university of groningen

faculty of science and engineering

Examining peptides and amino acids through different protein extracting steps using heat coagulation on potato fruit juice

Author: Tobias Mooy
Supervisors: prof. dr. GJW Euverink, dr. ing. H Kloosterman
Date: February 2022

Abstract

Peptides and amino acids in potato fruit juice (PFJ) were examined using HPLC analysis. The variety, brand, and country of origin of the potatoes used are Artemis, Lidl, and the Netherlands, respectively. Four different samples of PFJ were used in the analysis. The samples were taken after extracting starch from the potatoes by heating the PFJ to 70°C for 20 minutes, to 100°C for 20 minutes, and after evaporating the boiled PFJ in an oven for 24 hours. Pre-column derivation was done for the amino-acid analysis using ophthalaldehyde-o (OPA) and 3-mercaptoproprionic acid (3-MPA). Evidence for the existence of free peptides and amino acids was inconclusive. The potatoes' mass percentages were obtained for the components water, starch, and proteins. The corresponding mass percentages were 74.4% for water, 13.5% for starch, and 2.09% for proteins. A theoretical revenue yield of amino acids was determined to be 0.45 $/kg of potatoes. This value was determined by implementing the total amount of free amino acids in potatoes found in literature.

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Introduction

Plant-based proteins, peptides, and amino-acids have seen an increasing demand during the past decades. The potato is one of the sources for these nutrients as it has a comparable concentration of protein as wheat and a higher concentration than rice or maize (Bártová et al., 2015).. Potato Fruit juice (PFJ), the residual liquid after extraction of starch from potatoes, has an industrial usage by extracting the bulk of the protein from PFJ using heat coagulation and acid precipitation. Part of the soluble proteins turns into insoluble proteins by altering the temperature or pH values. The formal waste product PFJ is produced at a rate of approximately 0.5 – 0.7 m^3 for 1 ton of potatoes (Ralet & Guéguen, 2000). In this research, the remaining liquid after heat coagulation is further investigated by determining the presence of peptides and amino acids in the liquid. Furthermore, mass-balances are made for the potato components to provide an indication of the theoretical yield of peptides and amino acids in PFJ after heat coagulation. The core objective of the research was to establish the existence and determine the concentration of peptides and amino acids using HPLC analysis on PFJ after the extraction of proteins. The results of the analysis were applied to determine economic profitability.

On a dry-weight basis, protein content in potatoes is around 10% of the total weight (Bártová et al., 2015). Note that this percentage is an average of many different potato species on a dry basis. A 1951 study from the University of Maine researched the chemical composition of the white potato. The results of the composition can be found in Table 1 (Brautlecht & Getchell, 1951). Note that the composition can differ even within the same species of potato due to different growing conditions. The growing conditions relevant to the potato are weather, soil, fertilizer, water, and cultivation (Brautlecht & Getchell, 1951).

Table 1 – Composition of white potato (Brautlecht & Getchell, 1951)

Compound	**Percentage of the whole (%)**
Water	75.4 to 82.2
Protein	1.13 to 2.89
Fat or ether extract	0.02 to 0.18
Nitrogen-free extract	14.05 to 20.37
Fiber	0.28 to 0.85
Ash	0.78 to 0.95

Table 2 – Average composition of 100g of boiled potato tuber (Potatoes and Sweet Potatoes USDA., (2021).

Potato tuber 100g	
Water	77g
Carbohydrates	20.13g
Energy	87 kcal
Protein	1.87g
Fat	0.1g

Table 2 provides data on the composition of the average potato tuber (Potatoes and Sweet Potatoes USDA, (2021). These results are for a boiled potato tuber, including skin and peel. However, boiling the potato tuber does not influence the values from Table 2. When comparing Tables 1 and 2, it can be concluded that the average composition of water, protein, and fat has remained stable over the last seven decennia.

Potato proteins can be classified into three groups: patatin, high-molecular-weight proteins, and protein inhibitors. Patatin accounts for roughly 40% of the soluble protein in

potato tubers. The patatin is a group of glycoproteins with a molecular weight of 40kDa to 45 kDa (Kärenlampi & White, 2009). Patatin is a protein chain that is 366 amino acids long and made up out of 20 different amino acids. The protease inhibitor accounts for roughly 30-40% of the total protein of the potato tuber. The molecular weight of the protease inhibitor ranges from 5kDa to 25 kDa (Kärenlampi & White, 2009). In addition, protease inhibitors have more hydrophilic properties (Kärenlampi & White, 2009). Although, all three different protein groups can coagulate by heat. The last group of proteins, a variety of high-molecular-weight proteins, has not been extensively studied yet (Waglay & Karboune, 2016).

Specific proteins in PFJ denature when performing heat treatment. For example, patatin is a tertiary stabilized protein that unfolds at temperatures above 55°C and becomes insoluble and precipitates (Pots et al., 1999). Proteins lose their spherical shape during denaturation. This unfolding results in more exposed hydrophobic amino acids, lowering overall solubility. This exposure causes the precipitation of proteins. In general, the proteins in PFJ start to precipitate when the liquid is heated above 40 °C; at a temperature of 50°C, approximately 50% of the total precipitation has occurred, and above 70 °C complete denaturation of the proteins has taken place. Higher temperatures increase the speed of the denaturation process. Thus, boiling the PFJ will ensure the complete and rapid denaturation of the proteins.

High-Performance Liquid Chromatography (HPLC) is a technique that detects compounds in a sample vial by measuring the compound speed through a column (Figure 1). A solvent used to separate components from the sample allows for qualitative and quantitative analysis. The solvent (mobile phase) is delivered to the separation column (stationary phase). The detector measures the speed through the column. If the affinity between the component and the mobile phase is more substantial, the component will move through the column faster. If the component has a stronger affinity to the stationary phase, the component will move more slowly. The sensor measures both the time it takes for the components to go through the column as well as the intensity of the signal.

HPLC analysis is one of multiple techniques to analyze peptides. Another more trivial technique is sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). SDS-PAGE separates proteins with molecular masses between 5 and 250 kDa (Bury, 1981). The technique allows for separation by mass and eliminates the influence of structure and charge. A Tris-Tricine buffer is used to distinguish peptides, which enables the separation of proteins from 1 to 100 kDa (Schagger & von Jagow, 1987).Despite the effectiveness of SDS-PAGE, the research has been limited to HPLC analysis.

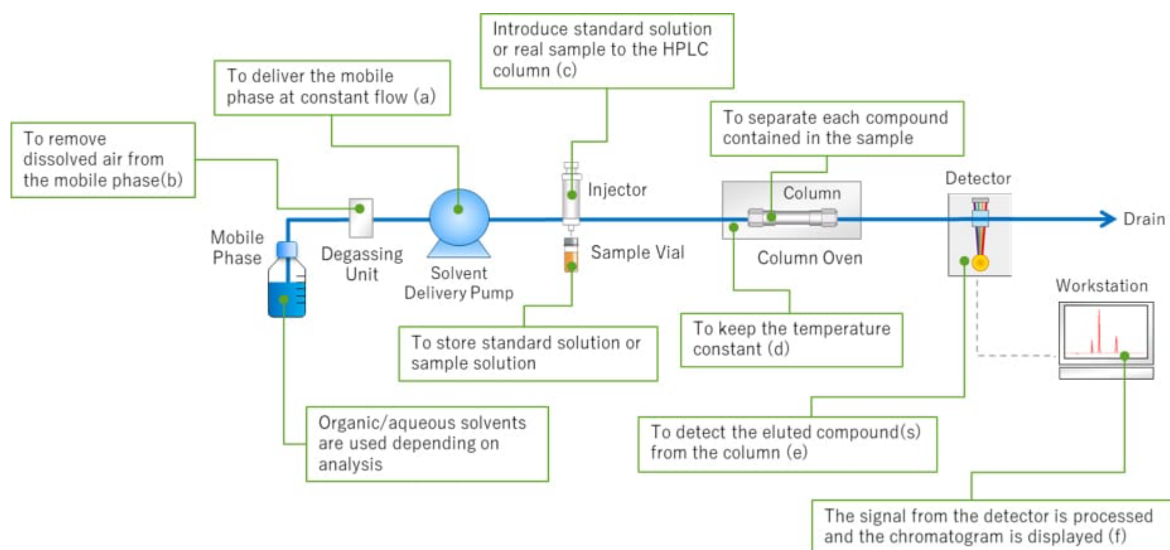

Figure 1: Flow diagram of an HPLC (What Is HPLC (High-Performance Liquid Chromatography), Shimazu., (2021)

The derivation of amino acids using ophthalaldehyde-o (OPA) is a commonly applied HPLC method for the quantitative determination of amino acids (R.C. Dorrestijn et al., 1996). This derivation is required to create a fluorescent molecule that are detectable for the HPLC method. Different combinations have been tried for the mobile phase consisting of acetonitrile, methanol, and water. In addition to OPA, 3-mercaptoproprionic (3-MPA) acid is often used for the derivation. Figure 2 depicts the chemical structure reaction of an amino acid with OPA and 3-MPA. Different ratios and concentrations are used for the derivation method. These differences are due to the possible chemical reactions that occur when using OPA and 3-mercaptoproprionic acid with amino acids.

Three conditions that are crucial for an optimal derivation are the concentration of 3-mercaptoproprionic acid, the pH value of the reaction mixture, and the reaction time (R.C. Dorrestijn et al., 1996). The reaction will have an optimal pH where the production rate of fluorescing derivatives is higher than the composition rate. The 3-mercaptoproprionic acid determines the stability of the OPA / 3-mercaptoproprionic acid complex. The reaction time should be sufficiently long to make sure the reaction of OPA with the amino acids is complete. The study of fluorometric excitation (λ_{ex}) and emission (λ_{em}) spectrum wavelength of OPA-amino acids provides a wavelength range to obtain high sensitivity and better resolution of amino acids. A range for the wavelength detection must be determined to detect all amino acids. Glycine, taurine, and GABA have the maximum signal peak at approximately 450nm. The best signal when experimenting with the 18 different amino acids studied was obtained with a minimum wavelength of 240. A wavelength of 240 to 450nm λ_{ex} -λ_{em} is most suitable for OPA-amino acids analysis to achieve the best signal response and maintain optimum chromatographic resolution (Perucho et al., 2015). Figure 3 depicts different peaks that were obtained using known amino acid concentrations and OPA derivation.

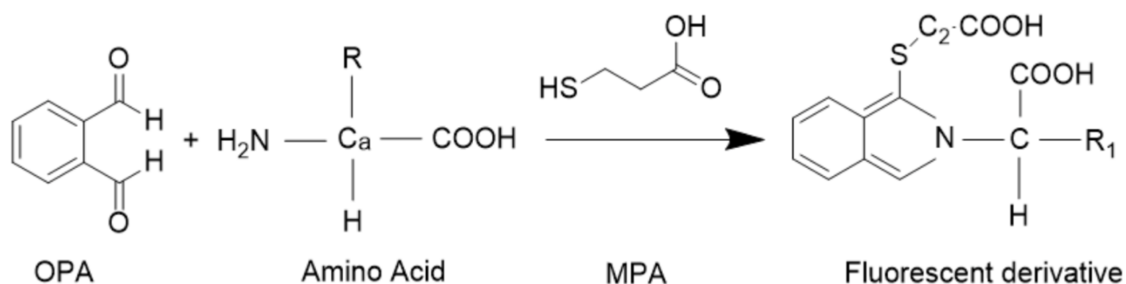

Figure 2: The reaction of OPA and amino acid together with 3-MPA to create a fluorescent derivative.

Figure 3 demonstrates a graph from an HPLC where the derivation mentioned above was used for 22 different amino acids. The intensity and placement of the various peaks can be compared with results obtained from the analysis of amino acids in PFJ.

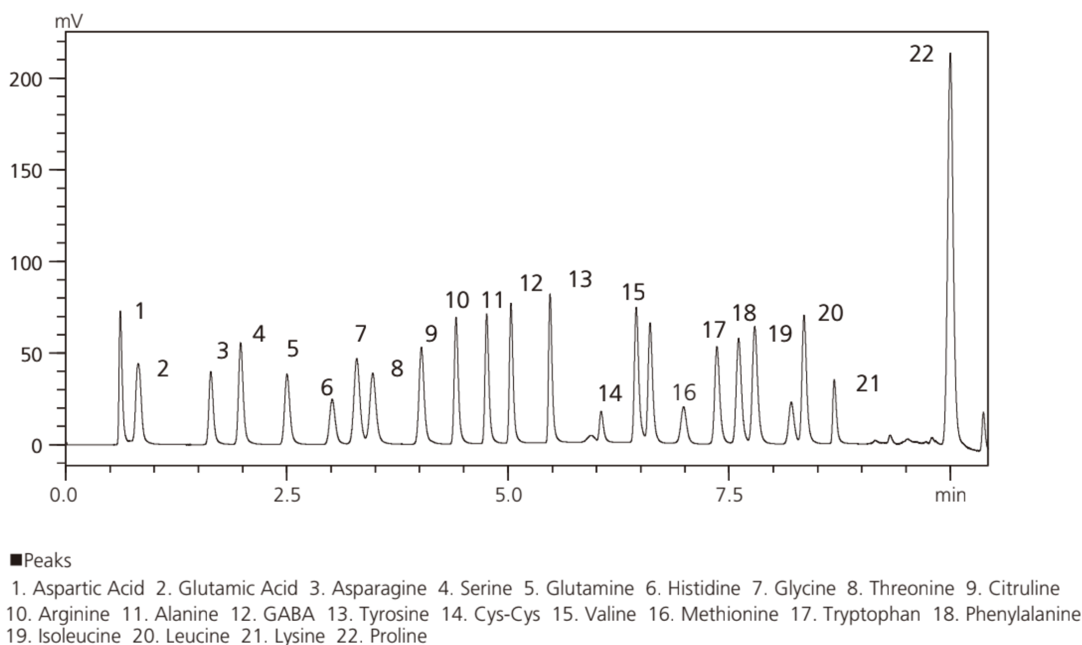

Figure 3: Chromatogram of 22 amino acids (10 μmol/L, 1 μl injection) (Shimadzu corporation, 2012)

Mass spectrometry (MS) is a tool to measure the mass-to-charge ratio (m/z) of molecules present in a sample. The exact molecular weight of a component in the sample can be found by obtaining these measurements. A mass spectrometer has three major components: Ionization Source, Mass Analyzer, and an Ion Detection System. The Ionization Source converts molecules to gas-phase ions. The Mass Analyzer separates the ions according to mass-to-charge (m/z). The Ion Detection System is used to find the relative abundance of the m/z ratio. Afterward, the information is a mass spectrum with the relative abundance on the vertical axis and the m/z ratio on the horizontal axis.

By combining HPLC and MS, a more advanced technique called Liquid chromatography-mass spectrometry (LC-MC) can be obtained for more a detailed analysis. The liquid chromatography separates mixtures with multiple components. For each separation, mass spectrometry provides information regarding molecular weight. An LC-MS system uses an interface that transfers separated components from the HPLC into an MS ion source (Dass, 2007). HPLC uses a pressurized liquid as a mobile phase; the MS on the other hand operates under a high vacuum. Therefore, the LC-MS systems ensure that during the transition from the HPLC to the MS, the identity of the chromatography products is preserved. Nonetheless the liquid does not interfere with the vacuum conditions and ionizing efficiency for the MS system.

Table 3 contains the minimum, maximum, and mean concentrations of the different amino acids in potatoes. The values are obtained by analyzing sixteen different potato varieties(Mant et al., 2007). The values for amino acids in Table 3 are not free amino acids. Thus, most of the amino acids measured come from proteins or peptides. Table 4 contains information on the free amino acids in a potato tuber. For the experiment, flour was prepared from individual freeze-dried tubers. The amino acids were derivatized and analyzed using gas chromatography-mass spectrometry (GC-MS) (Muttucumaru et al., 2015). The values were converted from mmol/kg to mg/g to match the values in Table 3. Table 5 compares the total mean concentration of amino acids in a potato tuber from Table 3 with the concentration of free amino acids from Table 4. The average fraction of free-amino acids from the total amount of amino acids is 0.29. Table 6 contains the averages prices of amino acids in $/kg retrieved from various Chinese suppliers.

Table 3: maximum, minimum, and mean concentration of different amino acids in potatoes without irrigation (Mant et al., 2007). Asparagine (Asn), Aspartic acid (Asp), Glutamine (Gln), Glutamic acid (Glu), Threonine (Thr), Serine (Ser), Proline (Pro), Glycine (Gly), Alanine (Ala), Cysteine (Cys), Valine (Val), Methionine (Met), Isoleucine (Ile), Leucine (Leu), Tyrosine (Tyr), Phenylalanine (Phe), Lysine (Lys), Histidine (His), and Arginine (Arg).

	Asn	**Asp**	**Gln**	**Glu**	**Thr**	**Ser**	**Pro**	**Gly**	**Ala**	**Cys**
Mean (mg/g)	22.1	19.9	10.9	21.8	3.0	4.4	2.5	2.7	2.8	1.0
Minimum (mg/g)	8.9	11.8	6.7	10.6	1.8	2.4	1.2	1.6	1.4	0.4
Maximum (mg/g)	34.5	38.6	16.2	45.0	5.2	7.2	3.3	3.8	4.6	1.8
	Val	**Met**	**Ile**	**Leu**	**Tyr**	**Phe**	**Lys**	**His**	**Arg**	
Mean (mg/g)	5.0	0.5	3.0	4.9	2.8	3.6	5.2	1.6	3.3	
Minimum (g/mg)	3.4	0.2	2.0	2.6	2.0	2.6	3.6	1.2	3.2	
Maximum (g/mg)	8.9	1.2	5.0	7.2	4.3	5.3	8.0	2.8	9.6	

Table 4: Mean values of free amino acids in mmol/kg and mg/g obtained from thirteen different species of potatoes grown in the UK without irrigation (Muttucumaru et al., 2015). With the exceptions of Cysteine, Valine, Leucine, Tyrosine, Histidine, and Arginine.

	Asn	**Asp**	**Gln**	**Glu**	**Thr**	**Ser**	**Pro**	**Gly**	**Ala**	**Cys**
Concentration (mmol/kg)	0.40	28.16	45.60	1.93	6.62	2.68	18.17	3.22	6.96	-
Molar mass	132.1	133.1	146.14	147.1	119.1	105.1	115.1	75.07	89.9	-
Concentration (mg/g)	0.1	3.7	6.7	0.3	0.8	0.3	2.1	0.2	0.6	-
	Val	**Met**	**Ile**	**Leu**	**Tyr**	**Phe**	**Lys**	**His**	**Arg**	
Concentration (mmol/kg)	-	2.27	7.03	-	-	4.76	8.31	-	-	
Molar mass	-	149.2	131.2	-	-	165.2	146.1	-	-	
Concentration	-	0.3	0.9	-	-	1.2	1.2	-	-	

Table 5: Fraction of free amino acids (AA) over the total AA amount in a table.

	Asn	**Asp**	**Gln**	**Glu**	**Thr**	**Ser**	**Pro**	**Gly**	**Ala**	**Cys**
Mean total AA (mg/g)	22.1	19.9	10.9	21.8	3.0	4.4	2.5	2.7	2.8	1.0
Free AA (mg/g)	0.1	3.7	6.7	0.3	0.8	0.3	2.1	0.2	0.6	-
Fraction free/total	**0.01**	**0.19**	**0.62**	**0.01**	**0.27**	**0.07**	**0.84**	**0.07**	**0,21**	**-**
	Val	**Met**	**Ile**	**Leu**	**Tyr**	**Phe**	**Lys**	**His**	**Arg**	
Total AA (mg/g)	5.0	0.5	3.0	4.9	2.8	3.6	5.2	1.6	3.3	

Free AA (mg/g)	-	0.3	0.9	-	-	1.2	1.2	-	-	
Fraction free/total	**-**	**0.60**	**0.30**	**-**	**-**	**0.33**	**0.231**	**-**	**-**	

Table 6: Free on-board prices of pure amino acids in dollar/kilogram. Prices were retrieved from various online Chinese suppliers.

	Asn	**Asp**	**Gln**	**Glu**	**Thr**	**Ser**	**Pro**	**Gly**	**Ala**	**Cys**
Price ($/kg)	32 - 39	30 - 45	1 – 10	1 – 10	5 - 60	10 - 50	2 – 15	8 – 10	3 – 6	35 - 45
	Val	**Met**	**Ile**	**Leu**	**Tyr**	**Phe**	**Lys**	**His**	**Arg**	
Price ($/kg)	5 – 30	4 – 5	5 – 50	10 – 50	9 – 30	8 – 25	1 – 1.5	1 – 1.8	20 – 45	

Materials & methods

Extracting protein from PFJ and performing weight measurements for mass balances

For the experiment, 2 kilograms of potatoes were purchased. The variety, brand and country of origin are Artemis, Lidl, and the Netherlands, respectively. The potatoes were thoroughly cleaned in the sink to eliminate potential mud and measured for weight. After cleaning, the potatoes were skinned using a potato peeler. Both the weight of the skinned potatoes and the weight of the peel were measured. Next, the potatoes were inserted into a kitchen blender one by one to extract the starch and blend into pulp. The potatoes were blended thoroughly so that no chunks were present, and the entire substance was converted into pulp. Next, the pulp was filtered through a metal sieve. This was done by some of the pulp into the sieve. After all the liquid went through, the thicker pulp was put into a separate bucket. The leftover pulp was put into an ordinary kitchen hand towel and squeezed thoroughly to extract more juice from the pulp. The liquid filtered using the hand towel was put into the bucket together with the liquid filtered using the metal sieve. Both the liquid and the solid matter were measured for their weight.

The remaining insoluble matter in the liquid was extracted using a laboratory centrifuge. The centrifuge was set on 5000 rounds per minute (RPM) for 20 minutes. After centrifuging, the liquid was carefully poured out. The liquid and the solid were measured for their weight. From the liquid, a 2 ml sample was taken for later investigation. The sample was put into an Eppendorf and labeled as '1'.

After weighing both the solid and liquid, the liquid was put into heat-resistant glass. The Liquid was heated on a metal plate to 70°C for 20 minutes. A thermometer was placed into the liquid and connected to the metal plate. The liquid was stirred by an RPM high enough to create a vortex. The emulsion was centrifuged using the laboratory centrifuge for 20 minutes at 5000RPM. The liquid was poured out carefully, and the solid matter was removed using a metal spoon. Both the solid and liquid were measured for weight. A 2 ml sample was extracted from the liquid and put into an Eppendorf. The sample was labeled with '2'.

After, heat treatment was performed. Similar af for the 70°C heat coagulation used with the first sample. However, now the liquid was heated to 100°C Celsius for 20 minutes. The emulsion was centrifuged using a laboratory centrifuge, and the solid matter was separated from the liquid. The solid and liquid were both measured for weight. A 2 ml sample was taken from the liquid and put into an Eppendorf labeled with '3' for later examination.

The remaining liquid was put into an oven at a temperature of 105°C for 20 hours to evaporate part of the H_2O and concentrate the substances in the liquid. After the evaporation

process, the liquid was measured for weight, and a two ml sample was taken and put into an Eppendorf labeled with '4'.

Performing moist analysis to the obtained substances

A moist analysis was performed to provide a mass balance of the substances obtained from the experiments. A sample of approximately 5 grams was taken from the potato peel, the solid matter, the substance after centrifuging the PFJ, the substance after heating to 70°C, and the substance after heating to 100°C. The samples were put into a moisture analyzer, and the result was noted.

Performing HPLC analysis of PFJ samples

HPLC analysis was done to locate peptides and amino acids. For peptide analysis, a PRP-C18 column was used. Mobile phase A was 0.1% formic acid solution, and B was acetonitrile. A gradient of 0-100% was taken over 20 minutes. The sensor's detection was set at 214 nm to correspond to the detectible range of peptides (Mant et al., 2007). An injection volume of 50 µL was used. With these settings, the four different Eppendorf samples were analyzed for the existence of peptides. The results were incorporated into graphs.

For HPLC analysis of amino acids buffers were made for the derivation of the amino acids. The first buffer used is a 0,1mol/L 3-mercaptopropionic acid (3-MPA) borate buffer (9,2pH). The second buffer used is a 0,1mol/L o – phthalaldehyde borate buffer (9,2pH). A 20 mmol/L phosphate potassium buffer (6,2pH) and a 45/40/15 acetonitrile/methanol/water mixture were used for the mobile phase. Six different solutions in water were made from various amino acids with a concentration of 5mM. Tyrosine, tryptophan, glycine, lysine, arginine, and cysteine were the amino acids used. The amino acids were to prepare a calibration line. A gradient for the HPLC from 0-70% was set for 30 minutes. The sensor was programmed to detect λ_{ex} of 266 nm and λ_{em} of 350nm. An injection volume of 1 µL and a flowrate of 1,6mL/min. The HPLC was set to insert 45 µL of 3-MPA, 22 µL of OPA, and 7.5 µL of a sample into an empty vial and mixed. After 2 minutes, 1 µL was inserted into the HPLC. The six different amino acids and four samples were all analyzed.

Results and discussion

Figure 4 demonstrates the results from the weight measurements done during the extraction of protein from PFJ. The PFJ fractions highlighted in red were sampled for further analysis. Tables 8, 9, and 10 were created to calculate the mass fraction of water, starch, and protein results. Table 7 shows the results from the moist analysis.

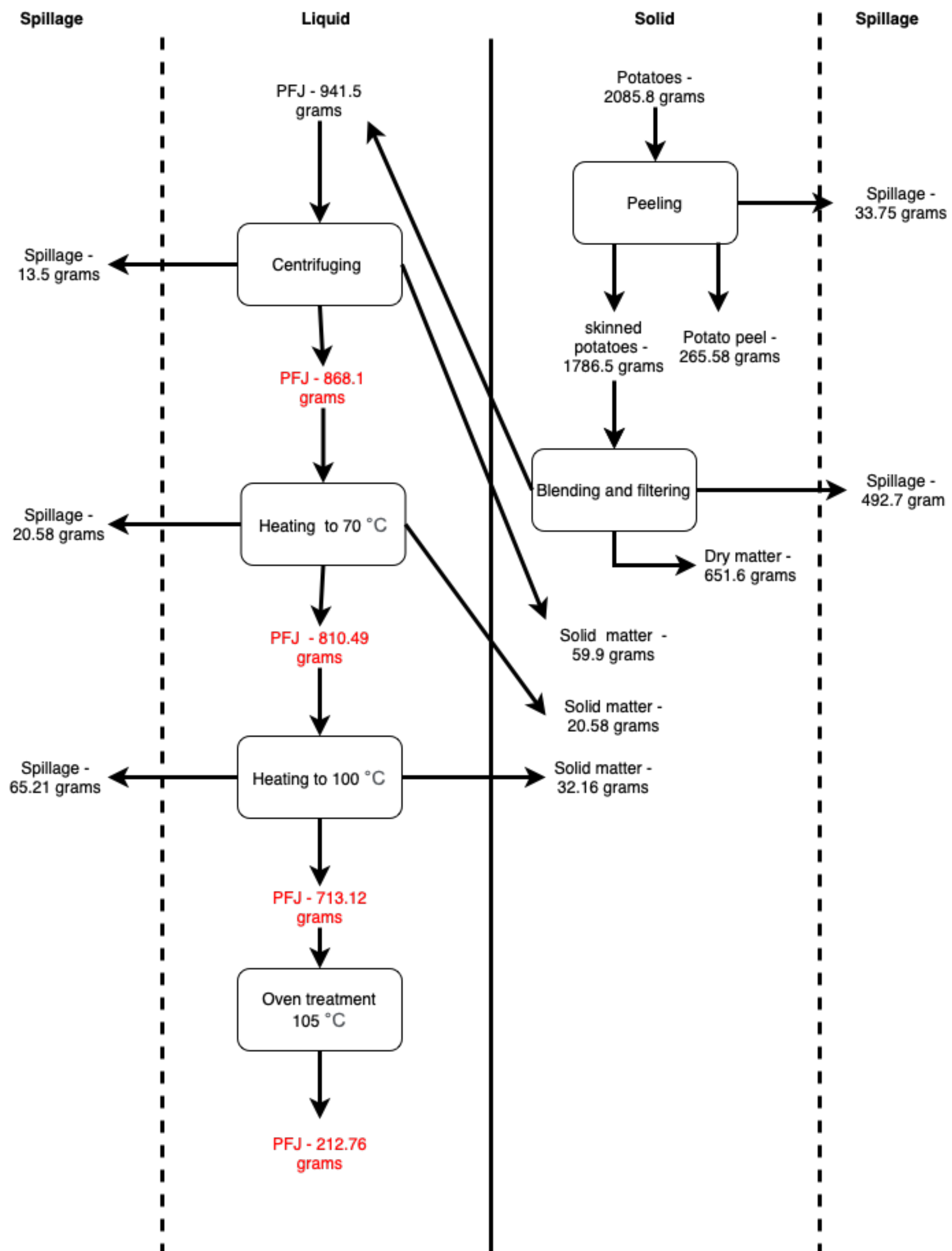

Figure 4: The results of the weight measurements during different temperatures of heat treatments.

Table 7: moist analysis of the potato extraction substances.

Substance	Result from moist analysis
Potato peel	66.2%
Solid potato matter leftover after liquid extraction	58.2%
Starch obtained after centrifuging PFJ	37.9%
Solid precipitated after 70°C heating	42.1%
Solid precipitated after 100°C heating	41.2%

To calculate the weight percentages of the measured substances, spillage must be considered. Therefore, the fraction of spillage was added equally to the weight measurement of the substances from that extraction step. Thus, the values were adjusted to calculate weight as if no spillage occurred. The calculations are presented in Table 8.

Table 8: Calculation for spillage fractions obtained from measurements depicted in Figure 4.

	Weight substance (g)	Spillage (g)	Spillage fraction
Peeling (potatoes)	2085.8	33.75	0.0162
Blending and filtering (Skinned potatoes)	1786.5	492.7	0.276
Centrifuging (PFJ)	941.5	13.5	0.0143
Heating to 70°C	868.1	20.58	0.0237
Heating to 100°C	810.5	65.21	0.0805

Table 9: Adjusted weight calculations after considering fractions of spillage. Each adjustment considers all fractions of spillage from previous steps.

	Substance	Weight (g)	Adjusted weight (g)	Moisture fraction	Dry adjusted weight (g)
Peeling (potatoes)	Skinned potatoes	1786.5	1815	-	-
	Potato peel	265.6	269.8	0.662	91.2
Blending and filtering (Skinned potatoes)	PFJ	941.5	1221	-	-
	Solid matter	651.6	844.9	0.582	353
Centrifuging (PFJ)	PFJ	868.1	1142	-	-
	Solid matter	59.92	78.78	0.379	48.9
Heating to 70°C	PFJ	810.5	1091	-	-
	Solid matter	20.58	27.71	0.421	16.0
Heating to 100°C	PFJ	713.1	1037	-	
	Solid matter	32.16	46.79	0.412	27.5

To assemble a mass balance for the potato tuber, assumptions had to be made. For the sake of simplicity, it was assumed that the total weight of the solid matter after both 70°C heat coagulation and 100°C heat coagulation was precipitated protein (Pots et al., 1999). In the following research, the precipitated substances could be analyzed for the presence of proteins by staining using a ninhydrin reaction (Friedman, 2004). The solid matter extracted after

centrifuging the PFJ before any treatments were assumed to be around 70% starch (Robertson et al., 2018). The solid value after blending and filtering was also assumed to be 70% starch. Thus, the total amount of starch was determined to be $(353 + 48.9) \times 0.7 = 281\ grams$. Iodine could be used to ensure that the solid matter after centrifuging contains starch. When starch is present, the substance should appear blue after mixing Iodine (Bhileyt & Whelans, 1961). Also, for the sake of simplicity, the assumption was made that the mass from the PFJ after heat coagulation is solely from water and the mass from other components in the PFJ can be neglected.

Table 9 contains results from the mass balance obtained from the measurements and calculations depicted in Figure 4 and Table 6. The water fraction was calculated by subtracting all adjusted, solid weight from the starting weight. The values match with the literature obtained from Tables 1 and 2. However, the protein in PFJ does not account for the total amount of protein in a potato. Around 25% of potato protein is insoluble and is most likely present in the 'solid matter' and 'spillage' obtained after blending and filtering from Figure 4 (Peksa et al., 2009). SDS-PAGE analysis could be used in further research to analyze the solid matter after blending and filtering. If the solid matter contains a significant percentage of proteins, this will decrease the mass percentage of starch in Table 9.

Table 10: Mass percentages of water, starch, and protein.

Substance	**Weight (g)**	**Mass percentage of total**
Water	1549	74.3%
Starch	281	13.5%
Protein in PFJ	43.5	2.09%

Table 11 uses the values from Tables 5 and 6 to calculate the revenue per kg for the amino acids. For the prices of amino acids, the price range averages were used. Using the values from the 13 amino acids from which the price and concentration of free amino acids are known, a total of 0.29 \$/kg can be yielded from amino acid extraction. The average yield for free amino acids is assumed to be 0.29 / 13 = 0.022 \$/kg per amino acid. Thus, the total revenue of free amino acids in potatoes is $0.022 \times 20 = 0.45\ \$/\text{kg}$.

Table 11: Theoretical revenue per kilogram from amino acids in potatoes.

	Asn	**Asp**	**Gln**	**Glu**	**Thr**	**Ser**	**Pro**	**Gly**	**Ala**	**Cys**
Revenue ($/kg)	0.0036	0.14	0.037	0.0017	0.026	0.0090	0.018	0.0018	0.0027	-
	Val	**Met**	**Ile**	**Leu**	**Tyr**	**Phe**	**Lys**	**His**	**Arg**	
Revenue ($/kg)	-	0.0014	0.025	-	-	0.020	0.0015	-	-	

Conclusion

The research goal was to determine the presence of peptides and amino acids using HPLC analysis after protein extraction. Unfortunately, based on this research we are yet unable to answer this question due to a lack of time. However, the research was successful in the following subjects: measuring the mass percentages of water, starch, and protein in potatoes, determining how to analyze peptides and amino acids using HPLC analysis, calculating the theoretical revenue gained from extracting the free amino acids. Finally, the determination of the economic feasibility requires further study of the cost factors.

The results from the mass percentage were found for water (74.3%), starch (13.5%), and proteins in PFJ (2.09%). To verify the results, the starch and protein analysis must be done in further research using SDS-PAGE for protein analysis and iodine staining for starch analysis. However, the results were partly verified by existing literature.

A method for HPLC analysis was provided to determine the existence of peptides and amino acids in PFJ after protein extraction. However, an HPLC-MS analysis must be carried out to discover the specific peptide. Also, it was determined that OPA derivation is needed to examine amino acids from PFJ.

Finally, the theoretical revenue from extracting free amino acids from potatoes was found using existing literature. The total yield was approximated to be 0.45 $/kg. However, no conclusions were made on how to extract the amino acids and if it is possible to extract all free amino acids. Further research must be done to establish this.

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