Pulsed electric field treatment of wheat bran to improve arabinoxylan extraction

Pulserad elektrisk fältbehandling av vetekli för att förbättra extraktionen av arabinoxylaner

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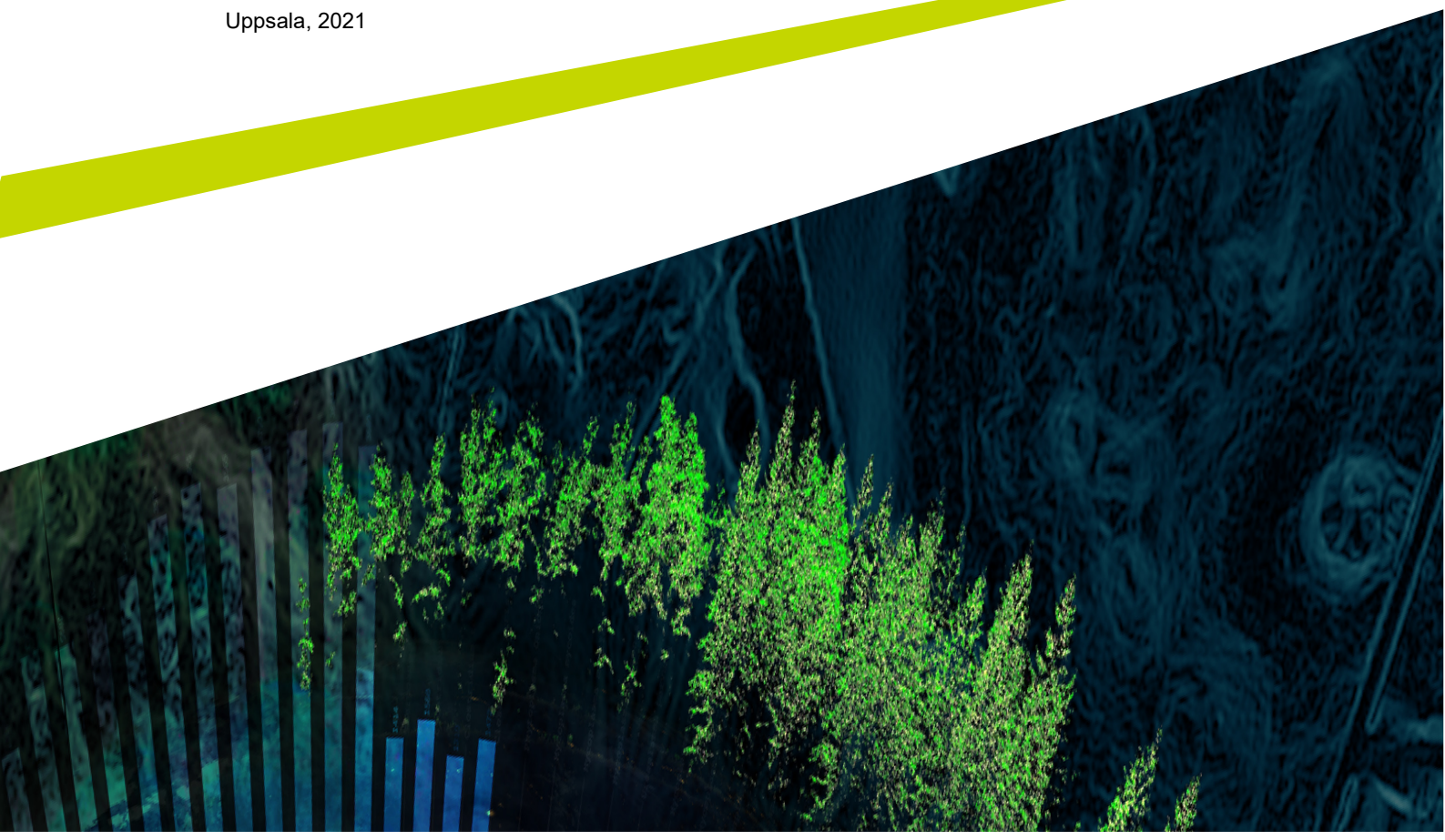

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Abstract

Valorization of agricultural and industrial by-products reduces the environmental impact of food production**.** Innovative low impact processes for food processing further limit the contribution and additionally enhances the generation of novel health-promoting foods.

Wheat bran (WB) is an industrial by-product produced during milling which currently have limited use for food applications, despite its exceptional nutritional content. Arabinoxylan is the main non-starch polysaccharide in WB and can be used to improve the quality and physiochemical properties of foods as well as improve the nutritional value.

The aim of this study was to determine if PEF enhances the extraction yield of extracted arabinoxylan and modulates the chemical composition of arabinoxylan extracts.

It was hypothesized that pulsed electric field technology (PEF) can aid extraction of arabinoxylans (AX) from WB. WB was treated with highest possible field strenght (3,7 kV/cm), a frequency of 2Hz, pulse width of 5 µ s and either 100, 250, 500. 750 or 1000 pulses.

PEF had a significant effect on total carbohydrate content of AX extracts ($p<0,05$). There was an increase in carbohydrate content for AX extracts treated with 250, 500 and 1000 pulses. PEF additionally decreased the A/X ratio. PEF treated AX extracts had ratios between 0,92-0,84.

The result confirms that PEF increases the purity and changes the chemical composition of alkaline extracted AX. PEF treatments with 1000 pulses is most effective in increasing purity. Further studies should examine the effect of different PEF parameters on extraction yield and physiochemical properties of AX extracts.

Keywords: Arabinoxylan, wheat bran, pulsed electric field technology, alkaline extraction

Sammanfattning

Valorisering av jordbruks- och industribiprodukter kan medverka till en minskning av livsmedelsproduktionens miljöpåverkan. Resurseffektiva innovativa processer för bearbetning av livsmedel minskar ytterligare miljöpåverkan samt förbättrar tillika genereringen av nya hälsofrämjande livsmedel.

Vetekli är en industriell biprodukt vid vetemjölsproduktion, som för närvarande används begränsat i livsmedel, trots dess exceptionella näringsinnehåll. Arabinoxylan är den dominerande icke-stärkelsepolysackariden i vetekli och kan användas för att förbättra livsmedlens kvalitet och fysiokemiska egenskaper samt förbättra näringsvärdet.

Syftet med denna studie var att fastställa om PEF förbättrar extraktionsutbytet av extraherat arabinoxylan samt om PEF modulerar den kemiska sammansättningen av arabinoxylan-extrakt.

Det antogs att pulserad elektrisk fältteknik (PEF) kan underlätta utvinningen av arabinoxylaner (AX) från vetekli. Vetekli behandlades med högsta möjliga fältstyrka (3,7 kV / cm), en frekvens på 2Hz, en pulsbredd på 5 µ s och antingen 100, 250, 500. 750 eller 1000 pulser.

PEF hade en signifikant effekt på det totala kolhydratinnehållet i AX-extrakt ($p < 0,05$). Det förekom en ökning av kolhydratinnehåll i AX-extrakt behandlade med 250, 500 och 1000 pulser. PEF minskade dessutom A/X-ratiot. PEF-behandlade AX-extrakt hade ration mellan 0,92-0,84.

Resultatet bekräftar att PEF ökar renheten samt förändrar den kemiska sammansättningen av alkaliskt extraherat AX. PEF-behandlingar med 1000 pulser var mest effektivt för att öka renheten. Ytterligare studier bör utföras för att undersöka effekten av olika PEF-parametrar på extraktionsutbyte och fysiokemiska egenskaper hos AX-extrakt.

Nyckelord: Arabinoxylan, vetekli, pulserad elektrisk fältteknik, alkalisk extraktion

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Abbreviations

PEF	Pulsed electric field technology
WB	Wheat bran
AX	Arabinoxylan
WUEAX	Water unextractable arabinoxylan
WEAX	Water extractable arabinoxylan
NCD	Non-communicable diseases
A/X ratio	Arabinose to xylose ratio
MW	Molecular weight
DW	Dry weight
DS	Supernatant of destarched wheat bran
DL	Supernatant of delignified wheat bran
AXE	Arabinoxylan extracts
AXR	Residue after the alkaline extraction

1. Introduction

Designing novel technologies for food processing can aid the transitioning to a more sustainable food system. Resource efficiency is important to limit the impact on the climate, including green gas emissions and other environmental impacts. Utilization of agricultural and industrial by-products combats side-stream losses and thus possesses potential to decrease the environmental impact of the food production**.** Valorization of by-products and low-valued agricultural waste using low impact processes can further limit the contribution by reducing the use of hazardous chemicals, increase the energy efficiency and reduce the water use.

The need of providing food with health promoting properties is increasing as diet related non-communicable diseases (NCDS) is becoming an elevated problem worldwide. NCDs are chronic diseases that are non-transmissible and include cardio-vascular diseases, diabetes, cancer and respiratory diseases which accounts for most NCD deaths (WHO 2020). The elevation is associated to dietary transitioning largely related to economic development, globalisation and urbanization. WHO assessed NCDs to be the leading cause of deaths globally by being responsible for 70 % of the total number of deaths worldwide (WHO 2020) Despite having well-developed national guidelines for disease management and unhealthy diet reduction measures, NCDs accounts for 90 % of the deaths in Sweden annually (WHO 2020). Health promotion and disease management are known to be very effective measures to reduce the mortality and tackling the burden of disease (Wang & Wang 2020).

Wheat (*Triticum aestivum*) production was assessed to 774,0 million tonnes 2020/2021 which is an increase of approximately 14 million tons compared to 2019/2020 (FAO 2021).
Wheat flour is a principal component in a large variety of staple foods. However, cereal processing generates continuously a large volume of underutilized by-products. During milling, wheat bran removal is necessary to create wheat flour with high quality and desirable physiochemical properties. Wheat milling produce approximately 0.15 ton wheat bran per ton processed wheat (Ruthers *et al.* 2017). Biomolecules in wheat bran have recently gained interest for nutritional and material applications, making wheat bran a valuable raw material for valorization. Exploring and designing novel efficient technologies for extraction are of interest

to accelerate the application and development of novel health promoting foods as well as sustainable biomaterials.

2. Background

2.1. Wheat bran

Wheat bran (WB) is an industrial by-product produced during milling when the germ and peripheral tissues are separated from the starchy endosperm. The germ and starchy endosperm are proceeding into further milling and purification to produce flour with an extraction yield of 73-77 % (Elliott *et al.* 2002). WB is a composite multi-layer material made up of several histological parts of the wheat kernel, including the pericarp, testa, hyaline layer and the peripheral layer of the endosperm also called the aleurone layer (Brauns *et al.* 2012) (Figure 1). WB consequently accounts for approximately 14-19 % of the wheat kernel (Brauns et al. 2012). The aleurone layer (6-9% of the wheat kernel and ~50 % of WB) is responsible for many of the reported health benefits of WB as most vitamins, minerals and bioactive compounds are allocated in this part of the kernel.

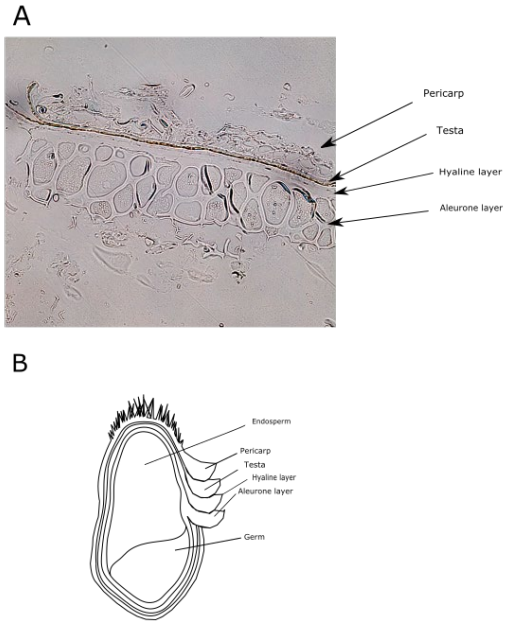

Figure 1. Histology and anatomy of Wheat (Triticum aestivum). (A) Wheat bran tissues obtained by Light microscopy (B) Wheat bran anatomy.

Conventional milling techniques aims to separate the starchy endosperm from the adjacent layers to produce a fraction with high purity and yield. The fractionation process determines the purity of WB resulting in differences in particle size, starch content, water content and fiber content between WB fractions (Hemery *et al.* 2007). General composition of WB has been reported by several authors (table 1).

Table 1 Wheat bran composition expressed in dw %

Compound	**Amount (%)**	**Reference**
Water	12,1	(Roberts *et al.* 1985)
Protein	13.2–18.4	(Di Lena *et al.* 1997, Dornez *et al.* 2006)
Fat	3.5–3.9	(Apprich et al 2004)
Phytosterols	0.16–0.17	(Fardet 2010, Hemery *et al.* 2007)
α-Linolenic acid	0.16	(Fardet, 2010)
Total Carbohydrates	56.8	(Apprich *et al.* 2004)
Starch	13.8–24.9	(Dornez *et al.* 2006, Hemery *et al.* 2007)
Cellulose	11	(Hemery *et al.* 2007)
Total arabinoxylans	10.9–26.0	(Dornez et al., 2006, Gebruers *et al*. 2008, Hemery *et al.* 2007)
a/x ratio*[1]	0.50- 0.65	(Gebruers *et al*. 2008; Kamal-Eldin *et al.* 2009,
WE a/x ratio*[2]	0.7-1.25	Shewry *et al.* 2013, Ruthes *et al.* 2017)
Total β-glucan	2.1–2.5	(Fardet 2010)
Phenolic acids	1.1	(Apprich *et al* 2004)
Ferulic acids	0.02–1.5	(Fardet, 2010, Hemery *et al.* 2007)
Phytic acid	4.2–5.4	(Gebruers *et al.* 2008; Shewry *et al.* 2013)
Lignin	3.3-4.9	(Babiker *et al.* 2009, Dornez *et al.* 2006, Fardet,
Ash	3.4–8.1	2010, Hemery *et al.* 2007)

*[1]*a/x: arabinoxylan/xylan ratio,*

*[2]WE a/x: water extractable arabinoxylan/xylan ratio

2.1.1 Cell walls of wheat bran

The high content of hemicelluloses and minor quantities of cellulose, xyloglucan and mannans is unique for cereal cell walls (Ruthes *et al.* 2017). The mechanical

properties of the bran are strongly related to the degree of crosslinking between polymers in the cell walls (Peyron *et al*. 2002). AX is the most abundant polymer in WB, as seen in table 1. Consequently, the structure and association behavior of AX is responsible for the recalcitrance of cereal bran (Ruthes *et al.* 2017). Ferulic acids in the cell wall induce polymer crosslinking resulting in a complex covalent network of heterogeneous molecules (Ruthers *et al.* 2017). AX residues with esterified ferulic acid can crosslink to neighboring AX chains and lignin monomers to generate ferulate-polysaccharide-lignin complexes (de O. Buanafina 2009). Lignification pattern and presence of ferulic acid mainly modulates the mechanical behavior of WB (Peyron *et al*. 2002).

2.1.2 Starch

Starch in cereals are encapsulated in granules in the endosperm (Maningat *et al.* 2009). Starch granules are composed of two polysaccharides, amylose and amylopectin (Maningat *et al.* 2009). Amylose is made up of linear chains of α-1,4-linked D-glucopyranose monomers whereas amylopectin consists of an identical backbone but have additional α-1.6-linked D-glucopyranose branches (Seung 2020).

Starch granules have crystalline and amorphous regions (Maningat *et al*. 2009). The amorphous regions are made up of the branched parts of amylopectin together with amylose (Seung 2020). Adjacent linear α-1,4-linked D-glucopyranose chains of amylopectin form double helices that creates the crystalline structure. Lipid complexed amylose is also consistently present in starch granules (Seung 2020). This assembling creates a rigid water-insoluble structure (Maningat *et al*. 2009).

Wheat starch consists of A-type (d>10 um) and B-type (d<10 um) starch granules with different functional and chemical properties (Maningat *et al.* 2009). WB consists of predominantly B-type granules compared to commercial wheat starch, which consists of ~70 % type A granules (Prückler *et al.* 2013). B-type granules have been observed to be digested more rapidly by α-amylase than A-type granules, due to less amylose content. Less amylose results in less crystallinity and hence increased accessibility by α-amylase (Maningat *et al.* 2009).

2.1.3 Protein

Wheat bran proteome mostly function as a defense to protect the inner layers (Jerkovic *et al.* 2010). The different layers have different roles and hence, proteins that are expressed are specific for each tissue due to their different functional properties. Main proteins in the pericarp are enzymes which protects and strengthens the cell wall by crosslinking with lignin (Jerkovic *et al*. 2010). The testa

have a similar composition as the outer layer of the bran and predominantly contains proteins which contribute to tissue strength and protection against pathogens (Jerkovic *et al*. 2010). The aleurone layer have a higher protein content (22.9 %) compared to the testa (5.7 %) and the pericarp (5.1 %) (D'Ovidio et al. 2009). However, the aleurone layer have predominantly (~58 %) globulin-like storage proteins. The remaining proteins are mostly enzymes involved in carbohydrate metabolism or protein synthesis (D'Ovidio *et al.* 2009).

2.1.4 Lignin

Lignin confers mechanical strength and tolerance to plant cells and is consequently an essential structural component of cell walls (Dominguez *et al.* 2017). However, the structure is complex and can vary considerably. Lignin is a phenolic heterogeneous polymer synthesized from three aromatic precursors; p-coumaryl, conyferyl and sinapyl alcohol (Dominguez *et al*. 2017). They create three different lignin units joined together by β-O-4 links. The resulting structure is a complex macromolecule with a large variety of functional groups and linkages. Lignin consequently creates molecular interactions with cellulose, hemicelluloses, protein and other phenolics (Peyron *et al.* 2002).

2.2 Arabinoxylan

The high content as well as functional and nutritional properties have generated a lot of interest towards arabinoxylan purification.
Cell walls of the aleurone tissue consist of 65 % arabinoxylan (AX), 30% β-(1,3;1,4) glucan and 2% glucomannan and cellulose (Freeman *et al*. 2017). AX is also a major component of pericarp cell walls (60% AX). Pericarp cell walls do however also contain 30% cellulose and 12% lignin (Shewry *et al.* 2014). Total AX content in bran varies depending on cultivar and cultivating conditions (Shewry *et al.* 2014).

2.2.1 Arabinoxylan structure

AX is a heterogeneous polysaccharide consisting of a linear backbone of β(1,4)-linked xylopyranose.The xylose monomer can be mono- or di-substituted with α-(1,3) linked arabinofuranose or α-(1,3) and α-(1,2) linked arabinose, respectively (Freeman *et al.* 2017). The size of the molecule and degree of substitution vary extensively. The xylose backbone can also be substituted with α-D-glucuronic acid or the derivative 4-O-methyl (Freeman *et al*. 2017).

Mono-substituted α-(1,3) linked arabinose can additionally be ester linked to ferulic acid and *p*-coumaric acid giving AX important physiochemical and structural properties (figure 3). Through oxidative dimerization can ferulic acid form intermolecular C-C crosslinks between neighboring AX molecules (figure 2). Dimerization can also occur between AX and glucuronoarabinoxylan's or lignin (figure 2). Crosslinking between AX and β-glucans, protein and cellulose can also occur (Zhang et al. 2019).

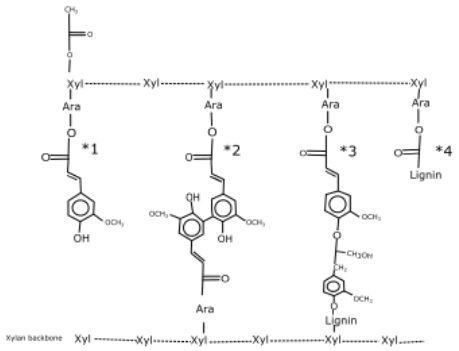

Figure 2 AX structure in cell walls [1]AX-FA, [2]AX-FA-FA-AX, [3]AX-FA-FA-lignin-AX, [4]AX-lignin. Adapted from (de O. Buanafina 2009)

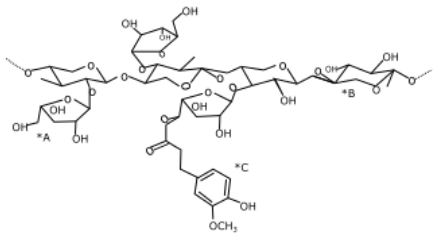

Figure 3 AX structure [A]Arabinose (Ara), [B]Xylose Backbone (Xyl), [C]Ferulic acid (FA).

2.2.2 Solubility

The degree of feruloynation, arabinose to xylose ratio as well as arabinose substitution pattern majorly effect the solubility of AX. The arabinose residues prevent hydrogen bonding between xylose residues of the backbone and hence provides steric hindrance between xylose chains (Freeman *et al.* 2017). This prevents aggregation of unsubstituted xylose regions (Zhang *et al.* 2019). AX with a higher A/X ratio is consequently more soluble. Low ratios of ~0.18–0.32 are associated with insolubility due to aggregation and non-covalent interactions with cellulose and β-glucans (Zhang *et al.* 2019). Additionally will more ferulic acids substitution result in more crosslinking and make AX less soluble (Freeman *et al.* 2017)

AX is commonly divided into water unextractable AX (WUEAX) and water extractable AX (WEAX). AX molecular architecture varies depending on histological origin resulting in differences in solubility between the tissues (Shewry *et al*. 2014). Bran WEAX tend to have a higher A/X ratio (~1), however this is also dependent on cultivar, cultivating conditions and milling procedure (Gebruers *et al.* 2008). Due to deficient crosslinking is WEAX loosely bound to cell wall components giving it water-extractable properties (Zhang *et al*. 2019).

2.2.4 Nutritional properties

AX classifies as a soluble dietary fiber as it is undigested in the stomach and small intestine and can only be degraded by colonic microbiota. A significant amount of studies have demonstrated a beneficial effect of soluble dietary fiber including reducing plasma cholesterol levels, improving gut health and improving long-term glycemic control (Zhong *et al*. 2000; Prückler *et al.* 2014). Additional studies show that AX specifically can reduce the postprandial glycemic response and hence reduce the risk of developing type 2 diabetes (EFSA 2010).
Ferulic acid has due to its radial scavenging properties been observed to have antioxidant, anti-microbial, anti-inflammatory, anti-thrombosis and anti-carcinogenic activity. The resonance-stabilized phenoxy radical structure can mitigate the presence of reactive oxygen species (ROS) in inflammatory processes (Ruthers *et al.* 2017).

2.2.5 Arabinoxylan applications

Wheat bran is currently underutilized for human consumption due to unattractive physiochemical and organoleptic properties. Consequently, it is mostly used as animal feed or to produce bio-fuel. WB addition to bread interferes with gluten formation and reduces the gas retention and hence leaves the dough less elastic and reduces the leavening potential (Zhang *et al.* 2019). Studies of <5 % AX enriched bread indicates it is having enhancing properties due to water absorption and increase in viscosity. Increase in molecular weight (MW) can enhance the effect. Zhang *et al*. (2019) showed that fortification with ~1 % high MW AX improved loaf volume and created bread which retained more water and had a softer crumb during storage compared to breads fortified with low MW AX.
AX can additionally be used as thickening or stabilizing agent due to its high viscosity and water-holding properties (Zhang *et al*. 2019).

AX can furthermore be valorized to design new biobased materials due to its favorable intrinsic properties and polymeric structure (Yilmaz-Turan *et al*. 2020). AX with higher MW as well as higher degree of substitution (DS) was observed to improve film formation and create biofilms with better thermal and mechanical properties (Yilmaz-Turan *et al*. 2020). Feronylated AX can create films with high antioxidant properties appropriate for preserving oxygen sensitive foods (Yilmaz Turan *et al.* 2020).

Studies have been examining the use of AX as a functional ingredient to suppress the development or improve metabolic diseases such as diabetes type 2 (Lu *et al.* 2000, Lu *et al*. 2004). The resistance towards degradation in the stomach and small intestine moreover makes AX a promising polymer for drug delivery systems. AX

drug delivery systems can be used to initialize colon-specific drug release as AX exclusively is degraded by anaerobic colonic microbiota (Oliveira *et al.* 2010)

2.3 Arabinoxylan extraction

Knowing the structural and physical properties of the extracts are crucial when optimizing the fractionation process (Hemery *et al.* 2007). The extraction method furthermore effect the molecular structure and composition of the extracts. Developing a method which enables tailoring of the chemical composition of the extracts is thus of value in addition to purity and yield. Conventional methods use alkaline conditions to extract AX from the cell wall. However, this process requires large amounts of water and consequently energy for dehydration, and results in significant waste production (Zhang *et al*. 2019).

Novel extraction techniques are investigated to by-pass the problem with extracting AX from the recalcitrant bran. Ruthers *et al.* (2017) recently developed an extraction and fractionation method, subcritical water extraction (SWE), optimized to fractionate hemicelluloses from industrial by-products. The method maintains the ferulic acid residues which retains the antioxidant properties of AX. However, a higher AX yield is still obtained with alkaline extraction.

2.3.1 Alkaline extraction

Alkaline extraction is the most common extraction technique used to obtain hemicelluloses with high molecular weight. The method is known to be highly efficient to produce a high extraction yield (Börjesson 2019). Yields reported by selected authors are presented in table 2.

Table 2 AX yield obtained with alkaline extraction.

AX Yield (%)	Mm	a/x ratio	Reference
20.8[1]	>650 kDa	1	Aguedo *et al*. (2014)
18.5[2]	350 kDa–7200 kDa	0.8	Zhou *et al*. (2010)
16-13[2] (6[4])			Bataillon *et al*. (1998)
11.7[3]	100–110 kDa	0.8	Hollmann & Lindhauer (2005)

[1]Yield calculated as % of destarched bran
[2] Yield calculated as % of destarched bran, with a delignification procedure.
[3] Yield calculated as % of untreated bran, with a delignification procedure.
[4] Yield calculated as % of total wheat bran

Alkaline extraction releases AX by alkaline saponification which hydrolyses the ester-bonds crosslinking AX and lignin and solubilizes AX. When using sodium hydroxide, the hydrogen bonds between cellulose and hemicellulose will additionally break and subsequently cause swelling of cellulose. This increases hemicellulose solubility but will result in degradation of its native structure. To improve AX yield, cleavage of lignin-carbohydrate complexes is important as well as increasing the solubility of AX by cleaving the hydrogen bonds between polysaccharides (Börjesson 2019, Giummarella *et al.* 2016, Nishimura *et al.* 2018). Alkali extraction yields highly substituted AX from the outer layer of the bran, as can be seen in table 2 (Hemery *et al.* 2007). The high molecular weight is important for AX nutritional properties (Zhang *et al.* 2019)

Depolymerization of hemicelluloses in alkaline conditions can be an issue during alkaline extraction (Börjesson 2019). This generates a lower yield than what is achievable with alkaline extraction. Börjesson (2019) observed a protecting effect of reducing agents towards polymerization resulting in a higher yield compared to extracts without reducing agents. Sodium dithionite, ($Na_2S_2O_4$) gave the highest yield when combined with 1 M NaOH. Using alkaline treatments in an industrial production is however limited due to cost, requirements of process safety and environmental effects (Zhang *et al.* 2019).

2.3.2 Pretreatment

Pretreatments are often conducted prior to the extraction. They are used to optimize the yield and purity of the extracts.

2.3.2.1 Destarching

Destarching is commonly used as an initial pre-treatment to simplify the extraction and purification (Ruthers *et al.* 2017). Destarching is known to effectively reduce starch whilst enriching the arabinoxylan content (Rudjito 2019). Gelatinization of starch by heating makes the starch water-soluble and susceptible for hydrolysis by hydrolytic enzymes (Rudjito 2019). α-amylase, an endoglycosidase, cleaves 1-4 α-linked D-glucan chains present in amylose and amylopectin (Rudjito 2019). It produces oligomers with different degree of branching and length which can be removed by washing with 70 % ethanol (Xie *et al.* 2008). Optimization of enzymatic treatments is done by adjusting pH, temperature, time and agitation (Msarah 2020).

2.3.2.2 Delignification

Pretreating WB with delignification can result in higher purity and lower polydispersity of AX extracts (Börjesson 2019). Purity and polydispersity are

important since it modulates the physiochemical properties of the extracts. Delignification of wheat bran prior to alkaline extraction generates hemicellulose yields of 13 % (Börjesson 2019). Glasser *et al*. (2000) observed an increase in purity of AX extracts when pretreating bran with chlorite. Bleaching changes the aromatic properties of lignin and makes it possible to isolate hemicelluloses by precipitation with an organic solvent or by ultrafiltration.

2.4 Pulsed electric field technology

Applying high voltage pulses to biological cells induces structural changes in the cell membrane (Toepfl 2005). Eventually, this can lead to breakdown of the cell membrane, disruption of intracellular organelles and similar structural changes (Janositz & Knorr 2010). Breaking the cell membrane means breaking the semipermeable membrane responsible for most of the fundamental activities of the cell, including mass transfer, nucleic acid synthesis and protein synthesis (Toepfl 2005). However, there is still a limited amount of research on the effects exerted on the cell wall and post-treatment effects (Janositz & Knorr 2010).

Electric fields make cell membranes electromechanically unstable by inducing a transmembrane potential ($\Delta\Phi$). The initial transmembrane potential is formed by accumulation of charges with opposite polarity at both sides of the nonconductive membrane, leading to a compressive force across the membrane (Zhang *et al.* 2019). Exposure to an external electrical field increases the potential and results in membrane thickness reduction (Janositz & Knorr 2010). Pores will be formed when exceeding a critical value due to local dielectric rupture of the membrane (Toepfl 2005). Pore formation is either reversible or irreversible depending on the formed pore geometry in relation to the membrane area (Toepfl 2005). Large pore formation leads to irreversible breakdown, mechanical destruction of the cell membrane and eventually cell death.

Janositz et al (2010) classified the effectiveness of PEF to permeable cell membranes in three contributing factors; technical and chemical processing conditions and biological product characteristics. Technical parameters include electric field intensity, treatment time, pulse shape and applied energy. The most important factors to control pore formation are treatment intensity and electric field strength (Janositz *et al.* 2010, Toepfl 2005, Zhang *et al*. 2019). Cell size are furthermore affecting cell permeabilization, where large plant cells are reported to be more susceptible to membrane permeabilization by PEF (Zhang *et al.* 2019). Temperature and conductivity of the medium are additionally important parameters regulating PEF effectivity.

Calculating the transmembrane voltage induced by the electric field estimates pore formation and cellular breakdown. Equation (1) can be used to determine the field strength needed to accomplish significant breakdown (Janositz & Knorr 2010).

$$\Delta\Phi = 3/2\ f\ E\ R\ (\varphi)$$

$\Delta\Phi$ is the induced transmembrane voltage, E is the external electric field, R is the radius of the cell and φ is the angle between the center of the cell and the direction of the field.

Bazhal et al. (2003) deduced that a field strength between 200-1100 V/cm was optimal to obtain cell disintegration of plant cells. Optimal field strength increased for cells with secondary cell walls (Bazhal et al. 2003). Furthermore, Angersbach et al (2010) concluded that field strength applied to plant cells with a cell size of 50–120 μm needs to be higher than 400–800 V/cm for significant membrane breakdown.
Several pulse waveforms are used depending on type of switch and configuration of the discharge unit (Toepfl 2005). Square wave pules are considered more efficient and reduces treatment time (Putri *et al*. 2010). Pulse width for square wave pules is defined as the time where the peak field is maintained. Increased treatment time, i.e. pulse width*number of pulses, increases the treatment effect. Saturation is observed after a certain amount of pulses (Baier *et al.* 2015).

PEF shows great potential to improve extraction of intracellular substances (Kumari *et al*. 2018). Permeabilization of cell membranes increases intracellular diffusion and hence increases mass transfer. Mass transfer processes such as extraction can thus be optimized with PEF (Kumari *et al.* 2018).
Electroporation by PEF can also permeabilize and reduce the structural components in plant cell walls (Baier *et al.* 2015). The treatment effect appears to depend on cell configuration (monolayer or tissue) and treatment protocol (Mahnič-Kalamiza *et al*. 2014). Janositz *et al.* (2010) observed a reduction in lignin content (~2,4%) in PEF treated asparagus. High voltage pulses were shown to break inter and intramolecular bonds between lignin, cellulose and hemicelluloses. Electrostatic dipole-dipole interactions between hydroxyl and methoxyl groups on the polymers are broken by high voltage electric pulses which consequently makes it easier to extract lignin and lignin-bound polymers. However, electropermeabilization is primarily used to increase diffusion-driven transmembrane transport of small molecules (Mahnič-Kalamiza *et al*. 2014). Extracting larger molecules requires formation of larger pores and subsequently higher energy input.

2.5 Analysis methods

HPAEC-PAD and Light microscopy were used to validate the effect of PEF on WB.

2.5.1 Light microscopy

Light microscopy (LM) is widely used in research and industry to examine food microstructures (Holgate *et al*. 2003). Moreover, it is the most common method in research to examine cell structures (Holik *et al*. 2003). LM is the simplest form of microscopy. Finer details can be visualized by creating a magnified image of an object (Holgate *et al*. 2003). Transmitted light rays are in LM allowed to pass through a thin object which enables parallel rays to meet at a certain point). The meeting point of the light rays is called the focus of the lens. The distance between the lens and its focus is referred to as the focal length and the magnitude of this distance determines the power of the objective (Holik *et al.* 2003). A higher power give rise to a more enlarged image.

2.5.2 HPAEC-PAD

High-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) is a frequently used method for carbohydrate analysis (Patil & Roher 2017). HPAEC- PAD detect and separates carbohydrates with specific interactions between their hydroxyl and carboxyl groups. The separation is based on charge, size, composition, isomerism, and linkages which makes the method appropriate to determine monosaccharide compositions (Patil & Roher 2017). Carbohydrates becomes oxyanions at high pH which enables separation by anion-exchange chromatography. A mobile phase (usually sodium hydroxide) with pH> 12 is normally used as some carbohydrates have a pKA>12 (Roher *et al.* 2013). PAD, which is a direct detection technique, is used to detect the carbohydrates after the separation. PAD detects carbohydrates by oxidizing them on a gold electrode (Roher *et al*. 2013). Carbohydrates can be detected from chromatograms by using standards for the monosaccharides (figure 4).

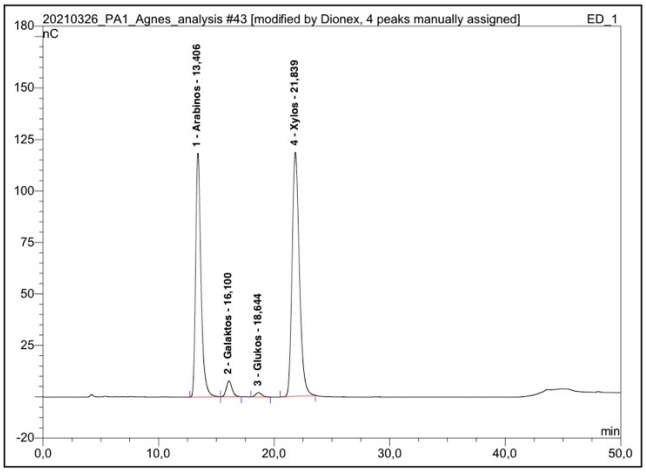

Figure 4 Chromatogram from the HPAEC-PAD analysis. Analyzed sample is hydrolyzed AX extract from WB treated with PEF (100 pulses).

3. Materials and methods

3.1 Materials

Milled wheat bran (kruskakli, <380 μm) was provided from Lantmännen (Stockholm, Sweden). Moisture content was measured gravimetrically in duplicates for the starting material. Chemicals and reagents used were of analytical grade. α-amylase (type IV−B from porcine pancreas), NaClO2 and sulfuric acid were obtained from Sigma Aldrich. NaH2PO4 and Na2HPO4 were from Labchem, NaCl and NaoH were from EMSURE. All monosugars used for the standard calibration curve were from sigma Aldrich. 95 % ethanol was from Solveco.

3.2 PEF treatment

Samples of 30 g dwb were weighted in glass bottles and soaked overnight (+4C) in water containing 0.184g NaCl/l.
The presoaked samples were transferred to the PEF test cell with added water containing 0.184g NaCl/l was added until the cell was completely full (approximatly 200 mL). The salt was added to mimic the conductivity of the water in Uppsala. Samples were poured back to the glass bottles from the test cell after the treatment. Same procedure was followed for the control samples. After the PEF, the bran was separated from the liquid by centrifugation (8000rpm 20min). Pellet was frozen -70C and the supernatants was collected and freeze-dried.

Highest possible field strenght, 950 V/cm was used with a frequency of 2Hz and pulse width of 5 μ s. This induced arching in one sample which argues for an adaptation of the method in future studies. The only changed treatment parameter was the number of pulses applied. WB was treated with either 100, 250, 500, 750 or 1000 pulses. Triplicates were used in all treatments.

PEF system was provided by Scandinova (Uppsala, Sweden). PEF treatments were performed discontinuously. Treatments were randomized and divided into two days and lasted for four hours each.

The PEF system consisted of three parts; a generator, an oscilloscope and a treatment chamber. The capacitor in the generator generated the voltage which consequently regulated the applicable electric field strength. The electric field strength was determined by force per unit charge. This was equal to the voltage moving through the electrodes in the treatment chamber. The oscilloscope measured and controlled the voltage, pulse width and intensity. The cylindrical treatment chamber had a sample volume of 200 mL. Temperature increase was recorded with a portable thermometer.

3.3 Moisture content

Moisture content was determined gravimetrically for all samples weighted in wet weight. Samples (duplicates) were dried in the oven overnight at 105 °C.

3.4 Arabinoxylan extraction

The alkaline extraction process was adapted from the method described by Ruthers *et al.* (2017). The extraction process used included destarching, delignification and alkaline extraction with membrane dialysis. Extraction process is presented in figure 5.

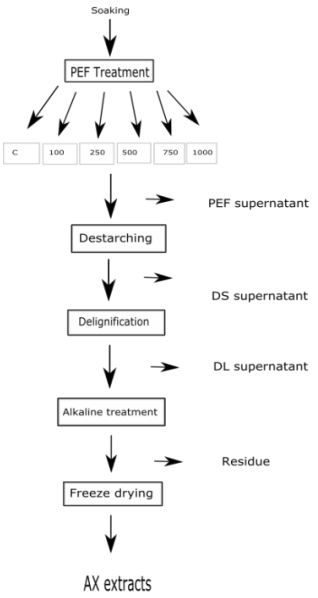

Figure 5: Flow chart of the extraction process

3.4.1 Destarching

Destarching was performed enzymatically by using α-amylase (Type IV−B from porcine pancreas). 20 mM sodium phosphate buffer was prepared by suspending 6.7 mM NaCl (pH 7), 1.12 g of NaH2PO4, 1.52 g of Na2HPO4 and 3.9 g of NaCl. 10 g (dwb) defrosted PEF treated bran was suspended in buffer in 1:10 (w/v) and put in water bath (5 min 85C) to gelatinize starch. Samples were cooled down to ~40°C and α-amylase (16U/g wheat bran) was added and left to destarch overnight (~16h) in 37C water bath under constant stirring.

The destarched bran was separated from the buffer by decanting. The liquids were collected and oven dried at 60°C overnight.
The starch was washed out by washing the bran with distilled water (50 mLx2). To precipitate the polysaccharides, 50 mL 95 % ethanol was added stirred and decanted. This was repeated once. The bran was then placed in oven overnight at 60°C.

3.4.2 Delignification

Destarched bran was suspended in distilled water 1:10 (w/v) dwb. Lignin was removed from the bran by adding NaClO2 (0.15 g/g wheat bran). pH was adjusted to 3.1 with HCl and samples were put in water bath 80°C for 3h under constant stirring. Delignification was stopped by removing the liquid through centrifugation 15min 5000rpm. The supernatant was collected and dried in oven at 60°C overnight. Distilled water was subsequently added to the pellet, mixed and separated from the bran by decanting. The procedure was repeated twice. Delignified bran was dried in oven at 60°C until completely dry.

3.4.3 Alkaline extraction

Dried delignified bran was suspended to 80 mL with 0.5 M NaOH (1:8 w/v) and put in water bath at 80°C for 16h.
The alkaline extraction was stopped by adjusting pH to 7. Extracted AX was separated from the residue by centrifugation. Supernatants was dialyzed with 3.5 kDa MWCO dialysis membrane and freeze-dried. Pellet was saved and dried in the oven at 60°C until completely dry.

A visual presentation of the extraction method is presented in figure 6. Precipitation of smaller particles were observed in samples during extraction as seen in picture D and E. This could potentially have affected extraction yields.

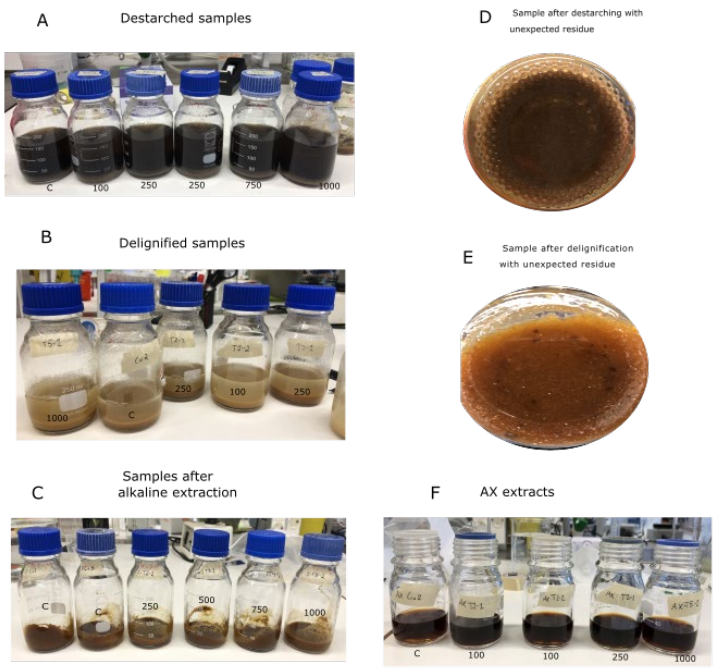

Figure 6 Visual presentation of the extraction process. (A) Samples after destarching (B) Samples after delignification (C) Samples after alkaline extraction (pH~7) (D) Destarched sample with precipitated bran particles (E) Delignified sample with precipitated bran particles (F) AX extracts after dialysis

3.5 HPAEC

For the HPAEC a Dionex ICS-3000 was used with a Carbonpac PA1 column for separation. An Ionpac AC3 was used as carbonate trap.
For the dialysis, a MWCO 3.5 kD membrane from spectrum labs was used.
To filtrate the hydrolyzed samples a vacuum filtration set was used. Glass fibre pre-filters from Merc Millipore Ltd was used to collect the Klason lignin.

3.5.1 Hydrolysis

70 mg (dwb) weighted samples was hydrolyzed by adding 1.05 mL of 73 % H2SO4 and left for 1.5 hours in vacuum. 29.4 mL of water was added to the samples and autoclaved at 125C for 1h. References were prepared following the same procedure as the samples. Glucose, Arabinose, Xylose, Galactose and mannose were used as Standards. Glucose, arabinose, galactose and mannose were the monosaccharides measured in the samples, the total carbohydrates measured in the samples were the sum of the measured monosaccharides.

1 mL single use syringes (codan medical Aps) with 25 mm syringe filters (vwr international) were used to filtrate the hydrolysed samples prior to the HPAEC analysis.

3.5.2 Klason lignin

Klason lignin content was obtained by vacuum filtration and determined gravimetrically. Samples were filtrated with 10 mL hot water and collected in glass bottles. Residues were collected on glass fiber filters. The filters were cleaned with 100 mL hot and 100 mL cold water, dried in the oven at 105 °C overnight and weighted to quantify Klason lignin content.

3.5.3 Monosaccharide analysis Dionex

Samples were diluted in Eppendorf tubes and transferred to vials through filtration with 1mL syringes and 25 mm syringe filters. The hydrolyzed monosaccharides were analyzed by HPAC-PAD using a Dionex ICS-3000 (Dionex, Sunnyvale, CA) using the following principles: 10 µL of each sample was injected using a 10 µL injection loop, flow rate 0.25 mL/min. Concentrations of monosaccharides were determined using HPAC-PAD (wave form standard quad) with CarboPac PA1 anion exchange column (separation: 2x250 mm, guard: 2x50 mm), T=30 °C, flow rate 0.25 mL min-1. Isocratic elution with 4 mM NaOH for 35 min was followed by 5 min of column regeneration consisting of a gradient (1 min) to 200 mM NaOH, which was kept for 5 min before switching back to 4 mM NaOH for 5 min. Before each sample injection the column was run for 2 min at 4 mM NaOH. A post-column addition of 0.3 M NaOH was applied at a flow of 0.125 mL min-1 to ensure a minimum of 0.1M NaOH in the detector. An Ionpac AC3 was used as carbonate trap placed before the guard and separation columns.

3.5.4 Light microscopy

Light microscope pictures were taken with a Nikon H550L Eclipse Ni-U microscope. Samples were prepared with a Leica EM C6 and a Leica MP hot plate. Glass knives were cut using a Leica EM KMRS knifemaker.

3.5.5 Statistical method

Data was analysed with R-studio (Version 1.4.1106, "Tiger Daylily" for macOS) One-way (two tailed) ANOVA was used to identify significant differences between the PEF treated AX extracts in yield and monosaccharide composition. A 95 % confidence interval was used for all statistical analyses. Raw data from the statistical analyses can be found in Appendices II.

4 Results & Discussion

4.1 Effect of process steps on chemical composition of wheat bran

The chemical composition of the untreated wheat bran (kruskakli) and control samples without PEF treatment after each extraction process step is shown in table 3.

Table 3 Chemical composition of untreated WB (Kruskakli), supernatans from the extraction process (PEF, DS, DL), AX extracts (AXE) and the residue after the extraction (AXR). Samples presented are control samples, they were not subjected to any PEF treatment.

	Kruskakli	PEF	DS	DL	AXE	AXR
Moisture content (%)	10.59 (± <0.1)					
Total Carbohydrate (mg g-1 DW)	292.7 (± 21)	339.69 (±45.44)	301(±45.57)	209(± 48.82)	601,6 (±<22.4)	633.58 (± 35.44)
Ara*	15.9 (± <0.1)	8.7(± 0.1)	2.6(±2.1)	34,5(± 1.5)	49.0 (± 1.4)	10.8(± 0.1)
Gal*	2.55 (± 0.5)	5,4(± 0.2)	2.0(± 0.2)	2.1(± 0.1)	1.8 (± 0.9)	0.9(± 0.1)
Glu*	57.48 (± 0.5)	55.1(± 0.3)	91.5(± 4.4)	23.9(± 5.7)	0.9(± 0.6)	49.9(± 5.7)
Xyl*	24 (± <0.2)	11.8(± 0.3)	3.2(± 1.9)	39.5(± 4.4)	48.3(± 0.9)	38.5(± 4.4)
Man*	17 (± <0.1)	0.7(± 0.1)	0.7(± 0.1)	0.0(± 0.1)	0.0(± <0.1)	0.0(± 0.1)
Klason lignin (mg/g DW)	147.14 (± 55.5)	51.4(±2.7)	75(±28.9)	120.2(±43.5)	100.24 (± 27.4)	181.8(±39.7)

**Kruskakli (untreated wheat bran), PEF supernatant (PEF), Supernatant after destraching (DS), Supernatant after delignification (DL), AX extracts (AXE), Residue after the extraction process (AXR)*

± indicates standard deviation

Kruskakli had the second lowest carbohydrate content (297.7 mg/g DW). Total carbohydrate content is low as untreated wheat bran in addition to carbohydrates also contains significant amounts of other compounds, including protein, fat and ash. WB also contains complementary monosaccharides and sugaracids in addition to those quantified in this analysis. AX can for instance be substituted with both

galacturonic acid and glucuronic acid (Fardet et al. 2010, Ruthers et al. 2017). Considerable amounts of mannans and arabinogalactans can also be present in WB cell walls which explains the high mannose content (17 %) (Ruthers el al. 2017).

Destarched supernatant (DS) and PEF supernatant (PEF) had a slightly higher carbohydrate content than the kruskakli (+8.3 and +46.99 mg/g DW respectively). Soaking of the bran releases carbohydrates in the PEF supernatant (Jacobs *et al*. 2014). Starch granules damaged during milling can in particular cause leaking and solubilisation of starch (Jacobs *et al*. 2014). Leaking of water-soluble compounds such as protein and ash explains the lower carbohydrate content in DS (301 mg/g DW). PEF supernatant had lower arabinose and xylose contents than the kruskakli (8.7 % and 11.8 % compared to 15.9 % and 24%). Soaking releases WEAX in PEF supernatants. WEAX is deduced to represent only 0.3-0.85 % of the bran content (Gebruers *et al*. 2008; Shewry *et al*. 2013). Hence, the lower content is a consequence of low AX solubility since Kruskakli contains both WUAX and WEAX (Fardet *et al*. 2010).

The supernatant from the destarching step (DS) did predominantly contain glucose (91.5 %), as expected from destarching with α-amylase. The low arabinose and xylose content indicate that this step not causes a noticeable reduction in AX content. The supernatant from the delignification (DL) had the lowest carbohydrate content (209 mg/g DW). Monosaccharides with highest content in DL were arabinose (34.5 %), xylose (39.5%) and glucose (23.9%). Arabinose and xylose appear to originate from AX crosslinked lignin and are probably released from the cell wall during the delignification (de O. Buanafina 2009).

The AX extract had both high carbohydrate content and AX purity. Very small amounts of galactose (1.8%) and glucose (0.9%) could be detected. Galactose and glucose could indeed be substituents or originate from other hemicelluloses such as arabinogalactan and B-glucan (Ruthers *et al*. 2019).

The residue after the extraction (AXR) had the highest carbohydrate content (633.58 mg/g). The very low A/X ratio (0.28) indicates that alkaline unextractable AX is bound to the cell wall by the xylose monomers (Zhang *et al.* 2019). Changes in A/X ratio during the extraction can be seen in figure 7.

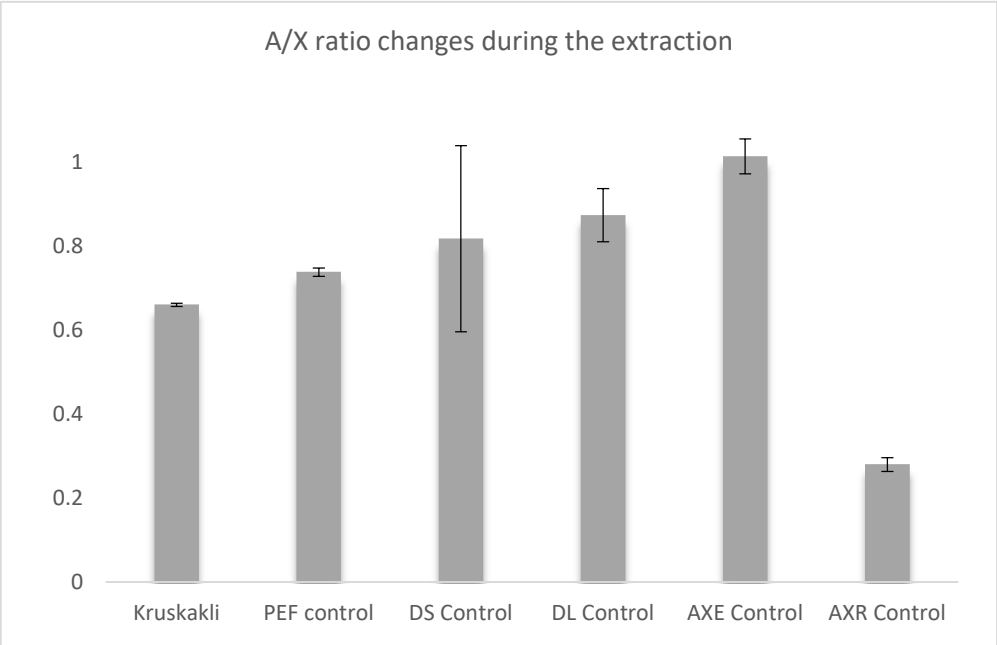

Figure 7 Arabinose to xylose ratio for untreated bran, supernatants and residue after each step of the extraction. Samples presented were not PEF treated

The A/X ratio in kruskakli (0.66) was similar to the AX ratio found in the endosperm (0.50-0.71) (Zhang *et al.* 2019). Hence, AX likely originated from parts of the endosperm that was not separated from the bran during milling.

The A/X ratio in PEF (0.74) was however higher than the ratio in the Kruskali (0.66) and indicates that soaking releases more substituted AX. The ratio was indeed similar to the ratio reported from WEAX (0.7-1.25).

Differences in A/X ratio between the samples suggests that AX derived from different histological parts of the bran. AX from the outer pericarp is observed to be more substituted and have a ratio around 1 while as low ratios as 0.44 and 0.42 have been reported for AXs from the aleurone and intermediate layers (Ruthers *et al.* 2017). It is previously known that alkaline extraction releases AX from the outerlayers of the bran (Ruthers *et al*. 2017). The high A/X ratio in DL confirms that AX from the outerlayer of the bran is released with lignin during delignification. This is expected as lignin is not present in the aleurone layer. The pericarp instead consists of 12 % lignin (Shewry *et al.* 2014).

Alkaline unextractable AX have very low ratios ~0.28. It is clear that arabinose residues are crucial for AX solubility. Consequently, highly substituted AX are more extractable with alkaline conditions.

4.2 PEF

4.2.1 Temperature

Temperature increased with number of pulses (table 4). Samples with a higher initial temperature had a higher temperature after the treatment. Samples treated with 1000 pulses had the highest temperature measured of 41.4 °C. A significant temperature increase can be problematic as high temperatures can affect molecular structures and hence degrade valuable nutritional substituents (Toepfl 2017). PEF treatments are additionally more energy efficient if temperatures are kept low (Kumari *et al.* 2018). Higher temperatures can however increase AX extractability (Ruthers *et al.* 2017). Temperatures >100 °C are observed to hydrolyse crosslinks between AX and cell wall components (Zhang *et al*. 2014).

Table 4 Temperature increase in PEF treated samples. Temperature increase is expressed as temperature differences between control samples and sample temperature after PEF treatment.

Treatment	ΔT
Control	0 (±0.7)
100	2.9 (±1.1)
250	8.63 (±1.9)
500	14.2 (±1.2)
750	16.6(±2.1)
1000	24.466(±2.8)

± indicates standard deviation

4.2.2 Yield

The PEF treatment did not result in a significant difference in total solids yield of AX extracts (p=0.6365) (figure 8). Yield ranged from 23.1-27.6 % of destarched wheat bran (dw).

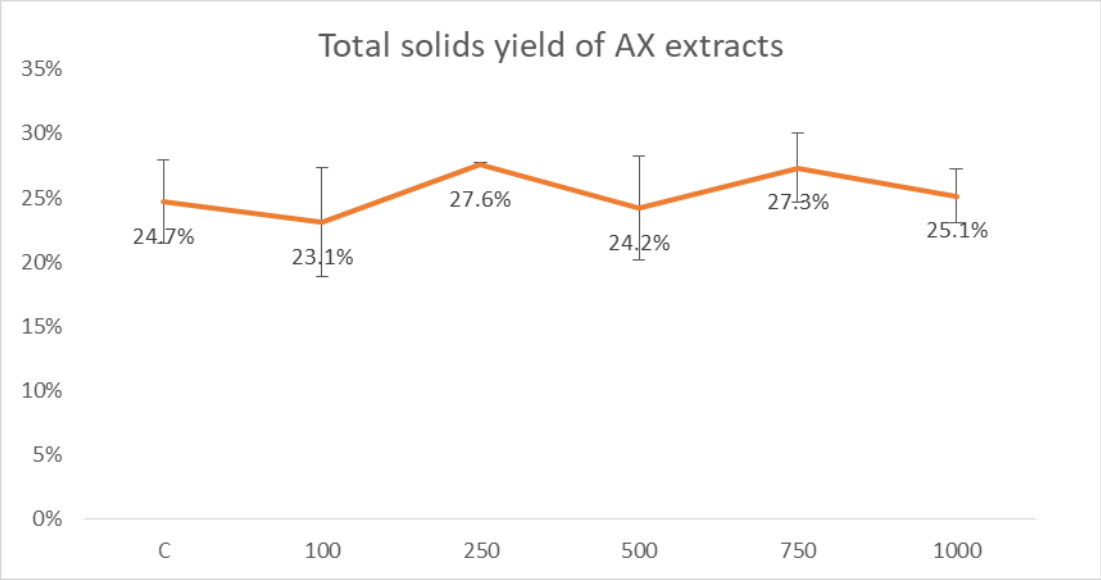

Figure 8 Total solids yield of AX extracts. Percentages are calculated based on destarched wheat bran (dw).

4.2.3 Chemical composition of AX extracts

PEF had a significant effect on total carbohydrate content of AX extracts (p=0.0005861). There was an increase in carbohydrate content for AX extracts treated with 250 (+28.131 mg/g DW), 500 (+14.979 mg/g DW) and 1000 (+78.172 mg/g DW) pulses (table 5). Thus, AX extracts from WB treated with 1000 pulses had the highest carbohydrate content (679.8 mg/g DW) and hence, had the highest AX purity.

Klason lignin content was lowest in AX extracts from WB treated with 1000 pulses (97.20 mg/g DW) (table 5). AX extracts from WB treated with 750 pulses had the highest lignin content (126.13 mg/g DW) but also the second highest carbohydrate

content (616.6 mg/g DW). Klason lignin content appear to not relate to carbohydrate content or be effected by PEF or increased treatment intensity.
PEF was shown to have a significant effect on the monosaccharide composition of the extracts (table 5). PEF induced differences in both arabinose (p=9e-04), xylose (p=0.002055) and glucose content (p=0.009286). The differences were related to differences in treatment intensity. Arabinose content decreased for AX extracts treated with 100, 500, 750 and 1000 pulses. Interestingly, arabinose content decreased with 2.3-5.2 % with increased number of pulses. 500 pulses generated the lowest arabinose content (43.7 %) whilst 1000 and 750 pulses generated similar arabinose content (46.1 % and 46.2%). 100 pulses decreased the arabinose content more than 1000 and 750 pulses. PEF significantly increased the xylose content for all treatments. The biggest increase was observed for AX extracts treated with 500 pulses (+3.9 %). Glucose content showed a significant increase in samples treated with 500 pulses (+9.17 %).

It appears that PEF changes the chemical composition of AX extracts and that the purity and composition are related to treatment intensity. The increase in purity is an interesting observation as it demonstrates that PEF releases compounds otherwise present in the extracts. It is generally known that electroporation increase diffusion driven membrane transport of smaller molecules. Hence, the treatment intensities used were likely sufficient to increase the extraction of smaller molecules but not sufficient to effect extraction of larger molecules as AX.

Accordingly, a/x ratio was significantly affected by PEF (p=0.0005145). PEF decreased the A/X ratio of the AX extracts compared to the control (figure 9). The decrease was statistically significant for all treatments. This implies that PEF changes the structure of AX and will allow us to modulate the properties of AX extracts as the A/X ratio determines the solubility, macromolecular conformation, physio-chemical and functional properties of AX (Ruthers *et al.* 2017). Further studies should be conducted to evaluate the substitution pattern and physiochemical properties of AX extracts.

Table 5 Chemical composition of PEF treated samples and control sample

	Control	T100	T250	T500	T750	T1000
Total Carbohydrate (mg g-1 DW)	601.6[a] (±<22.4)	588.0 [a](±<8.6)	629.8[a](±<1 1.6)	595.4[a] (±35.5)	616.6[a] (±<41.1)	679.8[b](±< 50.9)
Ara*	49.0[a] (± 1.4)	45.3[b](± 1.5)	46.7[a](±0.1)	43.7[c](±3.1)	46.1[d](± 1.0)	46.2[e](± <1.1)
Gal*	1.8 [a](± 0.9)	2.1[a](± <0.1)	1.9[a](± <0.1)	2.2[a](± 0.2)	2.1[a](± <0.2)	2[a](± <0.2)
Glu*	0.9[a](± 0.6)	1.1[a](± 0.5)	0..7[a](± 0.2)	1.8[b](± 0.4)	1.1[a](± 0.4)	1.0[a](± 0.3)
Xyl*	48.3[a](± 0.9)	51.5[b](± 1.3)	50.7[c](± 0.3)	52.3[d](±2,. 7)	50.7[e](± 0.5)	50.8[f](± 0.8)
Man*	0.0[a](± <0.1)	0.0[a](± <0.1)	0.0[a](± <0.1)	0.0[a](± <0.1)	0.0[a](± <0.1)	0.0[a](± <0.1)
Klason lignin (mg/g DW)	100,.24 (± <0.21)	111.095 (± <0.31)	126.42 (± <0.51)	98.50 (± <0.31)	128.13 (± <0.1)	97.20 (± <0.1)

± indicates standard deviation
a. Values followed by different letters (row) are significantly different at a 0.05 significance level.

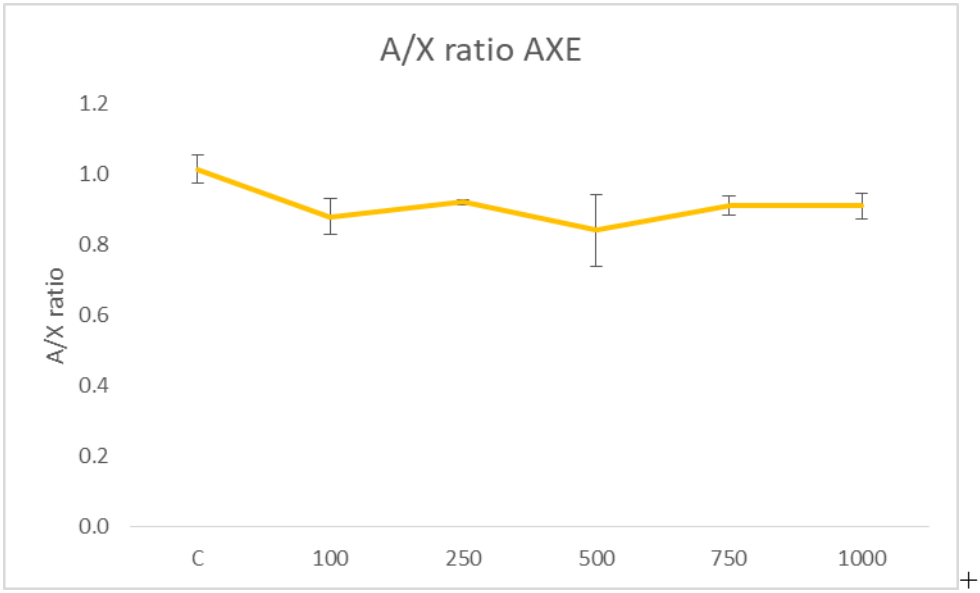

Figure 9 A/X ratio for AX extracts treated with increasing number of pulses and control sample.

1000 pulses and 750 pulses extracted AX with a similar ratio (0.91). 250 pulses generated AX with a 0.92 ratio. Lowest ratios were obtained with 100 (0.88) and 500 pulses (0.84). Thus, it was revealed that PEF probably generated AX from different bran tissues than the convential technique.
It is suggested that PEF extracted AX with higher molecular weight as AX with higher molecular weight is reported to have ratios<0.94 (Ruthers *et al.* 2017). AX

with higher molecular weight is additionally observed to have higher FA content which could be of value for nutritional or material applications (Ruthers *et al.* 2017).

The supernatant from the delignification (DL) had a ratio of 0.87, similar to AX extracts treated with 500 (0.88) and 100 pulses (0.84). This suggests that PEF treatments increases extraction of lignin-bound AX. It has previously been reported that PEF can increase extraction yields by removing lignin through breakage of covalent bonds and electrostatic dipole-dipole interactions between cellulose, hemicellulose and lignin (Janositz et al 2010).

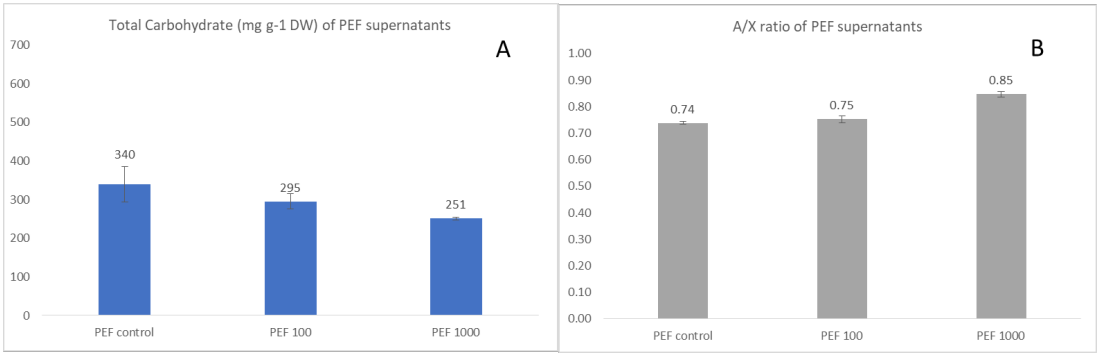

Figure 10 (A) Total carbohydrate content (mg/g DW) and (B) A/X ratio of PEF supernatants. Supernatants were collected from control sample and samples treated with 100 and 1000 pulses respectivly.

PEF supernatants from control samples and WB treated with 100 and 1000 pulses had different total carbohydrate content (figure 10 A). The carbohydrate content decreased progressively with increased number of pulses. PEF 100 had a carbohydrate content of 295 mg/g DW while PEF 1000 had a carbohydrate content of 251 mg/g DW. Increased leaking of protein, lipids and ash can be responsible for the demonstrated decrease (Baier *et al.* 2005, Chiang & Yeh 2002, Janositz & Knorr 2010). This further argues for an increased release of other, not analysed, compounds by PEF. The decrease may be related to the increase in purity of AX extracts. Thus, the protein, fat and ash content in the extracts should be analysed in upcoming research.

The increase in A/X ratio in PEF supernatants treated with 1000 pulses (0.85) compared to the PEF treated with 100 pulses (0.75) and the control (0.74) can furthermore confirm the conclusion that PEF changes the a/x ratio of AX extracts (figure 10 B).

4.3 Light microscopy

The morphology of WB is distinctively affected by PEF with different applied pulses as observed by light microscopy (Figure 11). LM images are however purposefully selected during the sample preparation and should thus only be considered as indicators of cellular changes. The histological parts of WB treated with 100 pulses (A) were still intact compared to WB treated with 750 pulses (C). The pericarp and testa were still attached to the aleurone layer. Most of the aleurone cells still had a cuboidal shape with distinct cell walls. Loss of the regular cuboidal shape of the aleurone cells indicate cell wall degradation (de O. Buanafina 2009). Degradation of the cell walls between the aleurone cells were observed in selected cells which can be confirmed by the increase in extraction yield and change in a/x ratio. The pericarp, testa and aleurone cells showed signs of progressive degradation for WB treated with 750 pulses. The pericarp tissue started to separate from the testa and had consequently been degraded into smaller fragments. The testa had additionally started to separate from the aleurone layer. Aleurone cells in picture B were much more irregular compared to picture A. Cell walls in B were indeed thinner and some were also degraded. WB treated with 750 pulses (picture C) showed an excessively degraded structure. The aleurone layer consisted of a short fragment with partly degraded cells. The pericarp and testa had additionally detached from each other. Picture D of WB treated with 1000 pulses showed a slightly more intact bran fragment. Same observations could however be made for the aleurone cells as in picture B and C. The cell wall line between the testa and aleurone layer showed a higher level of degradation compared to B and C.

These observations suggest that the outer layers are more affected by higher number of pulses (<100 pulses). Aleurone cells seem to be effected even by lower number of pulses (>100). This further confirms that different PEF intensities have different effects on cell wall permeabilization. The mechanical strength of the lignin-rich outerlayers may need higher treatment intensities for electroporation and cell wall permeabilization to occur. Cell wall permeabilization is dependent on several factors including cell configuration and treatment protocol (Mahnič-Kalamiza *et al*. 2014). Hence, more investigations need to be done to conclude the effect of PEF on WB.

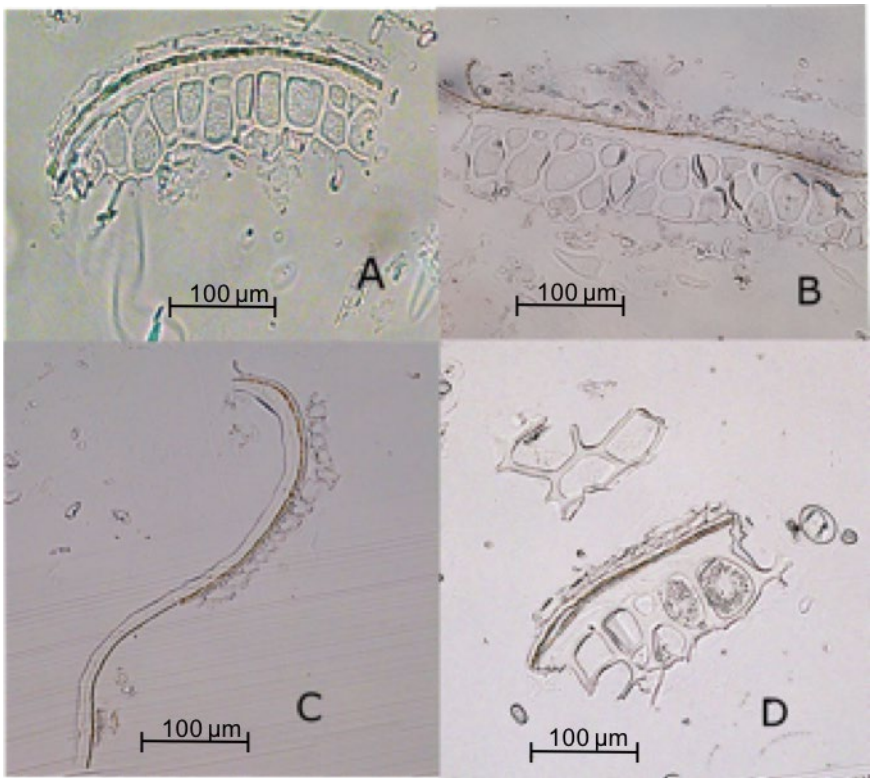

Figure 11 LMS pictures (20X) of PEF treated WB. A: 100 pulses, B: 250 pulses. C: 750 pulses. D:1000 pulses.

5 Conclusion

In this study the effect of PEF on extraction yield and monosaccharide composition of alkaline extracted AX was investigated. The aim was to examine the effect of PEF treatment intensity on AX yield and chemical composition of extracts. The result confirms that PEF can change the chemical composition of AX extracts. PEF increased the purity and changed the structure of AX in the extracts. The substitution of AX decreased with the PEF treatment and differed with number of pulses applied. It appeared that PEF extracted AX from different parts of the bran. The PEF treatment did not result in differences in total solids yield of AX extracts.

The result concludes that PEF can alter the fractionation of arabinoxylans. Determining the molecular structure and physiochemical properties of the AX extracts was not in the scope of this thesis and should therefore be evaluated in further research. The findings in this thesis comply with earlier findings about the potential of using PEF in novel food processing.

References

Aguedo, M., Fougnies, C., Dermience, M. & Richel, A. (2014). Extraction by three processes of arabinoxylans from wheat bran and characterization of the fractions obtained. *Carbohydrate Polymers*, 105, 317–324. https://doi.org/10.1016/j.carbpol.2014.01.096

Amami, E., Vorobiev, E. & Kechaou, N. (2005). Effect of Pulsed Electric Field on the Osmotic Dehydration and Mass Transfer Kinetics of Apple Tissue. *Drying Technology*, 23 (3), 581–595. https://doi.org/10.1081/DRT-200054144

Anderson, C. & Simsek, S. (2019). A novel combination of methods for the extraction and purification of arabinoxylan from byproducts of the cereal industry. *Journal of Food Measurement and Characterization*, 13 (2), 1049–1057. https://doi.org/10.1007/s11694-018-00020-4

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 (2), 222–231. https://doi.org/10.1016/j.lwt.2013.12.003

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 (4), 355–358. https://doi.org/10.3923/ijps.2009.355.358

Baier, A.K., Bußler, S. & Knorr, D. (2015). Potential of high isostatic pressure and pulsed electric fields to improve mass transport in pea tissue. *Food Research International*, 76, 66–73. https://doi.org/10.1016/j.foodres.2014.11.043

Bazhal, M., Lebovka, N. & Vorobiev, E. (2003). Optimisation of Pulsed Electric Field Strength for Electroplasmolysis of Vegetable Tissues. *Biosystems Engineering*, 86 (3), 339–345. https://doi.org/10.1016/S1537-5110(03)00139-9

Brouns, F., Hemery, Y., Price, R. & Anson, N.M. (2012). Wheat Aleurone: Separation, Composition, Health Aspects, and Potential Food Use. *Critical Reviews in Food Science and Nutrition*, 52 (6), 553–568. https://doi.org/10.1080/10408398.2011.589540

Chiang, P.-Y. & Yeh, A.-I. (2002). Effect of Soaking on Wet-milling of Rice. *Journal of Cereal Science*, 35 (1), 85–94. https://doi.org/10.1006/jcrs.2001.0419

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 (5), 285–288. https://doi.org/10.1002/food.19970410507

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 (22), 8521–8529. https://doi.org/10.1021/jf061728n

Elliott, D.C., Orth, R.J., Gao, J., Werpy, T.A., Eakin, D.E., Schmidt, A.J. & Neuenschwander, G.G. (2002). *Biorefinery Concept Development Based On Wheat Flour Milling*. (PNNL-SA-35705). American Chemical Society, Washington, DC, United States(US). https://www.osti.gov/biblio/15009662 [2021-05-25]

Fardet, A. (2010). New hypotheses for the health-protective mechanisms of whole-grain cereals: what is beyond fibre? *Nutrition Research Reviews*, 23 (1), 65–134. https://doi.org/10.1017/S0954422410000041

FAO Cereal Supply and Demand Brief | World Food Situation | Food and Agriculture Organization of the United Nations. http://www.fao.org/worldfoodsituation/csdb/en/ [2021-05-25]

http://www.fao.org/worldfoodsituation/csdb/en/ [2021-05-25]

Freeman, J., Ward, J.L., Kosik, O., Lovegrove, A., Wilkinson, M.D., Shewry, P.R. & Mitchell, R.A.C. (2017). Feruloylation and structure of arabinoxylan in wheat endosperm cell walls from RNAi lines with suppression of genes responsible for backbone synthesis and decoration. *Plant Biotechnology Journal*, 15 (11), 1429–1438. https://doi.org/10.1111/pbi.12727

Gebruers, K., Dornez, E., Boros, D., Dynkowska, W., Bedő, Z., Rakszegi, M., Delcour, J.A. & Courtin, C.M. (2008). Variation in the Content of Dietary Fiber and Components Thereof in Wheats in the HEALTHGRAIN Diversity Screen. *Journal of Agricultural and Food Chemistry*, 56 (21), 9740–9749. https://doi.org/10.1021/jf800975w

Glasser, W.G., Kaar, W.E., Jain, R.K. & Sealey, J.E. (2000). Isolation options for non-cellulosic heteropolysaccharides (HetPS). *Cellulose*, 7 (3), 299–317. https://doi.org/10.1023/A:1009277009836

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 (3), 327–347. https://doi.org/10.1016/j.jcs.2007.09.008

Holgate, J.H. & Webb, J. (2003). MICROSCOPY | Light Microscopy and Histochemical Methods. *Encyclopedia of Food Sciences and Nutrition*. Elsevier, 3917–3922. https://doi.org/10.1016/B0-12-227055-X/00778-1

Holik, A.S. (2001). Optical Microscopy. *Encyclopedia of Materials: Science and Technology*. Elsevier, 6458–6463. https://doi.org/10.1016/B0-08-043152-6/01142-6

Jerkovic, A., Kriegel, A.M., Bradner, J.R., Atwell, B.J., Roberts, T.H. & Willows, R.D. (2010). Strategic Distribution of Protective Proteins within Bran Layers of Wheat Protects the Nutrient-Rich Endosperm. *Plant Physiology*, 152 (3), 1459–1470. https://doi.org/10.1104/pp.109.149864

Kamal-Eldin, A., Lærke, H.N., Knudsen, K.-E.B., Lampi, A.-M., Piironen, V., Adlercreutz, H., Katina, K., Poutanen, K. & Åman, P. (2009). Physical, microscopic and chemical characterisation of industrial rye and wheat brans from the Nordic countries. *Food & Nutrition Research*, 53 (1), 1912. https://doi.org/10.3402/fnr.v53i0.1912

Kumari, B., Tiwari, B.K., Hossain, M.B., Brunton, N.P. & Rai, D.K. (2018). Recent Advances on Application of Ultrasound and Pulsed Electric Field Technologies in the Extraction of Bioactives from Agro-Industrial By-products. *Food and Bioprocess Technology*, 11 (2), 223–241. https://doi.org/10.1007/s11947-017-1961-9

Lu, Z.X., Walker, K.Z., Muir, J.G., Mascara, T. & O'Dea, K. (2000). Arabinoxylan fiber, a byproduct of wheat flour processing, reduces the postprandial glucose response in normoglycemic subjects. *The American Journal of Clinical Nutrition*, 71 (5), 1123–1128. https://doi.org/10.1093/ajcn/71.5.1123

Lu, Z.X., Walker, K.Z., Muir, J.G. & O'Dea, K. (2004). Arabinoxylan fibre improves metabolic control in people with Type II diabetes. *European Journal of Clinical Nutrition*, 58 (4), 621–628. https://doi.org/10.1038/sj.ejcn.1601857

Maningat, C.C., Seib, P.A., Bassi, S.D., Woo, K.S. & Lasater, G.D. (2009). Wheat Starch. *Starch*. Elsevier, 441–510. https://doi.org/10.1016/B978-0-12-746275-2.00010-0

Mahnič-Kalamiza, S., Vorobiev, E. & Miklavčič, D. (2014). Electroporation in Food Processing and Biorefinery. *The Journal of Membrane Biology*, 247 (12), 1279–1304. https://doi.org/10.1007/s00232-014-9737-x

Nishimura, H., Kamiya, A., Nagata, T., Katahira, M. & Watanabe, T. (2018). Direct evidence for α ether linkage between lignin and carbohydrates in wood cell walls. *Scientific Reports*, 8 (1), 6538. https://doi.org/10.1038/s41598-018-24328-9

de O. Buanafina, M.M. (2009). Feruloylation in Grasses: Current and Future Perspectives. *Molecular Plant*, 2 (5), 861–872. https://doi.org/10.1093/mp/ssp067

Oliveira, E.E., Silva, A.E., Júnior, T.N., Gomes, M.C.S., Aguiar, L.M., Marcelino, H.R., Araújo, I.B., Bayer, M.P., Ricardo, N.M.P.S. & Oliveira,

A.G. (2010). Xylan from corn cobs, a promising polymer for drug delivery: Production and characterization. *Bioresource Technology*, 101 (14), 5402–5406. https://doi.org/10.1016/j.biortech.2010.01.137

Peyron, S., Chaurand, M., Rouau, X. & Abecassis, J. (2002). Relationship between Bran Mechanical Properties and Milling Behaviour of Durum Wheat (Triticum durum Desf.). Influence of Tissue Thickness and Cell Wall Structure. *Journal of Cereal Science*, 36 (3), 377–386. https://doi.org/10.1006/jcrs.2002.0473

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 (2), 211–221. https://doi.org/10.1016/j.lwt.2013.12.004

Putri, R.I., Syamsiana, I.N. & Hawa, L.C. (2010). Design of High Voltage Pulse Generator for Pasteurization by Pulse Electric Field (PEF). *International Journal of Computer and Electrical Engineering*, 916–923. https://doi.org/10.7763/IJCEE.2010.V2.252

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 (1), 5–10. https://doi.org/10.1002/jsfa.2740360103

Rohrer, J.S., Basumallick, L. & Hurum, D. (2013). High-performance anion-exchange chromatography with pulsed amperometric detection for carbohydrate analysis of glycoproteins. *Biochemistry (Moscow)*, 78 (7), 697–709. https://doi.org/10.1134/S000629791307002X

Rudjito, R.C., Ruthes, A.C., Jiménez-Quero, A. & Vilaplana, F. (2019). Feruloylated Arabinoxylans from Wheat Bran: Optimization of Extraction Process and Validation at Pilot Scale. *ACS Sustainable Chemistry & Engineering*, 7 (15), 13167–13177. https://doi.org/10.1021/acssuschemeng.9b02329

Ruthes, A.C., Martínez-Abad, A., Tan, H.-T., Bulone, V. & Vilaplana, F. (2017). Sequential fractionation of feruloylated hemicelluloses and oligosaccharides from wheat bran using subcritical water and xylanolytic enzymes. *Green Chemistry*, 19 (8), 1919–1931. https://doi.org/10.1039/C6GC03473J

Seung, D. (2020). Amylose in starch: towards an understanding of biosynthesis, structure and function. *New Phytologist*, 228 (5), 1490–1504. https://doi.org/10.1111/nph.16858

Shewry, P.R., Hawkesford, M.J., Piironen, V., Lampi, A.-M., Gebruers, K., Boros, D., Andersson, A.A.M., Åman, P., Rakszegi, M., Bedo, Z. & Ward, J.L. (2013). Natural Variation in Grain Composition of Wheat and Related Cereals. *Journal of Agricultural and Food Chemistry*, 61 (35), 8295–8303. https://doi.org/10.1021/jf3054092

Shewry, P.R., Saulnier, L., Gebruers, K., Mitchell, R.A.C., Freeman, J., Nemeth, C. & Ward, J.L. (2014). Optimising the Content and Composition of Dietary Fibre in Wheat Grain for End-use Quality. I: Tuberosa, R.,

Graner, A., & Frison, E. (red.) *Genomics of Plant Genetic Resources*. Dordrecht: Springer Netherlands, 455–466. https://doi.org/10.1007/978-94-007-7575-6_19

Toepfl, S., Heinz, V., & Knorr, D. (2005). Overview of pulsed electric field processing of foods. In D. -W. Sun (Ed.), Emerging technologies for food processing. Oxford, UK:Elsevier.

Wang, Y. & Wang, J. (2020). Modelling and prediction of global non-communicable diseases. *BMC Public Health*, 20 (1), 822. https://doi.org/10.1186/s12889-020-08890-4

Weitzhandler, M., Rohrer, J., Thayer, J.R. & Avdalovic, N. (1998). HPAEC-PAD Analysis of Monosaccharides Released by Exoglycosidase Digestion Using the CarboPac MA1 Column. *Glycoanalysis Protocols*. New Jersey: Humana Press, 71–78. https://doi.org/10.1385/0-89603-355-4:71

WHO (2020). Non-communicable diseases progress monitor 2020. Geneva: World Health Organization. Licence: CC BY-NC-SA 3.0 IGO.

Yilmaz-Turan, S., Jiménez-Quero, A., Moriana, R., Arte, E., Katina, K. & Vilaplana, F. (2020). Cascade extraction of proteins and feruloylated arabinoxylans from wheat bran. *Food Chemistry*, 333, 127491. https://doi.org/10.1016/j.foodchem.2020.127491

Zhang, L., van Boven, A., Mulder, J., Grandia, J., Chen, X.D., Boom, R.M. & Schutyser, M.A.I. (2019). Arabinoxylans-enriched fractions: From dry fractionation of wheat bran to the investigation on bread baking performance. Journal of Cereal Science, 87, 1–8. https://doi.org/10.1016/j.jcs.2019.02.005

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Appendix 1 Raw data from PEF treatment

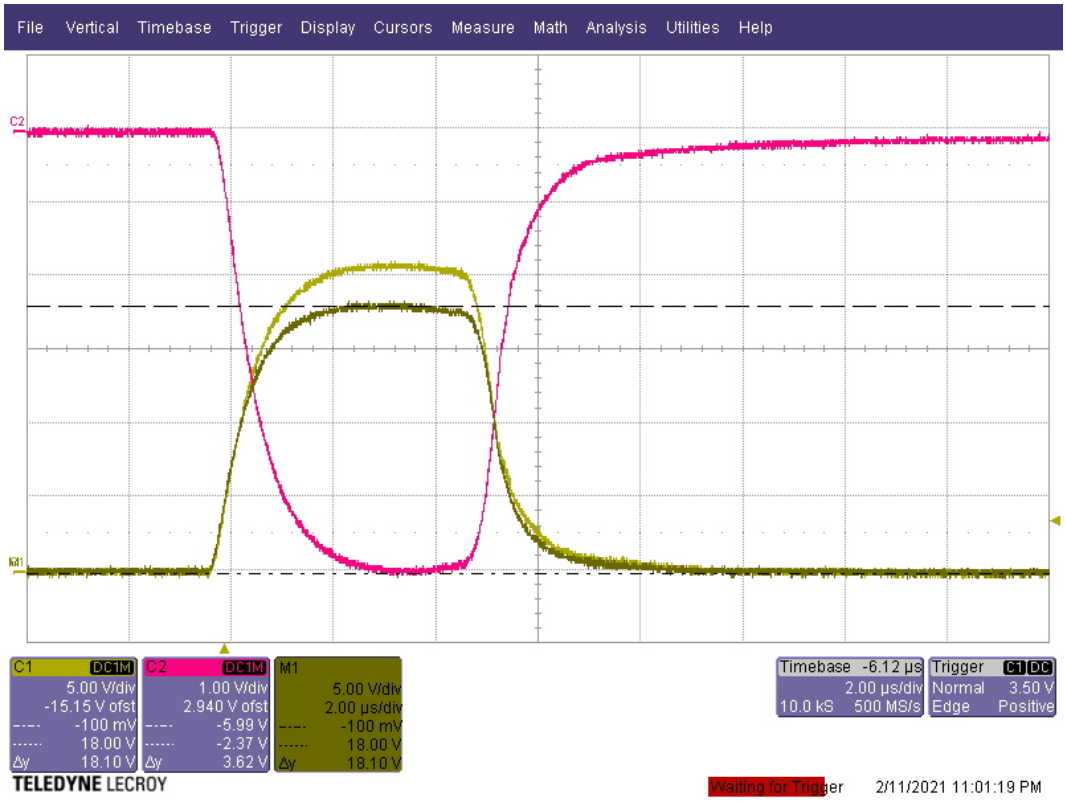

Figure 1: Output from PEF treatment with 100 pulses

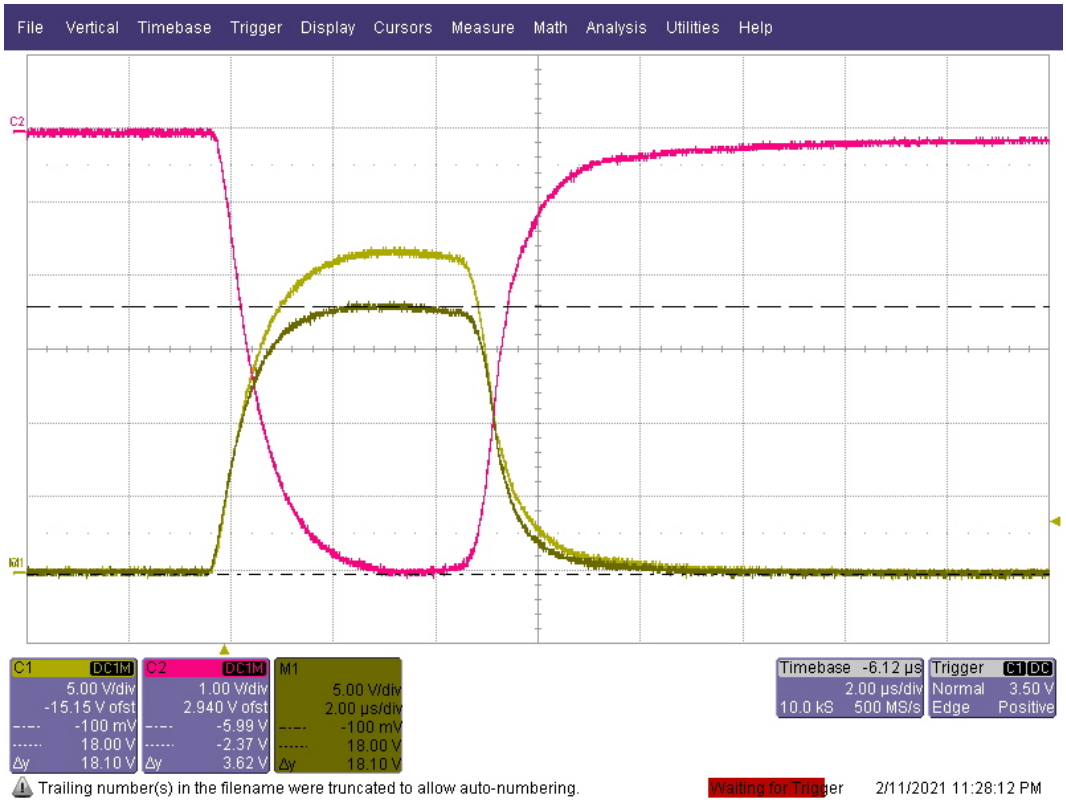

Figure 2: Output from PEF treatment with 250 pulses

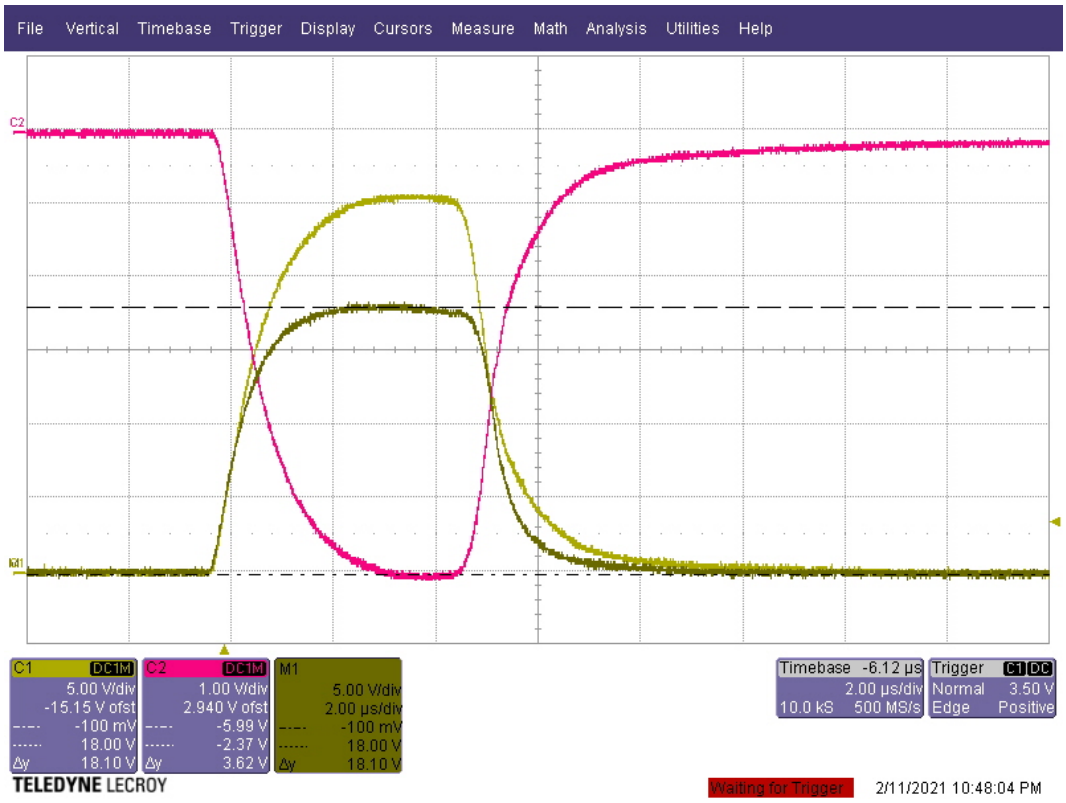

Figure 3: Output from PEF treatment with 500 pulses

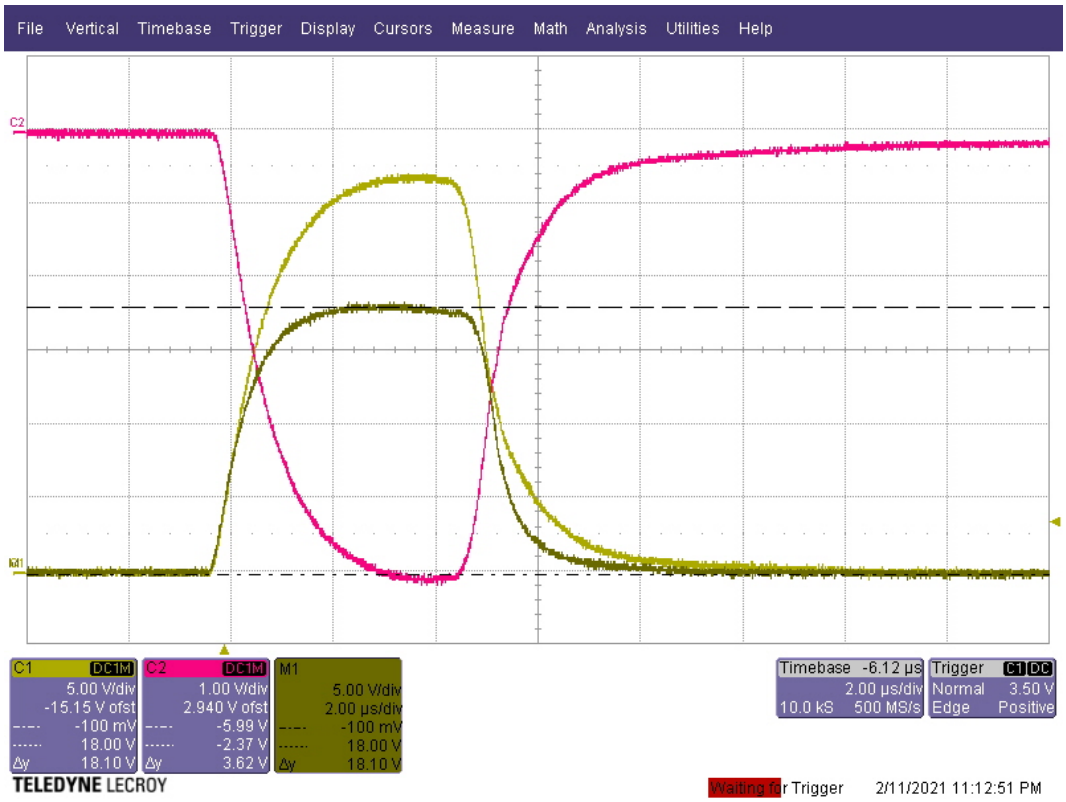

Figure 4: Output from PEF treatment with 750 pulses

Appendix II Raw data from statistical analysis

Table 1. Monosaccharide analysis

```
monosugars2 <- read_xlsx("SUGARS2.xlsx")
monosugars2.anova <- lm(Xyl ~ Treatment, data=monosugars2)
summary(monosugars2.anova)

##
## Call:
## lm(formula = Xyl ~ Treatment, data = monosugars2)
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.025820 -0.007456 -0.002087  0.005983  0.034651
##
## Coefficients:
```

```
##                Estimate Std. Error t value Pr(>|t|)
## (Intercept)    0.483253   0.005859  82.475  < 2e-16 ***
## TreatmentT100  0.032042   0.008286   3.867 0.000661 ***
## TreatmentT1000 0.024660   0.008286   2.976 0.006239 **
## TreatmentT250  0.023856   0.009265   2.575 0.016065 *
## TreatmentT500  0.039573   0.008286   4.776 6.08e-05 ***
## TreatmentT750  0.023532   0.009265   2.540 0.017403 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.01435 on 26 degrees of freedom
## Multiple R-squared:  0.4975, Adjusted R-squared:  0.4009
## F-statistic: 5.148 on 5 and 26 DF,  p-value: 0.002055

anova(monosugars2.anova)

## Analysis of Variance Table
##
## Response: Xyl
##           Df    Sum Sq    Mean Sq F value   Pr(>F)
## Treatment  5 0.0053027 0.00106054  5.1484 0.002055 **
## Residuals 26 0.0053559 0.00020599
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

plot(resid(monosugars2.anova) ~ fitted(monosugars2.anova))
```

```
monosugars2.anova <- lm(Ara ~ Treatment, data=monosugars2)
summary(monosugars2.anova)

##
## Call:
## lm(formula = Ara ~ Treatment, data = monosugars2)
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.038103 -0.010848  0.001207  0.008743  0.033518
##
## Coefficients:
##                 Estimate Std. Error t value Pr(>|t|)
## (Intercept)     0.489687   0.007084  69.129  < 2e-16 ***
## TreatmentT100  -0.036962   0.010018  -3.690  0.00104 **
## TreatmentT1000 -0.027960   0.010018  -2.791  0.00971 **
## TreatmentT250  -0.023020   0.011200  -2.055  0.05002 .
## TreatmentT500  -0.052503   0.010018  -5.241 1.78e-05 ***
## TreatmentT750  -0.028619   0.011200  -2.555  0.01681 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.01735 on 26 degrees of freedom
```

```
## Multiple R-squared:  0.5315, Adjusted R-squared:  0.4415
## F-statistic:   5.9 on 5 and 26 DF,  p-value: 9e-04

anova(monosugars2.anova)

## Analysis of Variance Table
##
## Response: Ara
##           Df    Sum Sq    Mean Sq F value Pr(>F)
## Treatment  5 0.0088819 0.00177638  5.9002  9e-04 ***
## Residuals 26 0.0078279 0.00030107
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

plot(resid(monosugars2.anova) ~ fitted(monosugars2.anova))
```


```
## Multiple R-squared:  0.5315, Adjusted R-squared:  0.4415
## F-statistic:   5.9 on 5 and 26 DF,  p-value: 9e-04

anova(monosugars2.anova)

## Analysis of Variance Table
##
## Response: Ara
##           Df    Sum Sq    Mean Sq F value Pr(>F)
## Treatment  5 0.0088819 0.00177638  5.9002  9e-04 ***
## Residuals 26 0.0078279 0.00030107
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

plot(resid(monosugars2.anova) ~ fitted(monosugars2.anova))
```

```
monosugars2.anova <- lm(Gal ~ Treatment, data=monosugars2)
summary(monosugars2.anova)

##
## Call:
## lm(formula = Gal ~ Treatment, data = monosugars2)
##
## Residuals:
##        Min         1Q     Median         3Q        Max
## -0.0181270 -0.0006031  0.0000137  0.0018545  0.0055250
##
## Coefficients:
##                Estimate Std. Error t value Pr(>|t|)
## (Intercept)    0.018127   0.001693  10.704 5.04e-11 ***
## TreatmentT100  0.003093   0.002395   1.291    0.208
## TreatmentT1000 0.001930   0.002395   0.806    0.428
## TreatmentT250  0.001265   0.002678   0.473    0.640
## TreatmentT500  0.003763   0.002395   1.571    0.128
## TreatmentT750  0.002536   0.002678   0.947    0.352
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.004148 on 26 degrees of freedom
```


```
monosugars2.anova <- lm(Gal ~ Treatment, data=monosugars2)
summary(monosugars2.anova)

##
## Call:
## lm(formula = Gal ~ Treatment, data = monosugars2)
##
## Residuals:
##        Min         1Q     Median         3Q        Max
## -0.0181270 -0.0006031  0.0000137  0.0018545  0.0055250
##
## Coefficients:
##                Estimate Std. Error t value Pr(>|t|)
## (Intercept)    0.018127   0.001693  10.704 5.04e-11 ***
## TreatmentT100  0.003093   0.002395   1.291    0.208
## TreatmentT1000 0.001930   0.002395   0.806    0.428
## TreatmentT250  0.001265   0.002678   0.473    0.640
## TreatmentT500  0.003763   0.002395   1.571    0.128
## TreatmentT750  0.002536   0.002678   0.947    0.352
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.004148 on 26 degrees of freedom
```


```
## Multiple R-squared:  0.1053, Adjusted R-squared:  -0.06673
## F-statistic: 0.6121 on 5 and 26 DF,  p-value: 0.6914

anova(monosugars2.anova)

## Analysis of Variance Table
##
## Response: Gal
##           Df     Sum Sq    Mean Sq F value Pr(>F)
## Treatment  5 0.00005266 1.0532e-05  0.6121 0.6914
## Residuals 26 0.00044736 1.7206e-05

plot(resid(monosugars2.anova) ~ fitted(monosugars2.anova))
```

```
## Multiple R-squared:  0.1053, Adjusted R-squared:  -0.06673
## F-statistic: 0.6121 on 5 and 26 DF,  p-value: 0.6914

anova(monosugars2.anova)

## Analysis of Variance Table
##
## Response: Gal
##           Df     Sum Sq    Mean Sq F value Pr(>F)
## Treatment  5 0.00005266 1.0532e-05  0.6121 0.6914
## Residuals 26 0.00044736 1.7206e-05

plot(resid(monosugars2.anova) ~ fitted(monosugars2.anova))
```

```
monosugars2.anova <- lm(Glu ~ Treatment, data=monosugars2)
summary(monosugars2.anova)

##
## Call:
## lm(formula = Glu ~ Treatment, data = monosugars2)
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.008933 -0.002311 -0.001158  0.004075  0.007741
##
## Coefficients:
##                 Estimate Std. Error t value Pr(>|t|)
## (Intercept)     0.008933   0.001852   4.825 5.34e-05 ***
## TreatmentT100   0.001827   0.002618   0.698  0.49145
## TreatmentT1000  0.001371   0.002618   0.523  0.60511
## TreatmentT250  -0.002101   0.002928  -0.718  0.47929
## TreatmentT500   0.009167   0.002618   3.501  0.00169 **
## TreatmentT750   0.002552   0.002928   0.872  0.39140
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.004535 on 26 degrees of freedom
```

```
## Multiple R-squared:  0.4272, Adjusted R-squared:  0.317
## F-statistic: 3.878 on 5 and 26 DF,  p-value: 0.009286

anova(monosugars2.anova)

## Analysis of Variance Table
##
## Response: Glu
##           Df     Sum Sq    Mean Sq F value   Pr(>F)
## Treatment  5 0.00039879 7.9759e-05  3.8777 0.009286 **
## Residuals 26 0.00053478 2.0569e-05
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

plot(resid(monosugars2.anova) ~ fitted(monosugars2.anova))
```

```r
monosugars2.anova <- lm(Man ~ Treatment, data=monosugars2)
summary(monosugars2.anova)

##
## Call:
## lm(formula = Man ~ Treatment, data = monosugars2)
##
## Residuals:
##    Min     1Q Median     3Q    Max
##      0      0      0      0      0
##
## Coefficients:
##                 Estimate Std. Error t value Pr(>|t|)
## (Intercept)            0          0      NA       NA
## TreatmentT100          0          0      NA       NA
## TreatmentT1000         0          0      NA       NA
## TreatmentT250          0          0      NA       NA
## TreatmentT500          0          0      NA       NA
## TreatmentT750          0          0      NA       NA
##
## Residual standard error: 0 on 26 degrees of freedom
## Multiple R-squared:    NaN,  Adjusted R-squared:    NaN
## F-statistic:   NaN on 5 and 26 DF,  p-value: NA
```

```r
anova(monosugars2.anova)

## Analysis of Variance Table
##
## Response: Man
##           Df Sum Sq Mean Sq F value Pr(>F)
## Treatment  5      0       0
## Residuals 26      0       0

plot(resid(monosugars2.anova) ~ fitted(monosugars2.anova))
```

```r
monosugars2.anova <- lm(AX ~ Treatment, data=monosugars2)
summary(monosugars2.anova)

##
## Call:
## lm(formula = AX ~ Treatment, data = monosugars2)
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.124599 -0.027743  0.008649  0.025072  0.106606
##
## Coefficients:
##                Estimate Std. Error t value Pr(>|t|)
## (Intercept)     1.01382    0.02270  44.652  < 2e-16 ***
## TreatmentT100  -0.13418    0.03211  -4.179 0.000293 ***
## TreatmentT1000 -0.10428    0.03211  -3.248 0.003200 **
## TreatmentT250  -0.09354    0.03590  -2.606 0.014976 *
## TreatmentT500  -0.17335    0.03211  -5.399 1.18e-05 ***
## TreatmentT750  -0.10385    0.03590  -2.893 0.007625 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.05562 on 26 degrees of freedom
```

```
## Multiple R-squared:  0.5531, Adjusted R-squared:  0.4671
## F-statistic: 6.434 on 5 and 26 DF,  p-value: 0.0005145

anova(monosugars2.anova)

## Analysis of Variance Table
##
## Response: AX
##           Df   Sum Sq   Mean Sq F value    Pr(>F)
## Treatment  5 0.099512 0.0199024  6.4345 0.0005145 ***
## Residuals 26 0.080420 0.0030931
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

plot(resid(monosugars2.anova) ~ fitted(monosugars2.anova))
```

```
monosugars2.anova <- lm(Tot ~ Treatment, data=monosugars2)
summary(monosugars2.anova)

##
## Call:
## lm(formula = Tot ~ Treatment, data = monosugars2)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -54.938 -20.616  -7.107  15.622  75.511
##
## Coefficients:
##                Estimate Std. Error t value Pr(>|t|)
## (Intercept)     601.622     13.311  45.199  < 2e-16 ***
## TreatmentT100   -13.575     18.824  -0.721 0.477238
## TreatmentT1000   78.172     18.824   4.153 0.000314 ***
## TreatmentT250    28.131     21.046   1.337 0.192916
## TreatmentT500    -6.222     18.824  -0.331 0.743657
## TreatmentT750    14.979     21.046   0.712 0.482972
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 32.6 on 26 degrees of freedom
```

```
## Multiple R-squared:  0.5481, Adjusted R-squared:  0.4612
## F-statistic: 6.308 on 5 and 26 DF,  p-value: 0.0005861

anova(monosugars2.anova)

## Analysis of Variance Table
##
## Response: Tot
##           Df Sum Sq Mean Sq F value    Pr(>F)
## Treatment  5  33529  6705.7  6.3081 0.0005861 ***
## Residuals 26  27639  1063.0
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

plot(resid(monosugars2.anova) ~ fitted(monosugars2.anova))
```

Appendix III Raw data from statistical analysis - Yield

```
yield <- read_xlsx("Yield3.xlsx")
yield.anova <- lm(PEF ~ treatment, data=yield)
summary(yield.anova)

##
## Call:
## lm(formula = PEF ~ treatment, data = yield)
##
## Residuals:
##        Min         1Q     Median         3Q        Max
## -0.0090782 -0.0031509  0.0000921  0.0035344  0.0073546
##
## Coefficients:
##                Estimate Std. Error t value Pr(>|t|)
## (Intercept)    0.148904   0.003558  41.848 1.46e-12 ***
## treatmentT100  0.006310   0.005032   1.254    0.238
## treatmentT1000 0.008904   0.005032   1.769    0.107
## treatmentT250  0.004597   0.005626   0.817    0.433
## treatmentT500  0.005800   0.005032   1.153    0.276
## treatmentT750  0.009220   0.005626   1.639    0.132
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.006163 on 10 degrees of freedom
## Multiple R-squared:  0.2919, Adjusted R-squared:  -0.06208
## F-statistic: 0.8246 on 5 and 10 DF,  p-value: 0.5597

anova(yield.anova)

## Analysis of Variance Table
##
## Response: PEF
##           Df     Sum Sq    Mean Sq F value Pr(>F)
## treatment  5 0.00015661 3.1321e-05  0.8246 0.5597
## Residuals 10 0.00037982 3.7982e-05

yield.anova <- lm(DS ~ treatment, data=yield)
summary(yield.anova)
```

```
##
## Call:
## lm(formula = DS ~ treatment, data = yield)
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.020076 -0.003217  0.000205  0.002507  0.013480
##
## Coefficients:
##                 Estimate Std. Error t value Pr(>|t|)
## (Intercept)     0.225380   0.005624  40.077 2.24e-12 ***
## treatmentT100  -0.006737   0.007953  -0.847    0.417
## treatmentT1000  0.000714   0.007953   0.090    0.930
## treatmentT250   0.010234   0.008892   1.151    0.277
## treatmentT500  -0.002919   0.007953  -0.367    0.721
## treatmentT750   0.005538   0.008892   0.623    0.547
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.00974 on 10 degrees of freedom
## Multiple R-squared:  0.3132, Adjusted R-squared:  -0.0302
## F-statistic: 0.912 on 5 and 10 DF,  p-value: 0.5106
```

```
anova(yield.anova)
```

```
## Analysis of Variance Table
##
## Response: DS
##           Df     Sum Sq    Mean Sq F value Pr(>F)
## treatment  5 0.00043265 8.6531e-05   0.912 0.5106
## Residuals 10 0.00094876 9.4876e-05
```

```
yield.anova <- lm(DL ~ treatment, data=yield)
summary(yield.anova)
```

```
##
## Call:
## lm(formula = DL ~ treatment, data = yield)
##
## Residuals:
##        Min         1Q     Median         3Q        Max
## -0.0200131 -0.0063966 -0.0004149  0.0042844  0.0278586
##
## Coefficients:
##                 Estimate Std. Error t value Pr(>|t|)
## (Intercept)     0.031732   0.008346   3.802  0.00347 **
## treatmentT100   0.006522   0.011803   0.553  0.59267
## treatmentT1000 -0.016693   0.011803  -1.414  0.18763
## treatmentT250  -0.012009   0.013196  -0.910  0.38421
## treatmentT500  -0.007683   0.011803  -0.651  0.52974
## treatmentT750  -0.013392   0.013196  -1.015  0.33408
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
##
## Residual standard error: 0.01446 on 10 degrees of freedom
## Multiple R-squared:  0.3463, Adjusted R-squared:  0.01949
## F-statistic:  1.06 on 5 and 10 DF,  p-value: 0.4364

anova(yield.anova)

## Analysis of Variance Table
##
## Response: DL
##           Df    Sum Sq    Mean Sq F value Pr(>F)
## treatment  5 0.0011071 0.00022142  1.0596 0.4364
## Residuals 10 0.0020895 0.00020895

yield.anova <- lm(AXR ~ treatment, data=yield)
summary(yield.anova)

##
## Call:
## lm(formula = AXR ~ treatment, data = yield)
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.008687 -0.003231  0.000000  0.003455  0.008055
##
## Coefficients:
##                 Estimate Std. Error t value Pr(>|t|)
## (Intercept)     0.034513   0.003523   9.797 1.92e-06 ***
## treatmentT100   0.001812   0.004982   0.364   0.7237
## treatmentT1000  0.003008   0.004982   0.604   0.5594
## treatmentT250  -0.000534   0.005570  -0.096   0.9255
## treatmentT500   0.009347   0.004982   1.876   0.0901 .
## treatmentT750   0.006664   0.005570   1.196   0.2592
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.006102 on 10 degrees of freedom
## Multiple R-squared:  0.3506, Adjusted R-squared:  0.02594
## F-statistic:  1.08 on 5 and 10 DF,  p-value: 0.4271

anova(yield.anova)

## Analysis of Variance Table
##
## Response: AXR
##           Df     Sum Sq    Mean Sq F value Pr(>F)
## treatment  5 0.00020104 4.0207e-05  1.0799 0.4271
## Residuals 10 0.00037233 3.7233e-05

plot(resid(yield.anova) ~ fitted(yield.anova))

qqnorm(residuals(yield.anova))
qqnorm(residuals(yield.anova))
```

```r
yield.anova <- lm(AXE ~ treatment, data=yield)
summary(yield.anova)

##
## Call:
## lm(formula = AXE ~ treatment, data = yield)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.04932 -0.01914  0.00000  0.02069  0.04583
##
## Coefficients:
##                 Estimate Std. Error t value Pr(>|t|)
## (Intercept)     0.246718   0.018783  13.135 1.24e-07 ***
## treatmentT100  -0.015785   0.026564  -0.594    0.566
## treatmentT1000  0.004538   0.026564   0.171    0.868
## treatmentT250   0.028843   0.029699   0.971    0.354
## treatmentT500  -0.005051   0.026564  -0.190    0.853
## treatmentT750   0.026333   0.029699   0.887    0.396
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.03253 on 10 degrees of freedom
## Multiple R-squared:  0.259,  Adjusted R-squared:  -0.1114
## F-statistic: 0.6992 on 5 and 10 DF,  p-value: 0.6365

anova(yield.anova)

## Analysis of Variance Table
##
## Response: AXE
##           Df    Sum Sq    Mean Sq F value Pr(>F)
## treatment  5 0.0037005 0.00074009  0.6992 0.6365
## Residuals 10 0.0105844 0.00105844

plot(resid(yield.anova) ~ fitted(yield.anova))

qqnorm(residuals(yield.anova))
qqnorm(residuals(yield.anova))
```

Appendix IV Percentage lost after each step of the extraction process

Table 1: Percentage lost after each step of the extraction process (dwb of initial sample)

	% lost after PEF		% lost after destarching		% lost after delignification		% lost after alkaline extraction		
	AV	STDV	AV	STDV	AV	STDV	AV	STDV	TOTAL
C	14,9	0,00681946	22,5	0,0015394	3,5	0,01405052	3,4	0,00792689	44,4
100	15,5	0,00664848	21,9	0,05654131	3,8	0,06105732	3,3	0,00743845	44,5
250	15,2	0,00244086	23,6	0,00132993	2,0	0,00499956	3,4	0,00426447	44,1
500	15,5	0,00524496	22,2	0,03498673	2,4	0,00758112	3,6	0,02100736	43,7
750	15,4	0,00690989	22,9	0,01837767	1,8	0,00266023	3,6	0,01335193	43,7
1000	15,8	0,00802175	22,4	0,01266694	2,5	0,04420584	3,8	0,04181497	44,4

Popular scientific summary

In the interface of climate crisis and a transpiring pandemic, the need of developing sustainable agri-food systems has never been more important. A threatened climate threatens global health and food security by elevating the frequency of infectious outbreaks. Developing a sustainable, resilient food system for improved production, nutrition and environment is crucial to mitigate climate change and future pandemics. One way of addressing these challenges is by utilizing by-products and waste from the food production.

Wheat production generated around 100 million tons underutilized by-products 2020/2021. Wheat bran (WB) is a fraction of the wheat kernel produced during wheat flour production which is primarily used as animal feed. The high nutritional and functional value of wheat bran makes it a by-product with potential for innovation. The main dietary fiber in wheat bran, arabinoxylan can reduce cholesterol levels, improve gut health and improve long-term glycemic control. Hence, it can be used to create novel foods with health promoting properties which can reduce diet related diseases such cardio-vascular diseases, diabetes, cancer and respiratory diseases.

Wheat bran is the outer parts of the wheat kernel and is a complex matrix of different tissues. The out coming structure is resistant towards many of the normally used mechanical, chemical and thermal treatments used in food processing. Pulse electric field (PEF) is an emerging food processing technique with great innovative potential. PEF acts by a mechanism called electroporation. Small pores are formed in cells by applying high voltage pulses. This can be utilized to increase extraction of valuable compounds from cells, to change the structure and mechanical behavior of cellular tissues or to kill microorganisms. PEF allows short time, energy efficient, waste-free food processing which subsequently allows development of cost-effective sustainable processing concepts in the food industry but also the pharmaceutical and biotechnology industry.

This study was a master thesis project conducted at SLU, Uppsala in collaboration with Lantmännen. Milled wheat bran (kruskakli) was subjected to PEF treatments with varying degrees of intensity. Intensities were varied with number of pulses applied (100, 250, 500, 750, 1000). Arabinoxylans were extracted from PEF treated wheat bran using NaOH a.k.a sodium hydroxide. Wheat bran was pre-treated prior to the extraction to increase the extraction yield and purity of the final extracts.

PEF changed the chemical composition of the extracts and increased the purity. The highest purity was obtained with the highest intensity (1000 pulses). Interestingly had the arabinoxylans in the extracts different structures depending on the treatment intensity. The structure determines the nutritional and functional properties of arabinoxylan which makes this an important observation for future applications.