Ultrasound pre-treatment of wheat bran to improve arabinoxylan extraction

Ultraljudbehandling av kruskakli för att förbättra extraktionen av arabinoxylan

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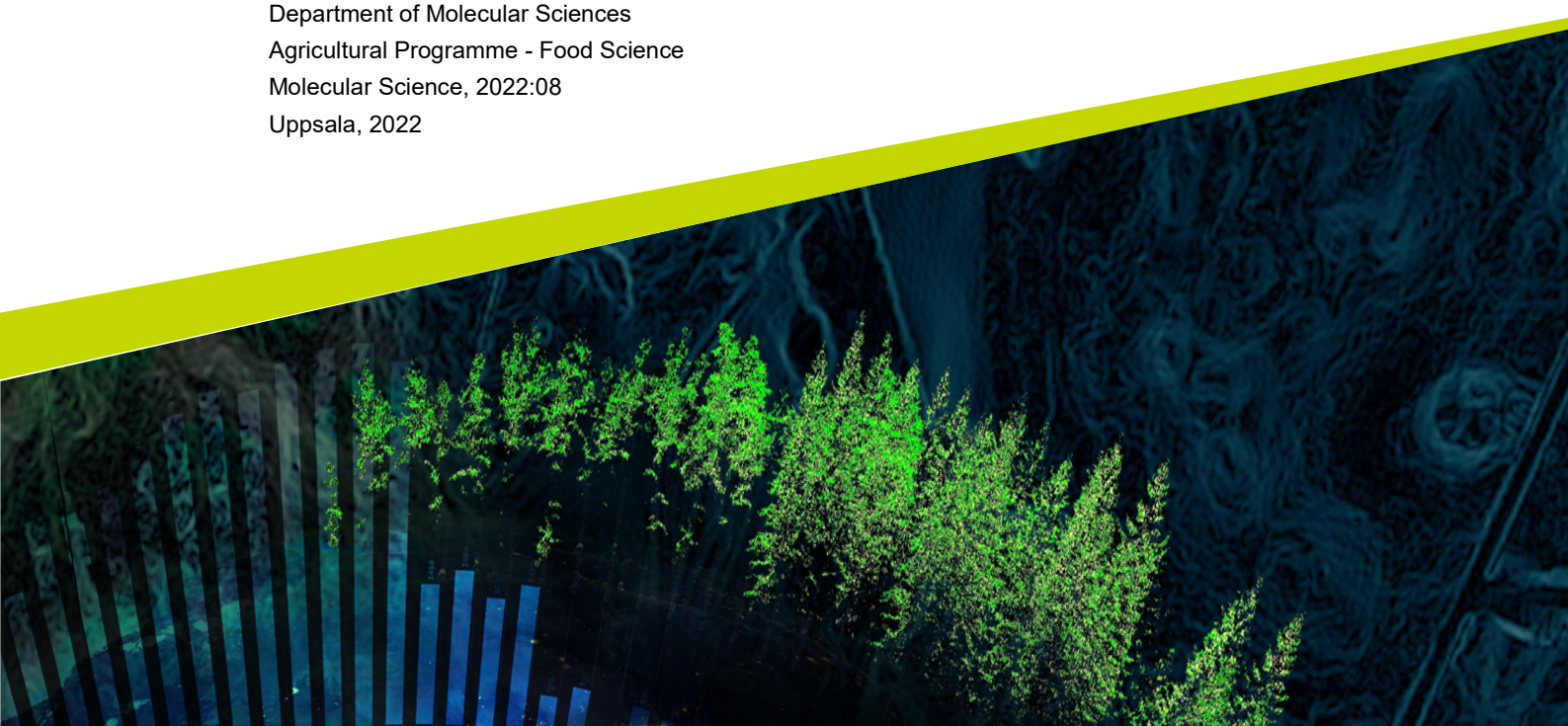

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Ultraljudsbehandling av vetekli for att förbättra extraktionen av arabinoxylan

Louise Lundquist

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Abstract

With increasing amounts of food waste and diet related diseases, the need to utilize our by-products of food for its health benefits and to limit the food waste has never been more prevalent. Wheat bran is a by-product from the wheat flour industry and contains a lot of dietary fibres which has a beneficial effect on human health. One of these dietary fibres is arabinoxylan (AX).

The aim of this thesis is to evaluate the effect ultrasonic pre-treatment has on arabinoxylan alkaline extraction from wheat bran. In parallel also evaluate the alkaline extraction process. Ultrasound can be applied in multiple ways with different power inputs, frequencies, and time durations. In this thesis, the studied parameter was time with 0.5-, 1-, 5-, and 10-minute-long treatments at 400 W power input. With the hypothesis that a longer treatment will give higher yields.

The extraction process, including the destarching, delignification and the alkaline extraction of the wheat bran, worked successfully. The destarching removed high amounts of starch while the delignification could have been more efficient. The alkaline extraction isolated both water extractable AX and water unextractable AX, which was determined by the high arabinose to xylose (A/X) ratio. Ultrasound showed potential to increase the total solid yield and total carbohydrate content, but was not statistically significant. Ultrasound did not seem to affect the monosaccharide composition or the A/X ratio to any greater extent.

Keywords: Ultrasound, arabinoxylan, wheat bran, alkaline extraction

Sammanfattning

Det blir allt mer vanligt med hälsoproblem kopplade till fettma och dibetes och mängden matsvinn ökar i världen. För att både minska på matsvinnet och öka folkhälsan kan vi använda oss utav bi-produkter som annars hade gått till spillo för deras hälsosamma egenskaper. Kruskakli är en biprodukt från vetemjölsproduktionen och innehåller stora mängder kostfibrer, som har en positiv inverkan på människors hälsa. En av dessa kostfibrer är arabinoxyan (AX).

Syftet med den här uppsatsen är att undersöka effekterna ultraljudsförbehandling har på en alkalisk extraktion av arabinoxylan från kruskakli. Parallellt evaluerades också den alkaliska extraktionen. Ultraljud kan appliceras på flera sätt med olika styrkor, frekvenser och tidsperioder. De studerade parametrarna var 0.5, 1, 5 och 10 minuters behandling med 400 W styrka, med hypotesen att en längre ultraljudsbehandlig ger en högre extraktionsavkastning.

Extraktionsprocessen, inklusive en stärkelseextraktion, delignifiering och alkalisk extraktion av AX var framgångsrikt. Stärkelseextraktionen utvann mycket av stärkelsen från kruskakliet medan delignifieringen kunde varit mer effektiv. Den alkaliska extraktionen extraherade både lösliga och olösliga AX, vilket man kunde uttyda i det höga arabinos till xylos (A/X) förhållandet. Ultraljudsförbehandlingen visade potential att öka extraktionsavkastningen och den totala kolhydrats mängden, dock så backades detta inte upp av den statistiska analysen. Ultraljud påverkade inte det kemiska förhållandet av sockerarter eller A/X förhållandet något märkbart.

Nyckelord: Ultraljud, arabinoxylan, kruskakli, alkalisk extraktion

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Abbreviations

To make it easier for the reader, you can make a list with common abbreviations in alphabetical order. Here you have a table you can use to make your list.
See example below:

AX	Arabinoxylan
DLS	Delignified supernatant
DSS	Destarched supernatant
DW	Dry weight
dwb	Dry weight basis
LDL	Low density lipoprotein
NCD	Non-communicable disease
US	Ultrasound
USS	Ultrasonicated supernatant
WB	Wheat bran

1. Introduction

In 2021, on July 29th, we surpassed the global resources our planet can sustain per year. That means that for the rest of the year we consume resources the planet cannot regenerate (Leighton 2021). At the same time around 1/3 of the food produced is wasted or lost and the world is divided in an overconsumption and underconsumption of food (Embrace Relief 2021; FAO n.d.). With all this in mind, we need a better way to utilize our agricultural resources and give value to by-products and food going to waste. Valorisation of by-products is one solution, among many needed, to a very complex problem to reduce some of the food loss and the global footprint we have on our planet (Strøm-Andersen 2020).

Parallel to overconsumption and underconsumption of food, non-communicable diseases (NCDs), have increased. NCDs include cardiovascular diseases, cancers, diabetes, and respiratory diseases all of which are chronic and non-transmittable. Most people affected live in low- and middle income countries and an unhealthy diet increases the risk as well as inactivity and alcohol consumption (WHO 2021). A more fibre rich diet has been proved to reduce the risk of getting NCDs as well as lower the mortality rate of cardiovascular diseases (Mayor 2019).

Wheat (*Triticum aestivum*) is one of the most produced crops over the world (*FAOSTAT* 2021) and wheat flour is the base of many staple foods around the world, including pasta, bread, pancakes, pizza and bakery goods such as cakes and biscuits (Arnarson 2019). White wheat flour is more commonly used than whole grain flour, leaving a substantial supply of wheat bran as a by-product from the milling process (Rizzello et al. 2017). Wheat bran as a by-product can be used as animal feed in Sweden (Jordbruksverket 2020), but in many countries it's burnt in open air (ElMekawy et al. 2013). Utilizing wheat bran both as a by-product and to include in healthy diets can be done by using whole grain flour or by adding value to the wheat bran by-product. Valorisation of wheat bran includes bioenergy (Levine 2003), animal feed (Jordbruksverket 2020) and enzyme production (Demir & Tarı 2014). More recent research include arabinoxylan extraction from wheat bran as a food additive or film forming agent (Bastos et al. 2018).

This thesis was done in collaboration with Lantmännen and performed at the Swedish University of Agricultural Sciences. **The aim** was to evaluate the effect ultrasonic pre-treatment has on arabinoxylan alkaline extraction from wheat bran and to evaluate the alkaline extraction process.

2. Background

2.1. Wheat

Wheat *(Triticum aestivum)* is one of the major crops cultivated around the world (*FAOSTAT* 2021), it was domesticated some 10000 years ago and has become a staple source of carbohydrates and calories for both humans and livestock (Dubcovsky & Dvorak 2007). The uses of wheat are versatile and about one fifth of the calories consumed by humans comes from wheat (Brenchley et al. 2012). The wheat kernel can be divided into three major parts: endosperm (80-85%), germ (3%), and bran (12-17%). The endosperm mainly consist of protein (gluten) and starch, the germ contains lipids, antioxidants, vitamins, minerals and enzymes while the bran is where the main part of the dietary fibres are located together with other proteins, enzymes, vitamins, minerals, and phenolic compounds (Onipe et al. 2015). Wheat has mainly been utilized because of its starchy endosperm producing wheat flour, where the wheat bran has been a by-product of the milling (Rizzello et al. 2017).

2.1.1. Wheat bran

When producing wheat flour, wheat bran is separated out by the milling process. About 14-19% of the total grain weight is bran, the amount will vary from different milling processes used by different mills. The wheat bran consists of an aleurone layer, testa and pericarp which encapsulates the endosperm and germ. The main dietary fibre found in wheat gran is arabinoxylan, minor dietary fibres include cellulose, lignin, fructan and β-glucan (Cui et al. 2013; Sibakov et al. 2013; Onipe et al. 2015). The wheat bran also constitutes of starch, proteins, minerals, vitamins and other organic compounds such as sterols, phytic acid, ferulic acid and phenolic acids (Onipe et al. 2015). About 95% of the dietary fibres in wheat bran is considered insoluble (Sibakov et al. 2013). With the increasing awareness of the beneficial role dietary fibres have to our health, wheat bran consumption has gradually increased in the form of whole grain food products (Ahluwalia et al. 2019).

2.1.2. Starch

Most of the wheat starch is found in the endosperm, but some also resides in the wheat bran layers. The main function of starch in the wheat kernel is to provide energy to the embryo of the seed if planted. However, the majority of wheat kernels produced are not planted, but milled into flour for human consumption (Debes n.d.).

Starch consists of two polysaccharides: amylose and amylopectin. Amylose contains linear chains of D-glucopyranose monomeric units with an α-1,4-linkage while amylopectin is branched with a linear backbone of α-1,4-lanked D-glucopyranose and α-1,6-linked D-glucopyranose branches (Seung 2020).

Wheat starch comes in A-type granules and B-type granules where the B-type has more amylopectin than the A-type. The granules have both amorphous and crystalline regions and different ratios of amylose and amylopectin. B-type granules are more abundant in the wheat bran than the A-type. Because of the lesser amylose content in B-type granules, the B-type granules has less crystalline structure and is therefore more easily digested by α-amylase (Maningat & Seib 2010).

2.1.3. Lignin

Lignin is the second most abundant biopolymer after cellulose and is found in the cell walls of plant cells. This non-carbohydrate phenolic polymer enhances cell wall rigidity and is an important barrier against pests and pathogens (Liu et al. 2018). The lignin structure is complex and vary considerably between species. It is biosynthesised by enzymatic dehydrogenation of *p*-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol to a randomly polymerized three-dimensional network. Common linkages between are β-O-4, β-β, β-5 and 5-5', but many others have also been observed (Katahira et al. 2018).

Lignin can crosslink with arabinoxylan through the phenolic subunits or ferulic acid which can be found as a component in the arabinoxylan (AX) molecule. The matrix of crosslinked lignin and AX increases the cell wall strength and gives the bran stiffness and rigidity (Härdelin 2018; Eugene et al. 2020). The matrix decreases the extractability of AX, thus, unlinking the crosslinkages should result in a higher AX yield (Eugene et al. 2020).

2.2. Arabinoxylan

Arabinoxylans (AX) are one of the most abundant types of non-starch polysaccharides in all cereal grains. It is located in the cell walls of the starchy endosperm, the aleurone layer, in the bran tissue as well as in the husk of some cereals (Izydorczyk 2021). In the wheat grain arabinoxylan stands for 70% of the cell walls of the starchy endosperm. In the aleurone cell walls 65% is arabinoxylan and it constitutes 60% of the pericarp cell walls (Freeman et al. 2017). The exact molecular structure of arabinoxylan vary between species and tissue type. Arabinoxylan consists of two pentose sugars: arabinose and xylose. The backbone is a linear backbone of β-1,4 linked xylose with α-1,3 and/or α-1,2 linked arabinose branches. Other sugar units, such as uronic acids and *O*-acetyl groups can also be substituted to various degrees to the backbone (Izydorczyk 2021).

The arabinose residue can be ester linked on the C-5 position with ferulic acid, which can cause covalent cross linkages between arabinoxylan chains. This occurs to a greater degree in the bran than in the endosperm and is partially why most bran arabinoxylan is insoluble (Izydorczyk 2021).

The solubility of arabinoxylan is also determined by the way arabinose is substituted onto the xylose backbone. Since the β-1,4 xylose backbone has various substitutions of arabinose on the C-2 and/or C-3 carbon position, it will cause a random helix formation, with varying degrees of flexibility and water solubility (Ramseyer et al. 2011). The ratio between arabinose and xylose also affects the water solubility. Water extractable arabinoxylan (WE-AX) has a typical average ratio of 0.5 – 0.6 and is more often located in the starchy endosperm (Cleemput et al. 1993), while a water unextractable arabinoxylan (WU-AX) has a higher ratio and are located in the outer bran fractions (Izydorczyk & Biliaderis 1995).

2.2.1. Health benefits of arabinoxylan

Arabinoxylan is a dietary fibre and as other dietary fibres it has beneficial effects on human health. Several biological effects have been seen for the consumption of AX. Positive effects of AX has been concluded to be antioxidant activity, prebiotic activity, cholesterol lowering agents, blood sugar modifiers and immunity enhancers (Bastos et al. 2018). Katapodis et al. (2003) have shown that arabinoxylan isolated feruloylated oligosaccharide (FAX_3) exhibits antioxidant activity. This antioxidative activity inhibit peroxidation of low-density lipoproteins (LDL) which in turn can reduce the risk of atherosclerosis and cardiovascular diseases.

AX has also been shown to have prebiotic effects. Reis et al. (2014) compared AX from brewers spent grains (BSG) to fructooligosaccharides and a control with no added carbohydrates. They confirmed *in vitro* that AX has a prebiotic potential due to the higher production of short chain fatty acids (SFCA) in bifidobacterial populations compared to the fructooligosaccharides and to the control. AX also produced the highest amount of the SCFA propionate, which is an indication that AX from BSG can be used in prebiotic treatments and prevention strategies on cardiovascular diseases and type II diabetes (Reis et al. 2014).

In an *in vivo* study by Lu et al. (2000) they concluded that the ingestion of AX-rich fibres improved the postprandial glucose and insulin responses. When measuring the plasma glucose and insulin levels, participants consumed a breakfast with whole wheat bread containing 0, 6 and 12g AX-rich fibres. After 30 minutes a significant decrease could be seen in plasma glucose levels in the participants that ate the 6 and 12g AX enriched breads. The same response was noticed for the plasma insulin. The mechanism for how AX lowers the glucose and insulin is not fully understood, but as with other dietary fibres it is likely that AX slows the rate of gastric emptying allowing delayed glucose absorptions (Lu et al. 2000)

AX can also lower cholesterol levels by preventing the reabsorption of bile salts in the gastrointestinal tract (Gunness et al. 2016). Cholesterols only way of leaving the body is through bile salt excretion. Bile salts are synthesized from cholesterol in the liver and aid food through the gastrointestinal tract. Dietary fibres require more bile salts and will therefore increase the bile salt excretion. The decrease of bile salts could lead to more cholesterol being absorbed from the blood plasma and lower the overall cholesterol levels (Ellegård & Andersson 2007).

2.2.2. Applications of arabinoxylan in the food industry

A reason for extracting arabinoxylan is to use it as a food additive. Arabinoxylan can be used in several ways to affect texture, nutritional status, and the functional properties of food. A difference must however made between WE-AX and WU-AX, since they can contribute with different properties (Izydorczyk 2021).

One of the main uses of AX is in bread making where AX affect the water absorption and structure of the dough. Both WE-AX and WU-AX increase the water absorption of a dough and increase the consistency and creates a stiffer dough. Thus, more water needs to be added to gain similar sensory qualities as a control bread (Courtin & Delcour 2002). Courtin & Delcour (2002) also investigated the effects on the dough structure and found differences between WE-AX and WU-AX. WE-AX increased the stability of the dough while WU-AX

destabilised the dough structure. They suggest that WE-AX form liquid films and help the dough structure stabilize while WU-AX can form physical barriers for the gluten network and prevent a proper dough development.

Within the food industry, AX can be used as a film formation, gelling agents, cryostabilizers and surface active agents (Izydorczyk 2021). AX is suitable for stabilizing emulsions and protein foam because of its surface active properties. Feruloylated AX can increase water holding capacity and shear resistance in sausages as demonstrated by Herrera-Balandro et al. (2019). Chanliaud (1995) created an arabinoxylan based films that had the prospect of replacing certain plastic films. The films had an effective barrier against CO_2 and O_2 as well as a good tensile resistance. Though the functional properties could not measure up to those of plastic. A proposal to improve the characteristics of the AX-film with lipid addition was tested by Péroval et al. (2002). The arabinoxylan-lipid film tested better than its predecessor, but still couldn't measure up to the characteristics of plastic.

2.3. Ultrasound

Ultrasound is a novel technology in the food industry which has many applications such as emulsification and microbial inactivation as well as many more applications that are currently being investigated (Gallo et al. 2018). Ultrasound works through a mechanism called cavitation and can be utilised with a horn transducer or through a water bath. Cavitation bubbles occur when sound waves are transmitted through a liquid system and thousands of microbubbles are produced. These bubbles implode and explode causing chemical reactions and physical damage (Bermudez-Aguirre 2017). The cavitation bubbles are created by the gas originating from the liquid medium. If the liquid medium is water, OH^- and H^+ radicals will be generated by the vacuum created by the ultrasound. The cavitation bubble will grow with the OH^- / H^+ gas before it collapses in on itself causing an explosion and a microjet of water with speeds up to 400 km/h. The microjet shoots out through the sample causing structural breakdowns and chemical reactions (Kentish 2017).

The effect of the ultrasound depends on the frequency, acoustic power, and sonication time as well as viscosity and volume of the sample. The frequencies used in the food industry are commonly between 20 and 40 kHz (Kentish 2017) which differs from ultrasound used clinically and diagnostically, as in sonography, which uses frequencies in the MHz range (Carovac et al. 2011). In food applications using a higher frequency is not always desirable. Higher frequencies (>70 kHz) generate more but smaller cavitation bubbles which leads to a decrease of energy released

when the bubble implodes (Belwal et al. 2020). The supplied power (W) will also influence the bubble distribution, especially if using a horn transducer. Too high power will cause the bubbles to coalesce and form a film around the horn transducer, thus limiting the acoustic effect (Kentish 2017).

2.3.1. Ultrasound assisted extraction

Ultrasound assisted extraction is a novel food processing technique but still has quite a few years in the industry dating back to the 1990s. It has been tested and used to increase extraction yield in multiple compounds and chemicals such as: polyphenols, flavonoids, polysaccharides, and essential oils (Belwal et al. 2020).

Hromádková & Ebringerová (2003) analysed the ultrasound assisted extraction of hemicelluloses from buckwheat hulls. They performed the sonication on the buckwheat suspended in the alkali solution and concluded that a short treatment was effective in breaking the cell wall structure and increasing the yield of hemicelluloses. The structure was not affected in any substantial way and certain extraction conditions allowed for xylan components to release from the starch and protein.

Ultrasound assisted extraction of hemicelluloses from wheat straw in 0.5 M NaOH in 60% ethanol was performed by Sun et al. (2002). They noticed an increase in hemicellulose yield by 2.9-9.2% when sonicating the sample 5-35 minutes at 100W. A slight increase in xylose was noticed however no significant difference in the structure of the hemicellulose could be determined. An optimum was found to be sonication for 20 minutes at 100W and 20kHz with the sample diluted 1:30 (w/V) in the 0.5 M NaOH in 60% ethanol.

2.3.2. Ultrasound in food processing

Ultrasound can be used in many different processing steps in the food industry, both in preservation and processing. A few of the most commonly used practices are emulsion, homogenisation, extraction, and enzymatic and microbial inactivation (Gallo et al. 2018).

Ultrasound has been used in food processing since the late 1990s. It started on a laboratory scale but has made it to commercial use in several applications. Some of the more commercially large scale uses of ultrasound includes wine barrel sanitation, mayonnaise emulsification, foam control in soft drinks, and viscosity

reduction for improving other downstream processes such as spray drying (Bates & Patist 2010).

2.4. Arabinoxylan alkaline extraction

The extraction of arabinoxylan is a multistep process and requires multiple days to perform. For a high AX purity, procedures are taking into place before the alkaline extraction. Possible steps to purify the bran include pre-treatments of destarching and delignification. Following the destarching and delignification steps the alkaline extraction can take place, generating a purer AX extract (Börjesson et al. 2018). Alkaline extraction has been shown to produce high yields and a high molecular weight of the AX (Aguedo et al. 2014).

2.4.1. Destarching

When extracting dietary fibre, a destarching step is necessary to reduce the glucose in the final yield. Destarching is done by gelatinizing the bran in water to solubilise the starch. The starch is then susceptible to enzymatic hydrolysis by a hydrolytic enzyme. α-amylase is added to cleave the amylose and amylopectin into smaller segments at the α-1,4 linkage (Sundarram & Murthy 2014).

2.4.2. Delignification

Lignin in WB can be removed using chlorite (Glasser et al. 2000; Börjesson et al. 2018). A pre-treating of WB with delignification can give a purer AX extract with lower polydispersity (Börjesson et al. 2018). The treatment with chlorite will change the aromatic properties of lignin and make it possible to isolate, either with an organic solvent precipitation or ultrafiltration (Glasser et al. 2000).

2.4.3. Alkaline extraction

Alkaline extraction is one of the most common procedures to obtain high molecular hemicelluloses. By increasing the pH to extreme alkaline conditions (pH 12-13) the AX will become soluble and can be separated from the bran (Glasser et al. 2000). The alkaline extraction de-esterifies the ferulic acid that crosslinks with the AX. The di- and triferulate crosslinks are removed and the AX can be liberated (Kale et al. 2013).

3. Material and methods

3.1. Materials

Milled wheat bran (<380 µm) was provided by Lantmännen (Stockholm, Sweden). Chemicals and reagents used were of analytical grade. α-amylase (type IV−B from porcine pancreas), $NaClO_2$, H_2SO_4, and all monosugars used for the standard calibration curve were obtained from Sigma Aldrich. NaH_2PO_4 and Na_2HPO_4 were from Labchem, while HCl, NaCl and NaOH were from EMSURE. 95 % ethanol was from Solveco.

3.2. Moisture content determination of wheat bran

The determination of moisture content of the wheat bran was measured gravimetrically in triplicates. Aluminium cups were dried in an oven at 105°C and a desiccator was used as a temporary storage out of the oven. The weight of each cup was noted and then filled with wheat bran to a set weight and noted. The content was dried overnight in an oven of 105°C, weighed again and moisture content was calculated.

3.3. Pre-trials with ultrasound

A pretrial was held to get accustomed to the ultrasonic processor and to determine the selected parameters for the main experiment.

The initial ultrasonication testing was done on 30 g wheat bran soaked in 170 g H_2O, three samples were treated at 80 W for 5 and 10 minutes and at 400 W for 5 minutes. The power input of 400 W resulted in a net power of 150-200 W. An increase in heat of the samples were noticed, correlating to longer time and higher power input. The ultrasound treated samples together with an untreated control sample were evaluated with a scanning electron microscope (SEM). No obvious

differences in the cell wall structure could be seen with the SEM and the pretrials therefore continued with a full arabinoxylan extraction.

In the second part of the pretrials five different combinations of time and power – 80 W for 2 minutes, 80 W for 10 minutes, 400 W for 2 minutes, 400 W for 5 minutes and 400 W for 10 minutes – were tested together with a control heated to 65°C. To minimize the heat increase, the samples were put in an ice bath during the sonication. Even regarding the ice bath, the sample treated the longest and with the highest power input still reached 65°C, therefore the control was also heated to 65°C. After the ultrasonication differences could already been seen visually. Samples that had undergone longer treatments had a distinct white layer between the supernatant and bran, which was believed to be starch (Picture 1).

Picture 1. To the left: 400 W for 5 minutes. To the right: 400 W for 10 minutes. A distinct white layer can be seen on top of the bran in the sample on the right.

After a full arabinoxylan extraction with destarching, delignification and alkaline extraction the samples were assessed both by total carbohydrate yield and with a HPAEC-PAD monosaccharide composition.

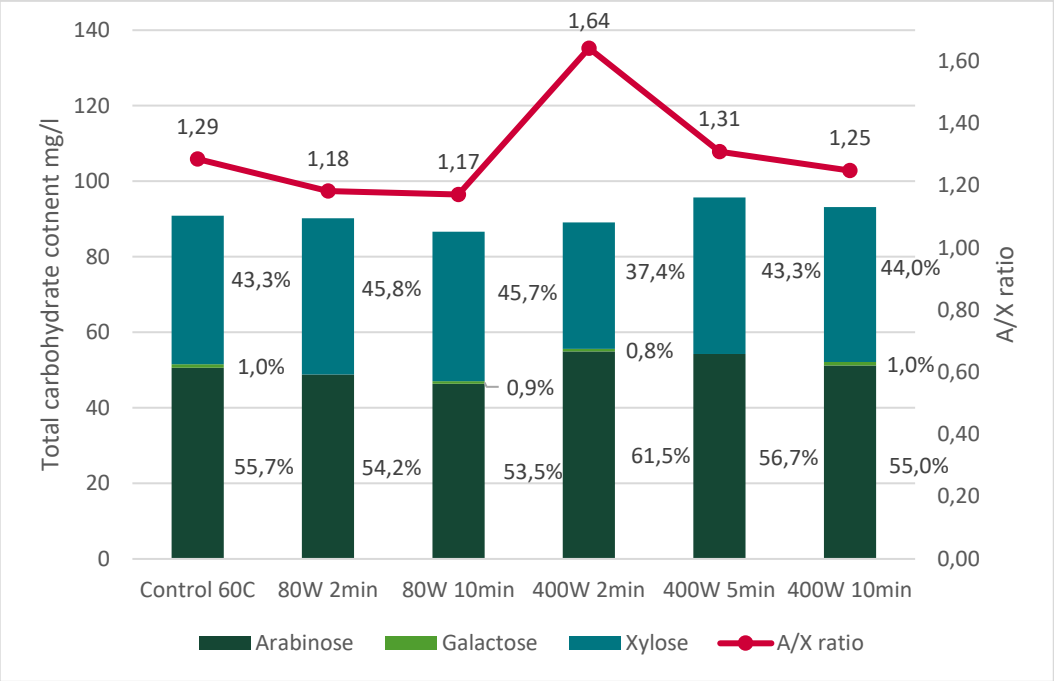


Figure 1 Total carbohydrate content, monosaccharide composition and A/X ratio of the pre-trial samples

A slight increase in total carbohydrate content could be seen from the 400 W 2 minute treatment to the 400 W 5 minute, while the opposite could be seen in the 80 W 2 minute treatment to the 10 minute (Figure 1). Greater differences were noticed in the A/X ratio among the samples treated with 400 W (1.64, 1.31 & 1.25), especially for the 2 minute sonication (Figure 1). With these results, together with consultations of previous users of the Ultrasonic processor, the power input of 400 W and treatments times 0.5, 1, 5 & 10 minutes, were set for the main experiment.

3.4. Pre-treatment with ultrasound

In a 250 ml Dalton bottle, 30 g dry weight basis (dwb) wheat bran was soaked overnight in 4°C with distilled water containing 0.184 g NaCl/L to a total weight of 200 g. The NaCl was added to mimic the mineral content of the water in Uppsala, Sweden.

The samples were treated with ultrasound using Hielscher UP400St Ultrasonic Processor with the S24d14D sonotrode. 4 different variations of time were tested (0.5, 1, 5, & 10 minutes) with the power input of 400 W. Because of the increase in temperature during the sonication both a cold control and a hot control (62.5°C)

were used as a standard. The sonotrode was submerged 3.8 cm into the sample and placed in the centre of the bottle. To decrease the rise in temperature the bottle was placed in an ice bath both before and during the sonication. After the ultrasound treatment the samples were separated by centrifugation at 8000 rpm for 20 minutes. The ultrasonicated supernatant (USS) was saved for chemical composition analysis (Figure 2). Moisture content of the ultrasonicated wheat bran was determined gravimetrically as in section 3.2. but was done only in duplicates.

3.5. Arabinoxylan extraction

3.5.1. Destarching of wheat bran

To gelatinize the starch, 10 g (dwb) of the ultrasonicated wheat bran was suspended (1:13 w/V) in 20 mM sodium phosphate buffer with 6.7 mM NaCl (pH 7) for 5 minutes at 85°C. The sodium phosphate buffer was made with 1 litre H_2O and 1.12 g of NaH_2PO_4, 1.52 g of Na_2HPO_4 and 3.9 g of NaCl. The samples were cooled down to ~40°C and α-amylase from porcine pancreas (16 U/g WB) was added and left to destarch for 16 hours in a 37°C water bath under constant stirring.

The next day the destarched bran and buffer was separated by decanting. The decanted destarched supernatant (DSS) was dialyzed and dried in 60°C overnight for further chemical composition analysis (Figure 2). The bran was washed twice with 95% ethanol to inactivate and remove the α-amylase and then dried at 60°C overnight.

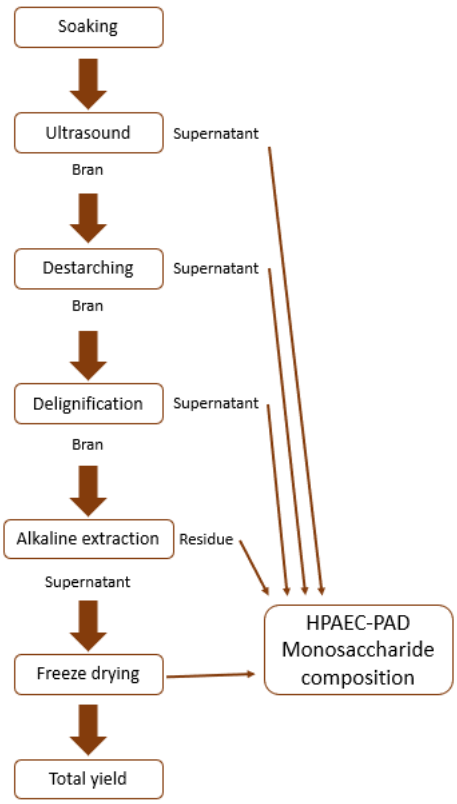


Figure 2 Experimental set up

3.5.2. Delignification of wheat bran

The destarched bran pellet was suspended 1:4 (w/V) in 50 ml distilled water and $NaClO_2$ (0.15 g/g) wheat bran was added. The pH was adjusted with HCl to 3.1 and

the samples were put in a water bath at 80°C for 3 hours. The samples were cooled down and separated through centrifugation for 15 minutes at 5000 rpm. The delignified supernatant (DLS) was saved for chemical composition analysis. The pellet bran was washed twice with distilled water and centrifuged for 15 minutes at 5000 rpm.

3.5.3. Alkaline arabinoxylan extraction

The delignified bran was suspended 1:8 (w/V) in a 0.5 M NaOH solution and put in a water bath for 16 hours at 80°C. The samples were cooled down and separated by centrifugation (5000 rpm 15min). The supernatant, now containing the extracted AX, was collected, adjusted to pH 7 with HCl and dialysed with 3.5 kDa MWCO dialysis membrane overnight. The pellet residue was liquidized with 50 ml water, adjusted to pH 7, then dried again in 60°C overnight. The extracted AX was freeze dried for 24 hours to get it in a powder format.

3.6. HPAEC to determine monosaccharide composition and Klason lignin determination

3.6.1. Hydrolysis

70 mg (dwb) of the USS, DSS, DLS, extracted AX and the residue was ground and put in a 50ml bottle together with 1.05 ml of H_2SO_4 (73%) and left for 1.5 hours in a desiccator vacuum. A monosaccharide reference was prepared in a 50 ml bottle with 17.7 mg of glucose, 10.3 mg arabinose, 10.3 mg xylose, 11.1mg mannose and 11.8 mg galactose together with 1.05 ml of H_2SO_4 (73%). 30 ml H_2O was added to the samples and the reference and they were autoclaved at 125°C for 60 minutes. The autoclaved samples were then filtrated through a glass fibre filter with 10 ml warm water, using vacuum filtration. The carbohydrate solution was saved, the filter was washed through with 100 ml warm water and 100 ml cold water and then dried at 105°C.

3.6.2. Monosaccharide composition analysis using HPAEC-PAD

173 µl of the carbohydrate solution was diluted in Eppendorf tube with 1.3 ml H_2O and the monosaccharide reference standard was diluted in to five concentrations 1, 2, 4, 8, 16, and 32%. All samples where then filtrated through 25 mm syringe filters (VWR International) into 1.5 ml glass vials and pre-slitted caps were put on. The samples were analysed by high performance anion-exchange chromatography

(HPAEC) with pulsed amperometric detection (PAD) in a Dionex ICS-3000 system (Dionex, Sunnyvale, CA)

The total carbohydrate content was overestimated by about 30%, which could be because of outdated HPAEC equipment. A standard with known carbohydrate content was run to confirm the overestimation. The total carbohydrate content in result has not been altered, the real measured numbers are shown, though when evaluating the result, the ~30% increase should be considered.

3.6.3. Lignin determination

The glass fibre filter used when filtrating the hydrolysed samples was weighed before and after to determine lignin content in the extracted samples.

3.7. Statistical analysis

The statistical analysis was done using R Studio version 2021.09.1 “Ghost Orchid” Release (8b9ced18, 2021-11-08) for Windows. A one-way ANOVA was used when comparing the hot and cold control, comparing the variance of the means. The results of total solid yield and chemical composition were analysed with fit linear model. Script and result from the analyses can be found in Appendix II.

4. Results & Discussion

4.1. Comparison of cold and hot control

During the sonication of the samples a noticeable increase in temperature was detected. Even with the samples in an ice bath both before, during and after the sonication a maximum increase to 62.5°C was seen in the 10 minutes duration samples. The samples sonicated for 5 minutes increased in temperature to 47°C. The samples treated for 1 and 0.5 minutes did not reach over room temperature. To evaluate the increase of temperature as a contributing factor, a heated control (62.5°C) done in duplicates, was included in the testing to see if the heat had any effect on the final yield.

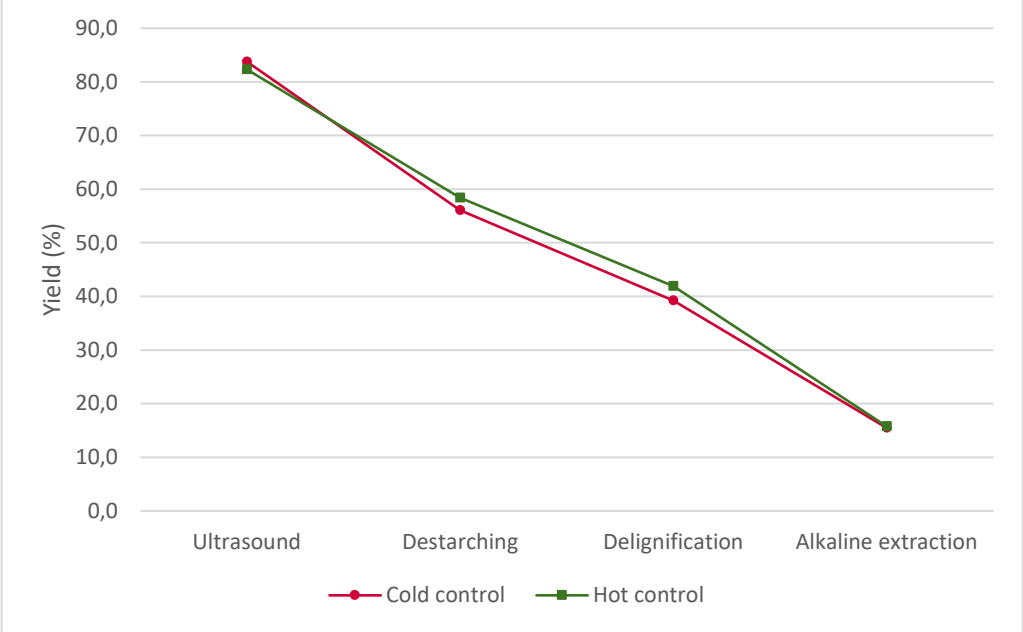


Figure 3 Total solid yield comparison of hot and cold control (%)

The total solid yield, containing the extracted AX, was not affected by the heat (Figure 3). No significant difference was found.

Small differences could be seen in the average monosaccharide composition of the extracted AX (Table 1), but no significant difference could be found. Therefore, all further analyses were done with the cold control only.

Table 1 Average monosaccharide composition of extracted AX in hot and cold control.

	Cold control	Hot control
Arabinose (%)	50.4 (±0.9)	52.1 (±0.8)
Galactose (%)	2.2 (±<0.1)	2.3 (±0.1)
Glucose (%)	2.5 (±0.3)	2.1 (±0.3)
Xylose (%)	44.9 (±0.6)	43.6 (±0.5)
Mannose (%)	0.0	0.0

4.2. Chemical composition and yield of the wheat bran through the alkaline extraction processing

4.2.1. Total solid yield after all steps in the extraction process

The extraction process included a destarching- and delignification step, as well as the pre-treatment of the bran. The supernatant of each extraction step has been analysed, while the treated bran has continued to the next step. After each step the original bran lost soluble components and the yield decreased. In this section the control is used to compare the different steps, therefore the ultrasound treatment was not performed. Instead, the first step is just the bran soaking in water over night.

After the overnight soaking 83.8% of the bran remained (Appendix I), 56.1% remained after the destarching step, 39.2% after the delignification and 15.5% was extracted to the total solid yield of extracted AX. The total solid yield contained not just AX, but other components as well. 54.9% of the total solid yield was pure AX (Table 2), where the rest most likely accounts for proteins (Buksa et al. 2016).

Table 2 Chemical composition of the control in the different extraction steps: wheat bran, soaked bran supernatant, destarched supernatant, delignified supernatant, extracted AX and residue in %. Total carbohydrates and lignin content in mg/g DW

	Wheat bran	Soaked bran supernatant	Destarched supernatant	Delignified supernatant	Extracted AX	Residue
Total carb. (mg/g DW)	639.6(±29.1)	314.8(±48.7)	771.0(±102.3)	460.3(±5.4)	576.1(±45.5)	664.6(±50.2)
Arabinose (%)	13.7(±0.2)	8.5(±0.2)	2.6(±0.2)	22.0(±<0.1)	50.4(±0.9)	20.2(±2.4)
Galactose (%)	1.5(±<0.1)	6.7(±0.1)	1.3(±<0.1)	2.4(±<0.1)	2.2(±0.0)	1.3(±0.1)
Glucose (%)	66.0(±0.3)	71.4(±0.4)	89.7(±0.2)	35.8(±<0.1)	2.5(±0.3)	43.0(±2.7)
Xylose (%)	18.5(±0.2)	11.6(±0.1)	6.4(±0.2)	39.4(±<0.1)	44.9(±0.6)	35.2(±0.1)
Mannose (%)	0.3(±0.1)	1.7(±0.3)	0.1(±0.1)	0.4(±<0.1)	0	0.4(±<0.1)
Klason Lignin (mg/g DW)	93.4(±5.1)	46.8(±2.4)	61.4(±34.3)	29.8(±24.7)	86.6(±10.3)	33.7(±14.1)

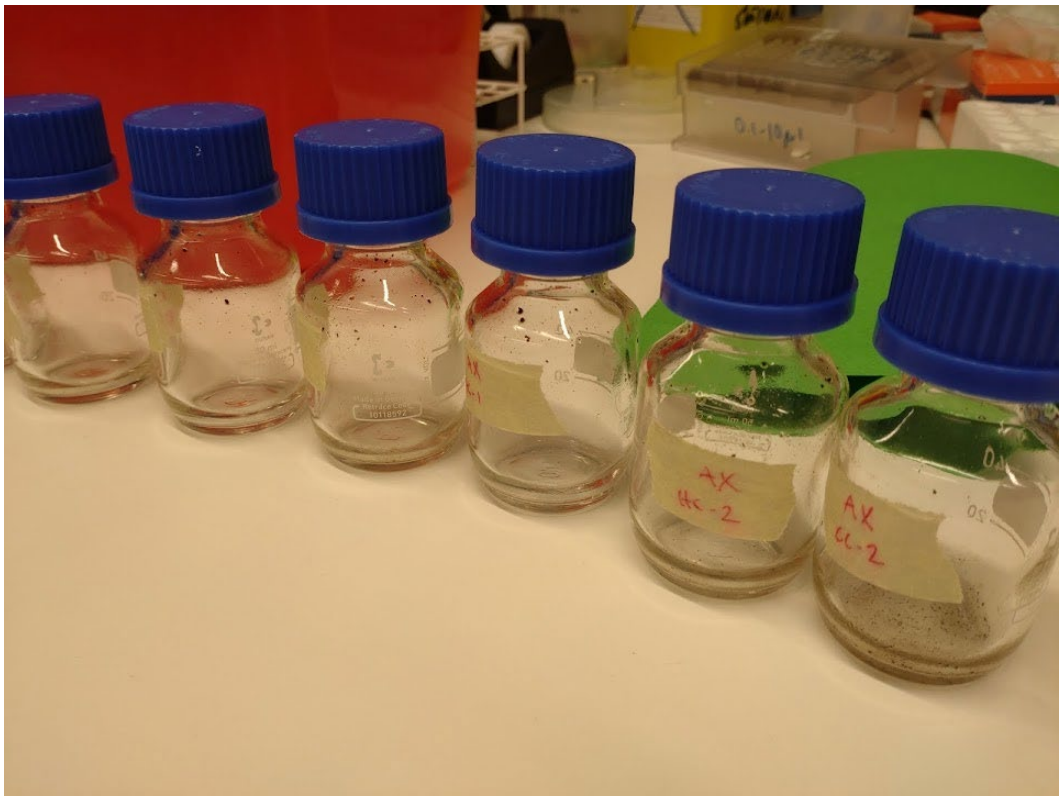
4.2.2. Chemical composition after each extraction step

The destarched supernatant (DSS) fraction had the highest glucose content (Table 2) with 89.7% of the total carbohydrates. This is reasonable since the α-amylase has been active and broken down the amylose and amylopectin in the sample generating a lot of water soluble sugars, indicating that the destarching works successfully. Second most glucose was found in the soaked bran supernatant, 71.4% versus 66% in the original wheat bran. Suggesting that the glucose originates from mostly water soluble carbohydrates in the bran (Harrison et al. 1997). The delignified supernatant (DLS) contained 39.4% xylose and 22% arabinose, compared to the 18.5% xylose and 13.7% arabinose in the wheat bran. Thus, indicating that the acidic delignification process releases some AX as well (Aguedo et al. 2014). Acid conditions cleaves the arabinofuranosyl and xylopyranolsyl links and solubilizes/debranching them from the AX molecule (Wallace et al. 1995).

The extracted AX contains 95.3% AX compared to the 32.2% AX in the bran. Which indicates that the extraction process was successful in removing starch and lignin generating a purer AX extract. Left in the residue is 43% glucose and 55.4% AX, suggesting that more WU-AX is present in the bran, and that the extraction can be optimised further.

Out of all the fractions, most lignin could be found in the extracted AX (Table 2). The least amount of lignin was found in the delignified supernatant, suggesting that the delignification step might not be very effective in solubilising lignin, but might still have an effect on the total solid yield. The lignin content method was affected by the filtration after the autoclaving of the hydrolysed samples. The cooler the bottle with the sample got, the more lignin got stuck on the inside of the bottle

(Picture 2). Therefore, samples filtrated last had a greater lignin loss in the bottle than the samples filtrated first. This could have been avoided by letting them all cool down to the same temperature, but this effect was first noticed at the end of the filtration.



Picture 2 Lignin (black residue) stuck on the inside of the glass bottle.

4.2.3. A/X ratio in the different extraction step fractions

The A/X ratio changes during the extraction process and is at its highest in the extracted AX at 1.12 in the control (Figure 4). The high ratio (>1) indicates that WU-AX from the pericarp has been released in the alkali extraction (Maes & Delcour 2002; Wang et al. 2015). WE-AX are mostly found in the starchy endosperm and aleurone layer and has a ratio of around 0.5-0.6 (Maes & Delcour 2002). The wheat bran had an A/X ratio of 0.74, implying that the bran used in this process contain amounts of both WE-AX and WU-AX.

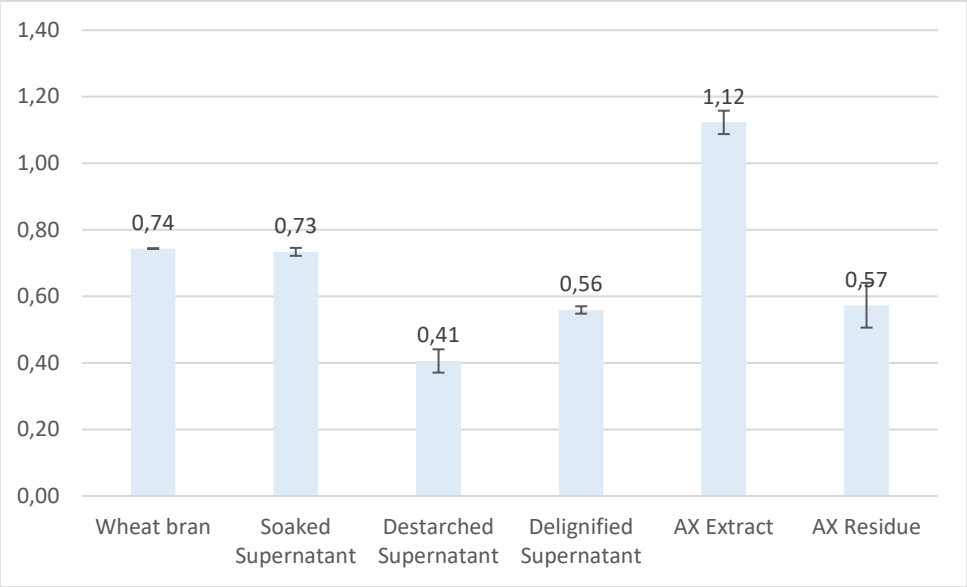


Figure 4 Changes in the A/X ratio during the extraction process for the control sample

4.3. Ultrasound treatments

4.3.1. Total solid yield & carbohydrate content of the different treatments

In Figure 5, the total solid yield is displayed from each US treatment. The highest total solid yield was found in the samples treated with ultrasound for 10 minutes, with a total of 21.3%, though only 55.3% of the yield was carbohydrates and 93.1% of the carbohydrates was arabinoxylan. The control had the lowest total solid yield with 19.2% indicating that the ultrasound treatment increases the total solid yield amount. The statistical analysis of the total solid yield showed an almost significantly difference with a p-value of 0.079 with the confident interval of 95%.

The samples treated for only 0.5 minutes, had the highest carbohydrate content of 76.9% followed by the samples treated for 1 minute with a carbohydrate content of 72.9% (Figure 5). The lowest carbohydrate content was found in the samples treated for 5 minutes. It looks like the shorter treatment times gave a higher carbohydrate content while the longer treatment times gave a higher total solid yield. Though this was not confirmed with the statistical analysis. The AX extract purity (total AX per total solid yield) was the highest in the 0.5 minute treated sample with 71.3% followed by 68.2% in the sample treated for 1 minute. Lowest AX purity (48.3%) was found in the sample treated for 5 minutes. The control had an AX purity of 54.0%. Bataillon et al. (1998) reported on similar results with an AX purity of

42.3% in extracted AX from destarched WB. The remaining amount could be assigned to proteins and small amounts of other carbohydrates. In wheat, a closely linked protein decreases the purification of AX (Buksa et al. 2016).

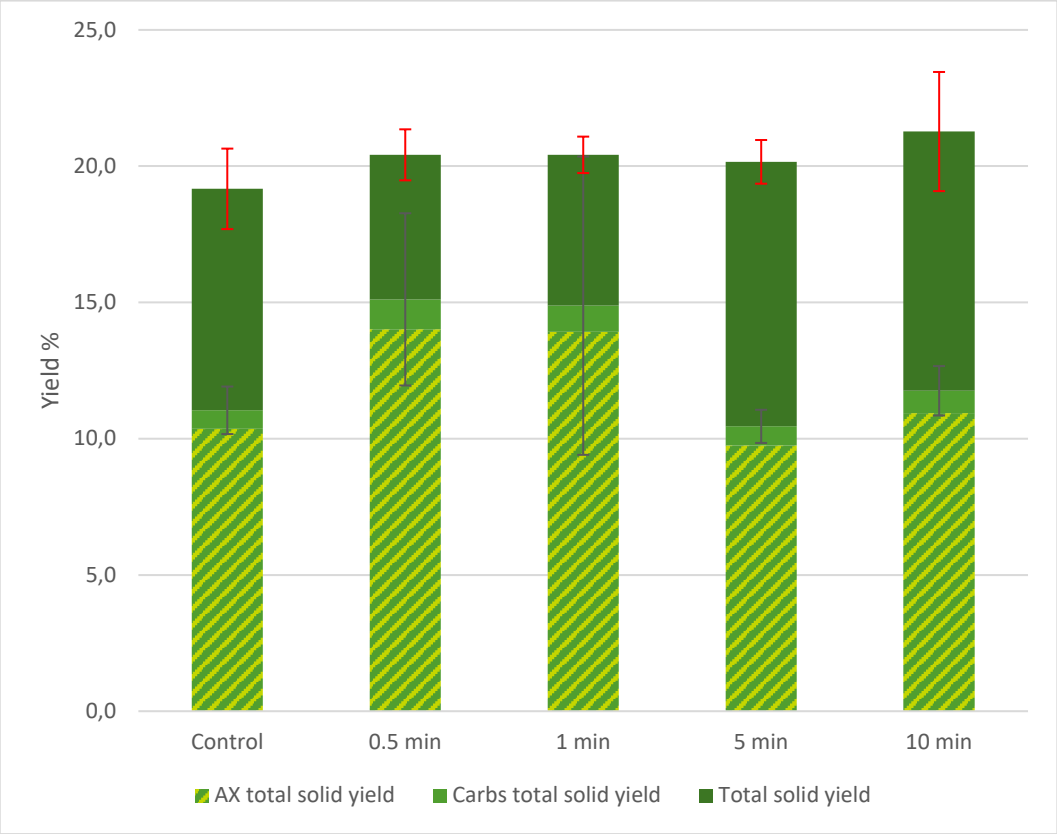


Figure 5 Total carbohydrate yield and total AX yield presented as a part of the total solid yield from ultrasound treated bran (%)

4.3.2. Chemical composition of the AX extracts of the different treatments

The monosaccharaide compositions were similar throughout the different treatments (Table 3) and no significant differences could be seen between the treatments (Appendix II). The extracted AX had a relatively high purity, ~95% of the total carbohydrates, indicating that the extraction process is working. The total carbohydrate content did vary between the treatments, higher contents were found in the 0.5 and 1 minute treatments, with 768.6 and 728.9 mg/g DW respectively while the two longer treatments gave more similar content as the control (Table 3). This suggests that low sonication time could yield higher carbohydrate contents, though this was not supported by the statistical analysis. This could be because of a too high power supply can cancel out the effect of the cavitation bubbles by

forming a film of bubbles around the sonotrode limiting the ultrasonic effect (Kentish 2017).

Table 3 Chemical composition of the extracted AX in all different treatments

	Control	0.5 min	1 min	5 min	10 min
Total carbs. (mg/g DW)	576.1(±45.5)	768.6(±160.7)	728.9(±268.1)	518.5(±30.2)	552.8(±42.6)
Arabinose (%)	50.4(±0.9)	50.1(±1.0)	50.1(±0.7)	49.6(±0.2)	50.1 (±1.0)
Galactose (%)	2.2(±<0.1)	2.2(±<0.1)	2.2(<0.1)	2.2(±<0.1)	2.2(±0.1)
Glucose (%)	2.5(±0.3)	2.9(±0.7)	2.7(±0.5)	3.0(±0.3)	3.0(±0.3)
Xylose (%)	44.9(±0.6)	44.8(±0.3)	45.1(0.2)	45.2(±0.5)	44.6(±0.7)
Mannose (%)	0	0	0	0	0
Klason Lignin (mg/g DW)	98.2(±21.5)	121.9(±26.3)	101.3(±24.6)	110.7(±42.9)	101.5(±23.7)

The lignin content among the treatments were in close range, the highest was found in the sample treated for 0.5 minutes (121.9 mg/g DW) and the lowest in the control (98.2 mg/g DW) (Table 3). Implying that the ultrasound treatment could release more lignin from the cell wall, though this was not confirmed with the statistical analysis (Appendix II).

4.3.3. A/X ratio of the treatments

Very little differences could be seen in the A/X ratio between the different treatments, as shown in Figure 6. The ratio range was only from 1.10 to 1.12 with very small standard deviations, with 0.039 being the highest for the 10-minute treatment. The statistical analysis showed no significant difference between the treatments. These results suggest that ultrasonic treatment, with 400 W power input for up to 10 minutes, does not affect the A/X ratio in wheat bran. Similar results were obtain by Sun et al. (2002), where sonication times up to 35 minutes only affected the A/X ratio by 3 hundredths.

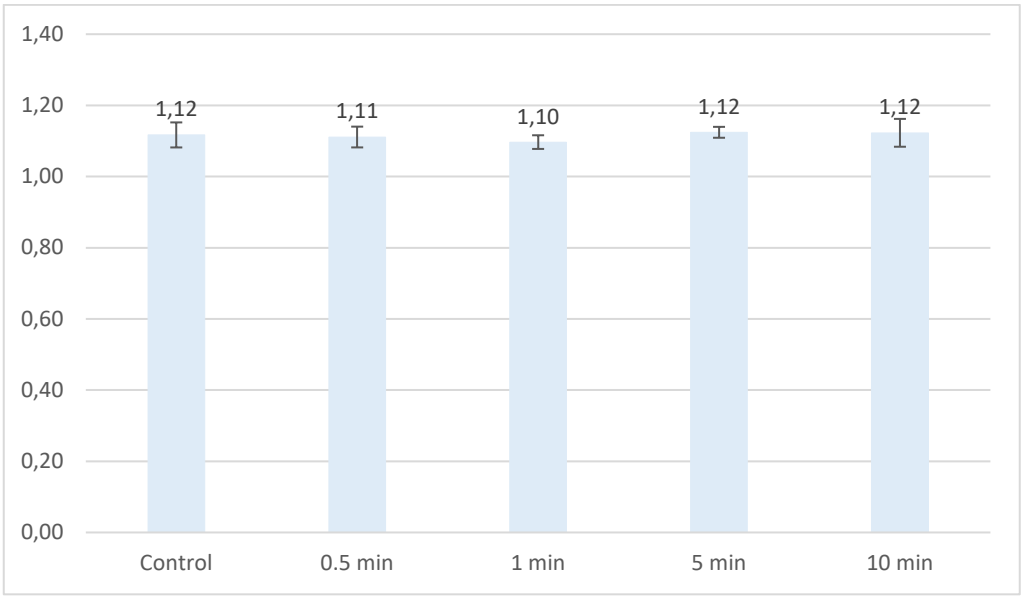


Figure 6 A/X ratio in the extracted arabinoxylan from the different treatments: control, 0.5, 1, 5 & 10 minutes.

5. Conclusion

The alkaline extraction worked successfully and was able to solubilize WU-AX, which can be concluded with the high A/X ratio. The destarching step was effective yielding high amounts of glucose in the DSS. The delignification step did not appear very effective since the delignified supernatant had the least amount of lignin while the extracted AX had the most.

400 W (150-200 W net power) ultrasonic pre-treatment of wheat bran showed a trend to increase the total solid yield with sonication for 10 minutes giving the highest yield. The ultrasound did affect the total carbohydrate content, more so in the lower treatment times, indicating that the ultrasound did have an impact on the samples. Though the monosaccharide composition and A/X ratio were unaffected.

For future research it is important to consider that the success of ultrasound treatment depends on many things, including the power input and viscosity of the treated sample. More extended testing with different power input and times would be needed to investigate the effects a pre-treatment with ultrasound could have on an AX alkaline extraction. Further research into ultrasound pre-treatment of wheat bran could include lower power input and longer treatment times as well as different bran to water ratio during the sonication.

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Appendix I – Raw Data

Yield from initial sample:

Sample treatment	Sample name	Ultrasound bran yield of initial sample (%)	Destarched bran yield of initial sample (%)	Delignified bran yield of initial sample (%)	Extracted AX yield of initial sample (%)
0,5 min	**0,5-2**	85,77	58,47	38,38	16,33
0,5 min	**0,5-3**	83,51	60,32	44,70	17,32
1 min	**1-1**	85,42	58,79	39,99	16,86
1 min	**1-3**	83,18	56,01	41,44	17,00
5 min	**5-1**	82,78	56,22	38,10	16,72
5 min	**5-3**	82,46	53,71	36,81	15,94
10 min	**10-1**	81,45	58,63	43,65	18,29
10 min	**10-3**	81,32	55,12	37,12	15,25
Cold control	**CC-1**	83,53	56,63	36,54	14,70
Cold control	**CC-2**	84,00	55,49	41,96	16,26
Hot control	**HC-1**	82,35	60,53	43,51	16,26
Hot control	**HC-2**	82,27	56,19	40,26	15,24

Yield from US treated sample:

Sample treatment	Sample name	Destarched yield of US treated sample (%)	Delignified yield from US treated sample (%)	Extracted AX yield from US treated sample (%)
0.5min	**0,5-1**	67,30	55,77	19,21
0.5min	**0,5-2**	68,17	49,48	19,04
0.5min	**0,5-3**	72,22	59,18	20,74
1min	**1-1**	68,82	51,76	19,74
1min	**1-2**	69,84	57,68	21,07
1min	**1-3**	67,34	55,08	20,44
5min	**5-1**	67,91	50,88	20,20
5min	**5-2**	68,89	56,29	20,94
5min	**5-3**	65,13	49,36	19,34
10min	**10-1**	71,99	59,25	22,46
10min	**10-2**	74,08	61,48	22,61
10min	**10-3**	67,78	50,47	18,75
Cold control	**CC-1**	67,80	48,37	17,60
Cold control	**CC-2**	66,06	55,23	19,36
Cold control	**CC-3**	66,98	55,95	20,54
Hot control	**HC-1**	73,50	58,42	19,74
Hot control	**HC-2**	68,30	54,11	18,53

Appendix II – Statistical Analysis

```
# Hot and cold control Ultrasonicated yield comparison:

Controls_US <- read_excel("C:/Users/elalu/Documents/Examensarbete
/R/Controls_US.xlsx")
Controls_US_results <- aov(values ~ ins, data = Controls_US)
summary(Controls_US_results)

##             Df Sum Sq Mean Sq F value Pr(>F)
## ins          1 2.1007  2.1007   36.93  0.026 *
## Residuals    2 0.1138  0.0569
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1


# Hot and cold control DeStarched yield comparison:

Controls_DS <- read_excel("C:/Users/elalu/Documents/Examensarbete
/R/Controls_DS.xlsx")
Controls_DS_results <- aov(values ~ ins, data = Controls_DS)
summary(Controls_DS_results)

##             Df Sum Sq Mean Sq F value Pr(>F)
## ins          1  5.293   5.293    1.05  0.413
## Residuals    2 10.084   5.042


# Hot and cold control DeLignified yield comparison:

Controls_DL <- read_excel("C:/Users/elalu/Documents/Examensarbete
/R/Controls_DL.xlsx")
Controls_DL_results <- aov(values ~ ins, data = Controls_DL)
summary(Controls_DL_results)

##             Df Sum Sq Mean Sq F value Pr(>F)
## ins          1  6.948   6.948   0.697  0.492
## Residuals    2 19.941   9.971


# Hot and cold control extracted AX yield comparison:

Controls_AE <- read_excel("C:/Users/elalu/Documents/Examensarbete
```

```
/R/Controls_AE.xlsx")
Controls_AE_results <- aov(values ~ ins, data = Controls_AE)
summary(Controls_AE_results)
```

```
##             Df Sum Sq Mean Sq F value Pr(>F)
## ins          1 0.0725  0.0725   0.084  0.799
## Residuals    2 1.7230  0.8615
```

```
# Hot and cold control arabinose % comparison:

Controls_Ara <- read_excel("C:/Users/elalu/Documents/Examensarbet
e/R/Controls_Ara.xlsx")
Controls_Ara_results <- aov(values ~ ins, data = Controls_Ara)
summary(Controls_Ara_results)
```

```
##             Df Sum Sq Mean Sq F value Pr(>F)
## ins          1  5.123   5.123   1.402  0.322
## Residuals    3 10.961   3.654
```

```
# Hot and cold control galactose % comparison:

Controls_Gal <- read_excel("C:/Users/elalu/Documents/Examensarbet
e/R/Controls_Gal.xlsx")
Controls_Gal_results <- aov(values ~ ins, data = Controls_Gal)
summary(Controls_Gal_results)
```

```
##             Df  Sum Sq Mean Sq F value Pr(>F)
## ins          1 0.01953 0.01953   1.621  0.293
## Residuals    3 0.03615 0.01205
```

```
# Hot and cold control glucose % comparison:

Controls_Glu <- read_excel("C:/Users/elalu/Documents/Examensarbet
e/R/Controls_Glu.xlsx")
Controls_Glu_results <- aov(values ~ ins, data = Controls_Glu)
summary(Controls_Glu_results)
```

```
##             Df Sum Sq Mean Sq F value Pr(>F)
## ins          1 0.1837 0.18368   2.136   0.24
## Residuals    3 0.2579 0.08598
```

```
#Hot and cold control xylose % comparison:

Controls_Xyl <- read_excel("C:/Users/elalu/Documents/Examensarbet
e/R/Controls_Xyl.xlsx")
```

```
Controls_Xyl_results <- aov(values ~ ins, data = Controls_Xyl)
summary(Controls_Xyl_results)
```

```
##             Df Sum Sq Mean Sq F value Pr(>F)
## ins          1  0.562  0.5623   0.184  0.697
## Residuals    3  9.159  3.0529
```

```
# Solid yield of destarched supernatant

yield_DS <- read_excel("C:/Users/elalu/Documents/Examensarbete/R/
yield_DS.xlsx")
options(contrast = c("contr.sum", "contr.sum"))
yield_DS1 <- lm(values ~ ins, data=yield_DS)
Anova(yield_DS1, type = "III", ddf = "Kenward-Roger")
```

```
## Anova Table (Type III tests)
##
## Response: values
##             Sum Sq Df   F value Pr(>F)
## (Intercept) 3.9214  1 7613.9774 <2e-16 ***
## ins         0.0016  1    3.0291 0.1054
## Residuals   0.0067 13
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
summary(yield_DS1)
```

```
##
## Call:
## lm(formula = values ~ ins, data = yield_DS)
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.040156 -0.010263 -0.002537  0.011242  0.042896
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept) 6.780e-01  7.770e-03   87.26   <2e-16 ***
## ins         4.485e-05  2.577e-05    1.74    0.105
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.02269 on 13 degrees of freedom
## Multiple R-squared:  0.189,  Adjusted R-squared:  0.1266
## F-statistic: 3.029 on 1 and 13 DF,  p-value: 0.1054
```

```
means.yield_DS <- emmeans(yield_DS1, ~ ins)
summary(means.yield_DS)
```

```
##  ins emmean      SE df lower.CL upper.CL
##  198  0.687 0.00586 13    0.674      0.7
```

```
##
## Confidence level used: 0.95

print(yield_DS1, correlation = TRUE)

##
## Call:
## lm(formula = values ~ ins, data = yield_DS)
##
## Coefficients:
## (Intercept)          ins
##   6.780e-01    4.485e-05



# Solid yield of delignified supernatant

yield_DL <- read_excel("C:/Users/elalu/Documents/Examensarbete/R/
yield_DL.xlsx")
options(contrast = c("contr.sum", "contr.sum"))
yield_DL1 <- lm(values ~ ins, data=yield_DL)
Anova(yield_DL1, type = "III", ddf = "Kenward-Roger")

## Anova Table (Type III tests)
##
## Response: values
##              Sum Sq Df   F value    Pr(>F)
## (Intercept) 2.45836  1 1412.4018 1.198e-14 ***
## ins         0.00106  1    0.6106    0.4486
## Residuals   0.02263 13
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

summary(yield_DL1)

##
## Call:
## lm(formula = values ~ ins, data = yield_DL)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.05437 -0.04111  0.01492  0.02810  0.05575
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept) 5.368e-01  1.428e-02  37.582  1.2e-14 ***
## ins         3.702e-05  4.738e-05   0.781    0.449
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.04172 on 13 degrees of freedom
## Multiple R-squared:  0.04486,    Adjusted R-squared:  -0.02861
## F-statistic: 0.6106 on 1 and 13 DF,  p-value: 0.4486
```

```r
means.yield_DL <- emmeans(yield_DL1, ~ ins)
summary(means.yield_DL)
```

```
##  ins emmean     SE df lower.CL upper.CL
##  198  0.544 0.0108 13    0.521    0.567
##
## Confidence level used: 0.95
```

```r
print(yield_DL1, correlation = TRUE)
```

```
##
## Call:
## lm(formula = values ~ ins, data = yield_DL)
##
## Coefficients:
## (Intercept)          ins
##   5.368e-01    3.702e-05
```

```r
# Total solid yield after the alkaline extraction

AX_yield <- read_excel("C:/Users/elalu/Documents/Examensarbete/R/
AX_yield.xlsx")
"AX_yield$ins <- as.factor(AX_yield$ins)"
```

```
## [1] "AX_yield$ins <- as.factor(AX_yield$ins)"
```

```r
options(contrast = c("contr.sum", "contr.sum"))
AX_yield1 <- lm(values ~ ins, data=AX_yield)
Anova(AX_yield1, type = "III", ddf = "Kenward-Roger")
```

```
## Anova Table (Type III tests)
##
## Response: values
##             Sum Sq Df   F value    Pr(>F)
## (Intercept) 3279.0  1 2143.0617 8.121e-16 ***
## ins            5.5  1    3.6239   0.07933 .
## Residuals     19.9 13
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
summary(AX_yield)
```

```
##       ins          values
##  Min.   :  0   Min.   :17.60
##  1st Qu.: 30   1st Qu.:19.27
##  Median : 60   Median :20.20
##  Mean   :198   Mean   :20.13
##  3rd Qu.:300   3rd Qu.:20.84
##  Max.   :600   Max.   :22.61
```

```r
means.AX_yield <- emmeans(AX_yield1, ~ ins)
summary(means.AX_yield)
```

```
##  ins emmean    SE df lower.CL upper.CL
##  198   20.1 0.319 13     19.4     20.8
##
## Confidence level used: 0.95

print(AX_yield1, correlation = TRUE)

##
## Call:
## lm(formula = values ~ ins, data = AX_yield)
##
## Coefficients:
## (Intercept)          ins
##   19.605523     0.002674



# Arabinose/xylose ratio in the different treatments after the al
kaline extraction

AX_ratio <- read_excel("C:/Users/elalu/Documents/Examensarbete/R/
AX_ratio.xlsx")
"AX_ratio$ins <- as.factor(AX_ratio$ins)"

## [1] "AX_ratio$ins <- as.factor(AX_ratio$ins)"

options(contrast = c("contr.sum", "contr.sum"))
AX_ratio1 <- lm(values ~ ins, data=AX_ratio)
Anova(AX_ratio1, type = "III", ddf = "Kenward-Roger")

## Anova Table (Type III tests)
##
## Response: values
##             Sum Sq Df   F value Pr(>F)
## (Intercept) 10.589  1 13816.138 <2e-16 ***
## ins          0.000  1     0.006 0.9392
## Residuals    0.010 13
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

summary(AX_ratio1)

##
## Call:
## lm(formula = values ~ ins, data = AX_ratio)
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.035591 -0.021627 -0.002235  0.017266  0.049639
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept) 1.114e+00  9.479e-03 117.542   <2e-16 ***
## ins         2.444e-06  3.144e-05   0.078    0.939
```

```
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.02768 on 13 degrees of freedom
## Multiple R-squared:  0.0004646,  Adjusted R-squared:  -0.07642
## F-statistic: 0.006043 on 1 and 13 DF,  p-value: 0.9392

means.AX_ratio <- emmeans(AX_ratio1, ~ ins)
summary(means.AX_ratio)

##  ins emmean      SE df lower.CL upper.CL
##  198   1.11 0.00715 13      1.1     1.13
##
## Confidence level used: 0.95

print(AX_ratio1, correlation = TRUE)

##
## Call:
## lm(formula = values ~ ins, data = AX_ratio)
##
## Coefficients:
## (Intercept)          ins
##   1.114e+00    2.444e-06



# Total carbohydrate content of the extracted AX

tot_carbs <- read_excel("C:/Users/elalu/Documents/Examensarbete/R
/tot_carbs.xlsx")
"tot_carbs$ins <- as.factor(tot_carbs$ins)"

## [1] "tot_carbs$ins <- as.factor(tot_carbs$ins)"

options(contrast = c("contr.sum", "contr.sum"))
tot_carbs1 <- lm(values ~ ins, data=tot_carbs)
Anova(tot_carbs1, type = "III", ddf = "Kenward-Roger")

## Anova Table (Type III tests)
##
## Response: values
##              Sum Sq Df  F value    Pr(>F)
## (Intercept) 2641825  1 126.9060 3.465e-06 ***
## ins           36485  1   1.7527    0.2221
## Residuals    166537  8
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

summary(tot_carbs)

##       ins          values
##  Min.   :  0   Min.   :497.2
##  1st Qu.: 30   1st Qu.:539.5
```

```
##  Median : 60   Median :563.4
##  Mean   :198   Mean   :629.0
##  3rd Qu.:300   3rd Qu.:643.3
##  Max.   :600   Max.   :918.5

means.tot_carbs <- emmeans(tot_carbs1, ~ ins)
summary(means.tot_carbs)

##  ins emmean   SE df lower.CL upper.CL
##  198    629 45.6  8      524      734
##
## Confidence level used: 0.95

print(tot_carbs1, correlation = TRUE)

##
## Call:
## lm(formula = values ~ ins, data = tot_carbs)
##
## Coefficients:
## (Intercept)          ins
##    681.5622      -0.2657



# Amount of AX purity(%) of the extracted AX

AX_purity <- read_excel("C:/Users/elalu/Documents/Examensarbete/R
/AX_purity.xlsx")
"AX_purity$ins <- as.factor(AX_purity$ins)"

## [1] "AX_purity$ins <- as.factor(AX_purity$ins)"

options(contrast = c("contr.sum", "contr.sum"))
AX_purity1 <- lm(values ~ ins, data=AX_purity)
Anova(AX_purity1, type = "III", ddf = "Kenward-Roger")

## Anova Table (Type III tests)
##
## Response: values
##             Sum Sq Df    F value Pr(>F)
## (Intercept) 4.9620  1 2.4609e+05 <2e-16 ***
## ins         0.0000  1 9.0270e-01 0.3699
## Residuals   0.0002  8
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

summary(AX_purity)

##       ins          values
##  Min.   :  0   Min.   :0.9274
##  1st Qu.: 30   1st Qu.:0.9290
##  Median : 60   Median :0.9324
##  Mean   :198   Mean   :0.9329
```

```
##  3rd Qu.:300   3rd Qu.:0.9363
##  Max.   :600   Max.   :0.9397

means.AX_purity <- emmeans(AX_purity1, ~ ins)
summary(means.AX_purity)

##  ins emmean      SE df lower.CL upper.CL
##  198 0.9329 0.00142  8   0.9296   0.9362
##
## Confidence level used: 0.95

print(AX_purity1, correlation = TRUE)

##
## Call:
## lm(formula = values ~ ins, data = AX_purity)
##
## Coefficients:
## (Intercept)          ins
##   9.341e-01   -5.934e-06



# Lignin content in extracted AX

AX_Lignin <- read_excel("C:/Users/elalu/Documents/Examensarbete/R
/AX_Lignin.xlsx")
"AX_Lignin$ins <- as.factor(AX_Lignin$ins)"

## [1] "AX_Lignin$ins <- as.factor(AX_Lignin$ins)"

options(contrast = c("contr.sum", "contr.sum"))
AX_Lignin1 <- lm(values ~ ins, data=AX_Lignin)
Anova(AX_Lignin1, type = "III", ddf = "Kenward-Roger")

## Anova Table (Type III tests)
##
## Response: values
##             Sum Sq Df  F value    Pr(>F)
## (Intercept) 166151  1 206.9288 5.481e-13 ***
## ins             63  1   0.0783    0.7822
## Residuals    18468 23
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

summary(AX_Lignin)

##       ins          values
##  Min.   :  0   Min.   : 50.85
##  1st Qu.: 30   1st Qu.: 88.16
##  Median : 60   Median :107.85
##  Mean   :198   Mean   :106.72
##  3rd Qu.:300   3rd Qu.:126.36
##  Max.   :600   Max.   :156.40
```

```r
means.AX_Lignin <- emmeans(AX_Lignin1, ~ ins)
summary(means.AX_Lignin)
```

```
##  ins emmean   SE df lower.CL upper.CL
##  198    107 5.67 23       95      118
##
## Confidence level used: 0.95
```

```r
print(AX_Lignin1, correlation = TRUE)
```

```
##
## Call:
## lm(formula = values ~ ins, data = AX_Lignin)
##
## Coefficients:
## (Intercept)          ins
##  108.102255    -0.006973
```

Could ultrasound improve the extractability of arabinoxylan in wheat?

: Louise Lundquist, 2022-01-16

About one third of all food produced end up as waste or discarded by-products. One of these by-products is wheat bran from the refined white flour production. Wheat bran is an excellent source of a dietary fibre called arabinoxylan (AX) which could be used in products as an additive to increase the dietary fibre content.

Background:

White flour products such as bread and pasta are often cheaper, more available, and liked than their whole grain counterpart, where the wheat bran is included. So, what if we could extract the AX from the wheat bran and incorporate it into food products? This would utilize the otherwise wasted wheat bran and create a more fibre rich product while remaining the white flour appearance and taste.

Source: Pixabay

To extract AX from wheat bran a high pH solution is needed, the alkaline conditions break the bonds between the AX and the bran and makes it soluble. The process leaves much to be desired and here is where ultrasound comes in. The idea is that by treating the bran with ultrasound, the bran structure will loosen up and leave more AX exposed for the alkaline extraction.

About the study:

This study is a master's thesis project in collaboration with Lantmännen and SLU. The study had two main objectives: to evaluate the alkaline extraction process and to investigate the effect ultrasound had on extraction. The ultrasound was applied as a pre-treatment of the wheat bran, the power applied was 400W during 0.5, 1, 5 and 10 minutes.

Results:

The extraction process was successful regarding both the yield and the AX quality. The ultrasound pre-treatment showed a trend to increase the yield with longer treatment times. Though it did not change the composition of the AX. Ultrasound treatment combinations of applied power and time durations are endless, and this study could only cover a small part of it. More testing is necessary to establish how effective ultrasound could be in the extraction of AX.