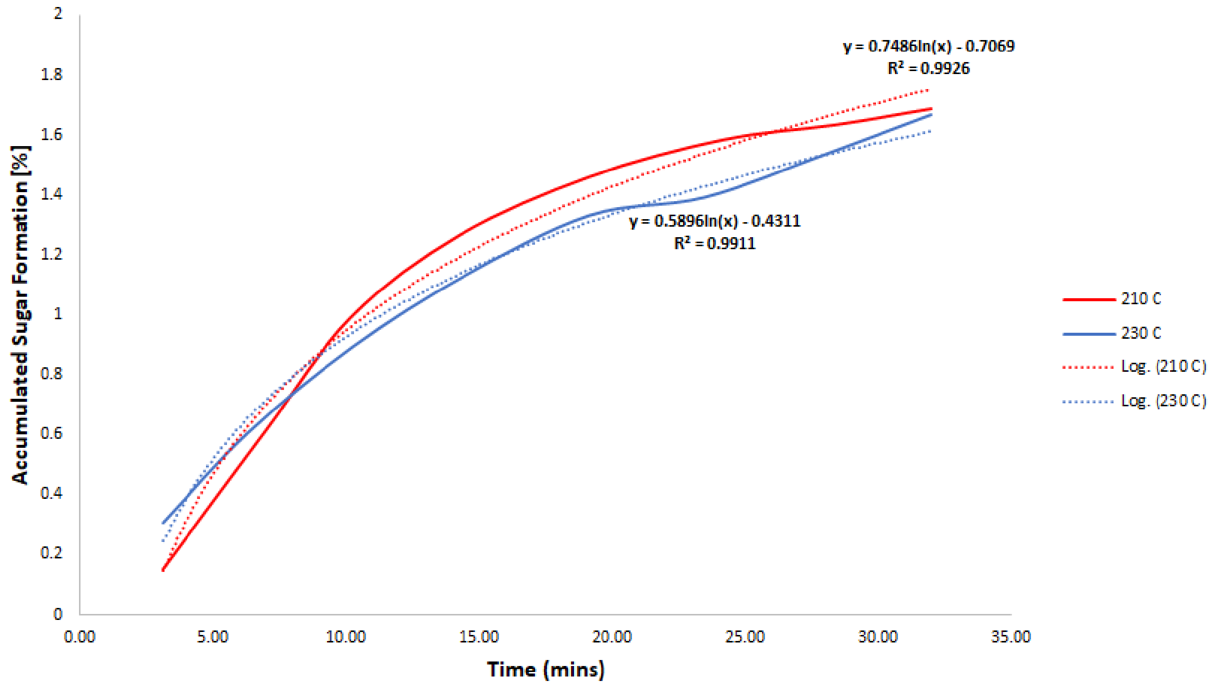

Figure 8. Sugar formation over the hydrolysis process at 210 and 230 C.

Chapter 5: Conclusions and Recommendations

Natural poultry feathers were hydrolyzed with subcritical water using a semi-continuous reactor. It was found through total nitrogen, chemical oxygen demand, and Nelson-Somogyi testing that the process successfully broke down the feathers, while maintaining the organic makeup. The poultry feathers were converted from being almost entirely protein to only about half protein, suggesting that most of the protein was successfully broken down into amino acids. Therefore, it can be inferred that amino acids are the main product from the hydrolysis reactions.

Furthermore, the operating conditions of the hydrolysis machine does play a role in the makeup of the hydrolysates themselves. For instance, the two runs that were done at 210 ℃, overall, showed to be higher in nitrogen, and thus protein, content than the run performed at 230 ℃. A higher nitrogen recovery of 17% from the raw poultry feathers was achieved at 210 ℃. In addition, the highest temperature the machine was operated at, 250 ℃, showed to be the highest in overall organic matter, which does not necessarily point to a high amino acid yield. Although no amino acid testing was done, it can be inferred that the protein content of the feathers were better able to be broken down into amino acids at 210 ℃ due to the higher nitrogen content at this point. While the amino acids continued to be broken down at higher temperature, which explains why the nitrogen content was lower with the overall organic content being relatively high.

In addition, it was evident that as the hydrolysis reaction proceeded the yield of amino acids was diminishing. The decrease in amino acids can be inferred from the fact that the amount of nitrogen in the hydrolysates dwindled with time. The results from the experiment suggest that an operating time less than or equal to 10 minutes would be best in obtaining optimum nitrogen yield. It was around 10 minutes into hydrolysate collection that the nitrogen content began to rapidly decrease, which continued until the process was completed. A higher flow rate than 10 mL/min would probably assist in running the machine for less time and collecting hydrolysates with increased amounts of nitrogen.

In the future, the experiment can better be improved by testing more temperatures along with different flow rates. Unfortunately, due to time constraints and the equipment, the machine operated under only a few temperatures and one flow rate. By testing temperatures lower than 210 ℃ and higher than 250 ℃, the effect of temperature can better be determined. Different flow rates would also reveal the impact of retention time on the organic yield of the hydrolysates and determine whether it is best to run the machine for longer or shorter periods of time. Another improvement that could be made is the addition of more hydrolysate analysis testing. For example, in this experiment the only elemental composition that was calculated was for total nitrogen. However, it could be additionally helpful to analyze the carbon and hydrogen content, which could be done using a CHN analyzer. A more detailed way of obtaining the elemental composition of the hydrolyzed poultry feathers, and thus indicate the presence of amino acids, would be to use energy dispersive x-ray analysis. Another useful implementation would be to analyze the microstructures of the poultry feather hydrolysates and see whether they change

depending upon the operating conditions as well as how they change before and after hydrolysis. The microstructures could be determined by using a Field Emission Scanning Microscope (FESEM)

This project will be continued by master's student Henrique Ziero in professor Tania Forster-Carneiro's lab. The project will expand to include more temperatures as well as more flow rates. Finally, the amino acid composition results will be available once the hydrolysis testing is completed and the hydrolysates are analyzed in professor Solange I. Mussatto's lab at the Technical University of Denmark. All these results will be available in a future publication.

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Appendices

Appendix A: Subcritical Water Hydrolysis Machine

Hydrolysis of feathers

Temperature	230°C	Pressure	15 Mpa	Flow rate	10 ml/min

Time (min:sec)	Temperature 1 - Bottom (°C)	Temperature 2 - Top (°C)		Reactor Heat Transfer (kW)
0	28	28		0
4	55	30		613.3615402
8	120	49	Heating	1741.946774
12	162	85		1889.153544
16	188	159		711.4993866
20	228	200		686.964925
20.3	230	204	Waiting	637.8960018
21.55	244	230		343.4824625
23	244	230		343.4824625
25.55	225	220		122.672308
29.55	230	227	Water	73.60338482
33.55	229	225		98.13784643
34				0
35.55	233	230		73.60338482
38.65	232	229		73.60338482
39.1	228	225		73.60338482
41.1	229	226		73.60338482
43.1	235	232		73.60338482
45.1	228	225		73.60338482
47.1	228	226		49.06892322
49.1	235	233		49.06892322
51.1	229	226	Hydrolysis	73.60338482
53.1	231	229		49.06892322
55.1	234	232		49.06892322
57.1	228	226		49.06892322
59.1	233	231		49.06892322
61.1	232	229		73.60338482

Heat Duty on Exchanger

LMTD	74.23621816	
Q	4489806.474	W
mass flow	0.000166667	kg/s
Cp =	147206769.6	J/kg K

hydrolyze 1 | Hydrolyze 2 | **Hydrolysis 3** | Hydrolysis 4 | Hydrolysis 5 | Hydrolysis 6

Temperature 1 is the temperature at the bottom of the reactor
Temperature 2 is the temperature at the top of the reactor

Heat Duty was found using the following Equation:

$$Q = U * A * LMTD$$

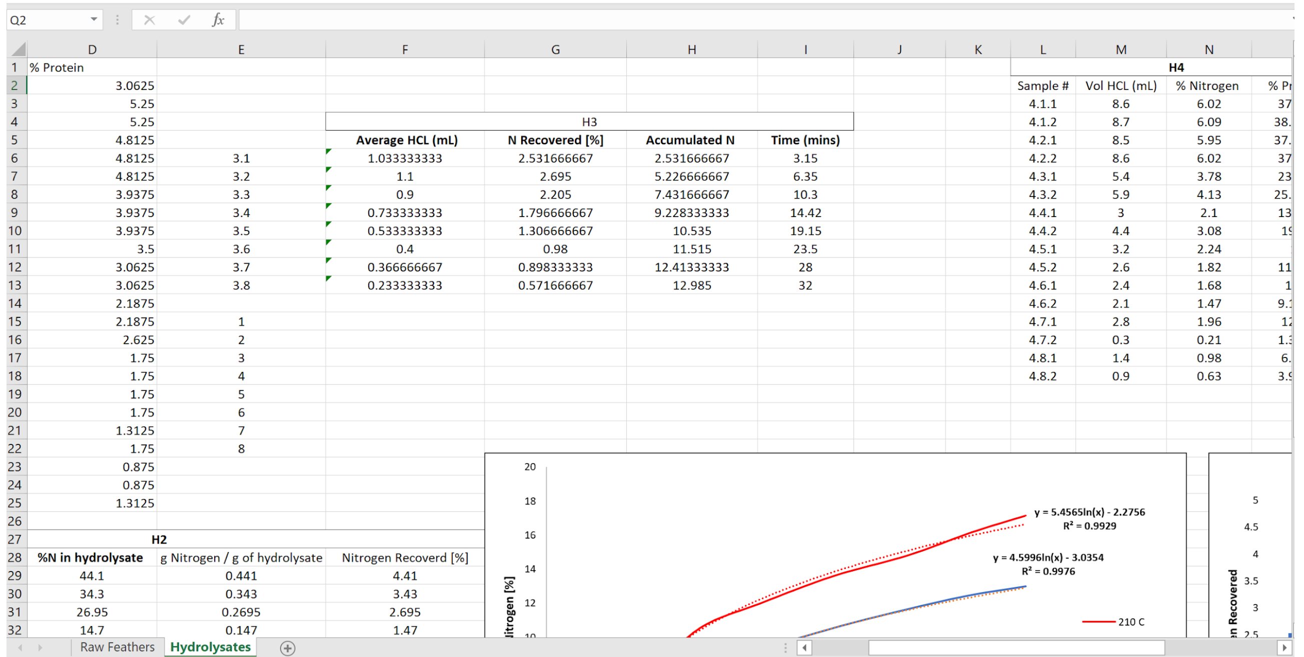
$$LMTD = \frac{\Delta T1 - \Delta T2}{ln(\frac{\Delta T1}{\Delta T2})}$$

According to Heat Exchanger Heuristics:

$U = 280\, W/m^2\, C°$ for liquid to liquid heat exchangers

Appendix B: Total Nitrogen Analysis

B.1 - Total Nitrogen of Hydrolysates



	D	E
1	% Protein	
2	3.0625	
3	5.25	
4	5.25	
5	4.8125	
6	4.8125	3.1
7	4.8125	3.2
8	3.9375	3.3
9	3.9375	3.4
10	3.9375	3.5
11	3.5	3.6
12	3.0625	3.7
13	3.0625	3.8
14	2.1875	
15	2.1875	1
16	2.625	2
17	1.75	3
18	1.75	4
19	1.75	5
20	1.75	6
21	1.3125	7
22	1.75	8
23	0.875	
24	0.875	
25	1.3125	

H3			
Average HCL (mL)	N Recovered [%]	Accumulated N	Time (mins)
1.033333333	2.531666667	2.531666667	3.15
1.1	2.695	5.226666667	6.35
0.9	2.205	7.431666667	10.3
0.733333333	1.796666667	9.228333333	14.42
0.533333333	1.306666667	10.535	19.15
0.4	0.98	11.515	23.5
0.366666667	0.898333333	12.41333333	28
0.233333333	0.571666667	12.985	32

H4			
Sample #	Vol HCL (mL)	% Nitrogen	% Pr
4.1.1	8.6	6.02	37
4.1.2	8.7	6.09	38.
4.2.1	8.5	5.95	37.
4.2.2	8.6	6.02	37
4.3.1	5.4	3.78	23
4.3.2	5.9	4.13	25.
4.4.1	3	2.1	13
4.4.2	4.4	3.08	19
4.5.1	3.2	2.24	
4.5.2	2.6	1.82	11
4.6.1	2.4	1.68	1
4.6.2	2.1	1.47	9.
4.7.1	2.8	1.96	12
4.7.2	0.3	0.21	1.
4.8.1	1.4	0.98	6.
4.8.2	0.9	0.63	3.9

H2		
%N in hydrolysate	g Nitrogen / g of hydrolysate	Nitrogen Recoverd [%]
44.1	0.441	4.41
34.3	0.343	3.43
26.95	0.2695	2.695
14.7	0.147	1.47

B.2 - Total Nitrogen of Raw Feathers

C26 | *fx*

	A	B	C	D	E	F	G
1	**Weight**						
2	***Processed (g)***	***Natural (g)***	natural	processed		M HCL	0.02
3	0.2097	0.1998	0.2626	0.2519			
4	0.2051	0.1992	0.2644	0.2537			
5	0.9992	0.2087	0.2508	0.2593			
6							
7	**Volume HCL**						
8	***Processed (ml)***	***Natural (ml)***	natural	processed			
9	82.4	59.3	27.5	24.7			
10	69.9	72.6	28	24.7			
11	6.5	86.2	26.6	25.5			
12							
13	**% N**						
14	***Natural***	***Processed***					
15	14.66	13.73					
16	14.83	13.63					
17	14.85	13.77					
18	14.78	13.71					
19	0.102330946	0.07073858					
20							
21							
22							
23	**% Protein (General)**						
24	***Natural***	***Processed***					
25	91.63	85.80					
26	92.66	85.19					
27	92.80	86.05					
28	92.37	85.68					
29	0.63956841	0.44211614					
30							

Appendix C: Nelson-Somogyi Raw Data

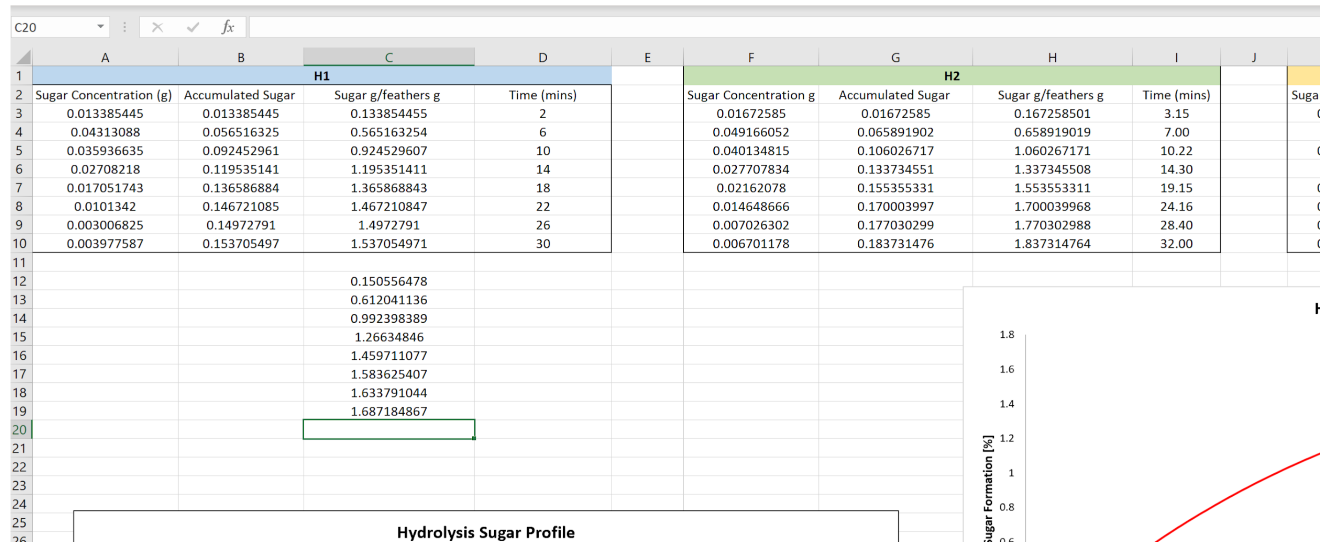
C.1 - Raw Data

	Abs (nm)				[Glucose] (g/L)			dilution	Concentration (g/L)		
	A1	A2	A3	A Average	C1	C2	C3	factor	C Average	STDEV (±)	[Feathers]
1.1	0.078	0.079	0.083	0.080	0.029	0.029	0.031	10	0.03	0.001	0.2975
1.2	0.223	0.231	0.216	0.223	0.096	0.099	0.092	10	0.10	0.003	0.9585
1.3	0.179	0.196	0.191	0.189	0.075	0.083	0.081	10	0.08	0.004	0.7986
1.4	0.155	0.144	0.139	0.146	0.064	0.059	0.057	10	0.06	0.004	0.6018
1.5	0.102	0.095	0.096	0.098	0.040	0.037	0.037	10	0.04	0.002	0.3789
1.6	0.065	0.069	0.059	0.064	0.023	0.025	0.020	10	0.02	0.002	0.2252
1.7	0.043	0.039	0.046	0.043	0.013	0.011	0.014	10	0.01	0.002	0.1253
1.8	0.036	0.036	0.032	0.035	0.009	0.009	0.008	10	0.01	0.001	0.0884
2.1			0.247	0.247	0.000	0.000	0.10676075	10	0.04	0.062	0.3559
2.2	0.248	0.239	0.24	0.242	0.107	0.103	0.10353256	10	0.10	0.002	1.0461
2.3	0.192	0.204	0.206	0.201	0.081	0.087	0.08785279	10	0.09	0.003	0.8539
2.4	0.144	0.141	0.145	0.143	0.059	0.058	0.05972145	10	0.06	0.001	0.5895
2.5	0.215	0.22	0.21	0.215	0.092	0.094	0.08969747	5	0.09	0.002	0.4600
2.6	0.131	0.199	0.122	0.151	0.053	0.085	0.04911455	5	0.06	0.019	0.3117
2.7	0.077	0.08	0.084	0.080	0.028	0.030	0.03159011	5	0.03	0.002	0.1495
2.8	0.081	0.074	0.077	0.077	0.030	0.027	0.02836193	5	0.03	0.002	0.1426
3.1	0.151	0.167	0.154	0.157	0.062	0.070	0.06387198	10	0.07	0.004	0.6541
3.2	0.151	0.148	0.165	0.155	0.062	0.061	0.06894484	10	0.06	0.004	0.6418
3.3	0.135	0.157	0.152	0.148	0.055	0.065	0.06294964	10	0.06	0.005	0.6110
3.4	0.123	0.122	0.124	0.123	0.050	0.049	0.05003689	10	0.05	0.000	0.4958
3.5	0.186	0.203	0.222	0.204	0.079	0.086	0.09523151	5	0.09	0.008	0.4339
3.6	0.07	0.072	0.074	0.072	0.025	0.026	0.02697842	5	0.03	0.001	0.1303
3.7	0.142	0.148	0.152	0.147	0.058	0.061	0.06294964	5	0.06	0.002	0.3040
3.8	0.152	0.131	0.131	0.138	0.063	0.053	0.05326508	5	0.06	0.006	0.2825

The sugar concentration, in grams of glucose per liter of solution, for each hydrolysis collection point for the initial three runs can be seen in column L. The values were obtained by multiplying the average concentration (column J) of the triplicate by the dilution factor (column I).

$$Glucose\ (g/L) = C_{avg} * DF$$

C.2 - Accumulated Sugar Calculations



C20

H1			
Sugar Concentration (g)	Accumulated Sugar	Sugar g/feathers g	Time (mins)
0.013385445	0.013385445	0.133854455	2
0.04313088	0.056516325	0.565163254	6
0.035936635	0.092452961	0.924529607	10
0.02708218	0.119535141	1.195351411	14
0.017051743	0.136586884	1.365868843	18
0.0101342	0.146721085	1.467210847	22
0.003006825	0.14972791	1.4972791	26
0.003977587	0.153705497	1.537054971	30
		0.150556478	
		0.612041136	
		0.992398389	
		1.26634846	
		1.459711077	
		1.583625407	
		1.633791044	
		1.687184867	

H2			
Sugar Concentration g	Accumulated Sugar	Sugar g/feathers g	Time (mins)
0.01672585	0.01672585	0.167258501	3.15
0.049166052	0.065891902	0.658919019	7.00
0.040134815	0.106026717	1.060267171	10.22
0.027707834	0.133734551	1.337345508	14.30
0.02162078	0.155355331	1.553553311	19.15
0.014648666	0.170003997	1.700039968	24.16
0.007026302	0.177030299	1.770302988	28.40
0.006701178	0.183731476	1.837314764	32.00

Appendix D: Chemical Oxygen Demand Raw Data

Hydrolysis Run	Sample #	Absorbance	DQO	Dilution Factor	Average DQO	STDEV	DQO Corrected (mgO2/L)	DQO Corrected g/L
H1	1.1.1	0.323	1070.333	5	689.2222222	330.1570894	37907.22222	37.90722222
	1.1.2	0.149	490.3333	5				
	1.1.3	0.154	507	5				
	1.2.1	0.139	457	5	937	423.9627866	51535	51.535
	1.2.2	0.38	1260.333	5				
	1.2.3	0.33	1093.667	5				
	1.3.1	0.257	850.3333	5	877	46.18802154	48235	48.235
	1.3.2	0.281	930.3333	5				
	1.3.3	0.257	850.3333	5				
	1.4.1	0.218	720.3333	5	685.8888889	32.03007846	37723.88889	37.72388889
	1.4.2	0.199	657	5				
	1.4.3	0.206	680.3333	5				
	1.5.1	0.171	563.6667	5	557	40.41451884	30635	30.635
	1.5.2	0.156	513.6667	5				
	1.5.3	0.18	593.6667	5				
	1.6.1	0.136	447	5	414.7777778	29.12298316	22812.77778	22.81277778
	1.6.2	0.119	390.3333	5				
	1.6.3	0.124	407	5				
	1.7.1	0.222	733.6667	1	668.1111111	140.3302982	7349.222222	7.349222222
	1.7.2	0.231	763.6667	1				
	1.7.3	0.154	507	1				
	1.8.1	0.158	520.3333	1	500.3333333	17.63834207	5503.666667	5.503666667
	1.8.2	0.148	487	1				
	1.8.3	0.15	493.6667	1				
	2.1.1	0.318	1053.667	5	1117	87.62292952	61435	61.435
	2.1.2	0.326	1080.333	5				
	2.1.3	0.367	1217	5				
	2.2.1	0.34	1127	5	1119.222222	35.64225541	61557.22222	61.55722222
	2.2.2	0.347	1150.333	5				
	2.2.3	0.326	1080.333	5				
	2.3.1	0.285	943.6667	5	1054.777778	151.2295288	58012.77778	58.01277778
	2.3.2	0.37	1227	5				
	2.3.3	0.3	993.6667	5				

Appendix E: Total Solids Raw Data

H25 | fx | not ready

	C	D	E	F	G	H	I	J
8			0.979	0.9303	97.9			
9			0.9398	0.9304	93.98			
10								
11		**Average =**	0.965566667	0.953566667				
12		**StDev =**	0.022321365	0.040212477				
13								
14	H1 Feathers Wt (g)	H2 Feathers Wt (g)	inroganic (g)	organic (g)	%organic	inorganic %		
15	0.3154	0.3481	0.3172	0.6828	68.28	31.72		
16			0.3481	0.6519	65.19	34.81		
17								
18								
19								
20					Crucible + sample			
21			Crucible mass	sample mass	mass after oven	mass after muffle		
22	Feathers	1.1	60.7453	1.1368	61.7908	60.7674	0.0221	
23		1.2	55.7715	1.1668	56.8399	55.7925	0.021	
24		1.3	54.4974	1.6443	55.9819	54.5576	0.0602	
25	feather meal	2.1	37.2598	2.1855	39.2675	not ready	#VALUE!	
26		2.2	35.0332	2.2456	37.0935	35.1029	0.0697	
27		2.3	40.5772	2.2124	42.6059	40.6468	0.0696	
28								
29		**ASH**						
30	Natural	1.0455	1.199466667	**<-- Average**				
31		1.0684	0.24711152	**<-- St. Dev**				
32		1.4845						
33	Processed	2.0077	2.032233333	**<-- Average**				
34		2.0603	0.026477412	**<-- St. Dev**				
35		2.0287						
36								
37								
38								
39								