



DIPLOMA THESIS

Characterisation of residues from the food industry: from the analytics to the biorefinery concept

Institute of Chemical, Environmental and Bioscience Engineering
Technische Universität Wien

CD-Labor für Optimierte Expression von Kohlenhydrat-aktiven Enzymen
Austria

Under supervision of:
Univ.Prof. Dipl.-Ing. Dr.techn. Anton Friedl
Laura Vanessa Daza Serna, MSc as collaborating project assistant

by

Alexander Rauch, BSc

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A handwritten signature in blue ink, which appears to read 'Alexander Rauch', is written over a horizontal line.

Date

Signature (student)

Note of thanks

First, I want express thanks to my parents who supported me through my studies.

I want to thank Laura Vanessa Daza Serna, MSc for the supervision of this work and together with Thomas Jung, MSc for their constructive inputs.

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Abstract

A lot of chemicals are derived from fossil fuel-based resources and there is a huge interest to use more sustainable raw materials that are processed in biorefineries. This thesis aims to design a strategy for the characterization of the agricultural by-products wheat bran and potato pulp for the use in a biorefinery by using and combining known methods. Currently, they are mainly used as a low-cost animal feed. The worldwide production of wheat bran is estimated to be between 101 and 233 million tons and the credible estimations for potato pulp range between 303000 and 380000 tons. The chemical composition, added to the availability, shows the huge potential for the application of these raw materials in biorefineries.

This work implemented different analytical methods for the determination of the particle size distribution, moisture, ash, starch, fat, pectin, protein, extractives, structural carbohydrates, monomeric and oligomeric sugars, ions and lignin. For wheat bran, the components with the highest share are structural carbohydrates, extractives, starch and protein, which make up more than 87 % of the dry mass. Xylose, arabinose and glucose are the three main monomers of the structural carbohydrates in wheat bran, which amount to more than 95 wt% of all structural carbohydrates. For potato pulp, the main components are structural carbohydrates, starch and extractives which cover more than 77 wt% of the mass. In contrast to wheat bran, the main monomers of the structural carbohydrates are glucose and mannose with a total share of 89 wt% in potato pulp.

In general, there are two main types of components in wheat bran and potato pulp for uses in biorefineries namely carbohydrates and non-carbohydrates. Due to the high carbohydrate content, the concept of a biorefinery built on the basis of a sugar platform was suggested. Starch is easier to degrade than hemicelluloses and cellulose, which makes it useful to hydrolyze starch separately. Also, hemicelluloses are degraded into pentoses. It must be noted that there are some commercially used microorganisms that cannot metabolize pentoses. The non-carbohydrate components of wheat bran and potato pulp make up a minor amount, but they can also be processed to valuable products like ferulic acid or in the case of protein, they could be used as animal feed.

Kurzfassung

Viele Chemikalien werden aus fossilen Brennstoffen gewonnen und es besteht ein großes Interesse an der Verwendung an nachhaltigen Rohstoffen, die in Bioraffinerien verarbeitet werden. Ziel dieser Arbeit ist es, eine Strategie zur Charakterisierung der landwirtschaftlichen Nebenprodukte Weizenkleie und Kartoffelpülpe für den Einsatz in Bioraffinerien zu entwickeln, indem bekannte Methoden eingesetzt und kombiniert werden. Derzeit werden sie hauptsächlich als kostengünstiges Tierfutter verwendet. Die weltweite Produktion von Weizenkleie wird auf 101 bis 233 Millionen Tonnen geschätzt und die glaubwürdigen Schätzungen für Kartoffelpülpe liegen zwischen 303000 und 380000 Tonnen. Die chemische Zusammensetzung und die Verfügbarkeit zeigen das große Potenzial für den Einsatz dieser Rohstoffe in Bioraffinerien.

In dieser Arbeit wurden verschiedene analytische Methoden zur Bestimmung der Partikelgrößenverteilung, Feuchtigkeit, Asche, Stärke, Fett, Pektin, Protein, Extraktstoffe, Strukturkohlenhydrate, monomere und oligomere Zucker, Ionen und Lignin eingesetzt. Bei Weizenkleie sind die Bestandteile mit dem höchsten Anteil Strukturkohlenhydrate, Extraktstoffe, Stärke und Eiweiß, die mehr als 87 % der Trockenmasse ausmachen. Xylose, Arabinose und Glucose sind die drei Hauptmonomere der Strukturkohlenhydrate in Weizenkleie, die mehr als 95 m% aller Strukturkohlenhydrate ausmachen. Die Hauptbestandteile von Kartoffelpülpe sind Strukturkohlenhydrate, Stärke und Extraktstoffe, die mehr als 77 % der Masse umfassen. Im Gegensatz zur Weizenkleie sind die Hauptmonomere der Strukturkohlenhydrate Glucose und Mannose mit einem Gesamtanteil von 89 m% im Kartoffelpülpe.

Im Allgemeinen gibt es in Weizenkleie und Kartoffelpülpe für die Verwendung in Bioraffinerien zwei Hauptbestandteile, nämlich Kohlenhydrate und Nichtkohlenhydrate. Aufgrund des hohen Kohlenhydratgehalts wurde das Konzept einer Bioraffinerie, die auf der Basis einer Zuckerplattform aufgebaut ist, vorgeschlagen. Stärke lässt sich leichter abbauen als Hemicellulosen und Cellulose, weshalb es sinnvoll ist, Stärke separat zu hydrolysieren. Außerdem werden Hemizellulosen zu Pentosen abgebaut. Es ist zu beachten, dass es einige kommerziell genutzte Mikroorganismen gibt, die Pentosen nicht verstoffwechseln können. Die nicht-kohlenhydrathaltigen Bestandteile von Weizenkleie und Kartoffelpülpe machen einen geringen Anteil aus, können aber auch zu wertvollen Produkten wie etwa Ferulasäure verarbeitet werden oder im Falle von Proteinen als Tierfutter verwendet werden.

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Abbreviations

AERS	Anion Electrolytically Regenerated Suppressor
AIL	Acid insoluble lignin
ASL	Acid soluble lignin
CERS	Cation Electrolytically Regenerated Suppressor
GC	Gas chromatography
GC-MS	Gas chromatography–mass spectrometry
H ₂ SO ₄	Sulfuric acid
HCl	Hydrochloric acid
HPAEC-PAD	High Performance Anion Exchange Chromatography with pulsed amperometric detector
IC	Ion chromatography
LAP	Laboratory Analytical Procedure
MS	Mass spectrometry
NaAc	Sodium acetate
NaOH	Sodium hydroxide
NREL	National Renewable Energy Laboratory
PP	Potato pulp
RDS	Readily digestible starch
RO-water	Reverse osmosis water
RS	Resistant starch
SDS	Slowly digestible starch
SRS	Sugar recovery standards
TDS	Total digestible starch
TS	Total starch
WB	Wheat bran

1 Introduction

1.1 Overview about the topic

Currently, a lot of chemicals, that are used in large quantities are produced from fossil fuels. This creates a problematic dependency and the products made from fossil fuels can cause CO₂-emissions at the end of their life. A solution to this problem are biorefineries, which cannot only produce chemicals, but also food and energy. A biorefinery is characterized by an integrative, multifunctional concept that uses biomass as a diverse source of raw materials for the sustainable production of a spectrum of different intermediate products and products. The aim is to use all components in the raw material as completely as possible.

In this thesis, the composition of the agricultural by-products WB and PP is examined and their possible contribution to the development of a biorefinery concept is discussed. A major part of the constituents of WB and PP are carbohydrates, which represents a substantial potential for a sugar platform (Serna-Loaiza et al., 2019). From this platform, many chemicals can be produced via fermentation. By-products, such as protein, can also be utilized. The carbohydrates can be divided into starch, cellulose, hemicellulose and for PP, pectin (Grommers and van der Krogt, 2009, Palmarola-Adrados et al., 2005).

1.2 Feedstocks

1.2.1 Wheat bran

Wheat is one the most important cereals harvested worldwide, which is why it plays a crucial role in food security. It is, after sugar cane and maize, the primary crop with the highest harvested amount. The annual worldwide production of wheat in 2021 was 778.0 million tons; in 2020 Austria produced 1.65 million tons. In 2020, China, India and the Russian Federation were the three production leaders. In the same year, 68 % of the global production was used as food and 16 % was used as feed. Non-food uses make up for 6 % and 1 % of the global production is further processed (FAO, 2022, FAO, 2023a, FAO, 2023b).

In Europe, 44 % of the domestic supply are used as food, 33 % as feed, 5 % for processing and 6 % for non-food uses (FAO, 2023b). There are various classes of wheat, including bread wheats and durum wheat, which is used for pasta and couscous. These bread wheat classes have different milling, baking and processing characteristics. Wheat is also used for the production of starch, malt, glucose, gluten and alcohol (Erenstein et al., 2022). Wheat grains are also used to produce malt, an ingredient for wheat beer and whiskey (Ssymank and Duell-Pfaff, 1999). Wheat gluten is produced by physical extraction from flour with water. The starch is removed while the gluten remains. Due to the

viscoelastic properties, wheat gluten improves dough strength, softness and shelf-life (Starch Europe, 2019).

During the harvest of wheat, the grains are removed from the rest of the plant. This is called threshing or trashing. The straw is the separated part of the plant. After threshing, the chaff is winnowed from the grain (FAO). The threshing and winnowing is done in a combine harvester (John Deere UK IE, 2018). In the following picture parts of the straw and the chaff that is surrounding the grain can be seen.

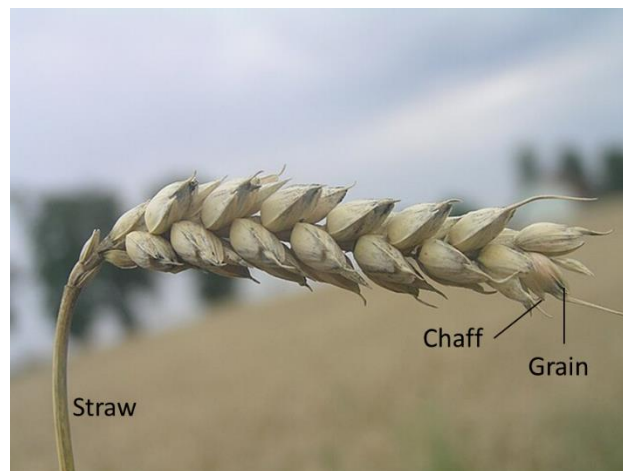


Figure 1: Straw and chaff surrounding the grain of a wheat plant (Filippov, 2010)

The grain, or the kernel consists of the bran, the germ and the endosperm. The multi-layered skin of the edible kernel is called the bran. The germ is the embryo that carries the potential to grow into a new plant. The essential energy for the young plant is supplied by the endosperm, which is also the largest part of the kernel (Oldways Whole Grains Council, 2019).

Wheat grains are milled into various flour fractions in the traditional wheat flour milling process. At first, the grains are cleaned to remove impurities and tempered with water to facilitate the separation of the outer bran layers from the endosperm. Then, the grains are subsequently processed through several break and reduction roller mills. In this way, flour fractions with the lowest possible bran impurities are produced (Koutinas et al., 2005).

The grain to flour conversion efficiency of wheat flour mills is between 70-80 %. The remaining 20-30 % are mainly WB and to a minor amount germ and flour. Therefore, a potential annual production of 155 to 233 million tons of WB can be estimated (Koutinas et al., 2005).

An important remark for this thesis is that the chemical composition of wheat is influenced by the variety of wheat, the growing season, precipitation and storage conditions (Kim et al., 2003).

1.2.2 Potato pulp

The global annual production of potatoes in 2020 was 359.1 million tons. The countries which produced the most potatoes were China, India and Ukraine. 107.7 million and 886 000 tons were produced in Europe and in Austria, respectively (FAO, 2022). On a global level, 69 % of potatoes are used as food, 10 % as feed and 2 % each for processing and other non-food uses. In Europe, 51 % of potatoes are used as food, 17 % as feed, 3 % go into processing and 5 % have other non-food uses. In Austria, while 46 % of potatoes are used as food, only 0.7 % as feed, but 32 % have other non-food uses and 6 % go into processing (FAO, 2023b).

For the production of starch, only certain varieties of potatoes are used, because they contain more starch than the potatoes grown for food (Grommers and van der Krogt, 2009). 1.6 million tons of potato starches and derivatives were produced in Europe 2021 (Starch Europe, 2021b). Globally, it can be estimated that there are around 3.9 million tons of potato starch produced in 2020 (Vilpoux and Santos Silveira Junior, 2023).

PP is produced during the starch production in huge amounts. Currently, it is used as a low-cost animal feed (Meyer et al., 2009, Werpy et al., 2005). It was also examined to extract starch and pectin from PP (Klingspohn et al., 1993, Abousteit and Kempf, 1974). An important disadvantage of PP compared to other raw materials is that fresh PP has a high water content of 87 wt% to 90 wt% and the removal of water by drying demands high amounts of energy, because the water is tightly bound to fibers as well as pectin and it is also in the residual intact cells. (Mayer, 1998, Mayer and Hillebrandt, 1997). Therefore, it is best to use fresh PP in processes that do not require dried PP.

The content of starch, protein and sugars can vary within a single species according to variety, growth conditions and the degree of maturity. After harvesting, the chemical composition can change during storage (Palmarola-Adrados et al., 2005).

For the starch production, the washed potatoes are ground, which causes the cells to break. This leads to a mixture of starch granules, broken cell walls and potato juice, which consists of water, proteins, amino acids, sugars and salts. The next step is to separate the starch, fibers and the potato juice. The potato juice is removed from the ground potatoes with a decanter centrifuge. Since starch granules have a diameter between 1 and 120 μm and fiber particles have one between 80 and 500 μm , they are separated by sieving (Grommers and van der Krogt, 2009).

Werpy et al. (2005) state that the Danish potato starch factories produce 200000 tons of starch annually, in which 150000 tons of PP are generated as well. This means that for every ton of starch, 750 kg of fresh PP are generated. 1.6 million tons of potato starches and derivatives were produced in

Europe 2021 and globally it can be estimated that there are around 3.9 million tons of potato starch produced in 2020. This leads to a global PP generation of 2.9 million tons. The dry matter content is around 13 wt%, therefore it can be estimated that there are around 380000 tons of dry PP generated (Mayer, 1998, Starch Europe, 2021a, Vilpoux and Santos Silveira Junior, 2023).

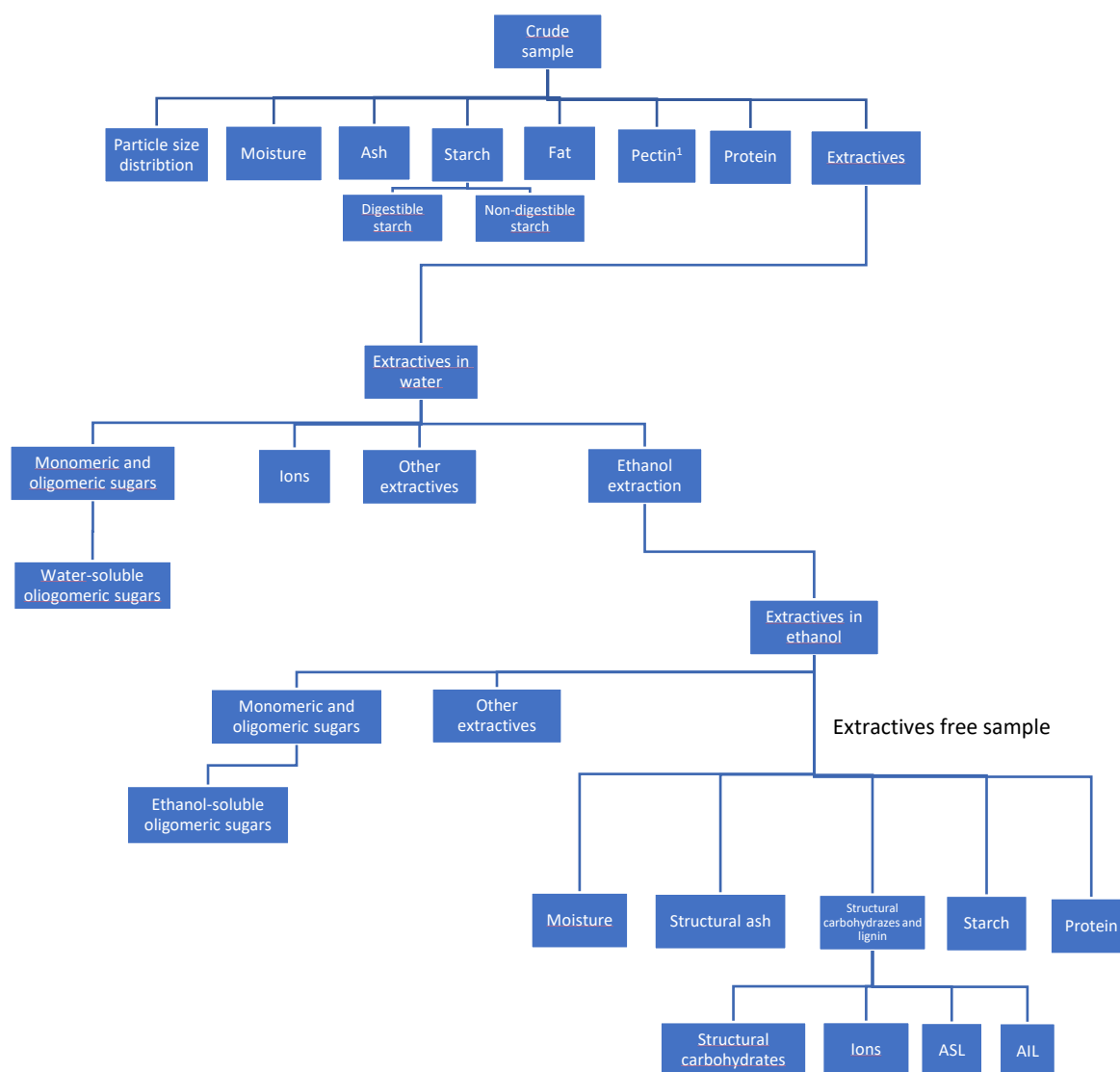
The starch is by far the most valuable material, making up more than 90 % of the sales value, while the fiber, protein and the deproteinized potato juice represent less than 10 % of the sales value (Grommers and van der Krogt, 2009). This is favorable for biorefineries because they can use a cheap raw material.

1.3 Discussion of the methods

As described in the previous sections, the composition of WB and PP can vary. Therefore, it is important to characterize the feedstock because the composition of it determines the uses in a biorefinery. Often, there is more than one method to determine the content of a specific substance in a biomass sample. Hence, it can be necessary to compare methods. However, in practice there are limitations that exclude certain methods or there are standard methods which are commonly used. It is useful to follow these methods, which was the case for most methods used in this work. In this section, most of the used methods for the determination of the components of WB and PP are discussed briefly.

Most of the components of WB and PP were determined via NREL LAPs, because NREL has developed a suite of LAPs which is built on a lot of experience. Additionally, it is ensured that interferences are avoided while allowing for a summative mass closure and a characterization of all components (Sluiter and Sluiter, 2010).

First, it was necessary to create a pathway for the characterization of the biomass samples to avoid interferences and to determine the amount of the constituents of interest. For this, the LAP Summative Mass Closure developed at the NREL was used and modified (Sluiter and Sluiter, 2010). In addition, a pathway of the characterization of the feedstocks in the form of a flow chart was created, which can be seen in Figure 2.



¹ only done for PP

Figure 2: Pathway for the analysis of PP and WB

1.3.1 Particle size characterization

Larger particles may lead to an incomplete hydrolysis of structural carbohydrates which causes a low bias in carbohydrate content and a high bias for AIL. A particle size of less than 180 µm may lead to an over hydrolysis of the polymeric sugars, resulting in a low bias in carbohydrate content. Additionally, the overproduction of sugar degradation products may lead to an overestimation in ASL (Sluiter and Sluiter, 2010, Hames et al., 2008). Gaining information about the grain size distribution by sieving is necessary because the NREL procedures used in this thesis have been optimized for a particle size range between 180 and 850 µm (Hames et al., 2008).

1.3.2 Determination of the moisture content

Knowledge about the moisture content is needed because the content of the components of the samples is referred to the dry mass of the samples. In addition, if the sample contains more than 10 wt% moisture, it may lead to an incomplete hydrolysis during the determination of the structural carbohydrates and lignin because the acid concentration will be lowered. This may result in a low bias in carbohydrate content and consequently in a high lignin bias (Hames et al., 2008).

1.3.3 Determination of the ash content

Ash is defined as the inorganic residue that is left after dry oxidation at 575 °C (Sluiter et al., 2008b). The determination of the ash content was done because ash is a part of biomass and an ash content above 10 wt% can lead to interferences with the acid concentration during the hydrolysis step of the determination of structural carbohydrates and lignin and possibly cause side reactions (Sluiter et al., 2008a).

1.3.4 Determination of the starch content

Starch is a polysaccharide and it is easier to break down to its monomer than hemicellulose and cellulose. Thus, the starch content is an important characteristic of a raw material regarding its use in a biorefinery. For the determination of the starch content, an enzymatic method was chosen, which is described in detail in section “2.4 Determination of the starch content”. With this used method, the starch can be divided into RDS, SDS, TDS and digestion resistant starch or amylase resistant starch (RS).

Since a high RS content reduces the conversion of starch into sugars, it is important for a biorefinery to know the amount of RS in biomass. It is possible to hydrolyze RS at higher temperatures, but this adds energy costs to a process (Sharma et al., 2010).

1.3.5 Determination of the fat content

The determination of the fat content was determined in accordance with the standard DIN ISO 11085:2015. This method differentiates between the raw and the total fat content. Raw fat is directly extractable with petrol ether. For the determination of the total fat content, an acid hydrolysis with HCl is necessary before the extraction with petrol ether. This is done to get access to the mechanically and chemically bound fats (DIN, 2016).

1.3.6 Determination of the pectin content

The pectin content was determined according to Yang et al. (2018) and Zaidel et al. (2015). Since the pectin in these papers was analyzed further, it was assumed that only pectin was extracted. Furthermore, the yield was in accordance with literature (Mayer and Hillebrandt, 1997, Klingspohn et

al., 1993). Some changes were made to facilitate the procedure, which are described in the methods and material part of this thesis.

1.3.7 Determination of the protein content

The Kjeldahl method was used to determine the nitrogen content because it is one of the suggested methods from the NREL and the AOAC. To convert it to the protein content, a conversion factor is used. To determine how much protein was removed during the extraction processes, the protein content of the extractives free sample was also determined.

1.3.8 Determination of extractives

For a complete quantification of extractives, a Soxhlet extraction with water followed by a Soxhlet extraction with ethanol were conducted. Thus, non-structural materials soluble in water were removed via the first extraction and non-structural materials soluble in ethanol were removed with the second extraction. The main reason for these extractions is to avoid interferences with subsequent analyses (Sluiter et al., 2008c). To identify valuable components of interest, the water and ethanol extractives were also examined via GC-MS.

1.3.9 Determination of structural carbohydrates

Structural carbohydrates, which are bound in the biomass, make up a significant part of biomass. They consist of cellulose and hemicellulose. The structural carbohydrates were hydrolyzed into the monomeric carbohydrates glucose, xylose, galactose, arabinose and mannose, which were measured. Since knowledge about the composition of them is important for a fermentation process, they were determined (Sluiter et al., 2008a).

1.3.10 Determination of ions

The ion content in WB and PP was determined because acetate because hemicellulose contains acetyl groups which are converted to acetate during hydrolysis (Delgenes et al., 1996, Bajpai, 2018). Acetate is a fermentation inhibitor, which is why information about the acetate content is valuable (Samson et al., 1955).

1.3.11 Determination of lignin

Lignin was determined according to the NREL LAP Determination of Structural Carbohydrates and Lignin which differentiates between ASL and AIL. The ASL is determined photometrically, which is why an absorptivity constant is needed for the calculation of the ASL content (Sluiter et al., 2008a). Thus, a literature search was conducted, which led to a value of $110 \text{ L g}^{-1} \text{ cm}^{-1}$ at a wavelength of 205 nm. The

AIL content is determined via the Klason method, which is very commonly used (Hatfield and Fukushima, 2005).

2 Methods and materials

In this chapter, the different methods for the characterization of PP and WB are described. At first, a pathway is presented according to how the samples were analyzed. After that, the methods to determine the constituents of the two feedstocks are described. WB and PP were analyzed with the same methods except for pectin, which was only determined in PP.

Dried PP and WB were obtained from a regional company and stored at room temperature in reclosable plastic bags.

The pathway of the characterization of the feedstocks in the form of a flow chart can be seen in Figure 2. The determination of moisture, ash, starch, fat, pectin and protein were done with the crude sample.

2.1 Particle size distribution

The particle size distributions of PP and WB were measured using a Retsch vibratory sieve shaker AS200 control (Retsch, Haan, Germany) with a sieve cascade. Both samples, WB and PP, were processed in the state they arrived and no further grinding or drying process was implemented. The mesh sizes of the sieves for the sieving of PP and WB are listed in Table 1:

Table 1: Parameters of the sieving analysis

Sieve number	Mesh size (μm)	Feedstock
1	0	WB, PP
2	224	WB, PP
3	280	WB, PP
4	315	WB, PP
5	400	WB, PP
6	500	WB, PP
7	630	WB, PP
8	800	WB
9	1250	WB

For both samples, PP and WB, the sieving time was 3 minutes and the sieving intensity was 0.7 mm g^{-1} . 64.74 g of PP and 62.99 g of WB were sieved.

With the known mesh sizes and the masses of the oversize particles, the distribution density and distribution sum are calculated. The respective equations are listed in section “7.1.1 Particle size distribution”.

2.2 Determination of the moisture content

The moisture content of the samples was determined using a Shimadzu MOC63u electronic moisture analyzer (Shimadzu Europa GmbH, Duisburg, Germany) in accordance with the instructions of the device. The Shimadzu MOC63u electronic moisture analyzer displayed the moisture content of the sample on a wet basis. The respective equation can be found in section “7.1.2 Determination of the moisture content”.

The mass of the oven dry sample was calculated according to Equation 7, which can be found in section “7.1.2 Determination of the moisture content”.

2.3 Determination of the ash content

The ash and structural ash content was measured conforming to the adapted NREL LAP Determination of Ash in Biomass (Sluiter et al., 2008b). For the determination of the ash content, an untreated sample was used, while for the determination of the structural ash content, an extractives-free sample was used. The procedure is the same for both samples.

According to the LAP, the marked empty porcelain crucibles were placed in a muffle furnace at 600 °C overnight and weighed after the crucibles reached room temperature. After that, the samples were weighed into the crucibles which were then covered with a lid to avoid a loss of sample during the ashing process. Subsequently, the crucibles were placed in a muffle furnace at 600 °C and ashed to constant weight.

To calculate the ash content on a dry matter basis, it is necessary to calculate the oven dry sample weight at first, as described in Equation 7 in section “7.1.2 Determination of the moisture content”. For the calculation of the total ash content, Equation 8 was used. To calculate the content of structural ash based on the untreated sample, Equation 9 was used. Both equations are listed in the appendix in section “7.1.3 Determination of the ash content”. With the known amount of total and structural ash, the content of extractable ash can be calculated, which is the difference between total and structural ash.

2.4 Determination of the starch content

2.4.1 Chemicals

For the starch determination, a “Digestible and Resistant Starch Assay Kit” (Megazyme, Bray, Ireland) was purchased, which included the following chemicals and enzymes:

- A mixture of purified pancreatic- α -amylase (PAA) with 40 KU g⁻¹ and amyloglucosidase (AMG) with 17 KU g⁻¹
- Amyloglucosidase (3300 U mL⁻¹)
- GOPOD Reagent Buffer: consisting of p-hydroxybenzoic acid and sodium azide
- GOPOD Reagent Enzymes: consisting of glucose oxidase, peroxidase and 4-aminoantipyrine
- D-Glucose Standard Solution (1 mg mL⁻¹) in 0.2 % (w/v) benzoic acid
- Digestible/Resistant Starch Control sample with an unknown type of starch

In addition, the following reagents were used:

- 96 vol% ethanol, from Sigma-Aldrich, Darmstadt, Germany
- Toluene, from Thermo Fisher Scientific, Geel, Belgium
- Maleic acid, from Sigma-Aldrich, Darmstadt, Germany
- Glacial acetic acid, from Sigma-Aldrich, Darmstadt, Germany
- Sodium hydroxide, from Merck KGaA, Darmstadt, Germany
- Calcium chloride dihydrate, from Sigma-Aldrich, Darmstadt, Germany

2.4.2 Method for starch determination

The starch determination of the samples was conducted according to the Digestible and Resistant Starch Assay Protocol K-DSTRS from NEOGEN and Megazyme with some modifications that are explained in the following (Megazyme, 2022). The moisture of the samples was determined as described in section “2.2 Determination of the moisture content”. For the determination, untreated WB and PP as well as extractives free PP was used.

For the starch determination, which started with the enzyme digestion of the sample, 0.5 g of sample were used. For the digestion, a 50 mL Schott flask was used. A water bath was put on a Rotilabo MH 15 heater and magnetic stirrer (Carl Roth, Karlsruhe, Germany). The Schott flask with the sample, a stirrer bar, 0.5 mL 96 vol% ethanol and 17.5 mL of 50 mM sodium maleate buffer were put in the water bath with a temperature of 35-39 °C, which was checked with a thermometer. The stirrer was set to 350 rpm. The equilibration lasted for 5 min and after that 2.5 mL of the PAA/AMG solution were added. After 20 min, 2 h and 4 h 1.0 mL of the reaction solution was removed and added to 20 mL of 50 mM

acetic acid for the determination of RDS, SDS and TDS, respectively. After mixing, 2 mL of each solution were filled into Eppendorf tubes and centrifuged at 13000 rpm for 5 min.

Each 0.1 mL of the centrifuged sample solution were transferred twice into two test tubes, mixed with 0.1 mL of diluted AMG and incubated at 50 °C for 30 min in a water bath type GFL-1083 (LAUDA DR. R. WOBSE GMBH & CO. KG, Lauda-Königshofen, Germany). Next, 3.0 mL of GOPOD reagent were added, and the samples were incubated at 50 °C for 20 min.

At the same time, a reagent blank solution and four D-glucose standards were prepared. The reagent blank solution was prepared by adding 3.0 mL of GOPOD reagent to 0.2 mL of 100 mM acetic acid and homogenizing the solution. Next, 3.0 mL of GOPOD reagent were added and the sample was incubated at 50 °C for 20 min. The D-glucose standards were prepared by adding 0.1 mL of the provided D-glucose solution and 0.1 mL 100 mM acetic acid to 3.0 mL of GOPOD reagent. All of these solutions were incubated at 50 °C for 20 min. The absorbance of the samples and standards was measured with a Thermo Fisher Scientific NanoDrop One^c (Thermo Fisher Scientific, Geel, Belgium).

Also, after 4 h, 4.0 mL of the solution were removed to determine the amount of RS. For this, 4.0 mL 96 vol% ethanol were filled in a FalconTM tube, to which were then added 4.0 mL of the stirring suspension. Afterwards the tube was inverted several times and centrifuged at 4000 rpm for 10 min. The supernatant solution was decanted and the pellet was resuspended in 2 mL of 50 vol% aqueous ethanol. Next, 6 mL of 50 vol% ethanol were added and the contents were vortexed and centrifuged at 4000 rpm for 10 min. The supernatant solution was removed again and the aforementioned procedure with adding ethanol, vortexing, centrifuging and decanting was repeated.

To the tube were added 2 mL of cold 1.7 M NaOH solution and the contents were stirred in an ice/water bath over a magnetic stirrer at 500 rpm for 20 min. After that, 8 mL of 1.0 M sodium acetate buffer were added under stirring. Promptly, 0.1 mL of AMG were added, everything was mixed and the tube was put in a water bath at 50 °C for 30 min. The tube was vortexed every 5 min.

The contents were transferred quantitatively to a 100 mL volumetric flask, the remaining volume was made up with deionized water and everything was homogenized. 2 mL were transferred into an Eppendorf vial and centrifuged at 13000 rpm for 5 min.

Each 0.1 mL of the sample solution were transferred into two glass test tubes, mixed with 0.1 mL of 100 mM sodium acetate buffer and 3.0 mL GOPOD reagent. The solution was incubated at 50 °C for 20 min. The reagent blank and the D-glucose standards were prepared as described for the digestible starch samples as well as the measurement of the absorbance of the sample and the standards.

The equations to calculate the content of RDS, SDS, TDS, RS and TS are listed in section “7.1.4 Determination of the starch content”.

2.5 Determination of the fat content

2.5.1 Chemicals

- Petroleum ether, boiling range from 40-60 °C, from Sigma-Aldrich, Darmstadt, Germany
- 37 wt% HCl, from Sigma-Aldrich, Darmstadt, Germany
- Diatomaceous earth, from Merck KGaA, Darmstadt, Germany
- Acetone, from Sigma-Aldrich, Darmstadt, Germany

2.5.2 Experimental procedure

Adapted from DIN ISO 11085:2015, the fat content was determined (DIN, 2016). Both the crude and raw fat content were determined.

2.5.2.1 Preparations

For the filtering, the diatomaceous earth was boiled in 6 M HCl for 30 min, then rinsed until it was acid free and dried at 130 °C, as described in the standard.

2.5.2.2 Hydrolysis for the determination of the total fat content

Approximately 5 g of PP and WB, were put in a 250 mL round-bottomed flask along with a magnetic stirrer bar and 100 mL of 3 M HCl. Two hydrolyses of the same sample type were done at the same time. A Dimroth condenser was put on top of the round bottomed flask. The flask was heated with a heating mantle and a magnetic stirrer was put underneath. The mixture was boiling for 1 h under continuous stirring. After that, 6 to 7 g of the prepared diatomaceous earth were put in the flask and it was left to cool to room temperature. The next steps were done as described in the standard: The suspension was filtered through a Büchner funnel containing two sheets of filter paper and the residue was washed with cold water, until the filtrate was neutral. All surfaces were cleaned with cotton soaked in acetone. For the drying, the Büchner funnel with its contents was put overnight in a Lyophilizer Christ 1-4 LD (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany).

2.5.2.3 Determination of the raw fat content

The raw fat content was determined according to the instructions of the standard and the requirements of the used Büchi-B811 extraction system (BÜCHI Labortechnik AG, Flawil, Switzerland) (DIN, 2016, BÜCHI Labortechnik AG, 2016). The total fat content was determined in the same way as the raw fat content, but the hydrolyzed sample was used instead of the untreated sample. In detail, the solvent cups containing two boiling stones were weighed and the dried residue of the filtration

with the cotton and filter papers were put in a cellulose thimble (Cytiva, Vienna, Austria). The Randall extraction with petrol ether lasted for 50 min.

The solvent cups with the extracted fat were dried in a drying oven at 70 to 100 °C for a short period (20 to 160 min). After this period, the solvent cups were put in a desiccator to cool to room temperature and weighed afterwards. The drying and weighing continued until the weight increased again which was attributed to oxidation of the fat.

2.6 Determination of the pectin content

2.6.1 Materials

- H₂SO₄, from Sigma-Aldrich, Darmstadt, Germany
- HCl, from Sigma-Aldrich, Darmstadt, Germany
- Ethanol from Merck KGaA, Darmstadt, Germany
- Soluble starch from Merck KGaA, Darmstadt, Germany
- Citric acid monohydrate from Merck KGaA, Darmstadt, Germany
- Pectin from citrus peel from Thermo Fisher Scientific, Geel, Belgium
- GAMALPHA SPEZIAL α -amylase, from AB Enzymes GmbH, Darmstadt, Germany
- GAMMADEX CAL glucoamylase, from AB Enzymes GmbH, Darmstadt, Germany

2.6.2 Appliances

- Sorvall RC 6+ high speed centrifuge (Thermo Scientific, Geel, Belgium)
- Lyophilizer Christ 1-4 LD (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany)
- Bruker Vertex 70 FTIR spectrometer (Bruker, Vienna, Austria), software used for evaluating the spectra: OPUS 7.0.129

2.6.3 Overview of the method

The pectin content was only determined for PP, because in contrary to PP, in literature it was not reported that WB contained pectin. Pectin was isolated following the methods of Zaidel et al. (2015) and Yang et al. (2018) with some modifications. Two types of PP samples were analyzed, one was enzymatically treated with α -amylase and glucoamylase and the other one remained untreated. This was done to check if starch interferes with the pectin determination. A detailed description of the enzymatic treatment is given in section “2.6.4 Enzymatic treatment of potato pulp”. The untreated sample is called the blank in the following. After the treatment, pectin was extracted with HCl and citric acid, respectively. This leads to a total of 4 different pectin samples:

- pectin extracted with HCl from enzymatically treated PP, in short EHCl
- pectin extracted with citric acid from enzymatically treated PP, in short ECA
- pectin extracted with HCl from non-enzymatically treated PP, in short BHCl
- pectin extracted with citric acid from non-enzymatically treated PP, in short BCA

After that, the extracted and dried pectin was weighed and analyzed via FTIR-spectroscopy.

The whole process is described in the following section and structured into the enzymatic treatment and the actual extraction of pectin with the analysis in the FTIR spectroscope.

2.6.4 Enzymatic treatment of potato pulp

11.25 g of PP were put in a 500 mL Schott bottle along with 250 mL deionized water. For the gelatinization, it was heated in a water bath with a temperature of 75 °C. After 15 minutes, 22.5 µL of thermoresistant α -amylase (GAMALPHA SPEZIAL) were added to the flask to start the liquefaction. This corresponds to a dosage of 2 µL of enzyme solution per g substrate. The reaction mixture was kept in the water bath for 60 minutes. In the next step, it had been cooled in an ice bath for 10 minutes before the pH was adjusted to 5.5 using 1 M H₂SO₄. For the saccharification, 22.5 µL of amyloglucosidase (GAMMADEX CAL) were added and the flask was incubated for 3 h in a water bath at 55 °C. After that, the PP was washed twice with each 740 mL of water and twice with each 500 mL of 96 vol% ethanol. Lastly, the PP was dried thoroughly at 50 °C for 40 h and the moisture content was determined as described in section “2.2 Determination of the moisture content”.

2.6.5 Extraction and analysis of pectin

Pectin was extracted using two similar methods that are described in the following. For the first method, 5 g of enzymatically treated PP were mixed with 175 mL of deionized water and 3 g of the blank sample of PP were mixed with 105 mL of deionized water in a beaker. The pH was adjusted to 2.00 with citric acid and 10 M HCl, respectively.

In the next step, the suspension was heated under continuous stirring with a magnetic stirrer to around 90 °C for about 1 h. Due to the high viscosity of the mixture, it was difficult to maintain the desired temperature. Then, the samples were filled into centrifugation flasks. To ensure a complete transfer of the samples, 40 mL of deionized water were used for the blank samples to rinse the beakers, while for the enzymatically treated samples, the beakers were rinsed twice with each 25 mL of deionized water. The samples were centrifuged for 30 min at 10000 rpm and 10 °C using a Sorvall RC 6+ high speed centrifuge. After the centrifugation, the liquid that was above the sludge was carefully moved into a 250 mL Schott flask and neutralized with 5 M NaOH solution. The samples BHCl and EHCl were neutralized with 1 M HCl. 75 mL of 96 vol% ethanol were added to the sample BCA, which was thrice

the amount of the liquid in the Schott flask. As a result, the pectin formed a white precipitate. All samples and the sludges were stored in a refrigerator for 16 h.

The precipitated pectin was moved onto a filter paper, which had been weighed and put onto a Büchner funnel before. The pectin was washed twice with 25 mL of 70 vol% ethanol each. This washing step was repeated with 80 and 90 vol% ethanol. Lastly, the extracted pectin was dried with the filter paper in a lyophilizer to constant weight and then weighed.

For the second method, 3 g of raw or untreated PP were used for each of the four samples. Firstly, 105 mL of deionized water were added. The next two steps, the pH adjustment and the heating were identical to the first method. However, instead of centrifuging, the suspension was separated by filtration using filtering clothes and the liquid was filled into a 50 mL Falcon tube, was then centrifuged for 10 minutes at 13000 rpm and 10 °C. The now clear liquid was neutralized with 5 M NaOH and filled into a Schott flask. After that, three volumes of 96 vol% ethanol were added and the precipitated pectin in ethanol was stirred on a magnetic stirrer for 5 minutes. The last steps were the same as in the first procedure.

The isolated and dried pectin was analyzed in a FTIR spectrometer for which it had been ground before. To check for residual amounts of starch and citric acid in pectin, spectra from soluble starch and citric acid were also acquired.

The pectin content was calculated according to Equation 14, which can be found in section “7.1.5 Determination of the pectin content”.

2.7 Determination of the protein content

The protein content was determined by the Kjeldahl method, which measures the total nitrogen in a sample and is briefly described in the following paragraph. The procedure was conducted by Dipl.-Ing. Zdravka Saracevic and Dr.techn. Ernis Saracevic.

For the Kjeldahl method, the organic components of the sample are broken down in the presence of a catalyst with H_2SO_4 . After alkalization of the reaction product, the distillation of the released ammonia takes place, which reacts with H_2SO_4 in the receiving flask during the distillation. Lastly, the remaining H_2SO_4 in the receiving flask is titrated with NaOH. The same procedure is repeated with a blind sample that does not contain any nitrogen. The nitrogen content of the sample can be calculated from the consumption of NaOH for the titration of the remaining H_2SO_4 after the reaction of the H_2SO_4 with ammonia and the H_2SO_4 from the blank sample respectively. To get from the nitrogen content to the protein content, a conversion factor of 6.02 for WB and 6.25 for PP is used by which the nitrogen

content is multiplied (DIN, 2005). Since the protein content of the untreated sample and of the extractives free sample were determined, the content of extractable protein from the raw sample can be calculated by forming the difference between the protein content of the untreated sample and the protein content of the extractives free sample.

2.8 Determination of extractives

The determination of extractives is separated into the determination of water extractives and ethanol extractives. Water extractives were obtained by a Soxhlet extraction with water. In the next step, a Soxhlet extraction with ethanol was conducted to obtain the ethanol extractives.

2.8.1 Appliances and materials

- Büchi-B811 extraction system (BÜCHI Labortechnik AG, Flawil, Switzerland)
- Lyophilizer Christ 1-4 LD (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany)
- Direct-Q® 3 UV Water Purification System (Merck KGaA, Darmstadt, Germany)
- Extraction thimbles from Cytiva, Vienna, Austria and BÜCHI Labortechnik AG, Flawil, Switzerland

2.8.2 Water extractives

The Soxhlet extraction of WB and PP with water as a solvent was conducted following the instructions of the used Büchi-B811 extraction system and the NREL LAP Determination of Extractives in Biomass to obtain the water extractives (Sluiter et al., 2008c, BÜCHI Labortechnik AG, 2016). 9.5 g of sample, and glass wool were put in a tared extraction cellulose thimble with known weight. Four samples were extracted with RO water in a single extraction run.

The extraction time was 24 h, as stated in the NREL LAP Determination of Extractives in Biomass (Sluiter et al., 2008c). Since water was used as a solvent, the maximum heating power was set.

After the extraction, the thimbles with the samples were dried to constant weight using a drying oven or a lyophilizer, respectively. The initial drying was done in the drying oven to avoid a formation of mold. The temperature of the drying oven was 60 °C and the pressure in the lyophilization chamber was set to 1 mbar. The weight of the dried thimbles was noted. Next, the samples in the thimbles were extracted with ethanol, what is described in section “2.8.3 Ethanolic extractives”.

The water extracts were frozen and stored at -22 °C until further use.

2.8.3 Ethanolic extractives

The dried thimbles from the water extraction were continued to use for the ethanol extraction. Four samples were extracted with absolute ethanol in a single extraction run.

The chosen extraction mode was “Soxhlet standard” and the extraction time was 24 h, as stated in the NREL LAP Determination of Extractives in Biomass (Sluiter et al., 2008c).

After the extraction, the thimbles with the samples were dried to constant weight using a drying oven or a lyophilizer, respectively. The initial drying was done in the drying oven to avoid a formation of mold. The temperature of the drying oven was 60 °C and the pressure in the lyophilization chamber was set to 1 mbar. The weight of the dried thimbles was noted. The extracted sample was directly used for the determination of moisture, ash and protein. For PP the starch content was also determined. It was also hydrolyzed to determine the amount of structural carbohydrates, ASL and AIL. Furthermore, the ions in the hydrolysis liquor were also measured as described in section “2.11 Determination of ions”.

The ethanol extracts were frozen and stored at -22 °C until further use. The ethanol extracts were analyzed in the same way as the water extracts except for ions.

To calculate the content of water and ethanol extractives, Equation 17 was used, which can be found in section “7.1.7 Determination of extractives”.

2.9 Determination of structural carbohydrates

To determine the structural carbohydrates of PP and WB, the extractives free samples had to be hydrolyzed to break the polymeric carbohydrates down into the monomeric forms. The monomers were then analyzed via High Performance Anion Exchange Chromatography with pulsed amperometric detection (HPAEC-PAD). The procedure as well as the used chemicals and appliances are described in the following section.

2.9.1 Chemicals:

The following chemicals were used to create the calibration curves, while the H₂SO₄ was used for the hydrolysis:

- D-(+)-Mannose ≥ 99 %, from Sigma Aldrich, Darmstadt, Germany
- D-(+)-Xylose ≥ 99 %, from Sigma Aldrich, Darmstadt, Germany
- L-(+)-Arabinose ≥ 99 %, from Sigma Aldrich, Darmstadt, Germany
- D-(+) Galactose ≥ 99 %, from Sigma Aldrich, Darmstadt, Germany
- D-(+)-Glucose ≥ 99.5 %, from Sigma Aldrich, Darmstadt, Germany
- D-(-)-Fructose ≥ 99.0 %, from Merck KGaA, Darmstadt, Germany
- H₂SO₄, from Sigma-Aldrich, Darmstadt, Germany

2.9.2 Experimental Procedure

The determination of structural carbohydrates was done in accordance with the adapted NREL LAP Determination of Structural Carbohydrates and Lignin in Biomass (Sluiter et al., 2008a). 3 mL 72 wt% H₂SO₄ were added to 300 mg extractives-free sample in a reclosable Pyrex tube. The suspension was stirred with a spatula and incubated in a shaking bath at 30 °C during 1 h. After the incubation, a graduated cylinder was used to measure 84 mL of water used for diluting the H₂SO₄ to 4 wt%. For the second part of the hydrolysis, SRS were prepared, as described in the following paragraph:

To correct for the degradation of sugars during hydrolysis, SRS were also hydrolyzed with the samples in the second hydrolysis (Sluiter et al., 2008a). The SRS consist of arabinose, galactose, glucose, xylose and mannose and have a concentration of 1, 5, 25 and 50 mg L⁻¹ of each sugar before the hydrolysis. They were prepared, lyophilized and stored in a freezer at -20 °C until use. After they were thawed, 100 µL of 4 wt% H₂SO₄ were added.

The SRS and the diluted sample were autoclaved at 121 °C for 1 h in a Certoclav CV-EL 12L/18L tabletop autoclave (CertoClav Sterilizer GmbH, Leonding, Austria).

The hydrolysis liquor was filtered through a 0.2 µm Phenomenex syringe filter (Phenomenex Inc., Aschaffenburg, Germany). After that, the sample was diluted 1:10 and 1:100 with RO water and measured with the calibration standards and the SRS in the HPAEC-PAD system. With this system, the concentration of the monomeric sugars arabinose, galactose, glucose, xylose and mannose in PP and WB could be determined. Since fructose was degraded during hydrolysis, it could not be measured. The parameters of the HPAEC-PAD are described in the following table:

Table 2: Description of the used HPAEC-PAD-system

HPAEC-PAD	Thermo Fisher Dionex ICS-5000 ⁺ DC
Pump	Thermo Fisher Dionex ICS-5000 ⁺ DP; an isocratic and a gradient pump
Analytical column	Dionex CarboPac PA1, 2x250 mm Analytical
Guard column	Dionex CarboPac PA1, 2x50 mm Guard
Sampler	Dionex AS-AP Autosampler
Detector	ICS-5000 Electrochemical Detector with ICS-5000 Electrochemical Cell (Ag/AgCl)
Software	Chromeleon 7.2 SR5
Other	EO gas regulator for helium loading of the solvent

The method used five different eluents, namely eluent A consisting of degassed RO water, eluent B consisting of 200 mM NaOH, eluent C, consisting of 200 mM NaOH and 500 mM NaAc, eluent D consisting of RO water and eluent A-iso, consisting of 200 mM NaOH. The separation took place in pure water (eluent A) and post-column dosing of NaOH (A-Iso) with a flow rate of 0.13 mL min⁻¹ was necessary for the electrochemical detection. The flow rate of all eluents was 0.26 mL min⁻¹ and the column temperature was 30 °C. The flow rates of the individual eluents are listed in Table 23 in section “7.1.8 Determination of structural carbohydrates”.

10 µl of a sample were injected after the equilibration at minute 0.

The calibration standards used for the HPAEC-PAD had a concentration of each 1, 5, 10, 25 and 50 mg L⁻¹ of arabinose, galactose, glucose, xylose, mannose and fructose.

The software Chromeleon 7.2 SR4 was used for the peaks integration and data processing. The determined monomeric sugars were expressed in mg L⁻¹. The necessary calculations to compute the content of structural carbohydrates are found in the appendix in section “7.1.8 Determination of structural carbohydrates”.

2.10 Determination of the monomeric and oligomeric sugars

The source for the determination of the water soluble monomeric and oligomeric sugars were the water and ethanol extracts. These sugars were determined via HPAEC-PAD with the same parameters as described in section “2.9.2 Experimental Procedure” Since the HPAEC-PAD can only measure monomeric sugars, the oligomeric sugars had to be broken down into monomeric sugars via hydrolysis.

The ethanol extractives were lyophilized before further analysis. Water and ethanol extractives were hydrolyzed in the same way. To correct for the degradation of sugars during hydrolysis, SRS were added (Sluiter et al., 2008a).

The preparation of the oligomeric sugar samples described below also took place in airtight sealable Pyrex tubes. 1 mL of water extractives and 1 mL of 8 wt% H_2SO_4 were added, while the lyophilized ethanol extractives were dissolved in 1 mL water and then 1 mL of 8 wt% H_2SO_4 was added as well. In the next step, the samples and the SRS were autoclaved at 121 °C for 1 h in a Certoclav CV-EL 12L/18L tabletop autoclave. After the autoclaving, the water extracts were diluted 1:20 and 1:100 and the ethanol extracts were diluted 1:20. The following HPAEC-PAD for the determination of the sugars was carried out as described in the following paragraph. Both the hydrolyzed and diluted oligomeric sugars as well as the monomeric sugars were analyzed with the same parameters.

For the determination of the monomeric sugar content of the water and ethanol extracts, the water extracts were diluted 1:10 and 1:100 with RO water, while 1 mL of the ethanol extracts was at first dried in a lyophilizer and then 2 mL of RO water were added so that the analytes were within the calibration range.

The calculations for the determination of the monomeric and oligomeric sugars can be found in section “7.1.9 Determination of the monomeric and oligomeric sugars”.

2.11 Determination of ions

The following chemicals were used to create the calibration curves:

- Roti® Star Single Element IC-Standard-Solution Acetate 1000 mg L⁻¹; from Carl Roth, Karlsruhe, Germany
- Roti® Star Single Element IC-Standard-Solution Propionate 1000 mg L⁻¹; from Carl Roth, Karlsruhe, Germany
- Roti® Star Single Element IC-Standard-Solution Formiate 1000 mg L⁻¹; from Carl Roth, Karlsruhe, Germany
- Roti® Star Single Element IC-Standard-Solution Succinate 1000 mg L⁻¹; from Carl Roth, Karlsruhe, Germany
- Roti® Star Single Element IC-Standard-Solution Citrate 1000 mg L⁻¹; from Carl Roth, Karlsruhe, Germany
- Ion chromatography anion multi-element standard solution I, (3 elements in water) 1000 mg L⁻¹: F⁻, Br⁻, PO₄³⁻ Certipur®; from VWR International GmbH, Darmstadt, Germany
- Ion chromatography anion multi-element standard solution II, (3 elements in water) 1000 mg L⁻¹: Cl⁻, NO₃⁻, SO₄²⁻ Certipur®; from VWR International GmbH, Darmstadt, Germany
- Ion chromatography cation multi-element standard solution VI with 10 mg L⁻¹ NH₄⁺, 50 mg L⁻¹ K⁺, 100 mg L⁻¹ Na⁺, 100 mg L⁻¹ Ca²⁺, 100 mg L⁻¹ Mg²⁺ in 0.1 wt% HNO₃; from VWR International GmbH, Darmstadt, Germany

The chemicals used for the preparation of the eluents were:

- Methanesulfonic acid for pharma synthesis; from Merck KGaA, Darmstadt, Germany
- NaOH solution 50 wt% in water; from Fisher Chemical, Geel, Belgium

The anions fluoride, acetate, propionate, formate, chloride, bromide, nitrate, succinate, sulfate, phosphate, citrate and oxalate and the cations sodium, ammonium, potassium, magnesium and calcium were measured with the Thermo Fisher Dionex ICS-5000⁺ DC IC system using diluted hydrolysis liquors and diluted water extracts of PP and WB. With the ion concentration in the hydrolysis liquor the composition of the ions in the extractives free WB and PP can be calculated, which is done in section “7.1.10 Determination of ions”. How to obtain the hydrolysis liquors is described in section “2.9.2 Experimental Procedure”. The hydrolysis liquors were diluted 1:10 and 1:200, while the water extracts were diluted 1:20 and 1:500. The chromatography system consists of two IC systems that are in one housing. The autosampler loads samples in both systems. The specifications of the IC system are listed in the following table:

Table 3: Description of the used IC system

IC system	Thermo Fisher Dionex ICS-5000 ⁺ DC
Pump	Thermo Fisher Dionex ICS-5000 ⁺ DP-5, an isocratic and a gradient pump
Analytical columns	Anions: Dionex IonPac AS11-HC 4x250 mm Analytical
	Cations: Dionex CS12A 4x250 mm Analytical
Guard columns	Anions: Dionex ATC-HC 9x75 mm TrapColumn
	Cations: CG12A 4x50mm Guard
Sampler	Dionex AS-DV Autosampler with 25 µL sample loops each for anions and cations
Detectors	Thermo Fisher Conductivity Detector each for anions and cations
Suppressors	Anions: self-regenerating Dionex AERS 500 4 mm
	Cations: self-regenerating Dionex CERS 500 4 mm
Software	Chromeleon 7.2 SR4
Other	EO gas regulator for helium loading of the solvent

The gradient program with the respective eluent ratios for the separation and quantification of the organic anions is described in section “7.1.10 Determination of ions”.

There were four eluents used, A, B, C and D. Eluent A consisted of RO water and it was only used to clean the guard column. Eluent B consisted of 50 mM NaOH, eluent C of degassed RO water and eluent D of 5 mM NaOH. The flow rate was 1.5 mL min⁻¹, the AERS current was set to 19 mA, the temperature of the suppressor was 20 °C and the column temperature was 30 °C.

The elution of the cations was performed isocratically, in which 20 mM methanesulfonic acid was used as the eluent with a flow rate of 1 mL min⁻¹. The run time was 28 min, the column temperature was 30 °C, the temperature of the suppressor was 20 °C and the CERS current was set to 59 mA.

How to calculate the ion content is described in section “7.1.10 Determination of ions”.

2.12 Characterization of other extractives

Other extractives in the water and ethanol extracts were characterized via GC-MS. A comparison of the amount of the identified substances was done via FID that was connected with the used GC system. However, a complete quantification of the substances found via GC-MS was not the aim of this thesis.

2.12.1 Chemicals

- N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) ≥ 98 % for GC, from Carl Roth
- Pyridine water free 99.8 %, from Sigma Aldrich
- Chlorotri(methyl)silane (TMCS) ≥ 99.0 % for GC, from Sigma Aldrich

2.12.2 Experimental procedure

Besides the constituents shown in Figure 2, other substances that are included in water and ethanol extracts of PP and WB were determined via GC-MS after a derivatization. How to obtain the ethanol extracts, is described in section "2.8.3 Ethanolic extractives". For this, 50 µL of the water and ethanol extracts were lyophilized in airtight screwable Pyrex tubes. Then, 80 µL of BSTFA, 20 µL of pyridine and 20 µL of Chlorotri(methyl)silane (TMCS) were added to each tube. After that, the samples were derivatized in a drying oven for 45 minutes at 70 °C. For the GC-MS measurement, the samples were filled into vials with inlets. The specifications of the GC-MS system are listed in Table 4:

Table 4: Specifications of the used GC-MS system

GC system	Agilent Technologies 7890A GC-System
Detectors	Agilent Technologies 5975C-MSD and Agilent Technologies 7890A FID
Column	Agilent HP5-MS (30 m, 0.25 mm diameter, 0.25 µm film thickness)
Sampler	CTC Analytics PAL System (CombiPAL)
Software	Chemstation B.04.03 SP1 and Masshunter
Databases	NIST17 and Wiley-W10N11_Full

The flow rate of the carrier gas helium was 1.3419 mL min⁻¹. For the initiation, the temperature was 50 °C, the holding time and the run time were 1 min each. For the ramp phase, the heating rate was 10 °C min⁻¹, the temperature was 325 °C, the holding time was 10 min and the run time was 38.5 min.

2.13 Determination of lignin

The lignin determination consists of two parts, the determination of AIL and ASL. The procedure was done in accordance with the NREL LAP Determination of structural carbohydrates and lignin (Sluiter et al., 2008a). In detail, an extractives-free sample was hydrolyzed in the same way as described in section 2.9.2 Experimental Procedure. The hydrolysis liquor was vacuum filtered through a previously weighed filtering crucible. For the determination of AIL, the filtering crucible with the residue was dried at 100 °C for 16 h. After cooling to room temperature, the crucibles were weighed again. Next, the crucibles were put in a muffle oven at 650 °C for 49 h. ASL was determined by measuring the absorbance of the diluted filtered hydrolysis liquor at a wavelength of 205 nm with RO water as a blank. For this, a Shimadzu UV-1800 spectrophotometer (Shimadzu Europa GmbH, Duisburg, Germany) was used. The equations to calculate the content of AIL and ASL are listed in section “7.1.11 Determination of lignin”.

3 Results

In this chapter, the results of the determinations are presented. Furthermore, the calculations, which were necessary to get to the results are shown. If not otherwise stated, the masses in the equations are given in grams.

3.1 Particle size distribution

All the measured and calculated values are listed in section “7.2.1 Particle size distribution”. In the following diagrams, $q_3(x_{i,\text{mean}})$ and $Q_3(x_{0,i})$ of both WB and PP are depicted:

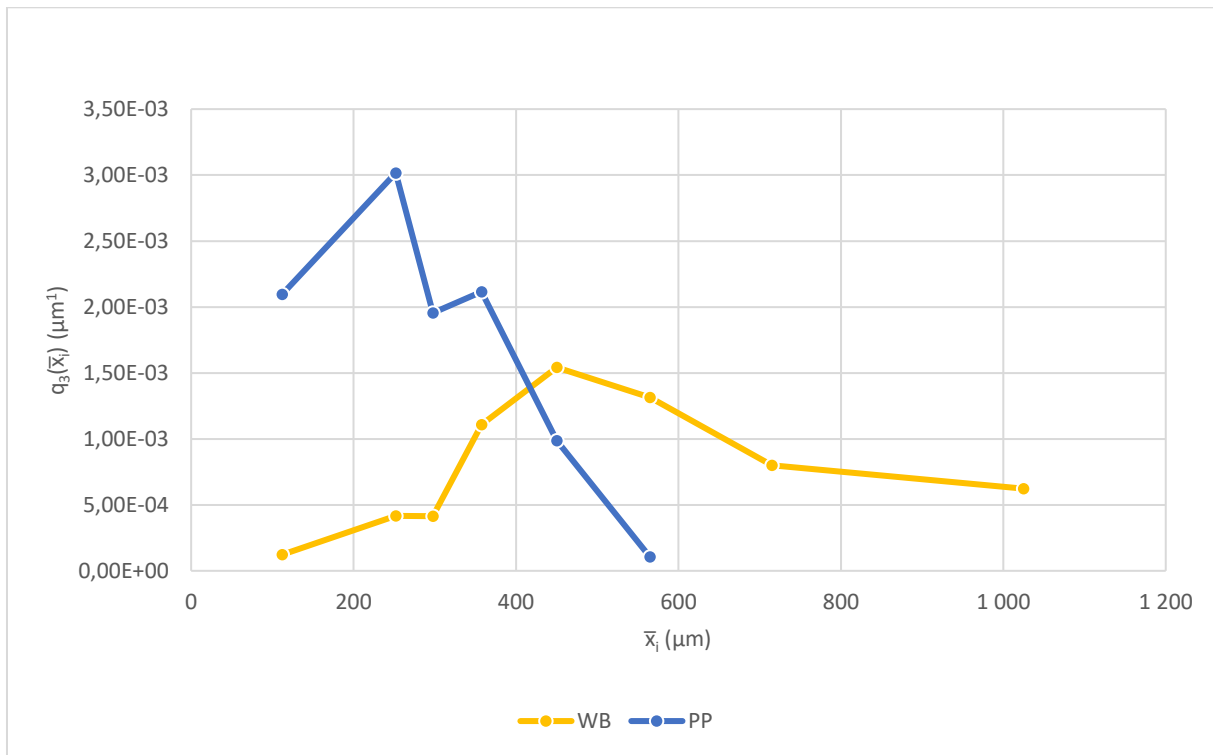


Figure 3: Distribution density of WB and PP

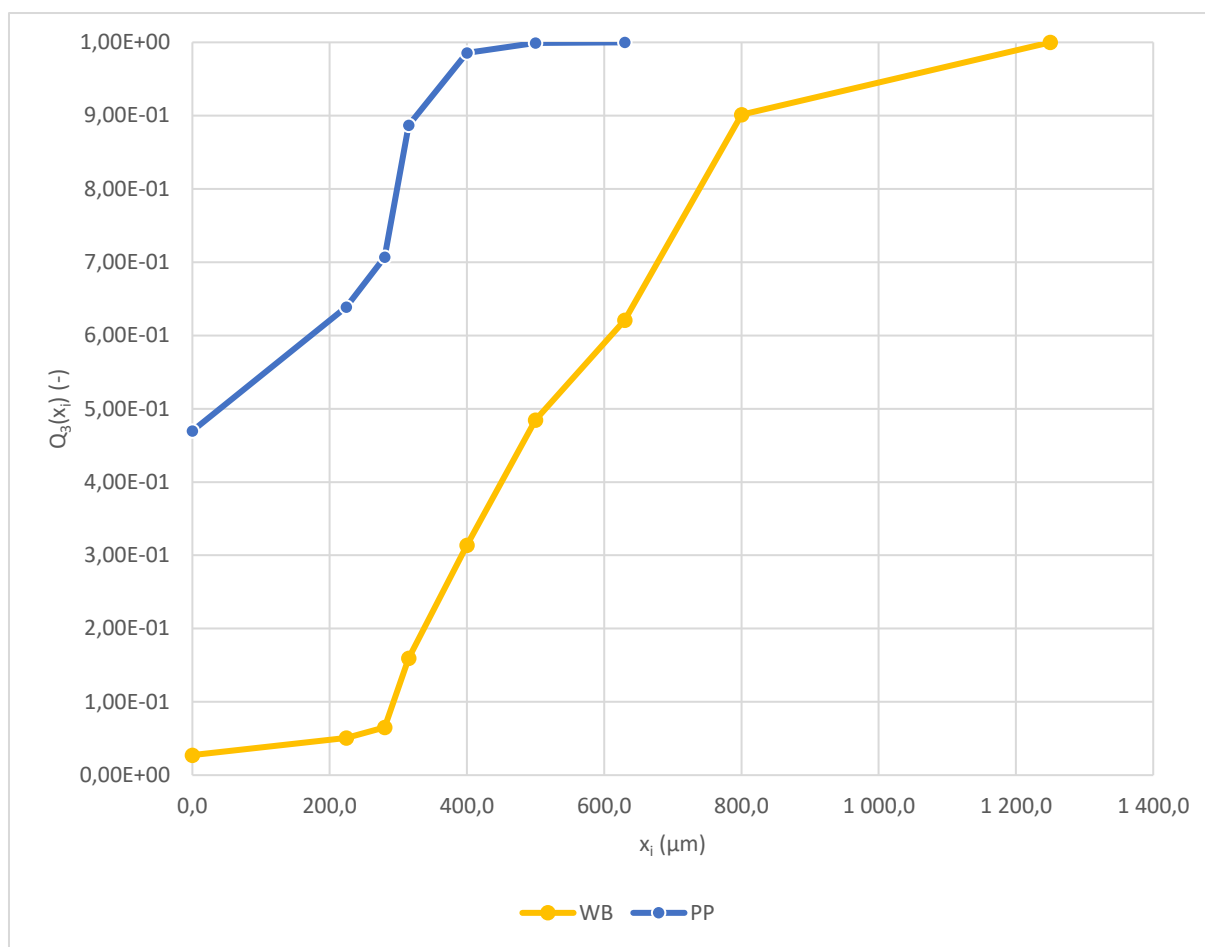


Figure 4: Distribution sum of WB and PP

From these diagrams can be seen that PP has on average a smaller particle size. For PP, sieves with smaller mesh sizes would have been necessary to gain more information about the distribution of the grain size. Furthermore, it is interesting that the distribution density of PP has another local maximum at 357.5 μm because one would expect that the distribution density only has one maximum. It has to be noted that the small particle size of PP may lead to a low value for the carbohydrate content and a high lignin bias (Hames et al., 2008). However, for the compositional analysis, an unsieved sample was used because the aim was to gain information about the whole composition of PP and not about a certain fraction.

For WB, the particle size distribution is more even and most of the grains have a mean particle diameter between 180 and 850 μm which is required for the compositional analysis. In the sieve with the grain size of 1250 μm was still sample left which means that a sieve with a larger mesh size would have given more information. WB could have been milled to avoid interferences with the determination of lignin and structural carbohydrates but it was not done because only a small fraction of WB was larger than 850 μm . Since the samples already have a particle size distribution suitable for further processing in the state they arrived, might make WB and PP favorable for further processing.

3.2 Moisture content

The values of every determination, the average moisture content, the standard deviation and the RSD of WB and PP are listed in the following table:

Table 5: Moisture content of PP and WB

	WB	PP
Values of the individual runs (wt%)	11.05	10.82
	10.96	10.83
	11.32	11.07
Average moisture content (wt%)	11.095	10.907
Standard deviation (wt%)	0.187	0.284
RSD (%)	1.69	2.58

For WB the measured moisture content is the initial moisture content, which means that WB does not have to be dried to be stored at room temperature. This could be favorable for industrial scale valorization. PP on the other hand was dried before it arrived at TU Wien, which means that the initial moisture content of PP is higher, as stated in section “1.2.2 Potato pulp”. The drying makes it possible for PP to be stored, with the disadvantage of additional energy consumption.

3.3 Ash content

The results of the determination of the ash content are listed in the following table and the individual values to calculate the results are in the appendix.

Table 6: Contents of structural ash, extractable ash and total ash for WB and PP, based on dry matter

		Structural ash content	Extractable ash content	Total ash content
WB	Average (wt%)	1.374	4.668	6.042
	Standard deviation (wt%)	0.145	0.175	0.030
	RSD (%)	10.6	3.75	0.50
PP	Average (wt%)	1.13	3.33	4.463
	Standard deviation (wt%)	0.08	0.60	0.109
	RSD (%)	7	0.19	2.44

There is less structural ash than extractable ash in both samples. Since both samples have an ash content below 10 wt%, there is no interference with the determination of structural carbohydrates and lignin (Sluiter et al., 2008a).

For comparison, the literature values for the ash content of WB vary between 4.6 and 10.4 wt% (Dornez et al., 2006, Roberts et al., 1985, Ye et al., 2021, Di Lena et al., 1997, Babiker et al., 2009, van

den Borne et al., 2012). Therefore, it can be said that the measured values match with the values reported in literature for WB.

For PP, the literature values of the ash content vary between 1.5 and 4.8 wt% (Yang et al., 2018, Antusiewicz and Święch, 2006, Mayer and Hillebrandt, 1997, Trabert et al., 2022). Like for WB, the measured values are in the same range as the values reported in literature.

3.4 Starch content

The following table shows the starch contents of WB, PP and extractives free PP, while the individual results are listed in the appendix:

Table 7: Results of the starch determination for WB, PP and extractives free PP, based on dry matter

Sample		RDS	SDS	TDS	RS	TS
WB	Average (wt%)	6.57	10.51	17.89	0.00	17.89
	Standard deviation (wt%)	0.49	0.86	0.62	0.00	0.62
	RSD (%)	7.4	8.1	3.5		3.5
PP	Average (wt%)	3.35	4.22	11.32	8.41	19.73
	Standard deviation (wt%)	0.89	1.07	1.70	1.75	3.45
	RSD (%)	26	25.3	15.0	20.8	17.5
Extractives free PP	Average (wt%)	18.74	1.541	19.02	0.664	19.69
	Standard deviation (wt%)	0.09	1.074	0.36	0.100	0.46
	RSD (%)	0.5	69.7	1.9	15.1	2.3

The sum of RDS and SDS is larger than TDS for extractives free PP, but considering the standard deviation, the sum of RDS and SDS is mostly comparable with the TDS. In addition, the method for the determination of glucose is very sensitive because it is based in spectrophotometry. The high standard deviation, especially for PP, is a general problem with this method.

The values for SDS and RS are lower for the extractives free PP than the untreated PP, while the TS the same for both samples. This means that during the extraction with water and ethanol, the RS was converted to RDS and no starch was extracted. Potato starch contains RS 2, which is a certain type of RS. It consists of RS granules. RS 2 is gelatinized in water at around 60 °C, which happened during the extraction with water (Champ, 2004). The gelatinization of starch increases the accessibility for the enzymes, which is why the RS was converted to digestible starch (Baks et al., 2008).

To check the results, a control sample with an unknown starch type that was supplied with the kit with a known amount of RDS, SDS, TDS and RS was measured thrice. The results are listed in Table 8:

Table 8: Measured and true values for RDS, SDS, TDS, RS and TS of the control sample from an unknown starch type, based on dry matter

	RDS	SDS	TDS	RS	TS
True value (wt%)	8.0	16.2	35.9	47.4	83.3
Average measured value (wt%)	9.684	19.33	42.29	39.07	81.36
Deviation of the measured value from the true value (wt%)	21	19.3	17.8	-17.6	-2.33
Standard deviation (wt%)	0.322	0.97	4.85	4.83	9.68
RSD (%)	3.32	5.0	11.5	12.4	11.9

The measured values of RDS and SDS of the control sample are with 21 and 19 wt% higher than the true values given by the manufacturer of the kit. There is a big absolute difference between the value of the manufacturer and the measured value for TDS which equates to a relative difference of 18 wt%. Furthermore, the standard deviation for this value is also high. It is unclear why the deviation to the true value is so high as well as the high standard deviation. If there was an error during the procedure, the RSD should be comparable for all samples. For WB and PP this means that the contents of RDS, SDS and especially TDS are lower than measured. However, the measured values of WB and PP were not adjusted because for TDS, RS and TS the standard deviations of the measured values of the starch control sample are quite high.

For RS, there is a similar problem, however the measured values are too low and the standard deviation remains high. Specifically, the measured value for RS is 18 wt% lower than the true value and the RSD is 12 wt%. For the case of RS, errors during the procedure can be the reason for the deviations, because it was difficult to remove the ethanol while keeping the starch between the centrifuging steps.

The TS values are comparable because the measured value is only 2 wt% lower than the true value. The high standard deviation comes from TDS and RS. This means that the used assay is suitable to determine the amount of TS in a sample, but it is not suitable for an accurate determination of the individual starch types. However, it must be kept in mind that the used experimental setup is slightly different to the setup described in the instructions of the kit.

For comparison, the starch content reported in literature for WB is in the range between 3.8 and 30.0 wt% (Amrein et al., 2003, Roberts et al., 1985, Reisinger et al., 2013, Hemery et al., 2007, Belitz et al., 2008, van den Borne et al., 2012, Xie et al., 2008, Ye et al., 2021, Dornez et al., 2006). It is probable that the wide range comes from different milling techniques which causes a different composition of WB. Additionally, the methods to determine the starch content vary. The measured value for RS does not match with the value reported in literature, which is 9.49 wt%. It was determined with the same method as in this work. However, the reported digesting time was 16 h instead of 4 h in this work, what could explain the difference (Xie et al., 2008).

For the starch content of PP, the literature values vary between 15.1 and 37 wt% (Brunt and Drost, 2010, Serena and Knudsen, 2007, Schmitz et al., 2021, Trabert et al., 2022, Mayer and Hillebrandt, 1997). The differences can be explained by different methods for the determination of the starch content and different technologies for the extraction of starch from potatoes.

3.5 Fat content

The fat content was calculated by dividing the difference between the mass of the solvent cup with the fat extract and the mass of the empty solvent cup by the oven dry weight of the sample. The results are listed in Table 9 and depicted in Figure 12:

Table 9: Results of the determination of the raw and total fat content, based on dry matter

		Raw fat	Total fat
WB	Average (wt%)	2.210	4.8359
	Standard deviation (wt%)	0.187	0.0679
	RSD (%)	8.44	1.41
PP	Average (wt%)	0.134	0.7501
	Standard deviation (wt%)	0.037	0.1058
	RSD (%)	28	14.10

For WB, the total fat content is about 2.2 times the raw fat content and for PP the total fat content is 5.6 times the raw fat content. In the used standard DIN EN ISO 11085, there is a comparison between the raw and the total fat content for 11 samples and the highest relative difference between the raw and total fat content is 160 %. For whole wheat grains, the total fat content is 46 % higher than the raw fat content (DIN, 2016). For comparison, the total fat content of WB and PP is 119 % and 460 % higher than the raw fat content.

The standard deviation of the raw fat content of PP can be attributed to one out of four values. Two out of four values are responsible for the high standard deviation of the total fat content. However, an explanation cannot be given for these deviations.

In literature, the raw fat content of WB ranges from 3.7 wt% to 5.5 wt% (Ye et al., 2021, Amrein et al., 2003, Reisinger et al., 2013, Babiker et al., 2009, Lee and Stenvert, 1973, van den Borne et al., 2012). For the total fat content, no literature sources were found. This means that the measured raw fat content is lower than what is reported in literature. This could be attributed to different milling or an incomplete extraction.

For PP, the raw fat content was reported in literature with 0.03 to 0.74 wt% (Abousteit and Kempf, 1974, Yang et al., 2018, Antusiewicz and Świąch, 2006). One paper reports the total fat content with 0.6 wt% (Serena and Knudsen, 2007).

3.6 Pectin content and identification of pectin

3.6.1 Pectin content

The different procedures for the pectin determination yielded different amounts of pectin, as can be seen in Table 10. The number 1 after the sample stands for method 1, number 2 for method 2.

Table 10: Pectin content of PP, based on dry matter

Input	Sample	Pectin content (wt%)
1	BCA1	22.55
2	ECA2	88.75
3	EHCl2	10.45
4	BCA2	112.18
5	BHCl2	21.00

Due to the unusual extremely high yield of pectin that was extracted with citric acid, the extraction with citric acid can be neglected. However, no IR spectrum was recorded for the pectin that was extracted with citric acid via the first method described in section “2.6.5 Extraction and analysis of pectin”. Therefore, it cannot be said if this method is suitable for the pectin extraction.

The content of pectin that was extracted with HCl varies a lot. Therefore, the isolated pectin samples were analyzed via IR spectroscopy. To compare with literature, the pectin content was stated with 15-20 wt% and 9.7 wt% with HCl used as an extracting agent. The papers that mention a pectin content with 15-20 wt% do not describe the extraction method (Mayer and Hillebrandt, 1997, Klingspohn et al., 1993). Thus, 10.45 wt% is the most reliable content compared to what is reported in literature. Impurities in the isolated pectin lead to higher yields which is why IR spectra of the pectin samples were obtained.

3.6.2 Identification of pectin

For the samples ECA2, EHCl2, BCA2 and BHCl2 IR spectra were acquired, as well as for citric acid, soluble starch and pectin from citrus peel. Therefore, it is possible to compare the spectra of the samples EHCl2 and BHCl2 with starch, which is done in Figure 5. These two samples were chosen because the pectin content is the closest to what is reported in literature. However, since starch and pectin have a similar structure, it is not easy to spot differences in an IR spectrum.

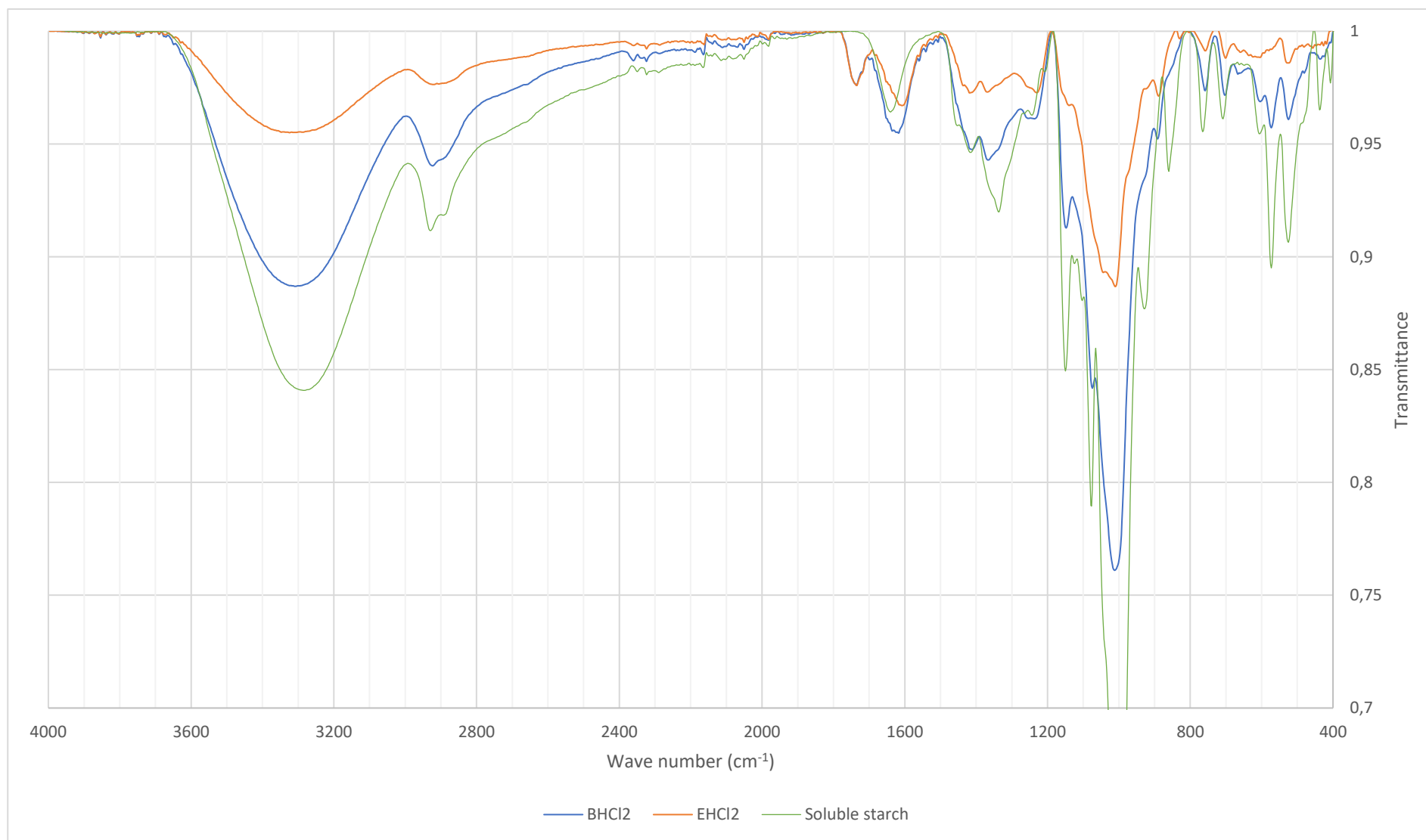


Figure 5: IR spectra of BHC12, EHC12 and soluble starch

Figure 5 shows that all samples have a band at around 3300 cm^{-1} coming from an O-H stretching vibration. The band from a C-H stretching vibration at 2900 cm^{-1} of BHCl2 looks similar to the band of starch, while the one from EHCl2 looks a bit differently. This is an indication that there is still starch left in the sample BHCl2. The band at 1730 cm^{-1} only appears at the pectin samples because it is the result of a stretching vibration of a carbonyl group, which does not appear in starch. All samples show a band at 1630 cm^{-1} which comes from a C-O bending vibration that is associated with an OH group, however, the band of the sample EHCl2 is shifted to slightly lower wavenumbers than the starch sample, while the band of the sample BHCl2 lies in the middle between starch and EHCl2. This could be due to an overlap between the band from starch and pectin if both substances are present in the BHCl2 sample. Another sign that starch is left in the BHCl2 sample is the band at 1150 cm^{-1} that comes from a C-O-C asymmetric stretching vibration. This band is strong in the starch sample, but non-existent in the EHCl2 sample and weak in the BHCl2 sample. Therefore, it can be assumed that this band in the sample BHCl2 comes from residual amounts of starch. Similar to this band, is the one at 1070 cm^{-1} which is clearly visible at the soluble starch sample, small at the BHCl2 sample, but does not appear at the EHCl2 sample. However, the lack of this band can also be explained by an overlap with the next band at around 1010 cm^{-1} . The latter two are caused by a C-O stretching vibration. The last band that suggests that there is starch left in the sample BHCl2 is at a wavenumber of 860 cm^{-1} because the transmission of the sample EHCl2 is higher than of the sample BHCl2, while the soluble starch has a clear band there coming from C-O-C ring vibration of the carbohydrate. This vibration also leads to a band at 760 cm^{-1} which appears at all three samples (Abdullah et al., 2018, Merck KGaA).

However, the sample EHCl2 should also have bands at the aforementioned wavenumbers in the range between 1150 cm^{-1} and 860 cm^{-1} because these C-O-C and C-O bonds that are the reason for these bands also exist in pectin. Actually, the sample EHCl2 shows bands at these wavenumbers, except at 860 cm^{-1} , but not to the same extent as the other samples. The different structure of pectin might cause intermolecular interactions that lead to slightly different bands, although the bond responsible for the band is the same but has a different interaction with the IR radiation.

To check if the sample EHCl2 is actually pectin, the IR spectra of EHCl2 and citrus peel pectin are compared in Figure 6.

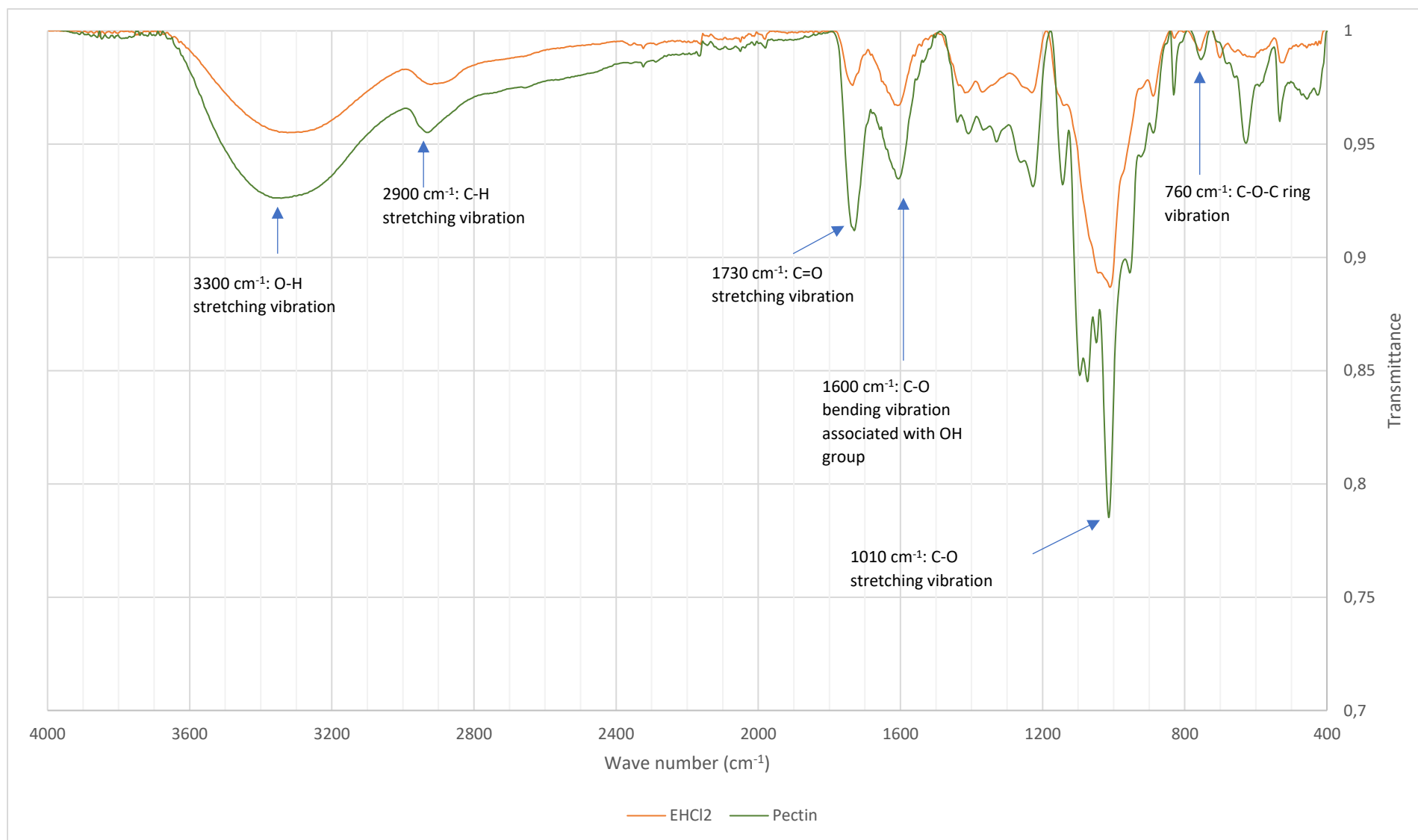


Figure 6: IR spectra of EHCI2 and citrus peel pectin

Gnanasambandam and Proctor (2000) states that the band around 1600 cm^{-1} indicates the carboxylate stretching band and the ones between 1300 and 1000 cm^{-1} are the result of C-O stretching vibrations. However, there are also some differences between the two samples shown in Figure 5, starting with the bands at 1070 to 1080 cm^{-1} which do not appear as clearly in the EHCl2 sample as in the pectin sample. There is an unidentified band at 630 cm^{-1} at the citrus peel pectin that does not appear in the EHCl2 sample. The differences can be attributed to a slightly different chemical structure of the two pectin samples, what can be seen in the paper from Yang et al. (2018). However, the carbonyl groups which make pectins distinguishable from other carbohydrates are visible in the EHCl2 sample and therefore it can be stated that this sample is pectin.

3.6.3 Conclusion

For the second extraction method, the best result gave enzymatically treated PP in which the pectin was extracted with HCl. It has been shown that the enzymatic treatment is necessary to get rid of starch. The pectin yield of $10.45\text{ wt\%}$ is in accordance with what is reported in literature with the same extracting method used.

3.7 Protein content

The protein content was determined for the raw and the extractives free sample. For the determination, a conversion factor was used to convert the nitrogen content determined via the Kjeldahl method to the protein content as described in Equation 15, which can be found in section „7.1.6 Determination of the protein content“. For WB, a conversion factor of 6.02 was used, while for PP the conversion factor was 6.25 . The factor 6.02 was used because it is the average of the conversion factors for WB that was found literature with a range between 5.26 and 6.82 (Roberts et al., 1985, Di Lena et al., 1997, Dornez et al., 2006, Beaugrand et al., 2004, Lee and Stenvert, 1973, Reisinger et al., 2013, Ye et al., 2021, van den Borne et al., 2012). A conversion factor for PP of 6.25 was used in literature, therefore 6.25 was also chosen in this work (Choi et al., 2016, Serena and Knudsen, 2007, Trabert et al., 2022, Brunt and Drost, 2010, Yang et al., 2018).

For the determination of the protein content, one sample was used in each case. The results are listed in the following table and depicted in Figure 13

Table 11: Total protein content in the raw sample, extractable protein content in the raw sample and protein content in the extractives free sample in WB and PP, based on dry matter

Input		Protein content in the raw sample (wt%)	Extractable protein content in the raw sample (wt%)	Protein content in the extractives free sample (wt%)
1	WB	18.86	6.91	11.95
2	PP	7.39	2.19	5.21

From Table 11 can be seen that the extractable protein content in untreated WB and PP is 6.91 wt% and 2.19 wt%, respectively. This means that 36.63 % and 29.6 % of the protein content of WB and PP could be extracted with a Soxhlet extraction as described in section “2.8 Determination of extractives” The protein content in literature varies between 10.2 and 18.69 wt% (Roberts et al., 1985, Di Lena et al., 1997, Dornez et al., 2006, Amrein et al., 2003, Babiker et al., 2009, Beaugrand et al., 2004, Lee and Stenvert, 1973, Reisinger et al., 2013, Ye et al., 2021, van den Borne et al., 2012). The protein content in the WB sample is within the range reported in literature.

For PP, the average protein content reported in literature is between 5.13 to 10.62 wt% (Choi et al., 2016, Serena and Knudsen, 2007, Trabert et al., 2022, Yang et al., 2018, Antusiewicz and Świąch, 2006). The protein content of the PP is in accordance the literature values.

3.8 Extractives

The results of the determination of extractives are listed in Table 12.

Table 12: Results of the determination of extractives, based on dry matter

		Water extractives	Ethanol extractives	Total extractives
WB	Average (wt%)	25.54	10.10	35.63
	Standard deviation (wt%)	0.92	4.98	5.89
	RSD (%)	3.6	49.3	16.5
PP	Average (wt%)	18.19	0.92	18.72
	Standard deviation (wt%)	2.57	0.32	3.72
	RSD (%)	14.1	35	19.9

For WB the determination of extractives in water and ethanol were conducted in triplicate and quadruplicate respectively. Different values of ethanol extractives (5.49 wt% to 14.71 wt%) were reported, this could be connected to the presence of foam going to the extraction thimble and dragging solid material.

The extraction of PP was done in two batches with water and in one batch with ethanol. As can be seen from Table 12, with ethanol only a small part of PP could be extracted and therefore the ethanol extraction was done in only one batch. One batch contained four samples, except the second batch for the water extraction which contained only three samples because one thimble had a rupture during the extraction. In section “3.10.4 Other extractives” it is assumed that the extraction of PP with water as a solvent was not exhaustive because sugars were found in the ethanol extract. Hence, it is suggested to use less sample what may lead to a better penetration of the sample, thus enhancing the yield of the extraction.

3.9 Structural carbohydrates

The concentration of the structural carbohydrates were estimated from the concentration of the monomeric sugars, multiplying by the anhydro correction factor of 0.88 and 0.9 for C-5 and C-6 sugars respectively (Sluiter et al., 2008a). The results are shown in Table 13.

Table 13: Content of polymeric sugars of the structural carbohydrates of WB and PP, based on dry matter

		Arabinans	Galactans	Glucans	Xylans	Mannans	Sum
WB	Average (wt%)	9.04	0.85	9.13	17.53	0.74	37.30
	Standard deviation (wt%)	0.27	0.03	0.70	1.19	0.03	1.85
	RSD (%)	3.04	3.81	7.63	6.80	4.24	4.96
PP	Average (wt%)	3.50	18.66	31.32	1.85	0.99	56.33
	Standard deviation (wt%)	0.26	1.75	3.19	0.14	0.13	3.66
	RSD (%)	7.45	9.40	10.20	7.71	12.62	6.49

In literature, for WB the arabinose content is reported with 9-11 wt%, the galactose content with 0.9 wt%, the glucose content with 2.9-7.5 wt%, the xylose content with 14.8-20 wt% and the mannose content with 0.5 wt% (Knudsen, 1997, Palmarola-Adrados et al., 2005). In general, the values are in accordance with the values reported in literature.

For PP, the values in literature range from 3.6 to 5.8 wt% for arabinose, 15.6 to 25.4 wt% for galactose, 23.2 to 26.4 wt% for glucose, 1.4 to 2.7 wt% for xylose and 0.1 to 0.8 wt% for mannose (Schmitz et al., 2021, Meyer et al., 2009, Ravn et al., 2015, Serena and Knudsen, 2007).

Compared with PP, WB has a higher content of arabinans and xylans, whereas PP has a higher content of galactans, glucans and in general a higher carbohydrate content, which makes it favorable for fermentations, especially if the additional starch content is considered. The proportion of mannans in WB and PP is the same.

3.10 Characterization of the extractives fraction

The extractives fraction is separated into the water and ethanol extractives. Water extractives comprise biomass material that is soluble in water during an exhaustive Soxhlet extraction. Ethanol extractives comprise biomass material that is soluble in ethanol during an exhaustive Soxhlet extraction. The characterization of extractives is important because extractives make up around 19 and 36 wt% of PP and WB, respectively. Therefore, the characterization of extractives gives information about the composition of the sample. Besides sugars, which are of interest to fermentation, there are also other compounds in the extractive fraction, that may be valuable.

This chapter is subdivided into the monomeric sugar content in water and ethanol extractives, the oligomeric sugar content in the water extractive fraction, the ion content in the water extractive fraction and other extractives which were determined via GC-MS.

3.10.1 Monomeric sugar content in water and ethanol extractives

The concentration of the monomeric sugars in the ethanol extract was below the lowest calibration standard which is why they were not evaluated. The results are listed in the following table:

Table 14: Content of monomeric sugars in WB and PP, based on dry matter

		Arabi- nose	Galactose	Glucose	Xylose	Mannose	Fructose	Sum
WB	Average (wt%)	1.08	0.07	0.66	0.29	0.03	0.81	2.93
	Standard deviation (wt%)	0.06	0.01	0.04	0.02	0.00	0.04	0.15
	RSD (%)	5.41	7.61	6.64	6.34	8.87	4.47	4.97
PP	Average (wt%)	0.14	< cal. std	0.60	< cal. std.	< cal. std.	0.66	1.40
	Standard deviation (wt%)	0.01		0.06			0.06	0.13
	RSD (%)	9.69		9.35			8.90	9.14

For WB the total monomeric sugar content of the extractive fraction is reported in literature with 0.41 and 0.7 wt% on a percent dry weight basis (Knudsen, 1997, U.S. Department of Agriculture, 2019). The difference from the measured values to the values in literature can be attributed to different extraction methods. For PP there are no values for monomeric sugars reported in literature.

3.10.2 Oligomeric sugar content in water extractive fraction

Oligomeric sugars were determined after hydrolysis with 4 wt% H₂SO₄. The monomeric composition of the oligomeric sugar content is calculated similarly to the monomeric sugar content, but the degradation of sugars during hydrolysis must be considered. A correction factor must be calculated like for the determination of the structural carbohydrates as described in Equation 18. The sugar content can be calculated as described in section “7.1.9 Determination of the monomeric and oligomeric sugars” with Equation 25 and Equation 26. To calculate the composition of the oligomeric sugars, the monomeric sugars must be subtracted from the total sugars. Table 15 shows the composition of the oligomeric sugars based on the dry untreated sample.

Table 15: Composition of the oligomeric sugars of WB and PP based on the dry untreated sample, based on dry matter

	Arabinose	Galactose	Glucose	Xylose	Mannose	Sum
WB						
Average (wt%)	0	0.61	5.26	0.81	0.10	5.61
Standard deviation (wt%)		0.05	0.32	0.08	0.01	0.19
RSD (%)		7.85	6.14	9.45	7.98	3.30
PP						
Average (wt%)	0.52	3.60	2.24	< cal. Std.	< cal. Std.	5.71
Standard deviation (wt%)	0.05	0.35	0.02			0.41
RSD (%)	10.05	9.77	0.83			7.24

The value for arabinose for WB is 0 because the value for the total arabinose content is smaller than the monomeric arabinose in the water extract. Both samples, WB and PP were hydrolyzed simultaneously, therefore errors should be minimized as the hydrolysis conditions were the same.

Compared with PP, WB has a higher content of glucose, while PP has more galactose. The sum of the sugars is very similar. Since fructose is completely degraded during hydrolysis, it cannot be measured and therefore the actual sugar content could be higher.

In literature, for WB the total extractable sugar content is reported with 5.3 wt% and for PP with 1.8 wt%, based on dry sample mass (Knudsen, 1997, INRAE CIRAD AFZ). In contrast to WB, the literature values of PP regarding the total sugar content do not match with the values determined in this work. The reason for that could be a different extraction method. For this work, the main point of the characterization of extractives was to identify a small portion that can be extracted easily and contains sugars.

3.10.3 Ion content in extractives-free and water extractives samples

The ion content was determined by analysing the extractives-free solids and the water-extractive fraction. For the extractives-free solids, the supernatant recovered after hydrolysis with 4 wt% was diluted and analyzed. On the other hand, the water extractive fraction was analysed without further treatment. The determined anions were fluoride, acetate, propionate, formate, chloride, bromide, nitrate, succinate, sulfate, phosphate, citrate and oxalate. The measured cations were sodium, ammonium, potassium, magnesium and calcium. Acetate is coming from the acetyl substituents of hemicellulose and can inhibit the bioconversion of lignocellulose at certain concentrations (Delgenes et al., 1996, Bajpai, 2018). Formate is also a degradation product and fermentation inhibitor (Larsson et al., 1999). Phosphate, potassium, calcium and magnesium are essential minerals for fermentations. Ammonium salts serve as a source of nitrogen (Lallemand Inc., 2022, Kounbesiou et al., 2011).

3.10.3.1 Ion content of the extractives-free samples

The results of the determination of the ion content of the extractives-free samples are listed in the following table:

Table 16: Ion content of hydrolyzed WB and PP, based on dry matter

		Acetate	Phosphate	Ammonium	Sum (wt%)
WB	Average (wt%)	0.875	0.303	0.116	1.242
	Standard deviation (wt%)	0.282		0.026	0.308
	RSD (%)	32.2		23	24.8
PP	Average (wt%)	1.788	< cal. std.	< cal. std.	1.788
	Standard deviation (wt%)	0.747			0.747
	RSD (%)	41.8			41.8

The reason why there is no standard deviation and RSD for phosphate for the WB sample is that there is only one sample that is within the calibration range. For PP all samples had phosphate and ammonium concentrations that were below the concentration of the calibration standards. The same applies for both samples for all the other ions.

The concentration of formate was below the detection range. Formic acid is generated by the degradation of hexoses, which are degraded to hydroxymethylfurfural and then further to levulinic acid and formic acid (Saeman, 1945). This is an indicator of the reliability in the characterization of the structural carbohydrates.

The hydrolyzed PP samples also contained an anion in a concentration that was within the calibration range of the other ions, but it could not be identified. However, it could be concluded that it was not butyrate, pyruvate, valerate or quinate. The retention time was 7.73 min.

3.10.3.2 Ion content of the water extractives

The results are listed in the following table:

Table 17: Anion content of the water extractives of WB and PP, in relation to the dry sample mass

		Acetate	Succinate	SO ₄ ²⁻	Cl ⁻	PO ₄ ³⁻	Citrate	Oxalate	K ⁺
WB	Average (wt%)	0.184	0.149	< cal. std.	< cal. std.	1.018	0.132	0.012	> cal. std.
	Standard deviation (wt%)	0.005	0.008				0.010	0.002	
	RSD (%)	3	6				8	16	
PP	Average (wt%)	0.230	0.225	0.111	< cal. std.	0.100	0.474	0.007	> cal. std.
	Standard deviation (wt%)	0.010	0.015	0.009		0.001	0.037	0.001	
	RSD (%)	4.5	6.6	8		0.9	7.9	12	

The cation concentration of the water extractives was too low to evaluate, except for potassium which had a concentration above the highest concentrated calibration standard. This applies for both WB and PP.

In summary, the extractives-free solid and the water extractives fraction were characterized for containing acetate, succinate, sulfate, chloride, phosphate, citrate, oxalate, potassium and ammonium ions distributed mostly in the water extractives fraction.

3.10.4 Other extractives

This section deals with the substances found in the water and ethanol extractives. At first, the evaluation is described and then the results are presented.

3.10.5 GC-MS screening of water and ethanol extractives

GC-MS was used for the qualitative screening and identification of the different components present in water and ethanol extractives. A lot of substances in the ethanol and water extractives could be identified via the databases from Wiley and NIST coupled to the chromatography device, although the limitation regarding the identification of stereoisomers must be considered. For the ethanol extractives of PP and WB as well as for the water extracts of WB, four extracts were analyzed, while for the water extracts of PP, three extracts were analyzed.

The areas for a given substance were compared among the sample replicates. The aim was to check how much the amounts of a certain substance differed within the samples of one extractive. It showed that there are deviations between the amounts of a certain substance within the samples of one type

of extract. For the same extract, but from a different sample, the lowest amount was often much lower than the highest amount, sometimes by as much as 70 %. Several times the lowest amount was only around 30 % of the highest amount of the same extract, but from a different sample.

The substances in the following table appeared in more than one type of extract. The relative amount of a substance in a sample in relation to the highest amount of that substance is shown. It was assumed that the disaccharide with the highest amount was sucrose. The substances are not ordered. The abbreviation PE stands for PP ethanol extract, PH for PP water extract, WE for WB ethanol extract and WH stands for WB water extract.

Table 18: Substances that appeared in at least two types of extracts

Substance	Type of extract	Relative amount (%)
Malic acid	PE	100
	PH	41
	WH	79
5-Oxoproline	PE	100
	PH	41
Ketohexose	PE	100
	PH	20
	WH	52
Ketohexose	PE	100
	WH	44
Aldohexose	PE	100
	PH	14
	WH	53
Aldohexose	PE	100
	PH	22
	WH	84
Palmitic acid	PE	19
	WE	100
Stereoisomer of inositol	PE	23
	WH	100
9,12-Octadecadienoic acid (Z,Z) or 9(E),11(E)-Conjugated linoleic acid	PE	4
	WE	100
Acids with the molecular formula $C_{18}H_{34}O_2$ and one double bond at position 6 to 13	PE	10
	WE	100
Sucrose	PE	51
	PH	3
	WH	100
Citric acid	PH	58
	WH	100
Quininic acid	PE	100
	PH	12

Since only a qualitative analysis was performed, the absolute amount of any substances cannot be stated.

Although the water extraction was conducted before the ethanol extraction, there are still sugars in the ethanol extract of PP and the concentration of the monomeric sugars in the ethanol extract of PP is higher than in the water extract. This shows that the water extraction was not exhaustive, because otherwise all the water-soluble substances should have been extracted with water and therefore the ethanol extractives should be free of water-soluble substances. It is sure that the extracts were not mixed up, because in the ethanol extracts was palmitic acid found, but not in the water extracts. Furthermore, the ethanol extracts of PP do not contain a lot of extractives in total, as shown in section “3.8 Extractives”.

Interestingly, in the water extracts appeared only two aldohexoses with an amount of at least 5 % of the amount of the most abundant substance, but with HPAEC-PAD other sugars were also found in the extracts, including arabinose and xylose which are pentoses. Sucrose was the most abundant substance in some PE and in all WH samples. This means that if sucrose could be quantified, more information about the composition of the extractives would be available.

In the PE samples, two ketohexoses and stearic acid were also found. In general, the major amount of the constituents found via GC-MS were sugars, and to a lesser extent malic acid, quinic acid and 5-Oxoproline.

Citric acid had the highest amount in the PH samples, followed by sugars and malic acid. There was one substance, that appeared in a significant amount, but could not be identified. Other components that appeared are aspartic acid, asparagine and a ketohexose.

Additional substances found in the WE samples were 5-Nonadecylresorcinol and 5-Henicosylresorcinol. The main substances in WE were organic acids with 9,12-Octadecadienoic acid (Z,Z) or 9(E),11(E)-conjugated linoleic acid being the most abundant one, respectively.

Besides the substances listed in Table 18, a ketohexose, and three other disaccharides were found in the WH samples. The highest amount of all substances was sucrose and to a lesser extent hexoses as well as citric acid. Sucrose is the most common sugar and it is mostly used as a sweetener (Clarke and Singh, 2023). Sucrose is used as a carbon source for fermentations, especially for the fermentation of ethanol. Sucrose can be metabolized by *Saccharomyces cerevisiae* just like glucose, therefore the sucrose in WB and PP can serve as a carbon source for fermentations (Pepin and Marzzacco, 2015).

Citric acid is present in nearly all plants. It is used as a flavouring agent in food products and in metal-cleaning compositions. The organic acid is produced via fermentation of cane sugar or molasses using the fungus *Aspergillus niger* (Britannica, 2023). It has been shown that citric acid hinders the metabolic growth of *S. cerevisiae* at a concentration of 50 mM (Deshavath et al., 2021).

3.11 Lignin

The determination of lignin consists of the determination of ASL and AIL. The results are listed in the following table and in Figure 17:

Table 19: Content of ASL, AIL and total lignin in WB and PP, based on dry matter

		ASL	AIL	Total lignin
WB	Average (wt%)	3.25	6.53	9.78
	Standard deviation (wt%)	0.15	0.77	0.92
	RSD (%)	4.5	12	9.4
PP	Average (wt%)	1.75	4.34	6.09
	Standard deviation (wt%)	0.03	0.10	0.13
	RSD (%)	2	2.3	2.1

The standard deviation of AIL of WB is higher than the standard deviation of AIL of PP, leading to a higher standard deviation of the total lignin content. It must be noted that due to the small amounts of sample used for the lignin determination, even small deviations lead to notable relative deviations.

In literature, the content of ASL in WB is reported with 0.91 wt%, the content of AIL with 4.1 wt% (van den Borne et al., 2012). The total lignin content varies between 4.8 and 5.01 wt% (van den Borne et al., 2012, Lee and Stenvert, 1973, Palmarola-Adrados et al., 2005). In this work, all three lignin values are higher than the literature values. This could be due to different methods for the determination of lignin or a different composition of the samples.

For PP there is no ASL reported in literature, but two sources give an AIL content with 3.5 and 3.8 wt% (Serena and Knudsen, 2007, Schmitz et al., 2021). One web page states a total lignin content of 5.9 wt% (INRAE CIRAD AFZ). Interestingly, the measured values of lignin in PP in this work are closer to the literature values than the measured values of WB.

3.12 Chemical characterization of wheat bran and potato pulp

In this section the contents of WB and PP are summarized and the composition of the extractives is shown as well. Table 20 presents the normalized compositions of WB and PP.

Table 20: Normalized composition of WB and PP, based on dry matter

	WB	PP
Structural carbohydrates (wt%)	29.37	46.03
Extractives (wt%)	28.06	15.29
Pectin (wt%)	not determined	8.54
Starch (wt%)	14.09	16.12
Protein (wt%)	14.86	6.04
Lignin (wt%)	7.70	4.98
Fat (wt%)	3.81	0.61
Structural ash (wt%)	1.08	0.92
Ions (wt%)	1.02	1.46
Sum (wt%)	99.99	99.99

The high content of structural carbohydrates and starch is useful as a carbon source for fermentations. They can be hydrolyzed to monomeric sugars which are then metabolized by microorganisms. The high protein content in WB could be interesting for animal feed. The pectin in PP can also be utilized as an emulsifier. The possible applications will be discussed in the next chapter in detail.

With the known amounts of the substances in the water extracts, it is also possible to characterize them. The composition of the water extracts is listed in Table 21. The unknown components were calculated by subtracting the sum of the known components from 100 wt%.

Table 21: Composition of the water extractives of WB and PP, based on dry matter

	WB	PP
Extractable ash (wt%)	18.28	18.31
Protein (wt%)	27.1	12.0
Sugars (wt%)	33.44	39.08
Ions (wt%)	5.86	6.30
Unknown (wt%)	15.4	24.3

In general, the composition of the water extracts of WB and PP looks very similar regarding the values for extractable ash, sugars and ions. However, the sugar content of PP is a bit higher than that of WB and the content of the unknown substances is a bit lower in WB than in PP. The protein content in the water extractives for WB is higher than for PP, which is in accordance with the solid samples. The unknown substances in the water extracts of WB are probably sucrose and to a lesser extent other disaccharides. For PP, they could be malic acid, sucrose and a substance that could not be identified via GC-MS. These assumptions are based on the results from the GC-MS.

4 Potential uses of wheat bran and potato pulp in biorefineries

The composition of the raw material determines the possible platforms and products that can be acquired. With the known composition of WB and PP it is possible to identify opportunities for the uses of these by-products in biorefineries. The products obtained from WB and PP can be derived into starch, products derived from carbohydrates which were produced by fermentation, products derived from the non-carbohydrate fraction and pectin. Also, the current uses of WB and PP are discussed briefly.

4.1 WB

4.1.1 Current uses

Currently, WB is mainly used as animal feed while a minor amount is used as an ingredient in food products (Koutinas et al., 2005, Prückler et al., 2014). WB can be broken down and divided into pure parts to synthesize chemicals which can be used as precursors for higher polymerized substances. Furthermore, WB comprises valuable substances that can be accessed after separation and purification (Aprich et al., 2014).

4.1.2 Starch

As described in section “2.4 Determination of the starch content”, WB has a starch content of around 18 wt% of the basis in dry matter. This is far less than in maize, which has a starch content of around 72 wt% in dry matter (U.S. Grains Council, 2021, Hamaker et al., 2019). From this point of view, it seems uneconomical to produce starch from WB. Additionally, considering the raw materials used for starch production it shows that by far the largest amount of starch is produced from maize, resulting in maize starch accounting for 74.7 % of the total starch production of 88.1 million tons in 2020 (Vilpoux and Santos Silveira Junior, 2023). Therefore, it is more economical to use the starch from WB for processing.

Apart from this, WB starch has some unique properties compared to wheat starch. In WB starch, the content of small granule, which have a diameter of less than 10 μm , is lower than in wheat starch. Furthermore, the RS and amylose content is higher in WB starch than in wheat starch (Xie et al., 2008). This contrasts with what has been examined in this work, because there was no RS found in the examined WB, as described in section “3.4 Starch content”. Additionally, WB starch shows a higher crystallinity, swelling power and melting enthalpy, whereas the gelatinization temperature, pasting peak, final viscosities and retrogradation rate are lower in WB starch than in wheat starch. Due to the higher RS content and lower retrogradation rate, WB starch may play a role in the functional food industry (Xie et al., 2008).

A procedure for the extraction of starch from WB was published by Xie et al. (2008). In short, WB was soaked in 70 vol% ethanol and water, ground, filtered, washed, extracted with NaOH and a mixture of four parts water and one part toluene. A recovery rate of 90 % was achieved.

However, the starch from WB should be seen as an intermediate to produce other substances which are portrayed in the following sections. For this, the starch is enzymatically hydrolyzed to glucose, resulting in a sugar feedstock for fermentation processes. A more detailed description of the hydrolysis is given in section “4.1.3.2 Ethanol”. The remaining WB has a higher protein content, thus gaining a value as animal feed (Elliott et al., 2002).

4.1.3 Products derived from carbohydrates

Carbohydrates appear in WB as starch, hemicelluloses and cellulose. Starch can be processed easily enzymatically, while hemicelluloses and cellulose are more recalcitrant and therefore need harsher pre-treatment steps, like acid hydrolysis (Apprich et al., 2014). The following diagram shows a possible route for the utilization of the carbohydrates in WB. At first, the starch is enzymatically hydrolyzed to glucose. Then, the starch-free residue is hydrolyzed to the monomeric sugars. In many papers, acid hydrolysis is used in this step.

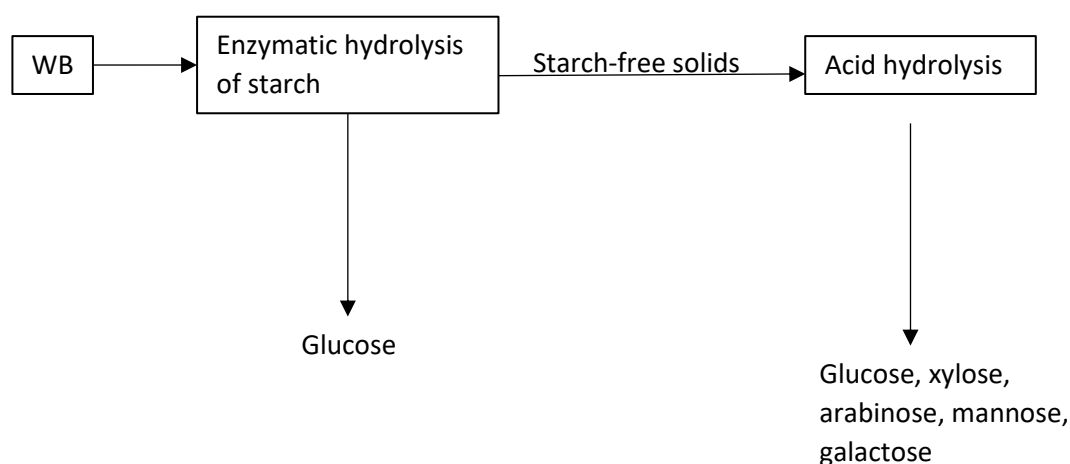


Figure 7: Overview of the utilization of the carbohydrates in WB

4.1.3.1 Potential fermentative products from sugars platforms based on WB and PP sugars platforms

The substrate (carbon source) for the fermentations are the C5 and C6 sugars that are obtained from WB and PP after hydrolysis of the carbohydrate fraction. The steps necessary to break down the polysaccharides are described briefly in section “4.1.3.2 Ethanol”. The presented fermentative products are lactic acid, succinic acid, ethanol and n-butanol and amino acids. However, the universe of potential cases of studies is nearly infinite.

4.1.3.1.1 Lactic acid

Lactic acid is mainly used as an acidulant, preservative and flavour compound in the food, cosmetic, pharmaceutical and chemical industry (Apprich et al., 2014). It is a platform chemical with the possibility of the practical and efficient production of green solvents, fine chemicals, commodity chemicals and fuel precursors (Dusselier et al., 2013).

Lactic acid can be produced via microbial fermentation as well as via chemical synthesis. However, the fermentation process is of overriding importance compared to the chemical synthesis because the latter leads to a racemic mixture of lactic acid, while fermentative production yields only one isomer, depending on the strain of lactic acid bacteria used (Hofvendahl and Hahn-Hägerdal, 2000, Abdel-Rahman et al., 2011). Other advantages are the possibility to use renewable and low cost raw materials, low energy consumption and mild operating conditions (Komesu et al., 2017).

Elliott et al. (2002) described in their paper a pilot-scale fermentation process to produce lactic acid from WB-millfeed. At first, the millfeed was washed with water in a tank, and dewatered with 400 and 600 micron filter bags. The filtrate was liquefied with α -amylase. After that, the temperature and pH were adjusted and glucoamylase was added for the conversion of the liquefied starch to glucose. For the subsequent lactic acid fermentation, the hydrolysate of the millfeed was used as the carbon source. Three strains of *Lactobacillus* were examined to produce lactic acid.

L. rhamnosus (ATCC 11443) performed best due to shorter fermentation time and was more tolerant towards higher sugar concentrations. The pure hydrolysate supplemented with manganese showed a higher yield in lactic acid, leading to better results than a medium with reagent grade glucose.

Pentoses can also be used for lactic acid fermentation, however, for 1 mol of lactic acid, 1 mol of acetic acid is produced, which is unfavorable because the yield of lactic acid is decreased and it raises costs for purification processes (Apprich et al., 2014).

4.1.3.1.2 Succinic acid

Succinic acid is applied in the food industry as an acidulant, flavouring agent or as a preservative, while in the pharmaceutical industry it serves as an acidity regulator. Furthermore, succinic acid is used as a platform chemical for the synthesis of 1,4 butanediol, tetrahydrofuran, γ -butyrolactone, adipic acid and for biodegradable polymers, for instance polybutyrate succinate (PBS) (Apprich et al., 2014).

Bio based succinic acid is produced via microbial fermentation of various glucose sources (Pinazo et al., 2015). Andersson et al. (2007) have shown that succinic acid can also be produced from fructose and xylose using the *Escherichia coli* strain AFP184. They used fructose, glucose, xylose, sucrose and

an equal mixture of glucose and fructose as well as an equal mixture of glucose and xylose as a substrate. For every fermentation, the initial concentration of the substrate was 100 g L⁻¹. When sucrose was used as a substrate, no succinic acid was produced, nor sucrose was consumed. Acetic acid was a by-product for all three substrates, while pyruvic acid was formed with fructose and xylose. The final concentrations and the mass yields of succinic acid are reported in the following table:

Table 22: Final concentrations and mass yields of succinic acid using different sugars, data from Andersson et al. (2007)

Substrate	Final concentration of succinic acid (g L ⁻¹)	Mass yield of succinic acid (g g ⁻¹ sugar)
Glucose	40	0.83
Fructose	32	0.66
Xylose	25	0.50
Mixture of glucose and fructose	25	0.58
Mixture of glucose and xylose	27	0.60

To convert the starch of WB to glucose, an enzymatic hydrolysis can be used, as described in section “4.1.3.2 Ethanol”. There are several microorganisms used for the commercial production of succinic acid, such as yeasts or modified *E. coli* (Roquette Frères, 2022, U.S. Department of Energy, 2012). There are three bio-based succinic acid production processes, the direct crystallization process, the electro-dialysis process and the ammonium sulfate process. For the latter two, CO₂ is needed as an additional carbon source. A description of these processes is given in Cok et al. (2014).

4.1.3.2 Ethanol

Ethanol is used as a disinfectant, an organic solvent, a chemical feedstock and as a biofuel (Hoang and Nghiem, 2021). The global production in 2019 were 111 million m³, in which 54 % were produced in the USA, 30 % in Brazil and 5 % in the EU (Renewable Fuels Association, 2022). The vast majority of ethanol produced is first generation bioethanol with around 94 % of the global ethanol production, while the remaining 6 % are second generation bioethanol (Iram et al., 2022).

For the production of first-generation bioethanol, sugar-based feedstocks like sugar cane and sugar beet as well as starch-based feedstocks like corn, barley or wheat are used. In comparison, to produce second-generation bioethanol, lignocellulosic feedstocks like agricultural residues, forest residues or dedicated energy crops are used. Cellulose and hemicellulose in these raw materials are hydrolyzed to sugars which are used in the subsequent fermentation to ethanol. Another possibility is the route via gasification of the biomass. In this process, synthesis gas is produced which is then converted to ethanol (Hoang and Nghiem, 2021). For second generation bioethanol, the conversion of the lignocellulosic biomass to fermentable sugars is difficult due to the recalcitrance of the biomass. Consequently, there is a need for high capital investment and severe pretreatment methods. Also, the

cost of the enzymes is another major bottleneck (Iram et al., 2022). Yet, there were some facilities for the production of second generation bioethanol built in recent years (BEST GmbH, 2023).

Wild-type yeast *Saccharomyces cerevisiae*, which is used for ethanol fermentation, cannot metabolize pentose sugars (Bondesson and Galbe, 2016, Zabed et al., 2017). This is why genetically engineered strains were developed that can metabolize C6 sugars as well as xylose (Lallemand Biofuels & Distilled Spirits, 2019). The yeast strain XyloFerm can reach ethanol concentration of 103 g L⁻¹ using corn cobs as a substrate. However, for wheat straw the ethanol titer is only 17 g L⁻¹ (Taurus Energy AB, 2015). In comparison, the fermentation of glucose coming from the starch in corn using yeast leads to an ethanol concentration of 10-12 vol% (Bothast and Schlicher, 2005).

For WB, this means that the lignocellulose needs to be hydrolyzed to sugars before the fermentation to ethanol. Chotěborská et al. (2004) evaluated several methods for the hydrolysis of polysaccharides in WB. The aim was to maximize the yield of monosaccharides and minimize the amount of furfural and 5-hydroxymethylfurfural which are inhibitors for the fermentation. At first, the starch was converted to glucose with starch degrading enzyme preparates containing amylase and amyloglucosidase. After that, the starch-free residue was hydrolyzed with diluted H₂SO₄ under various conditions concerning the concentration of the acid, the duration of the hydrolysis and the temperature. It has been shown that the highest yield of sugars was achieved with 1 wt% H₂SO₄, at 130 °C for 40 min. This generated 52.1 g total sugars per 100 g starch-free residue with 17.3 g arabinose, 31.3 g xylose, 1.8 g glucose and 1.7 g galactose. The concentrations of the degradation products furfural and 5-hydroxymethylfurfural were too low to cause any inhibitory effects for the fermentation.

The effect of different types of hydrolyses of WB with and without pretreatments on the sugar yield is discussed in the paper from Palmarola-Adrados et al. (2005). For the hydrolysis of starch, the enzymes Termamyl and amyloglucosidase were used. After filtration and washing, a solid residue, starch-free bran was obtained. For the highest total sugar yield, it was pretreated with 0.2 wt% H₂SO₄ at 160 °C for 20 min and then enzymatically hydrolyzed with Celluclast and Utraflow. Celluclast is mixture containing hemicellulose-degrading enzymes and Utraflow is a β-glucanase preparation. This procedure yielded 13.3 g arabinose, 23.3 g xylose and 16.4 g glucose, thus leading to a total sugar yield of 53.0 g per 100 g of starch-free bran. The concentration of the degradation products 5-hydroxymethylfurfural and furfural were too low to influence fermentation. Acid hydrolysis of the starch-free bran led to higher pentose yields, but very low glucose yields. However, higher glucose yields were achievable with only acid hydrolysis, but this led to a sharp decrease in pentoses and furfural.

Comparing the results of these two papers, it shows that to hydrolyze the glucans, an enzymatic hydrolysis is necessary because acid hydrolysis leads to a low glucose yield and higher concentration of inhibitory compounds.

Since WB has a lower carbohydrate content than for example corn, the other contents of wheat bran must be valorized to be competitive with other raw materials (U.S. Grains Council, 2021, Hamaker et al., 2019). The potential of WB to produce ethanol is difficult to compare with other lignocellulosic feedstocks because the composition of them can vary a lot. In general, a high content of cellulose and hemicellulose is desired (McKendry, 2002, Ssymank and Duell-Pfaff, 1999).

4.1.3.3 *n*-Butanol

n-Butanol, in the following butanol, as a fuel is superior compared to ethanol because it is more hydrophobic and has a higher energy density. However, there are some hindrances such as low product yield and high recovery costs. (Apprich et al., 2014). Butanol is also a widely-used industrial solvent and as an intermediate in the manufacturing process of glycol ether, acrylate esters, acetates and amines (Gomes, 2019). Liu et al. (2010) showed that WB hydrolysate can be used as a fermentation medium for the microbial production of butanol. WB was hydrolysed at 121 °C with 0.75 wt% H₂SO₄, then neutralized, detoxified by adding Ca(OH)₂ and filtrated. For the inoculation of the supernatant, the mutant strain *Clostridium beijerinckii* 55025 was used. The fermentation was done at 37 °C for 72 h. Besides butanol, acetone and ethanol were produced as well with concentrations of 8.8, 2.2 and 0.8 g L⁻¹, respectively. It was showed that glucose, xylose and arabinose were metabolized, thus utilizing most of the sugars contained in WB. However, the fermentation came to a halt because of the end product inhibition and the toxicity of butanol (Ezeji et al., 2007). It was concluded that WB hydrolysate is a suitable medium for the fermentation of butanol, but the strain must be modified to improve the production and sugar utilization ratio (Liu et al., 2010). To establish an efficient production of large-scale biobutanol at a competitive price, major technological innovations in fermentation, microbial culture, and waste biomass are needed. Biobutanol must be sold as a top-quality product in the chemical market to achieve economic viability (Abo et al., 2019).

4.1.3.4 *Amino acids*

Amino acids are used as additives in food as well as in feed, but also to a minor amount in pharmaceutical and cosmetic products (Novalin-Canoy, 2011). Strains of *Corynebacterium glutamicum* have been used for more than 50 years to produce amino acids (Wendisch, 2007). Specifically, more than two million tons of lysine and glutamate are produced every year using the *C. glutamicum*. The used carbon sources for the fermentation are glucose, fructose or sucrose (Wittmann and Becker, 2007, Shimizu and Hirasawa, 2007, Zahoor et al., 2012). Amino acids can also be produced by

fermenting WB hydrolysate with recombinant pentose-utilizing microorganisms such as *C. glutamicum*. Genetically engineered *C. glutamicum* can produce glutamate, lysine, phenylalanine, aspartate, tryptophan, arginine and valine. More L-glutamate and L-lysine was produced by the recombinant strains than the control strains, which metabolized glucose. The recombinant strains co-utilized arabinose, xylose and glucose. An advantage of this microorganism is its resistance against the inhibitors furfural and 5-HMF. The highest lysine concentration was 42 mM and the highest glutamate concentration was 96 mM (Gopinath et al., 2011, Gopinath et al., 2012).

4.1.4 Products derived from the non-carbohydrate fraction

4.1.4.1 Ferulic acid and vanillin

Ferulic acid is bound in the WB via ester linkage. It has antioxidant, antimicrobial, anti-inflammatory, antithrombosis and anticarcinogenic activities, which is why ferulic acid is a bioactive compound. There are possible applications in the food, health and cosmetic industry (Ou and Kwok, 2004). Ferulic acid can be released via acid or alkaline hydrolysis or via enzymatic treatment using ferulic acid esterase (Ferreira et al., 2007, Kim et al., 2006, Apprich et al., 2014). After the release of ferulic acid, a purification step is done. Due to complicated adsorption and desorption processes, this step is problematic and adds cost to the product (Apprich et al., 2014). Alternatives could be subcritical water extraction or extraction with ethanol (Buranov and Mazza, 2009, Pourali et al., 2010).

Ferulic acid can be converted to vanillin via biotechnological processes which can be considered as natural vanillin (Di Gioia et al., 2009). Vanillin is mostly used as flavouring agent in foods and beverages and to a minor degree as a fragrance in perfumes or for pharmaceuticals (Priefert et al., 2001). A solution to the expensive isolation of ferulic acid with ion exchange resins is solid phase extraction with commercial solvents. The isolated free ferulic acid is converted to vanillin by different fungi and bacteria (Di Gioia et al., 2009).

4.1.4.2 Proteins

Due to the increasing population, there is a rising demand for dietary protein from plants and microorganisms. The proteins in WB contain a higher amount of lysine, arginine, alanine, asparagine and glycine and a lower amount of glutamine, proline, phenylalanine and sulfur containing amino acids compared to the proteins in the endosperm. Hence, the proteins in WB have a higher biological and nutritional value, which may be useful for food ingredients (Di Lena et al., 1997).

The extraction of the proteins can be conducted via alkaline extraction, but also acidic extraction is possible, however, alkaline extraction has higher yields with a yield of 83 % of the protein with a pH of 12 at a temperature of 60 °C and a duration of 16 h. A more economically favorable extraction with a

pH of 6.5, at 20 °C and a duration of 16 h recovered 72 % of the total protein (Roberts et al., 1985). As described in section “4.1.2 Starch”, protein can also be a by-product and can be used as an animal feed.

4.1.4.3 Alkylresorcinols

In the ethanol extracts of WB were 5-Nonadecylresorcinol and 5-Henicosylresorcinols found, which are alkylresorcinols. These compounds show antigenotoxic and antioxidant activity under in vitro conditions (Parikka et al., 2006). Additionally, they also exhibit anticancer activity against various cancer cells in vitro (Guo et al., 2019). Another observed health benefit is the reduction of blood cholesterol concentration in animal trails (Oishi et al., 2015).

4.2 PP

PP is currently used as a low cost animal feed (Meyer et al., 2009, Werpy et al., 2005). In general, the carbohydrate fraction of PP can be utilized in the same way as that of WB, but since PP contains pectin, the possible utilization of pectin is described. The lower protein content makes the extraction of it less economically viable compared to WB. PP does not contain ferulic acid or alkylresorcinols.

4.2.1 Pectin

Pectin is mostly obtained from residues of the production of fruit juices, in particular from peels of citrus fruits and apple pomace (Peter et al., 2015). Compared with citrus and apple pectin, which are used as gelling agents in the food processing industry, potato pectin has a shorter homogalacturonan domain, which is why it has a bad gelling ability. Potato pectin has more acetyl groups and neutral sugar side chains, leading to slightly better emulsifying properties (Funami et al., 2011, Leroux et al., 2003). Hence, potato pectin may be used as an emulsifier (Yang et al., 2018).

5 Conclusions

The aim of this thesis is to characterize the composition of the agricultural by-products WB and PP for a potential use in biorefineries. These feedstocks are available in substantial quantities in Austria. For a summative mass closure of these materials, several known methods were used and combined in a certain defined way, leading to a pathway that allows for a determination of all components. With the known composition, it is possible to determine what products can be obtained from biorefineries. Furthermore, several potential uses of WB and PP in biorefineries were presented.

The used methods for the characterization of these materials can be grouped into gravimetric, photometric and chromatographic methods. Gravimetric methods were used for the determination of moisture, ash, fat, pectin, extractives and AIL. Starch as well as ASL were determined via photometric methods while ions, sugars, other extractives and structural carbohydrates were determined via chromatographic methods. The Kjeldahl method was used for the determination of proteins, which does not fall into one of the categories mentioned above.

In general, the gravimetric methods had a low RSD, except for the fat content in PP and the extractives in both samples. Concerning both the water and ethanol extractives, it is better to use less sample to avoid a formation of foam which causes problems during the extraction and to ensure an exhaustive extraction. The photometric method for the determination of starch had a high RSD and for the determination of the individual types of starch this method is not accurate. However, a reason for that could be deviations from the protocol. The RSDs of the content of structural carbohydrates and sugars were low. Regarding the ion content, the RSD for the extractives free samples is too high to be reliable. In contrast, the ion content of the water extractives had a low RSD, which is a sign of a trustworthy measurement. Most of the measured values align with the values reported in literature, except for the fat content in WB, some sugar values and the lignin values. Therefore, it can be stated that the used methods are suitable. For lignin, it must be noted that the values depend on the used absorptivity constants for WB and PP, which were not determined but they were based on literature values. The quantification of sucrose in WB and PP, which was not done in this work, gives additional information about the composition of these two agricultural by-products. Since this disaccharide can be used in fermentations, knowledge about the sucrose content helps to evaluate the feedstocks. With an improved extraction by using less sample it should be possible that all of the sucrose is in the water extracts. However, a different quantification method than the used one in this thesis must be employed to determine the amount of sucrose.

This work describes a pathway that allows for a summative mass closure of WB and PP. All important constituents can be determined accurately with the methods used in this thesis. It could be shown that

for WB and PP the structural carbohydrates make up 30 and 46 wt% of the total mass, respectively. Most of the structural carbohydrates of WB consist of xylans, glucans and arabinans. The structural carbohydrates of PP are mostly made up of glucans and galactans. The high amount of glucans is favorable for fermentation because they can be hydrolyzed to glucose which is an important carbon source for fermentations. Glucose can be used as a substrate for the fermentation of lactic acid, while for the biotechnological production of succinic acid, ethanol and butanol all the monomeric sugars can be used. It must be kept in mind that the hydrolyzing conditions are not too harsh to avoid a formation of furfural and HMF which act as inhibitors for the subsequent fermentation. Starch, which is easily degradable to glucose, has a share of 14 wt% in WB and 16 wt% in PP. 15 wt% of WB and 6 wt% of PP is protein, which can be used as animal feed. This is important if the residue that is remaining after the fermentation is used as animal feed. Pectin from PP can be extracted after the starch hydrolysis. Ferulic acid, which can be further converted to vanillin, only occurs in WB.

These findings show that WB and PP have a potential as a feedstock for a biorefinery where valuable chemicals can be produced. Also, protein-rich residues can be valorized as animal feed. The pathway that is shown in this work can serve as an instruction for the summative mass closure of agricultural by-products. Companies where WB and PP are generated have an economic advantage if they manage to sell or process them themselves.

The general economics of a biorefinery may need more attention, starting with the amount of available PP and WB at a site or in a region, the transportation costs, if transportation is needed or which chemicals are the most promising ones that can be produced in a biorefinery. The economic assessment of a biorefinery using WB or PP must also be seen in connection with extractable compounds that were identified in this work via GC-MS because these compounds can have a high market value. Therefore, an interesting topic for future works is the quantification of these compounds. In a further step, it can be assessed whether the extraction and purification of these components is economically viable. Within this context it may also be useful to examine different extraction methods and solvents, for example supercritical CO₂.

6 Bibliography

- ABDEL-RAHMAN, M. A., TASHIRO, Y. & SONOMOTO, K. 2011. Lactic acid production from lignocellulose-derived sugars using lactic acid bacteria: Overview and limits. *Journal of Biotechnology*, 156, 286-301.
- ABDULLAH, A. H. D., CHALIMAH, S., PRIMADONA, I. & HANANTYO, M. H. G. 2018. Physical and chemical properties of corn, cassava, and potato starches. *IOP Conference Series: Earth and Environmental Science*, 160, 012003.
- ABO, B. O., GAO, M., WANG, Y., WU, C., WANG, Q. & MA, H. 2019. Production of butanol from biomass: recent advances and future prospects. *Environmental Science and Pollution Research*, 26, 20164-20182.
- ABOUSTEIT, O. & KEMPF, W. 1974. Pektingewinnung aus Kartoffelpülpe und ihre mögliche Bedeutung für die Kartoffelstärkeindustrie. *Starch - Stärke*, 26, 417-421.
- AMREIN, T. M., GRÄNICH, P., ARRIGONI, E. & AMADÒ, R. 2003. In vitro digestibility and colonic fermentability of aleurone isolated from wheat bran. *LWT - Food Science and Technology*, 36, 451-460.
- ANDERSSON, C., HODGE, D., BERGLUND, K. A. & ROVA, U. 2007. Effect of Different Carbon Sources on the Production of Succinic Acid Using Metabolically Engineered *Escherichia coli*. *Biotechnology Progress*, 23, 381-388.
- ANTUSZEWICZ, A. & ŚWIĘCH, E. 2006. Apparent ileal digestibility of amino acids in pig diets containing various sources of dietary fibre. *Journal of Animal and Feed Sciences*, 15, 53-56.
- APPRICH, S., TIRPANALAN, Ö., HELL, J., REISINGER, M., BÖHMDORFER, S., SIEBENHANDL-EHN, S., NOVALIN, S. & KNEIFEL, W. 2014. Wheat bran-based biorefinery 2: Valorization of products. *LWT - Food Science and Technology*, 56, 222-231.
- BABIKER, M. S., KIJORA, C., ABBAS, S. A. & DANIER, J. 2009. Nutrient composition of main poultry feed ingredients used in Sudan and their variations from local standard tables values. *International Journal of Poultry Science*, 8, 355-358.
- BAJPAI, P. 2018. Chapter 2 - Wood and Fiber Fundamentals. In: BAJPAI, P. (ed.) *Biermann's Handbook of Pulp and Paper (Third Edition)*. Elsevier.
- BAKS, T., BRUINS, M. E., MATSER, A. M., JANSSEN, A. E. M. & BOOM, R. M. 2008. Effect of Gelatinization and Hydrolysis Conditions on the Selectivity of Starch Hydrolysis with α -Amylase from *Bacillus licheniformis*. *Journal of Agricultural and Food Chemistry*, 56, 488-495.
- BEAUGRAND, J., CRÔNIER, D., DEBEIRE, P. & CHABBERT, B. 2004. Arabinoxylan and hydroxycinnamate content of wheat bran in relation to endoxylanase susceptibility. *Journal of Cereal Science*, 40, 223-230.
- BELITZ, H.-D. Q. B. H.-D., GROSCH, W. Q. W. & SCHIEBERLE, P. Q. P. 2008. *Lebensmittelchemie*, Berlin, Heidelberg, Berlin, Heidelberg : Springer-Verlag.
- BEST GMBH. 2023. *Database on facilities for the production of advanced liquid and gaseous biofuels for transport* [Online]. Inffeldgasse 21b 8010 Graz: BEST GmbH. Available: <https://demoplants.best-research.eu/> [Accessed June 15th, 2023].
- BONDESSON, P.-M. & GALBE, M. 2016. Process design of SSCF for ethanol production from steam-pretreated, acetic-acid-impregnated wheat straw. *Biotechnology for Biofuels*, 9, 222.
- BOTHAST, R. J. & SCHLICHER, M. A. 2005. Biotechnological processes for conversion of corn into ethanol. *Applied Microbiology and Biotechnology*, 67, 19-25.
- BRITANNICA, T. E. O. E. 2023. *citric acid* [Online]. Encyclopedia Britannica. Available: <https://www.britannica.com/science/citric-acid> [Accessed September 11th, 2023].
- BRUNT, K. & DROST, W. C. 2010. Design, Construction, and Testing of an Automated NIR In-line Analysis System for Potatoes. Part I: Off-line NIR Feasibility Study for the Characterization of Potato Composition. *Potato Research*, 53, 25-39.
- BÜCHI LABORTECHNIK AG 2016. Extraktionssystem B-811. *Bedienungsanleitung*. CH-9230 Flawil: BÜCHI Labortechnik AG.

- BURANOV, A. U. & MAZZA, G. 2009. Extraction and purification of ferulic acid from flax shives, wheat and corn bran by alkaline hydrolysis and pressurised solvents. *Food Chemistry*, 115, 1542-1548.
- CHAMP, M. 2004. 20 - Resistant starch. In: ELIASSON, A.-C. (ed.) *Starch in Food*. Woodhead Publishing.
- CHOI, S.-H., KOZUKUE, N., KIM, H.-J. & FRIEDMAN, M. 2016. Analysis of protein amino acids, non-protein amino acids and metabolites, dietary protein, glucose, fructose, sucrose, phenolic, and flavonoid content and antioxidative properties of potato tubers, peels, and cortexes (pulp). *Journal of Food Composition and Analysis*, 50, 77-87.
- CHOTĚBORSKÁ, P., PALMAROLA-ADRADOS, B., GALBE, M., ZACCHI, G., MELZUCH, K. & RYCHTERA, M. 2004. Processing of wheat bran to sugar solution. *Journal of Food Engineering*, 61, 561-565.
- CLARKE, M. A. & SINGH, R. P. 2023. *sugar* [Online]. Encyclopedia Britannica. Available: <https://www.britannica.com/science/sugar-chemical-compound> [Accessed September 11th, 2023].
- COK, B., TSIROPOULOS, I., ROES, A. L. & PATEL, M. K. 2014. Succinic acid production derived from carbohydrates: An energy and greenhouse gas assessment of a platform chemical toward a bio-based economy. *Biofuels, Bioproducts and Biorefining*, 8, 16-29.
- DELGENES, J. P., MOLETTA, R. & NAVARRO, J. M. 1996. Effects of lignocellulose degradation products on ethanol fermentations of glucose and xylose by *Saccharomyces cerevisiae*, *Zymomonas mobilis*, *Pichia stipitis*, and *Candida shehatae*. *Enzyme and Microbial Technology*, 19, 220-225.
- DESHAVATH, N. N., GOUD, V. V. & VEERANKI, V. D. 2021. Curtailing citrate buffer inhibition effect on *S. cerevisiae* to enhance the fermentability of cellulosic hydrolysate. *Journal of Environmental Chemical Engineering*, 9, 105696.
- DI GIOIA, D., SCIUBBA, L., RUZZI, M., SETTI, L. & FAVA, F. 2009. Production of vanillin from wheat bran hydrolyzates via microbial bioconversion. *Journal of Chemical Technology & Biotechnology*, 84, 1441-1448.
- DI LENA, G., VIVANTI, V. & QUAGLIA, G. B. 1997. Amino acid composition of wheat milling by-products after bioconversion by edible fungi mycelia. *Food / Nahrung*, 41, 285-288.
- DIN 2005. Futtermittel – Bestimmung des Stickstoffgehaltes und Berechnung des Rohproteingehaltes – Teil 1: Kjeldahl-Verfahren (ISO 5983-1:2005). 10772 Berlin: Beuth Verlag GmbH.
- DIN 2016. Getreide, Getreideerzeugnisse und Futtermittel – Bestimmung des Rohfettgehalts und des Gesamtfettgehalts mit dem Extraktionsverfahren nach Randall (ISO 11085:2015). 10772 Berlin: Beuth Verlag GmbH.
- DORNEZ, E., GEBRUERS, K., WIAME, S., DELCOUR, J. A. & COURTIN, C. M. 2006. Insight into the Distribution of Arabinoxylans, Endoxylanases, and Endoxylanase Inhibitors in Industrial Wheat Roller Mill Streams. *Journal of Agricultural and Food Chemistry*, 54, 8521-8529.
- DUSSELIER, M., VAN WOUWE, P., DEWAELE, A., MAKSHINA, E. & SELS, B. F. 2013. Lactic acid as a platform chemical in the biobased economy: the role of chemocatalysis. *Energy & Environmental Science*, 6, 1415-1442.
- ELLIOTT, D., ORTH, R., WERP, T., GAO, J., EAKIN, D., SCHMIDT, A., NEUENSCHWANDER, G., MURRY, J., FLAGG, A., LAHMAN, L., MENNEL, D. & LIN, C. 2002. Biorefinery Concept Development Based on Wheat Flour Milling. *Fuel Chemistry Division Preprints*, 47.
- ERENSTEIN, O., JALETA, M., MOTTALEB, K. A., SONDER, K., DONOVAN, J. & BRAUN, H.-J. 2022. Global Trends in Wheat Production, Consumption and Trade. In: REYNOLDS, M. P. & BRAUN, H.-J. (eds.) *Wheat Improvement: Food Security in a Changing Climate*. Cham: Springer International Publishing.
- EZEJI, T. C., QURESHI, N. & BLASCHEK, H. P. 2007. Bioproduction of butanol from biomass: from genes to bioreactors. *Current Opinion in Biotechnology*, 18, 220-227.
- FAO. *Post-harvesting processing* [Online]. Available: <https://www.fao.org/3/au104e/au104e.pdf> [Accessed July 27th, 2023].

- FAO 2022. World Food and Agriculture – Statistical Yearbook 2022. *FAO Statistical Yearbook – World Food and Agriculture*. Rome.
- FAO 2023a. Crop Prospects and Food Situation. *Quarterly Global Report No. 1, March 2023*. Rome.
- FAO 2023b. Food Balances (2010-). Rome.
- FERREIRA, P., DIEZ, N., FAULDS, C. B., SOLIVERI, J. & COPA-PATÍÑO, J. L. 2007. Release of ferulic acid and feruloylated oligosaccharides from sugar beet pulp by *Streptomyces tendae*. *Bioresource Technology*, 98, 1522-1528.
- FILIPPOV, P. 2010. Available: https://upload.wikimedia.org/wikipedia/commons/thumb/6/65/Triticum_aestivum1.JPG/640px-Triticum_aestivum1.JPG?uselang=de [Accessed August 16th, 2023].
- FUNAMI, T., NAKAUMA, M., ISHIHARA, S., TANAKA, R., INOUE, T. & PHILLIPS, G. O. 2011. Structural modifications of sugar beet pectin and the relationship of structure to functionality. *Food Hydrocolloids*, 25, 221-229.
- GNANASAMBANDAM, R. & PROCTOR, A. 2000. Determination of pectin degree of esterification by diffuse reflectance Fourier transform infrared spectroscopy. *Food Chemistry*, 68, 327-332.
- GOMES, E. 2019. *What Is N-Butanol?* [Online]. Ecolink. Available: <https://ecolink.com/info/what-is-n-butanol/> [Accessed August 3rd, 2023].
- GOPINATH, V., MEISWINKEL, T. M., WENDISCH, V. F. & NAMPOOTHIRI, K. M. 2011. Amino acid production from rice straw and wheat bran hydrolysates by recombinant pentose-utilizing *Corynebacterium glutamicum*. *Applied Microbiology and Biotechnology*, 92, 985-996.
- GOPINATH, V., MURALI, A., DHAR, K. S. & NAMPOOTHIRI, K. M. 2012. *Corynebacterium glutamicum* as a potent biocatalyst for the bioconversion of pentose sugars to value-added products. *Applied Microbiology and Biotechnology*, 93, 95-106.
- GROMMERS, H. E. & VAN DER KROGT, D. A. 2009. Chapter 11 - Potato Starch: Production, Modifications and Uses. In: BEMILLER, J. & WHISTLER, R. (eds.) *Starch (Third Edition)*. San Diego: Academic Press.
- GUO, Y.-Z., YANG, X.-M. & LI, Y.-Y. 2019. Effect of Alkylresorcinols on Autophagy, Migration, and Invasion of HepG2 Cells. *Journal of Food Science*, 84, 3063-3068.
- HAMAKER, B. R., TUNCIL, Y. E. & SHEN, X. 2019. Chapter 11 - Carbohydrates of the Kernel. In: SERNA-SALDIVAR, S. O. (ed.) *Corn (Third Edition)*. Oxford: AACC International Press.
- HAMES, B., RUIZ, R., SCARLATA, C., SLUITER, A., SLUITER, J. & TEMPLETON, D. 2008. Preparation of Samples for Compositional Analysis: Laboratory Analytical Procedure (LAP). 1617 Cole Boulevard, Golden, Colorado 80401-3393: National Renewable Energy Laboratory.
- HATFIELD, R. & FUKUSHIMA, R. S. 2005. Can Lignin Be Accurately Measured? *Crop Science*, 45, 832-839.
- HEMERY, Y., ROUAU, X., LULLIEN-PELLERIN, V., BARRON, C. & ABECASSIS, J. 2007. Dry processes to develop wheat fractions and products with enhanced nutritional quality. *Journal of Cereal Science*, 46, 327-347.
- HOANG, T.-D. & NGHIEM, N. 2021. Recent Developments and Current Status of Commercial Production of Fuel Ethanol. *Fermentation*, 7, 314.
- HOFVENDAHL, K. & HAHN-HÄGERDAL, B. 2000. Factors affecting the fermentative lactic acid production from renewable resources1. *Enzyme and Microbial Technology*, 26, 87-107.
- INRAE CIRAD AFZ. *Potato pulp, dried* [Online]. Available: <https://www.feedtables.com/content/potato-pulp-dried> [Accessed July 28th, 2023].
- IRAM, A., CEKMECELIOGLU, D. & DEMIRCI, A. 2022. Integrating 1G with 2G Bioethanol Production by Using Distillers’ Dried Grains with Solubles (DDGS) as the Feedstock for Lignocellulolytic Enzyme Production. *Fermentation*, 8, 705.
- JOHN DEERE UK IE. 2018. *John Deere | S700 Complete Cropflow Animation* [Online]. Available: <https://youtu.be/z1UPnNf8nU?t=75> [Accessed August 15th, 2023].
- KIM, J. C., MULLAN, B., SIMMINS, H. & PLUSKE, J. 2003. Variation in the chemical composition of wheats grown in Western Australia as influenced by variety, growing region, season, and post-harvest storage. *Australian Journal of Agricultural Research*, 54, 541-550.

- KIM, K.-H., TSAO, R., YANG, R. & CUI, S. W. 2006. Phenolic acid profiles and antioxidant activities of wheat bran extracts and the effect of hydrolysis conditions. *Food Chemistry*, 95, 466-473.
- KLINGSPORN, U., BADER, J., KRUSE, B., VIJAI KISHORE, P., SCHÜGERL, K., KRACKE-HELM, H. A. & LIKIDIS, Z. 1993. Utilization of potato pulp from potato starch processing. *Process Biochemistry*, 28, 91-98.
- KNUDSEN, K. E. B. 1997. Carbohydrate and lignin contents of plant materials used in animal feeding. *Animal Feed Science and Technology*, 67, 319-338.
- KOMESU, A., OLIVEIRA, J., MARTINS, L. H., MACIEL, M. & FILHO, R. 2017. Lactic acid production to purification: A review. *BioResources*, 12, 4364-4383.
- KOUNBESIOU, M. K., SAVADOGO, A., BARRO, N., THONART, P. & SABADENEDY, A. S. 2011. Effect of Minerals Salts in Fermentation Process using Mango Residues as Carbon Source for Bioethanol Production. *Asian Journal of Industrial Engineering*, 3, 29-38.
- KOUTINAS, A. A., WANG, R., CAMPBELL, G. M. & WEBB, C. 2005. A Whole Crop Biorefinery System: A Closed System for the Manufacture of Non-food Products from Cereals. In: GRUBER, P. R., KAMM, M. & KAMM, B. (eds.) *Biorefineries-Industrial Processes and Products*. Weinheim, Germany: Wiley-VCH Verlag GmbH.
- LALLEMAND BIOFUELS & DISTILLED SPIRITS. 2019. *Fuel Ethanol Products* [Online]. 1815 Satellite Blvd., Bldg. 200 • Duluth, GA 30097: Lallemand Biofuels & Distilled Spirits. Available: https://www.lbds.com/wp-content/uploads/2019/05/LBDS_ProductPortfolioSheet_DRAFT_050319.pdf [Accessed July 16th, 2023].
- LALLEMAND INC. 2022. *Yeast nutrition fundamentals* [Online]. 1620 rue Prefontaine Montreal H1W 2N8 Quebec. Available: <https://www.lallemantbrewing.com/wp-content/uploads/2017/03/Nutrition-Booklet-ENG-Digital-AB-Vickers.pdf> [Accessed September 9th, 2023].
- LARSSON, S., PALMQVIST, E., HAHN-HÄGERDAL, B., TENGBORG, C., STENBERG, K., ZACCHI, G. & NILVEBRANT, N.-O. 1999. The generation of fermentation inhibitors during dilute acid hydrolysis of softwood. *Enzyme and Microbial Technology*, 24, 151-159.
- LEE, J. W. & STENVERT, N. L. 1973. Conditioning studies on Australian wheat. IV. Compositional variations in the bran layers of wheat and their relation to milling. *Journal of the Science of Food and Agriculture*, 24, 1565-1569.
- LEROUX, J., LANGENDORFF, V., SCHICK, G., VAISHNAV, V. & MAZOYER, J. 2003. Emulsion stabilizing properties of pectin. *Food Hydrocolloids*, 17, 455-462.
- LIU, Z., YING, Y., LI, F., MA, C. & XU, P. 2010. Butanol production by *Clostridium beijerinckii* ATCC 55025 from wheat bran. *Journal of Industrial Microbiology and Biotechnology*, 37, 495-501.
- MAYER, F. 1998. Potato pulp: properties, physical modification and applications. *Polymer Degradation and Stability*, 59, 231-235.
- MAYER, F. & HILLEBRANDT, J.-O. 1997. Potato pulp: microbiological characterization, physical modification, and application of this agricultural waste product. *Applied Microbiology and Biotechnology*, 48, 435-440.
- MCKENDRY, P. 2002. Energy production from biomass (part 1): overview of biomass. *Bioresource Technology*, 83, 37-46.
- MEGAZYME 2022. Digestible and Resistant Starch Assay Protocol. *K-DSTRS*. Megazyme Building 1, Bray Business Park, Bray, Co. Wicklow, A98 YV29 Ireland: NEOGEN.
- MERCK KGAA. *IR Spectrum Table & Chart* [Online]. Darmstadt, Germany: Merck KGaA. Available: <https://www.sigmaaldrich.com/AT/en/technical-documents/technical-article/analytical-chemistry/photometry-and-reflectometry/ir-spectrum-table> [Accessed July 23rd, 2023].
- MEYER, A. S., DAM, B. P. & LÆRKE, H. N. 2009. Enzymatic solubilization of a pectinaceous dietary fiber fraction from potato pulp: Optimization of the fiber extraction process. *Biochemical Engineering Journal*, 43, 106-112.
- NOVALIN-CANOY, P. 2011. *Grüne Bioraffinerie Phase III - Marktstudie zum Thema Aminosäuren und deren Anwendungen* [Online]. Bundesministerium für Verkehr, Innovation und Technologie. Available:

- https://nachhaltigwirtschaften.at/resources/fdz_pdf/endbericht_1102c_marktstudie.pdf [Accessed August 3rd, 2023].
- OISHI, K., YAMAMOTO, S., ITOH, N., NAKAO, R., YASUMOTO, Y., TANAKA, K., KIKUCHI, Y., FUKUDOME, S.-I., OKITA, K. & TAKANO-ISHIKAWA, Y. 2015. Wheat Alkylresorcinols Suppress High-Fat, High-Sucrose Diet-Induced Obesity and Glucose Intolerance by Increasing Insulin Sensitivity and Cholesterol Excretion in Male Mice^{1, 2, 3}. *The Journal of Nutrition*, 145, 199-206.
- OLDWAYS WHOLE GRAINS COUNCIL. 2019. *WHAT IS A WHOLE GRAIN?* [Online]. 266 Beacon Street, Suite 1
Boston, MA 02116, USA. Available: <https://wholegrainscouncil.org/what-whole-grain> [Accessed September 30th, 2023].
- OU, S. & KWOK, K.-C. 2004. Ferulic acid: pharmaceutical functions, preparation and applications in foods. *Journal of the Science of Food and Agriculture*, 84, 1261-1269.
- PALMAROLA-ADRADOS, B., CHOTĚBORSKÁ, P., GALBE, M. & ZACCHI, G. 2005. Ethanol production from non-starch carbohydrates of wheat bran. *Bioresource Technology*, 96, 843-850.
- PARIKKA, K., ROWLAND, I. R., WELCH, R. W. & WÄHÄLÄ, K. 2006. In Vitro Antioxidant Activity and Antigenotoxicity of 5-n-Alkylresorcinols. *Journal of Agricultural and Food Chemistry*, 54, 1646-1650.
- PEPIN, C. & MARZZACCO, C. 2015. *The fermentation of sugars using yeast: A discovery experiment* [Online]. Melbourne, Florida, USA: University of Waterloo. Available: <https://uwaterloo.ca/chem13-news-magazine/april-2015/activities/fermentation-sugars-using-yeast-discovery-experiment> [Accessed September 11th, 2023].
- PETER, M. G., RÖMPP REDAKTION & SEIBEL, J. 2015. Pektine. December 16th, 2015 ed. Stuttgart: Thieme Gruppe.
- PINAZO, J. M., DOMINE, M. E., PARVULESCU, V. & PETRU, F. 2015. Sustainability metrics for succinic acid production: A comparison between biomass-based and petrochemical routes. *Catalysis Today*, 239, 17-24.
- POURALI, O., ASGHARI, F. S. & YOSHIDA, H. 2010. Production of phenolic compounds from rice bran biomass under subcritical water conditions. *Chemical Engineering Journal*, 160, 259-266.
- PRIEFERT, H., RABENHORST, J. & STEINBÜCHEL, A. 2001. Biotechnological production of vanillin. *Applied Microbiology and Biotechnology*, 56, 296-314.
- PRÜCKLER, M., SIEBENHANDL-EHN, S., APPRICH, S., HÖLTINGER, S., HAAS, C., SCHMID, E. & KNEIFEL, W. 2014. Wheat bran-based biorefinery 1: Composition of wheat bran and strategies of functionalization. *LWT - Food Science and Technology*, 56, 211-221.
- RAVN, H. C., BANDSHOLM SØRENSEN, O. & MEYER, A. S. 2015. Time of harvest affects the yield of soluble polysaccharides extracted enzymatically from potato pulp. *Food and Bioproducts Processing*, 93, 77-83.
- REISINGER, M., TIRPANALAN, Ö., PRÜCKLER, M., HUBER, F., KNEIFEL, W. & NOVALIN, S. 2013. Wheat bran biorefinery – A detailed investigation on hydrothermal and enzymatic treatment. *Bioresource Technology*, 144, 179-185.
- RENEWABLE FUELS ASSOCIATION. 2022. *Annual Ethanol Production* [Online]. 601 Pennsylvania Ave, NW North Building - Suite 200 Washington, DC 20004. Available: <https://ethanolrfa.org/markets-and-statistics/annual-ethanol-production> [Accessed July 14th, 2023].
- ROBERTS, P. J., SIMMONDS, D. H., WOOTTON, M. & WRIGLEY, C. W. 1985. Extraction of protein and solids from wheat bran. *Journal of the Science of Food and Agriculture*, 36, 5-10.
- ROQUETTE FRÈRES. 2022. *Enabling sustainable materials with BIOSUCCINIUM® - bio succinic acid* [Online]. 62136 Lestrem, France: Roquette Frères. Available: <https://www.roquette.com/industries/selected-products/industry-performance-materials-biosuccinum> [Accessed June 20th, 2023].
- SAEMAN, J. F. 1945. Kinetics of Wood Saccharification - Hydrolysis of Cellulose and Decomposition of Sugars in Dilute Acid at High Temperature. *Industrial & Engineering Chemistry*, 37, 43-52.

- SAMSON, F. E., KATZ, A. M. & HARRIS, D. L. 1955. Effects of acetate and other short-chain fatty acids on yeast metabolism. *Archives of Biochemistry and Biophysics*, 54, 406-423.
- SCHMITZ, E., NORDBERG KARLSSON, E. & ADLERCREUTZ, P. 2021. Altering the water holding capacity of potato pulp via structural modifications of the pectic polysaccharides. *Carbohydrate Polymer Technologies and Applications*, 2, 100153.
- SERENA, A. & KNUDSEN, K. E. B. 2007. Chemical and physicochemical characterisation of co-products from the vegetable food and agro industries. *Animal Feed Science and Technology*, 139, 109-124.
- SERNA-LOAIZA, S., MILTNER, A., MILTNER, M. & FRIEDL, A. 2019. A Review on the Feedstocks for the Sustainable Production of Bioactive Compounds in Biorefineries. *Sustainability*, 11, 6765.
- SHARMA, V., RAUSCH, K. D., GRAEBER, J. V., SCHMIDT, S. J., BURIK, P., TUMBLES, M. E. & SINGH, V. 2010. Effect of Resistant Starch on Hydrolysis and Fermentation of Corn Starch for Ethanol. *Applied Biochemistry and Biotechnology*, 160, 800-811.
- SHIMIZU, H. & HIRASAWA, T. 2007. Production of Glutamate and Glutamate-Related Amino Acids: Molecular Mechanism Analysis and Metabolic Engineering. In: WENDISCH, V. F. (ed.) *Amino Acid Biosynthesis ~ Pathways, Regulation and Metabolic Engineering*. Berlin, Heidelberg: Springer Berlin Heidelberg.
- SLUITER, A., HAMES, B., RUIZ, R., SCARLATA, C., SLUITER, J., TEMPLETON, D. & CROCKER, D. 2008a. Determination of Structural Carbohydrates and Lignin in Biomass: Laboratory Analytical Procedure (LAP). 1617 Cole Boulevard, Golden, Colorado 80401-3393: National Renewable Energy Laboratory.
- SLUITER, A., HAMES, B., RUIZ, R. O., SCARLATA, C., SLUITER, J. & TEMPLETON, D. 2008b. Determination of Ash in Biomass: Laboratory Analytical Procedure (LAP). 1617 Cole Boulevard, Golden, Colorado 80401-3393: National Renewable Energy Laboratory.
- SLUITER, A., RUIZ, R., SCARLATA, C., SLUITER, J. & TEMPLETON, D. 2008c. Determination of Extractives in Biomass: Laboratory Analytical Procedure (LAP). 1617 Cole Boulevard, Golden, Colorado 80401-3393: National Renewable Energy Laboratory.
- SLUITER, J. & SLUITER, A. 2010. Summative Mass Closure: Laboratory Analytical Procedure (LAP). 1617 Cole Boulevard, Golden, Colorado 80401-3393: National Renewable Energy Laboratory.
- SSYMANK, A. & DUELL-PFAFF, N. 1999. *Weizen* [Online]. Heidelberg, Germany: Spektrum Akademischer Verlag. Available: <https://www.spektrum.de/lexikon/biologie/weizen/70571> [Accessed July 27th, 2023].
- STARCH EUROPE. 2019. *(Vital) Wheat Gluten* [Online]. Available: <https://starchinfo.eu/ingredient/wheat-gluten-vital-wheat-gluten/> [Accessed July 27th, 2023].
- STARCH EUROPE. 2021a. *EU Consumption of Starch & Derivatives* [Online]. Avenue des Arts, 43, B-1040 Brussels, Belgium. Available: <https://starch.eu/wp-content/uploads/2023/01/Starch-Europe-Market-Data-202111.jpeg> [Accessed June 16th, 2023].
- STARCH EUROPE. 2021b. *Main Production in EU Starch Products in Starch Equivalent* [Online]. Avenue des Arts, 43, B-1040 Brussels, Belgium. Available: <https://starch.eu/wp-content/uploads/2023/01/Starch-Europe-Market-Data-20218.jpeg> [Accessed June 15th, 2023].
- TAURUS ENERGY AB. 2015. *XyloFerm* [Online]. Chalmers Teknikpark, 412 88 Göteborg, Sweden: Taurus Energy AB. Available: <https://www.lbds.com/wp-content/uploads/2015/06/Xyloferm-product-sheet-2016-05-19.pdf> [Accessed July 16th, 2023].
- TRABERT, A., SCHMID, V., KELLER, J., EMIN, M. A. & BUNZEL, M. 2022. Chemical composition and technofunctional properties of carrot (*Daucus carota* L.) pomace and potato (*Solanum tuberosum* L.) pulp as affected by thermomechanical treatment. *European Food Research and Technology*, 248, 2451-2470.
- U.S. DEPARTMENT OF AGRICULTURE. 2019. *FoodData Central Search Results* [Online]. Available: <https://fdc.nal.usda.gov/fdc-app.html#/food-details/169722/nutrients> [Accessed July 28th, 2023].

- U.S. DEPARTMENT OF ENERGY. 2012. *Myriant succinic acid biorefinery (MySAB)* [Online]. Washington, DC 20585: U.S. Department of Energy. Available: https://www1.eere.energy.gov/bioenergy/pdfs/ibr_arra_myriant.pdf [Accessed June 20th, 2023].
- U.S. GRAINS COUNCIL 2021. 2021/2022 Corn Harvest Quality Report. 20 F Street NW, Suite 900, Washington, DC 20001: U.S. Grains Council.
- VAN DEN BORNE, J. J. G. C., KABEL, M. A., BRIENS, M., VAN DER POEL, A. F. B. & HENDRIKS, W. H. 2012. Effects of pretreatment of wheat bran on the quality of protein-rich residue for animal feeding and on monosaccharide release for ethanol production. *Bioresource Technology*, 124, 446-454.
- VILPOUX, O. F. & SANTOS SILVEIRA JUNIOR, J. F. 2023. Chapter 3 - Global production and use of starch. In: PASCOLI CEREDA, M. & FRANÇOIS VILPOUX, O. (eds.) *Starchy Crops Morphology, Extraction, Properties and Applications*. Academic Press.
- WENDISCH, V. F. 2007. *Amino Acid Biosynthesis – Pathways, Regulation and Metabolic Engineering*, Heidelberg, Springer.
- WERPY, T., FRYE, J. & HOLLADAY, J. 2005. Succinic Acid – A Model Building Block for Chemical Production from Renewable Resources. *Biorefineries-Industrial Processes and Products*.
- WITTMANN, C. & BECKER, J. 2007. The L-Lysine Story: From Metabolic Pathwaysto Industrial Production. In: WENDISCH, V. F. (ed.) *Amino Acid Biosynthesis ~ Pathways, Regulation and Metabolic Engineering*. Berlin, Heidelberg: Springer Berlin Heidelberg.
- XIE, X., CUI, S. W., LI, W. & TSAO, R. 2008. Isolation and characterization of wheat bran starch. *Food Research International*, 41, 882-887.
- YANG, J.-S., MU, T.-H. & MA, M.-M. 2018. Extraction, structure, and emulsifying properties of pectin from potato pulp. *Food Chemistry*, 244, 197-205.
- YE, G., WU, Y., WANG, L., TAN, B., SHEN, W., LI, X., LIU, Y., TIAN, X. & ZHANG, D. 2021. Comparison of six modification methods on the chemical composition, functional properties and antioxidant capacity of wheat bran. *LWT*, 149, 111996.
- ZABED, H., SAHU, J. N., SUELY, A., BOYCE, A. N. & FARUQ, G. 2017. Bioethanol production from renewable sources: Current perspectives and technological progress. *Renewable and Sustainable Energy Reviews*, 71, 475-501.
- ZAHOOR, A., LINDNER, S. N. & WENDISCH, V. F. 2012. METABOLIC ENGINEERING OF CORYNEBACTERIUM GLUTAMICUM AIMED AT ALTERNATIVE CARBON SOURCES AND NEW PRODUCTS. *Computational and Structural Biotechnology Journal*, 3, e201210004.
- ZAIDEL, D. N. A., ZAINUDIN, N. N., JUSOH, Y. M. M. & MUHAMAD, I. I. 2015. Extraction and characterisation of pectin from sweet potato (*Ipomoea batatas*) pulp. *Journal of Engineering Science and Technology*, 10, 22-29.

7 Appendix

7.1 Supplementary material of section “2 Methods and materials”

7.1.1 Particle size distribution

For the calculation of the distribution density and distribution sum, it was necessary to calculate the mass of the oversize particle Δm_i with Equation 1:

$$\Delta m_i = m_v - m_0 \quad \text{Equation 1}$$

Δm_i Mass of the oversize particle (g)

m_v Empty mass of the sieve with oversize particles (g)

m_0 Empty mass of the sieve (g)

The distribution density function and the distribution sum function can be set up using the following formulas based on the mass of the oversize particle and the grain sizes. The grain fraction width indicates the mesh size distance from sieve $i+1$ to the sieve with the smaller mesh size i , as indicated in the following Equation 2:

$$\Delta x_i = x_{i+1} - x_i \quad \text{Equation 2}$$

Δx_i Grain fraction width (μm)

x_i Grain size of fraction i (μm)

The fraction average (mean particle diameter) of the i -th grain class $x_{i,\text{mean}}$ is calculated using Equation 3:

$$x_{i,\text{mean}} = \frac{x_{i+1} - x_i}{2} \quad \text{Equation 3}$$

$x_{i,\text{mean}}$ fraction average (mean particle diameter) of grain fraction i (μm)

The distribution density and the distribution sum can be calculated on the basis of $x_{i,\text{mean}}$ and Δx_i according to Equation 4 and Equation 5:

$$q_3(x_{i,\text{mean}}) = \frac{\Delta m_i}{m_{\text{tot}} * \Delta x_i} \quad \text{Equation 4}$$

$$Q_3(x_{0,i}) = \sum q_3(x_{i,\text{mean}}) * \Delta x_i = \sum \frac{\Delta m_i}{m_{\text{tot}}} \quad \text{Equation 5}$$

$q_3(x_{i,\text{mean}})$ Mass-related distribution density (μm^{-1})

$Q_3(x_{0,i})$ Mass-related distribution sum (-)

$x_{0,i}$ Upper limit of fraction (largest particle diameter) of fraction i (μm)

m_{tot} Total mass of the sample (g)

$Q_3(x_{0,i})$ is summed up starting from the smallest diameter. The calculated values for the distribution sum and the distribution density of WB and PP are entered in Table 28 and Table 29.

7.1.2 Determination of the moisture content

The moisture content was determined according to the following equation:

$$M = \frac{U - D}{U} \times 100 \quad \text{Equation 6}$$

M Moisture content (wt%)

U Mass of the undried sample (g)

D Mass of the dried sample (g)

Equation 7 is used to calculate the ODW of the sample.

$$ODW = \frac{S_a * (100 - M)}{100} \quad \text{Equation 7}$$

ODW Mass of the oven dry sample (g)

S_a Mass of the air dry sample (g)

M Moisture content (wt%)

7.1.3 Determination of the ash content

For the determination of the total ash content, the following equation was used (Sluiter et al., 2008b):

$$\%Ash = \frac{m_{crucible+ash} - m_{crucible}}{ODW} \quad \text{Equation 8}$$

$m_{crucible+ash}$ Mass of the crucible with ash (g)

$m_{crucible}$ Mass of the empty crucible (g)

To calculate the content of structural ash based on the untreated sample, Equation 9 was used:

$$\%Ash_{structural} = \frac{m_{crucible+ash} - m_{crucible}}{ODW_{extractive-free}} \times \frac{100 - \%Extractives}{100} \quad \text{Equation 9}$$

7.1.4 Determination of the starch content

The following calculation are based on the Digestible and Resistant Starch Assay Protocol from (Megazyme, 2022). To calculate the starch content, it is necessary to calculate the conversion factor from the absorbance value to μg first, which was done via Equation 10 :

$$F = \frac{100 \mu g \text{ glucose}}{\bar{A}_s} \quad \text{Equation 10}$$

F Conversion factor from absorbance to μg

$\bar{A}_s$ Average absorption of D-glucose standards (-)

With the following Equation 11 the content of digestible starch in g per 100 g of untreated sample for RDS and TDS was calculated. For the calculation of SDS, the absorbance value measured after 20 min of incubation was subtracted from the absorbance value after 120 min. The rest of the equation remains the same.

$$\%Starch_{digestible} = A \times F \times \frac{EV}{W} \times \frac{D}{0.1} \times 100 \times 10^{-6} \times \frac{162}{180} \times \frac{100 - M}{100} \quad \text{Equation 11}$$

A..... Respective absorbance value against blank (-)
 EV..... Extraction volume; 20.5 mL
 W..... “as is” weight of the sample analyzed (g)
 D..... Dilution factor of the sample; 21
 M..... Moisture content (wt%)

0.1 comes from the 0.1 mL of sample analyzed, 100 from the conversion to g/100 g, 1,000,000 from the conversion from μg to g and 162/180 is the factor to convert from the determined free D-glucose to the anhydro-D-glucose in starch.

For the calculation of the RS content in the untreated sample, Equation 12 was used:

$$\%Starch_{resistant} = A \times F \times \frac{EV}{4} \times \frac{FV}{0.1} \times \frac{100}{W} \times 10^{-6} \times \frac{162}{180} \times \frac{100 - M}{100} \quad \text{Equation 12}$$

FV..... Final volume, 100 mL

4 comes from the 4 mL of solution taken from the reaction mixture for the analysis of RS. TS is the sum of TDS and RS.

For the determination of the starch content of the extractives free sample, Equation 13 was used:

$$\%Starch_{extractives\ free} = \%Starch \times \frac{100 - \%Extractives}{100} \quad \text{Equation 13}$$

7.1.5 Determination of the pectin content

$$\%Pectin = \frac{\text{Mass filter paper with pectin} - \text{Mass filter paper}}{ODW} \times 100 \quad \text{Equation 14}$$

7.1.6 Determination of the protein content

$$\%Protein = \frac{\%Nitrogen \times F}{100 - \%Moisture} \quad \text{Equation 15}$$

F..... Conversion factor, for WB 6.02, for PP 6.25

For the determination of the protein content of the extractives free sample, Equation 16 was used:

$$\%Protein_{extractives\ free} = \%Protein \times \frac{100 - \%Extractives}{100} \quad \text{Equation 16}$$

7.1.7 Determination of extractives

It is necessary to know the amount of the moisture in the raw and extractives free sample to subtract the moisture, as described in Equation 17. The moisture of the extractives free WB and PP was determined with 2.68 wt% and 2.52 wt%, respectively. For the same reason, the mass of the dried empty thimble is used in the equation.

$$\%Extractives = \frac{m_T + m_G + m_S - m_e}{ODW} \quad \text{Equation 17}$$

m_T Mass of the dried thimble (g)

m_G Mass of glass wool (g)

m_S Mass of the sample with 2.68 % or 2.52 % moisture (g)

m_e Mass of the thimble containing the extractives-free sample and the glass wool (g)

7.1.8 Determination of structural carbohydrates

Table 23: Used gradient program for the separation of monosaccharides

	Time (min)	Flow rate (mL min ⁻¹)	Eluent A (%)	Eluent B (%)	Eluent C (%)	Eluent D (%)
Equilibration	-17	0.26	0	87	13	0
	-7	0.26	0	87	13	0
	-6	0.26	100	0	0	0
Run	0	0.26	100	0	0	0
	18	0.26	100	0	0	0

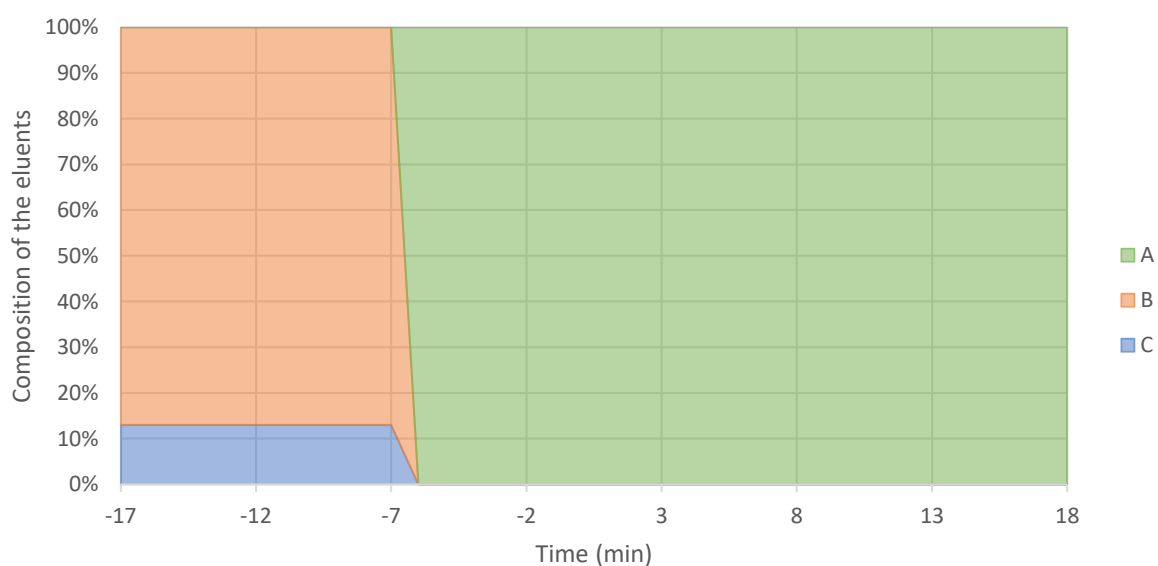


Figure 8: Graphic illustration of the multi-step gradient of the HPAEC-PAD in relation to time

To calculate the composition of the structural carbohydrate in the extractives free sample, the degradation of the sugars had to be considered. Therefore, correction factors for the SRS were calculated according to Equation 18.

$$CF = \frac{\text{concentration detected by HPAE} - \text{PAD}}{\text{known concentration of sugar before hydrolysis}} \quad \text{Equation 18}$$

CF Correction factor (-)

Each concentration of each SRS was measured thrice and the average of the measurements was used for the estimation of the correction factor.

In the next step, it was checked which dilution factor of the sample, 10 or 100, for which sugar is within the calibration range and is better suited for the evaluation. The chosen dilution factors are listed in the following table:

Table 24: Dilution factors for the evaluation of the monomeric sugars of the structural carbohydrates of WB and PP

	Arabinose	Galactose	Glucose	Xylose	Mannose
WB	10	10	100	100	10
PP	10	100	100	10	10

Now, the actual concentrations of the monomeric sugars of the structural carbohydrates can be calculated according to the following equation:

$$c_{\text{sugar}} = \frac{c_{\text{HPAE-PAD}}}{CF} \quad \text{Equation 19}$$

c_{sugar} Sugar concentration (mg L⁻¹)

$c_{\text{HPAEC-PAD}}$ Sugar concentration as measured via HPAEC-PAD (mg L⁻¹)

Then, the mass of the sugars in the sample was calculated, as described in Equation 20. The 86.73 mL is the volume of the hydrolysis liquor.

$$m_{\text{sugar in sample}} = \frac{c_{\text{sugar}} \times 86.73 \text{ mL}}{1000 \text{ mL L}^{-1}} \quad \text{Equation 20}$$

$m_{\text{sugar in sample}}$ Mass of sugar in the sample (mg)

Finally, the sugar content of the sample was calculated using Equation 21:

$$\% \text{Sugars} = \frac{m_{\text{sugar in sample}}}{1000 \text{ mg g}^{-1} \times \text{ODW}} \times (100 - \% \text{total extractives}) \quad \text{Equation 21}$$

%Sugars Sugar content of the sample (wt%)

Since starch is not a structural carbohydrate, but it is still present in the sample after the extraction, it must be subtracted from glucose. Therefore, the C-6 sugar glucose was at first converted to glucan using an anhydro correction of 162/180 according to the following equation:

$$\%Glucan = \%Glucose \times \frac{162}{180} \quad \text{Equation 22}$$

Then, the starch content was subtracted from the glucan content and in the last step the glucan was converted back to glucose:

$$\%Glucan \text{ without starch} = \%Glucan - \%Total \text{ starch} \quad \text{Equation 23}$$

$$\%Glucose \text{ from struct. carbohydrates} = \%Glucan \text{ without starch} \times \frac{180}{162} \quad \text{Equation 24}$$

7.1.9 Determination of the monomeric and oligomeric sugars

To calculate the monomeric sugar content from the water extracts, it is necessary to calculate the mass of the sugars in the water extract, for which Equation 25 was used. All dilution factors were 10 for both samples.

$$m_{\text{sugar in extract}} = c_{\text{sugar}} \times V_{\text{sample}} \quad \text{Equation 25}$$

$m_{\text{sugar in extract}}$ Mass of the sugar in the extract (mg)

V_{sample} Volume of the sample, 250 mL

After that, the content of monomeric sugars in the sample can be calculated, as described in the following equation:

$$\% \text{ monomeric sugars} = \frac{\frac{m_{\text{sugar in extract}}}{1000 \text{ mg g}^{-1}}}{ODW} \times 100 \quad \text{Equation 26}$$

7.1.10 Determination of ions

To separate and quantify the organic anions, a multi-step gradient was used for the AS11-HC column, which is described in the subsequent table:

Table 25: Gradient program with the respective eluent ratios

Time (min)	Flow rate (ml min ⁻¹)	Eluent A (%)	Eluent B (%)	Eluent C (%)	Eluent D (%)
0	1.5	0	0	70	30
5	1.5	0	0	0	100
18	1.5	0	40	0	60
20	1.5	0	100	0	0
25	1.5	0	100	0	0
27	1.5	0	0	70	30

The following chart illustrates the composition of the eluents:

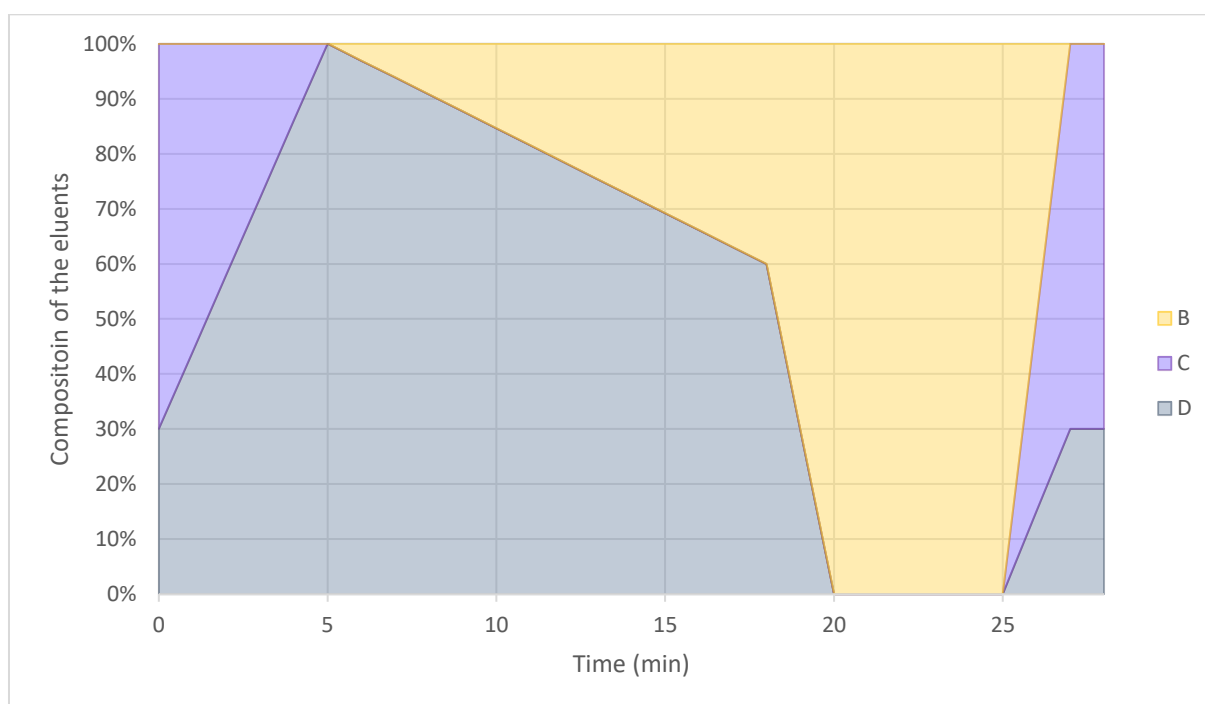


Figure 9: Graphic illustration of the multi-step gradient of the anion chromatography in relation to time

To calculate the ion content of the extractives free sample, it was necessary to calculate the mass of the ions in the sample according to Equation 27:

$$m_{ion\ in\ sample} = \frac{c_{ion} \times 86.73\ mL}{1000\ mL\ L^{-1}} \quad \text{Equation 27}$$

$m_{ion\ in\ sample}$ Mass of the ion in the sample (g)

c_{ion} Concentration of the ion ($mg\ L^{-1}$)

In the next step, the ion content of the sample was calculated with the following equation:

$$\%ion = \frac{m_{ion\ in\ sample}}{1000\ mg\ g^{-1} \times ODW} \times (100 - \%total\ extractives) \quad \text{Equation 28}$$

To determine the ion content of the water extractives, it necessary to calculate the mass of the ions in the water extract at first, which is done via Equation 29:

$$m_{ion\ in\ extract} = c_{ion} \times V_{sample} \quad \text{Equation 29}$$

$m_{ion\ in\ extract}$ Mass of the ion in the extract (mg)

V_{sample} Volume of the sample, 250 mL

After that, the content of ions in the sample can be calculated, as described in the following equation:

$$\%ion = \frac{\frac{m_{ion\ in\ extract}}{1000\ mg\ g^{-1}}}{ODW} \times 100 \quad \text{Equation 30}$$

7.1.11 Determination of lignin

The content of ASL is calculated according to the following equation (Sluiter et al., 2008a):

$$\% ASL = \frac{UVabs \times Volume_{filtrate} \times Dilution}{\varepsilon \times ODW \times Pathlength} \times 100 \quad \text{Equation 31}$$

UVabs UV-Vis absorbance at 205 nm, average of three repetitions (-)

Volume_{filtrate} Volume of filtrate, 86.73 mL

Dilution Dilution of the hydrolysis liquor in the cuvette

ODW Mass of the oven dry sample (mg)

ε Absorptivity constant of biomass at specific wavelength, 110 L g⁻¹ cm⁻¹

Pathlength Pathlength of the UV-Vis cell, 1 cm

Equation 32 is used to calculate the content of AIL in the samples (Sluiter et al., 2008a):

$$\% AIL = \frac{m_{crucible\ with\ AIR} - m_{crucible}}{ODW} \times 100 \quad \text{Equation 32}$$

7.2 Single values of the results and additional figures

7.2.1 Particle size distribution

Table 26: Results of the sieving process for WB

Sieve number /grain fraction	Mesh size/grain size (μm)	Empty mass of the sieve (g)	Empty mass of the sieve with oversize particles (g)	Mass of the oversize particle (g)
i	x_i (mm)	m_0 (g)	m_v (g)	Δm_i (g)
1	0	192.52	194.24	1.72
2	224	451.11	452.58	1.47
3	280	345.36	346.27	0.91
4	315	224.23	230.15	5.92
5	400	236.88	246.57	9.69
6	500	243.92	254.67	10.75
7	630	255.21	263.77	8.56
8	800	383.35	400.98	17.63
9	1250	410.77	416.97	6.20
Sum				64.59

The original WB sample weight was 62.99 g and the outsize was 0.14 g.

Table 27: Results of the sieving process for PP

Sieve number /grain fraction	Mesh size/grain size (μm)	Empty mass of the sieve (g)	Empty mass of the sieve with oversize particles (g)	Mass of the oversize particle (g)
i	x_i (mm)	m_0 (g)	m_v (g)	Δm_i (g)
1	0	192.58	222.92	30.34
2	224	451.18	462.09	10.91
3	280	345.40	349.82	4.42
4	315	224.33	235.95	11.62
5	400	236.93	243.30	6.37
6	500	243.89	244.77	0.88
7	630	255.23	255.28	0.05
Sum				64.59

The original PP sample weight was 64.74 g and the outsize was 0.15 g.

Table 28: Determination of $q_3(x_{i,\text{mean}})$ and $Q_3(x_{0,i})$ from WB

Mesh size/grain size (μm)	Δm_i (g)	Δx_i (μm)	$x_{i,\text{mean}}$ (μm)	$q_3(x_{i,\text{mean}})$ (μm^{-1})	$Q_3(x_{0,i})$ (-)
0	1.72	224	112	1.22E-04	2.74E-02
224	1.47	56	252	4.18E-04	5.08E-02
280	0.91	35	297,5	4.14E-04	6.52E-02
315	5.92	85	357,5	1.11E-03	1.59E-01
400	9.69	100	450	1.54E-03	3.14E-01
500	10.75	130	565	1.32E-03	4.85E-01
630	8.56	170	715	8.01E-04	6.21E-01
800	17.63	800	1025	6,23E-04	9.01E-01
1250	6.20				1.00E+00

Table 29: Determination of $q_3(x_{i,\text{mean}})$ and $Q_3(x_{0,i})$ from PP

Mesh size/grain size (μm)	Δm_i (g)	Δx_i (μm)	$x_{i,\text{mean}}$ (μm)	$q_3(x_{i,\text{mean}})$ (μm^{-1})	$Q_3(x_{0,i})$ (-)
0	30.34	224	112	2.10E-03	4.70E-01
224	10.91	56	252	3.02E-03	6.39E-01
280	4.42	35	297.5	1.96E-03	7.07E-01
315	11.62	85	357.5	2.12E-03	8.87E-01
400	6.37	100	450	9.86E-04	9.86E-01
500	0.88	130	565	1.05E-04	9.99E-01
630	0.05				1.00E+00

7.2.2 Determination of the ash content

Table 30: Total ash content of WB

Sample name	Proportion of dry matter (wt%)
WB_Crucible 1	6.01
WB_Crucible 2	6.07
WB_Crucible 3	6.05
Average	6.042
Standard deviation	0.030
RSD	0.50

Table 31: Structural ash content of WB

Sample name	Proportion of dry matter of untreated sample (wt%)
WB_Crucible E1	1.25
WB_Crucible E2	1.58
WB_Crucible E3	1.33
WB_Crucible E4	1.32
Average	1.374
Standard deviation	0.145
RSD	10.6

Table 32: Total ash content of PP

Sample name	Proportion of dry matter (wt%)
PP_Crucible 1	4.40
PP_Crucible 2	4.40
PP_Crucible 3	4.59
Average	4.463
Standard deviation	0.109
RSD	2.44

Table 33: Structural ash content of PP

Sample name	Proportion of dry matter of untreated sample (wt%)
PP_Crucible E1	1.1
PP_Crucible E2	1.1
PP_Crucible E3	1.0
PP_Crucible E4	1.2
Average	1.13
Standard deviation	0.08
RSD	7

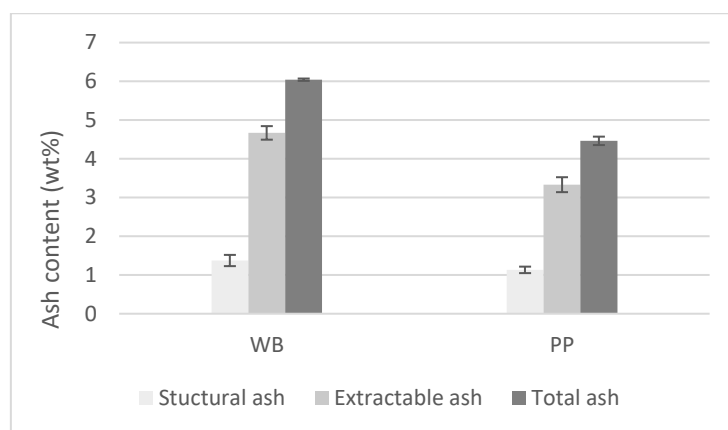


Figure 10: Graphic depiction of structural, extractable and total ash in WB and PP, based on dry matter

7.2.3 Determination of the starch content

Table 34: Starch content of WB, based on dry matter

Sample name	RDS (wt%)	SDS (wt%)	TDS (wt%)	RS (wt%)	TS (wt%)
WB_1	6.1	9.6	17.3	0	17.3
WB_2	6.5	11	17.8	0	17.8
WB_3	7.1	11	18.5	0	18.5
Average	6.57	10.5	17.89	0	17.89
Standard deviation	0.49	0.86	0.62	0	0.62
RSD	7.4	8.1	3.5		3.5
Control sample	10.0	19.4	42.2	42.0	84.3
True values for S (control)	8.0	16.2	35.9	47.4	83.3

Table 35: Starch content of PP, based on dry matter

Sample name	RDS (wt%)	SDS (wt%)	TDS (wt%)	RS (wt%)	TS (wt%)
PP_1	2.7	4.1	10.2	10.0	20.2
PP_2	3.0	1.9	9.01	11.8	20.8
PP_3	4.4	4.9	12.7	7.3	20.0
PP_4	4.0	4.5	12.1	8.2	20.4
PP_5	5.0	4.5	14.9	6.6	21.5
PP_6	3.0	5.3	11.0	7.7	18.7
PP_7	3.7	4.5	12.0	7.6	19.6
PP_8	3.8	6.0	12.3	5.5	17.9
PP_9	2.3	3.9	10.5	8.7	19.3
PP_10	2.2	3.6	9.66	9.82	19.5
PP_11	2.7	3.5	10.1	9.2	19.3
Control sample 1	9.73	18.3	37.5	41.7	79.2
Control sample 2	9.34	20.2	47.2	33.5	80.7
Average of the PP samples	3.35	4.22	11.32	8.41	19.73
Standard deviation	0.89	1.07	1.70	1.75	1.00
RSD	26	25.3	15.0	20.8	5.05
True values for S (control)	8.0	16.2	35.9	47.4	83.3

Table 36: Starch content of extractives free PP, based on dry matter

Sample name	RDS (wt%)	SDS (wt%)	TDS (wt%)	RS (wt%)	TS (wt%)
PP_ES1	18.8	0.926	18.9	0.65	19.6
PP_ES2	18.6	0.917	18.7	0.57	19.3
PP_ES3	18.7	2.78	19.4	0.77	20.2
Average	18.74	1.541	19.02	0.664	19.69
Standard deviation	0.09	1.074	0.36	0.100	0.46
RSD	0.5	69.67	1.9	15.1	2.3

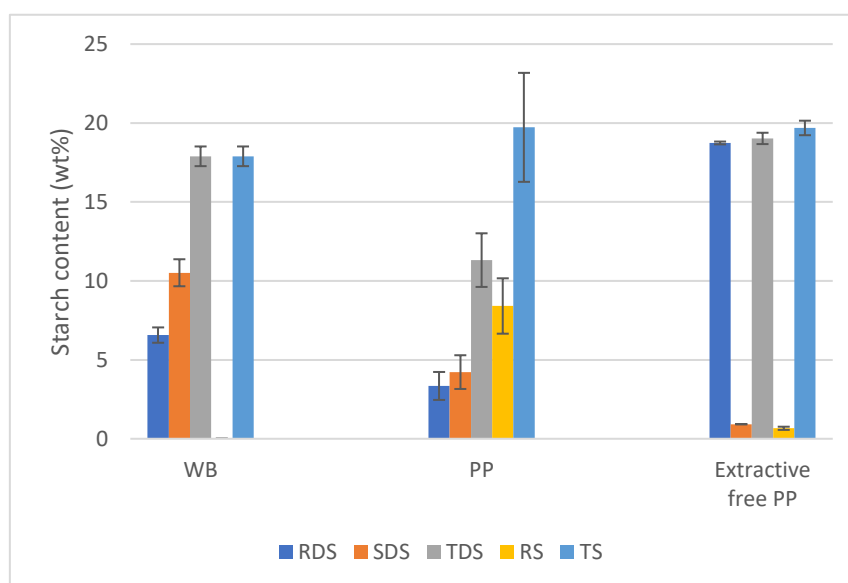


Figure 11: RDS, SDS, TDS, RS and TS contents of WB, PP and extractives free PP, based on dry matter

7.2.4 Determination of the fat content

Table 37: Fat content of WB

	Sample name	Fat content in proportion of dry matter (wt%)
Raw fat	WB_RF1	2.435
	WB_RF2	2.22
	WB_RF3	2.21
	WB_RF4	1.98
	Average	2.210
	Standard deviation	0.187
	RSD	8.44
Total fat	WB_TF1	4.870
	WB_TF2	4.910
	WB_TF3	4.755
	WB_TF4	4.808
	Average	4.8359
	Standard deviation	0.0679
	RSD	1.41

Table 38: Fat content of PP

	Sample name	Fat content in proportion of dry matter (wt%)
Raw fat	PP_RF1	0.19
	PP_RF2	0.10
	PP_RF3	0.12
	PP_RF4	0.13
	Average	0.134
	Standard deviation	0.037
	RSD	28
Total fat	PP_TF1	0.659
	PP_TF2	0.662
	PP_TF3	0.812
	PP_TF4	0.867
	Average	0.7501
	Standard deviation	0.1058
	RSD	14.10

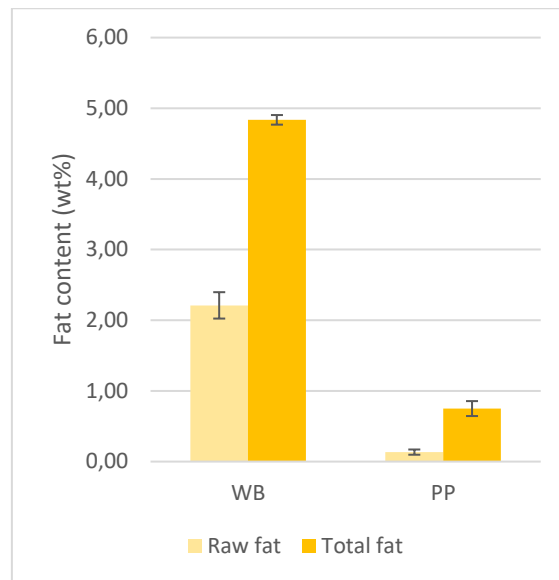


Figure 12: Raw and total fat content of WB and PP, based on dry matter

7.2.5 Determination of the protein content

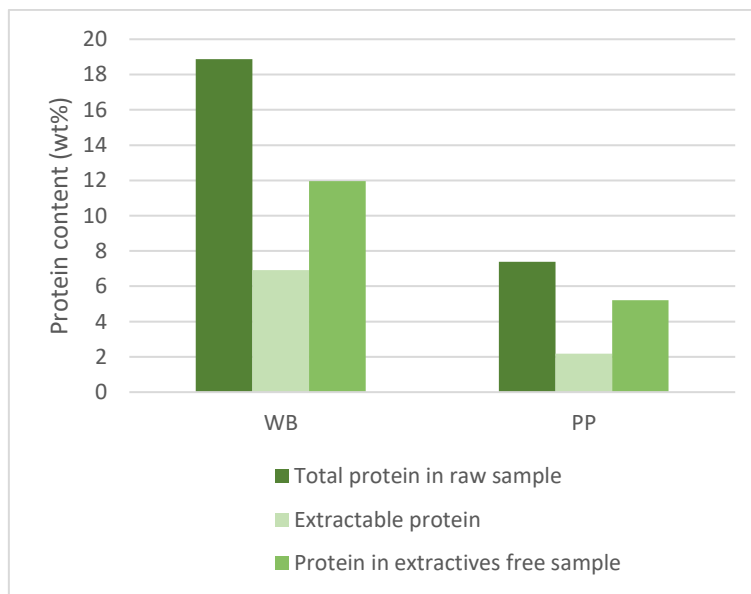


Figure 13: Total protein content in the raw sample, extractable protein content in the raw sample and protein content in the extractives free sample in WB and PP, based on dry matter

7.2.6 Determination of extractives

Table 39: Content of extractives in WB

		Sample name	Proportion of dry matter (wt%)
1 st batch	Water extractives	WB_Thimble 1	25.216
		WB_Thimble 2	26.530
		WB_Thimble 3	26.574
		WB_Thimble 4	25.841
		Average	26.0402
		Standard deviation	0.6437
		RSD	2.472
	EtOH extractives	WB_Thimble 1	5.097
		WB_Thimble 2	5.079
		WB_Thimble 3	6.553
		WB_Thimble 4	5.226
		Average	5.4888
		Standard deviation	0.7127
		RSD	12.98
	Total extractives	WB_Thimble 1	30.314
		WB_Thimble 2	31.608
		WB_Thimble 3	33.127
		WB_Thimble 4	31.067
		Average	31.5290
		Standard deviation	1.1904
		RSD	3.7756
2 nd batch	Water extractives	WB_Thimble 5	19.497
		WB_Thimble 6	18.627
		WB_Thimble 7	17.177
		WB_Thimble 8	17.847
		Average	18.2868
		Standard deviation	1.0011
		RSD	5.4744
3 rd batch	Water extractives	WB_Thimble 9	25.722
		WB_Thimble 10	25.665
		WB_Thimble 11	25.016
		WB_Thimble 12	23.724
		Average	25.0315
		Standard deviation	0.9287
		RSD	3.710
	EtOH extractives	WB_Thimble 9	13.834
		WB_Thimble 10	14.271
		WB_Thimble 11	15.685
		WB_Thimble 12	15.036

		Average	14.7066
		Standard deviation	0.8201
		RSD	5.576
	Total extractives	WB_Thimble 9	39.555
		WB_Thimble 10	39.936
		WB_Thimble 11	40.701
		WB_Thimble 12	38.760
		Average	39.7381
		Standard deviation	0.8075
		RSD	2.032

Table 40: Content of extractives in PP

		Sample name	Proportion of dry matter (wt%)
1 st batch	Water extractives	PP_Thimble 1	18.831
		PP_Thimble 2	13.778
		PP_Thimble 3	21.764
		PP_Thimble 4	19.802
		Average	18.5438
		Standard deviation	3.4037
		RSD	18.355
	EtOH extractives	PP_Thimble 1	1.01
		PP_Thimble 2	0.724
		PP_Thimble 3	0.604
		PP_Thimble 4	1.32
		Average	0.92
		Standard deviation	0.32
		RSD	35
	Total extractives	PP_Thimble 1	19.844
		PP_Thimble 2	14.502
		PP_Thimble 3	22.368
		PP_Thimble 4	21.122
		Average	19.4590
		Standard deviation	3.4620
		RSD	17.791
2 nd batch	Water extractives	PP_Thimble 5	16.352
		PP_Thimble 6	ruptured
		PP_Thimble 7	19.104
		PP_Thimble 8	17.722
		Average	17.7257
		Standard deviation	1.3760
		RSD	7.7629

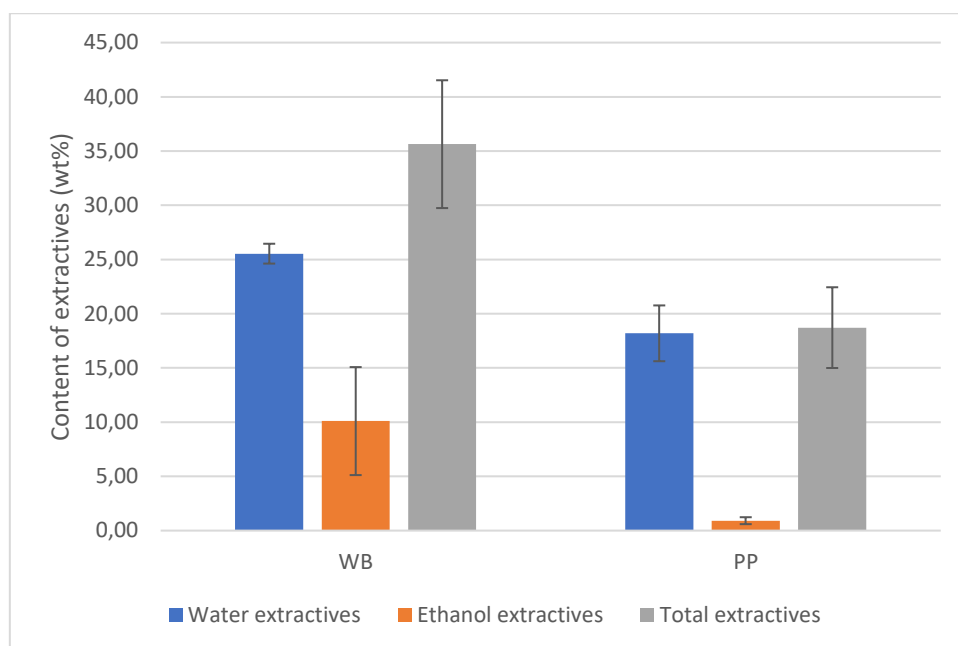


Figure 14: Content of water, ethanol and total extractives in WB and PP, based on dry matter

7.2.7 Determination of structural carbohydrates

Table 41: Content of monomeric sugars of the structural carbohydrates of WB, based on dry matter

Sample name	Arabinose (wt%)	Galactose (wt%)	Glucose (wt%)	Xylose (wt%)	Mannose (wt%)	Sum (wt%)
WB_H1	10.54	0.99	10.08	20.18	0.85	42.63
WB_H2	10.47	0.96	31.11	20.89	0.86	44.41
WB_H3	10.23	0.92	29.30	20.69	0.79	42.04
WB_H4	9.85	0.91	29.75	17.94	0.80	39.37
Average	10.27	0.94	30.03	19.93	0.82	42.11
Standard deviation	0.31	0.04	0.77	1.35	0.03	2.09
RSD	3.04	3.81	2.58	6.80	4.24	4.96

Table 42: Content of monomeric sugars of the structural carbohydrates of PP, based on dry matter

Sample name	Arabinose (wt%)	Galactose (wt%)	Glucose (wt%)	Xylose (wt%)	Mannose (wt%)	Sum (wt%)
PP_H1	4.10	21.84	36.10	1.94	0.94	64.92
PP_H2	4.32	24.13	38.27	1.89	0.93	69.53
PP_H3	3.51	20.00	39.59	1.91	0.95	65.96
PP_H4	3.65	18.21	37.43	2.15	1.11	62.55
Average	3.90	21.04	37.85	1.97	0.98	0.98
Standard deviation	0.38	2.53	1.46	0.12	0.09	0.09
RSD	9.71	12.04	3.87	6.03	8.89	8.89
PP_H5	4.14	21.43	33.97	2.26	1.23	63.02
PP_H2.2	4.30	21.83	30.13	2.18	1.20	59.64
PP_H7	3.80	18.65	31.47	2.28	1.24	57.45
PP_H8	4.01	19.78	31.48	2.21	1.21	58.68
Average	4.06	20.42	31.76	2.23	1.22	1.22
Standard deviation	0.21	1.48	1.60	0.05	0.02	0.02
RSD	5.14	7.24	5.04	2.02	1.64	1.64

The final calculated sugar contents are presented in the following table:

Table 43: Content of monomeric sugars of the structural carbohydrates of WB and PP, based on dry matter

		Arabinose	Galactose	Glucose	Xylose	Mannose	Sum
WB	Average (wt%)	10.27	0.94	10.15	19.93	0.82	42.11
	Standard deviation (wt%)	0.31	0.04	0.77	1.35	0.03	2.09
	RSD (%)	3.04	3.81	7.63	6.80	4.24	4.96
PP	Average (wt%)	3.98	20.73	34.80	2.10	1.10	62.72
	Standard deviation (wt%)	0.30	1.95	3.55	0.16	0.14	4.06
	RSD (%)	7.45	9.40	10.2	7.71	12.62	6.48

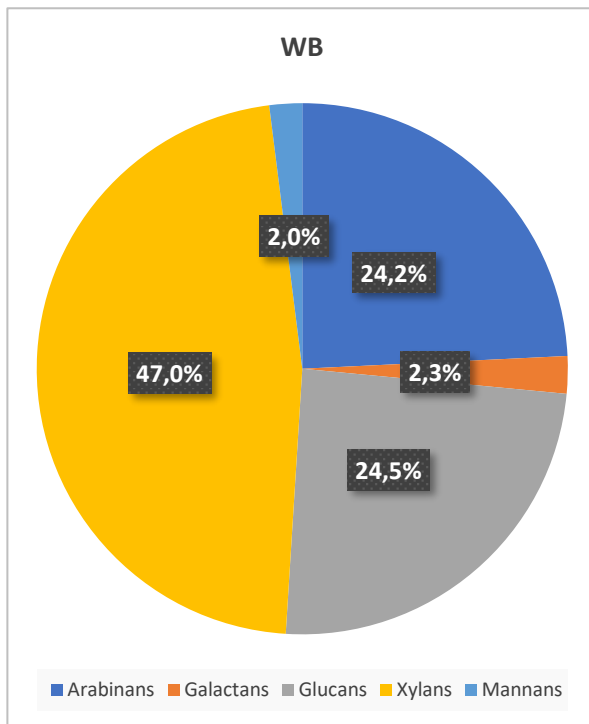


Figure 15: Composition of the structural carbohydrates of WB, based on dry matter

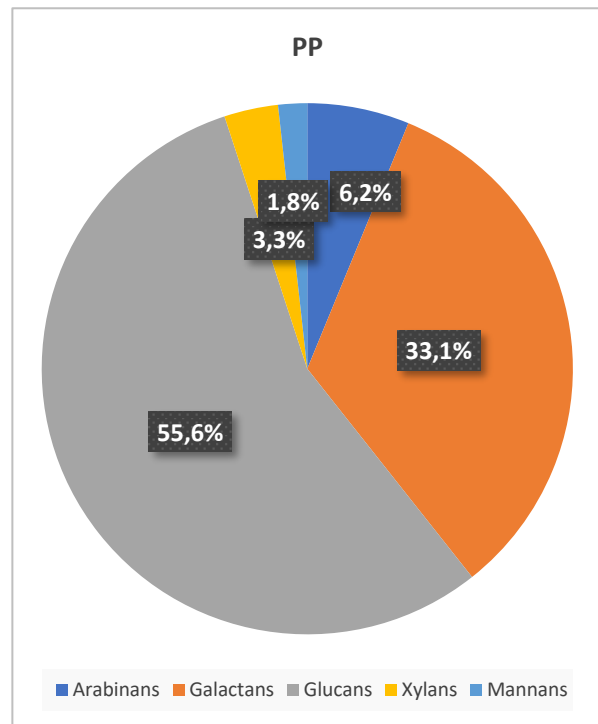


Figure 16: Composition of the structural carbohydrates of PP, based on dry matter

7.2.8 Determination of monomeric sugars

Table 44: Monomeric sugar content of WB, based on dry matter

Sample name	Arabinose (wt%)	Galactose (wt%)	Glucose (wt%)	Xylose (wt%)	Mannose (wt%)	Sum (wt%)
WB_SE1	1.09	0.07	0.64	0.29	0.03	0.80
WB_SE2	1.00	0.06	0.63	0.26	0.03	0.76
WB_SE3	1.13	0.07	0.66	0.30	0.02	0.83
WB_SE4	1.10	0.07	0.73	0.30	0.03	0.84
Average	1.08	0.07	0.66	0.29	0.03	0.81
Standard deviation	0.06	0.01	0.04	0.02	0.00	0.04
RSD	5.41	7.61	6.64	6.34	8.87	4.47

Table 45: Monomeric sugar content of PP, based on dry matter

Sample name	Arabinose (wt%)	Galactose (wt%)	Glucose (wt%)	Xylose (wt%)	Mannose (wt%)	Sum (wt%)
PP_SE5	0.14	< cal. std	0.61	< cal. std	< cal. std	0.66
PP_SE7	0.13	< cal. std	0.54	< cal. std	< cal. std	0.59
PP_SE8	0.16	< cal. std	0.65	< cal. std	< cal. std	0.71
Average	0.14		0.60			0.66
Standard deviation	0.01		0.06			0.06
RSD	9.69		9.35			8.90

7.2.9 Determination of oligomeric sugars

Table 46: Oligomeric sugar content of WB, based on dry matter

Sample name	Arabinose (wt%)	Galactose (wt%)	Glucose (wt%)	Xylose (wt%)	Mannose (wt%)	Sum (wt%)
WB_SEH1	-0.46	0.58	5.70	0.71	0.09	5.83
WB_SEH2	-0.32	0.56	5.30	0.80	0.09	5.68
WB_SEH3	-0.39	0.64	5.13	0.85	0.10	5.50
WB_SEH4	-0.35	0.66	4.94	0.90	0.11	5.42
Average	-0.38	0.61	5.26	0.81	0.10	5.61
Standard deviation	0.06	0.05	0.32	0.08	0.01	0.19
RSD	-15.82	7.85	6.14	9.45	7.98	3.30

Table 47: Oligomeric sugar content of PP, based on dry matter

Sample name	Arabinose (wt%)	Galactose (wt%)	Glucose (wt%)	Xylose (wt%)	Mannose (wt%)	Sum (wt%)
PP_SEH5	0.46	3.22	2.22	< cal. std	< cal. std	5.24
PP_SEH7	0.54	3.68	2.26	< cal. std	< cal. std	5.89
PP_SEH8	0.57	3.91	2.24	< cal. std	< cal. std	6.01
Average	0.52	3.60	2.24			5.71
Standard deviation	0.05	0.35	0.02			0.41
RSD	10.05	9.77	0.83			7.24

7.2.10 Determination of ions

7.2.10.1 Ion content of the extractives free samples

Table 48: Ion content of extractives free WB, based on dry matter

Sample name	Acetate (wt%)	Phosphate (wt%)	Ammonium (wt%)	Sum (wt%)
WB_E1	1.264	0.303	0.152	1.719
WB_E2	0.775	< cal. std	0.114	0.889
WB_E3	0.862	< cal. std	0.109	0.972
WB_E4	0.598	< cal. std	0.089	0.687
Average	0.875	0.303	0.116	1.294
Standard deviation	0.282		0.026	0.308
RSD	32.2		22.8	23.8

Table 49: Ion content of extractives free PP, based on dry matter

Sample name	Acetate (wt%)
PP_E1	2.603
PP_E2	1.135
PP_E3	1.626
Average	1.788
Standard deviation	0.747
RSD	41.8

7.2.10.2 Ion content of the water extractives

Table 50: Anion content of the water extractives of WB, in relation to the dry sample mass, based on dry matter

Sample name	Acetate (wt%)	Succinate (wt%)	SO ₄ ²⁻ (wt%)	Cl ⁻ (wt%)	PO ₄ ³⁻ (wt%)	Citrate (wt%)	Oxalate (wt%)	K ⁺ (wt%)
WB_W1	0.182	0.151	< cal. std.	< cal. std.		0.134	0.013	> cal. std.
WB_W2	0.191	0.139	< cal. std.	< cal. std.		0.121	0.013	> cal. std.
WB_W3	< cal. std.	< cal. std.	< cal. std.	< cal. std.	1.018		0.010	> cal. std.
WB_W4	0.180	0.156	< cal. std.	< cal. std.		0.141	0.014	> cal. std.
Average	0.184	0.149			1.018	0.132	0.012	
Standard deviation	0.005	0.008				0.010	0.002	
RSD	3	6				8	16	

Table 51: Anion content of the water extractives of PP, based on dry matter

Sample name	Acetate (wt%)	Succinate (wt%)	SO ₄ ²⁻ (wt%)	Cl ⁻ (wt%)	PO ₄ ³⁻ (wt%)	Citrate (wt%)	Oxalate (wt%)	K ⁺ (wt%)
PP_W1	0.219	0.232	0.119	0.116	0.099	0.490	0.007	> cal. std.
PP_W2	0.234	0.208	0.102		0.100	0.432	0.006	> cal. std.
PP_W3	0.238	0.235	0.111		0.101	0.501	0.007	> cal. std.
Average	0.230	0.225	0.111		0.100	0.474	0.007	
Standard deviation	0.010	0.015	0.009		0.001	0.037	0.001	
RSD	4.5	6.6	8		0.9	7.9	12	

7.2.11 Determination of lignin

Table 52. Lignin content of WB, based on dry matter

Sample name	ASL (wt%)	AIL (wt%)	Total lignin (wt%)
WB_L1	3.13	7.37	10.5
WB_L2	3.21	6.97	10.2
WB_L3	3.47	5.68	9.14
WB_L4	3.19	6.11	9.30
Average	3.250	6.530	9.780
Standard deviation	0.147	0.774	0.92
RSD	4.527	11.851	9.42

Table 53: Lignin content of PP, based on dry matter

Sample name	ASL (wt%)	AIL (wt%)	Total lignin (wt%)
PP_L1	1.75	4.25	6.00
PP_L2	1.78	4.44	6.22
PP_L3	1.72	4.33	6.05
Average	1.749	4.341	6.090
Standard deviation	0.032	0.098	0.13
RSD	1.838	2.253	2.13

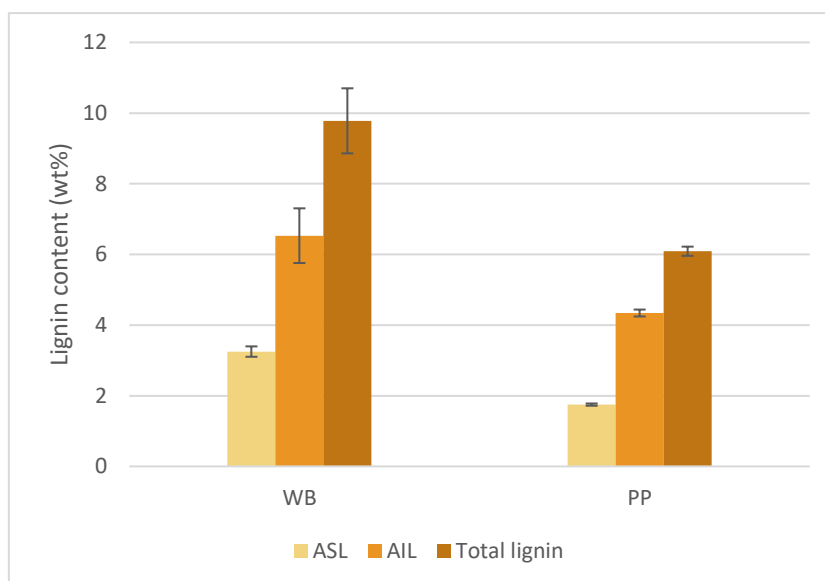


Figure 17: Content of ASL, AIL and total lignin in WB and PP, based on dry matter

7.2.12 Chemical composition of WB and PP

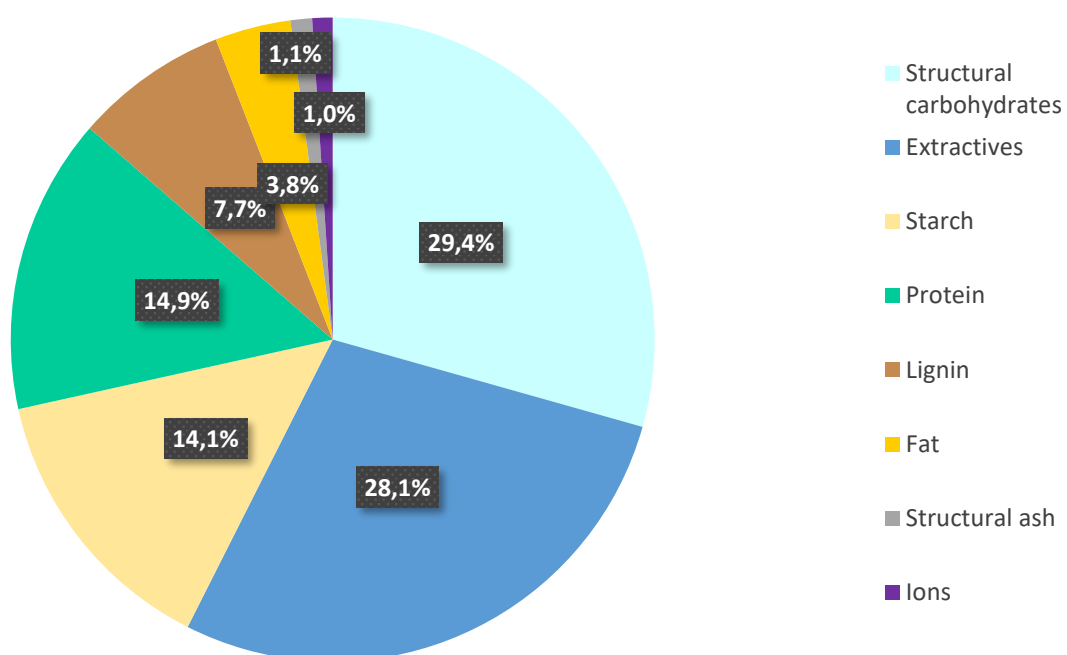


Figure 18: Composition of WB, based on dry matter

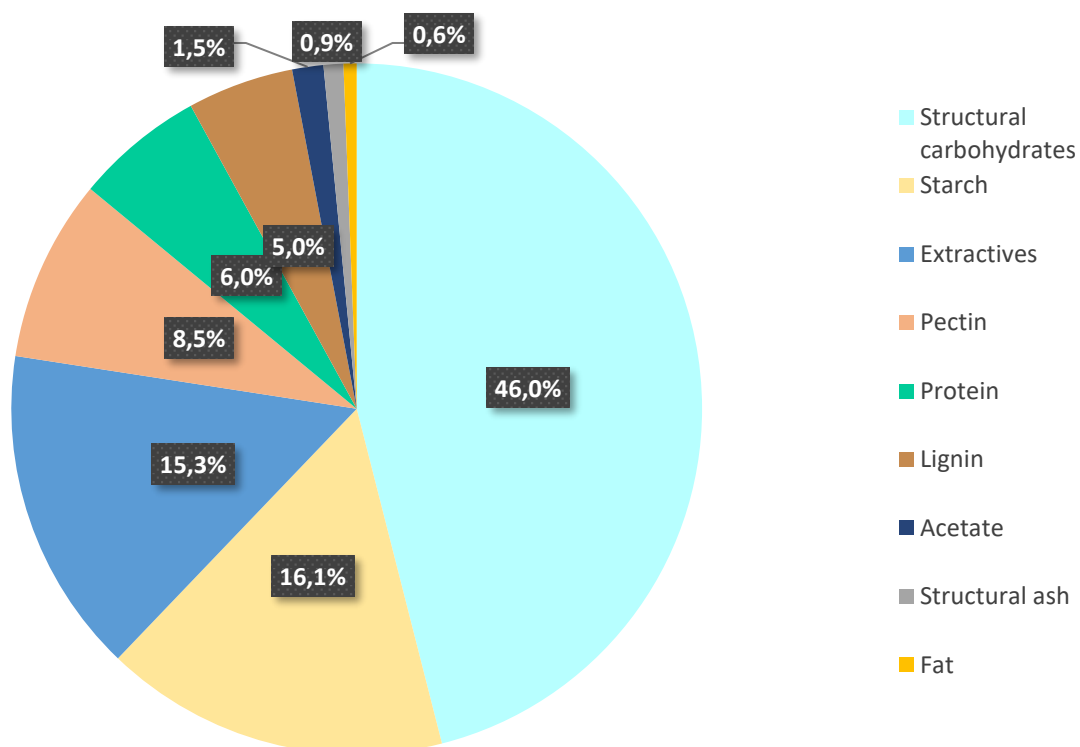


Figure 19: Composition of PP, based on dry matter

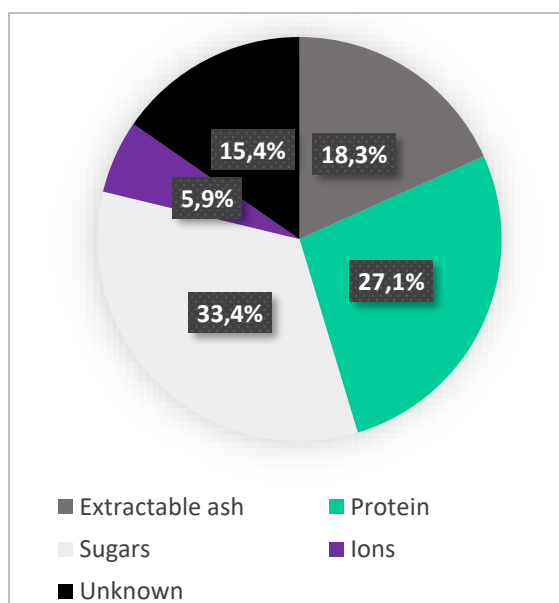


Figure 20: Composition of the water extracts of WB, based on dry matter

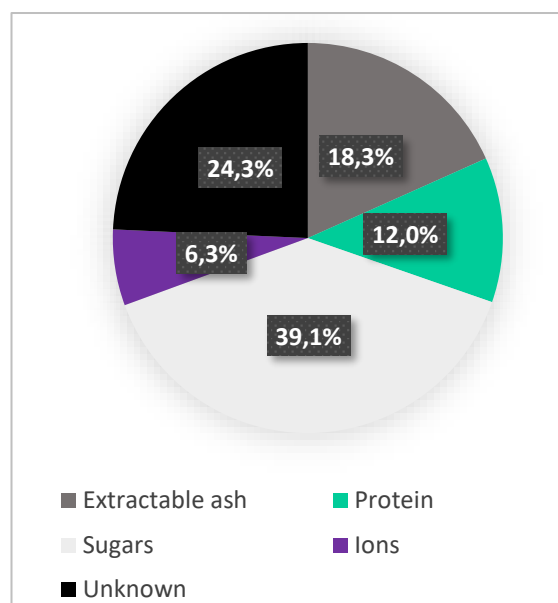


Figure 21: Composition of the water extracts of PP, based on dry matter