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Comparison of different twin-screw extraction conditions for the production of arabinoxylans

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ABSTRACT

The aim of this article is to compare two different sets of optimal conditions for twin-screw extraction of xylans and define their influence on the purification steps, combining ultrafiltration and industrial chromatography. Two xylan extracts were obtained by twin-screw extrusion of straw and bran. Condition 1 used a high straw/bran ratio (equal to 6) and high sodium hydroxide content, and condition 2 used a lower straw/bran ratio (equal to 2) and low sodium hydroxide content.

Arabinoxylan extraction yields are slightly higher for conditions with low straw content (5.1% versus 4.4%). Nevertheless, these recovery yields remain between 9% and 10%. Ultrafiltration is as efficient as evaporation for polysaccharide concentration, with lower energy consumption, but also demineralizes the solution. The combination of ultrafiltration and chromatography gives partial purification of the extract with a final arabinoxylan purity ranging from 16% to 26%. This is slightly higher than by direct precipitation, but limited because all the large molecules such as proteins and lignins were retained by ultrafiltration.

1. Introduction

Wheat bran and wheat straw are sources of cell-wall polysaccharides such as cellulose, hemicelluloses, and lignin. Arabinoxylans are the most abundant hemicellulose polymers in wheat bran. They mainly consist of a chain of D-xylopyranosyl with β-(1–4) links, substituted on position 2 and/or 3 with α-L-arabinofuranosyl residues (Spiridon & Popa, 2008). Hemicelluloses from bran have the potential to be incorporated into a wide variety of applications, such as thickeners, emulsifiers, stabilizers and binders in the food, pharmaceutical and cosmetics industries, but film-forming ability is one of their most attractive properties (Jain, Sjöstedt, & Glasser, 2000; Spiridon & Popa, 2008). Therefore, much

work has been undertaken investigating their possible fractionation.

A large range of procedures has been proposed for the extraction and purification of arabinoxylans. One of the most widespread consists of stirred extraction, with sodium hydroxide (Brillouet, Joseleau, Utille, & Lelievre, 1982; Bataillon, Mathaly, Numes Cardinali, & Duchironet, 1998; Maes & Delcour, 2001), but many others such as barium hydroxide (Bergmans, Beldman, Gruppen, & Voragenet, 1996), or dimethyl sulfoxide (Lawther, Sun, & Banks, 1995) can be used. Alkaline conditions disrupt the cell walls by hydrolyzing ester linkages between the hemicelluloses and the other cell-wall components thus dissolving these hemicelluloses, but also lignin. The liberated hemicelluloses can then be recovered in aqueous media (Fang, Sun, Salisbury, Fowler, & Tomkinson, 1999) with an extraction yield of 50% after 2 h in 1% NaOH solution (Sun, Tomkinson, Wang, & Xiao, 2000). Higher recovery yields can be obtained but with longer residence time (Chanliaud, Saulnier, & Thibault, 1995). However, other extraction technologies have been studied to improve recovery efficiency, such as steam explosion (Glasser & Wright, 1998; Montané, Farriol, Salvado, Jollez, & Chornet, 1998) or chemical extraction assisted by ultrasonication (Ebringerovà & Hromàdkovà, 2002; Hromàdkovà & Ebringerovà, 2003; Sun, Sun, Sun, & Su, 2004). Combined with the use of alkaline

agents in order to accelerate solubilization of the arabinoxylans instead of the lignin and cellulose, steam explosion is an efficient tool for monomeric sugar recovery (Sun, Sun, Xu, Geng, Fowler, 2005; Krawczyk, Persson, Andersson, & Jönsson, 2008). However, it results in hydrolytic depolymerization of hemicelluloses, which is attractive for alcoholic fermentation or xylose transformation (Puls & Saake, 2004), but not for isolation of hetero-polysaccharides (Glasser, Kaar, Jain, & Sealey, 2000).

All the treatments lead to the production of a complex extract that requires different purification steps, and alcoholic precipitation is the most useful and efficient procedure on a laboratory scale. Other purification treatments have been studied, such as microfiltration (Bataillon et al., 1998) and ultrafiltration (Jorda, Maréchal, Rigal, & Pontalier, 2002) in order to reduce the cost of the purification step. However, the production of solid arabinoxylans (in powder form) from an aqueous solution or suspension requires either a freeze–drying or a spray–drying step (Glasser et al., 2000).

Ultrafiltration, a well-established separation process in industry, was used to separate polysaccharides from co-extracted molecules, small oligosaccharides, monosaccharides and salts, in order to concentrate the solution and reduce the volume of alcohol used for precipitation (Schlesinger, Götzinger, Sixta, Friedl, & Harasek, 2006; Montané, Farriol, Salvado, Jollez, & Chornet, 1998; Nabarlatz, Torras, Garcia-Valls, & Montané, 2007; Persson & Jönsson, 2010). This is a very attractive process because it results in the partial demineralization and removal of small molecules (Glasser et al., 2000). Hemicelluloses are purified because large molecules are retained and low molecular mass solutes pass through the membrane. However, in some cases, coloration of the extract remains too dark after the ultrafiltration treatment, producing a dark-brown arabinoxylan powder, therefore anion-exchange chromatography can be combined with ultrafiltration to improve purification prior to powder recovery (Glasser et al., 2000; Andersson, Persson, Zacchi, Stalbrand, & Jönsson, 2007).

Nonetheless, even if only a few studies have dealt with pilot scale extraction of hemicelluloses (Annison, Choct, & Cheethamet, 1992; Bataillon et al., 1998; Zeitoun, Pontalier, Maréchal, & Rigal, 2010; Montané, Farriol, Salvado, Jollez, & Chornet, 1998), it needs to be developed on an industrial scale (Puls & Saake, 2004). As twin-screw extrusion is a continuous process, which could be scaled up to an industrial scale, it was investigated for straw/bran co-extraction using sodium hydroxide. This technique combines chemical, thermal and mechanical actions to remove the dissolved molecules from the plant tissue by continuous squeezing in a screw press (Zeitoun et al., 2010), and it has proved to be an efficient method for alkaline extraction of hemicelluloses from wheat bran (Maréchal, Jorda, Pontalier, & Rigal, 2003). It gives lower extraction yields and selectivity than batch extraction but has the advantage of being continuous and requiring smaller quantities of reagents and water (Montané et al., 1998; Maréchal et al., 2003). The extrusion process has been studied on a laboratory scale, using an experimental design, covering the influence of the straw/bran ratio, the bran/sodium hydroxide ratio and screw rotation speed. The results showed that two optimal extraction conditions exist, the first with a high straw/bran ratio (equal to 6) and high sodium hydroxide content, and the second with a low straw/bran ratio (equal to 2) and low sodium hydroxide content (Maréchal et al., 2003), but the comparison was only made using the recovery yield with alcoholic precipitation.

Twin-screw fractionation leads to the production of very complex solutions containing the biopolymers, but also their hydrolyzed products and co-extracted molecules (proteins, lignin, and inorganic salts). Hence, it is important to develop efficient purification steps regarding efficiency, cost and environmental impact (Jorda et al., 2002). For example, precipitation of the hemicellulose alkaline extract using acids and alcohols to obtain a certain
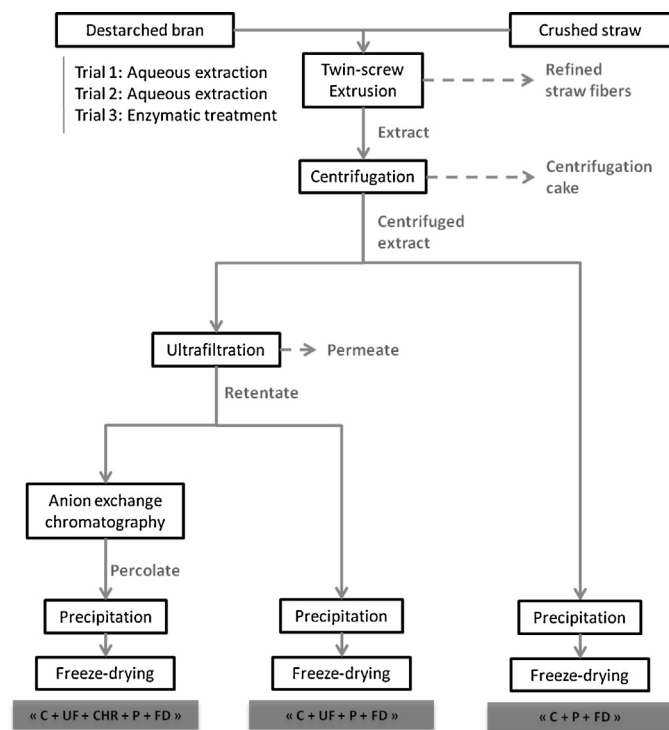
Fig. 1. Schematic representation of the processes. C—centrifugation; UF—ultrafiltration; CHR—chromatography; P—precipitation; FD—freeze drying.

degree of purification, remains expensive because it requires several volumes of alcohol for one volume of solution. Thus, work is needed to devise more cost-effective separation methods.

In this study, extraction has been performed on a larger scale (a few hundred kilos), using the first set of optimal extraction conditions above, in order to evaluate the performance of the purification process, including ultrafiltration and anion exchange chromatography. Thus, the aim of the present work is to compare both optimal extraction conditions on a larger scale and define their influence on the efficiency of the refining procedure.

2. Materials and methods

2.1. General description of the process

Two sets of large-scale optimal extrusion conditions were evaluated in order to produce a large volume of extract. Each extract was refined using three refining pathways (Fig. 1) in order to study the influence of the extraction conditions on purification performance. The three pathways were:

- fractionation of straw and bran and direct extract precipitation: ("C + P + FD");
- extract precipitation after concentration by ultrafiltration: ("C + UF + P + FD"); and
- as above but with an ion exchange chromatography purification step: ("C + UF + CHR + P + FD").

In addition, because the destarching step was derived from a laboratory scale protocol, it also had to be adapted, and so three different procedures have been tested, as shown below.

Table 1
Extrusion conditions.

	Conditions used for trial 1	Conditions used for trials 2 and 3
Bran/NaOH	2	7
Straw/bran	6.2	2
Liquid/solid	5.9	9.4
Screw rotation speed (rpm)	150	150
Wash water flow (kg/h)	92	92
Induction belt temperature (°C)	50	50

2.2. Extraction process

2.2.1. Raw material pre-treatments

Wheat straw was crushed before extrusion with a hammer-mill (Electra BC-P) fitted with a 6 mm-screen. However, because it contains around 20% starch, which could hinder subsequent extraction and purification steps, the bran had to be destarched.

Starch was removed using two different procedures:

– Three hot water extractions by mixing 15 kg of dry bran with 150 L of water at 40 °C for 15 min. The liquid/solid separation was made either by aspiration of the liquid from the top (trial 1) or by bottom filtration (trial 2).
– Enzymatic hydrolysis using BAN 480L (Novozyme, Denmark), 1 h stirring at 60 °C followed by water washing for 15 min at 40 °C by top filtration (trial 3).

For each procedure, the bran was then dried at 45 °C for 24 h. All wheat bran and straw was from the same origin (Arteris, France).

2.2.2. Twin screw extraction

Experiments were conducted using a co-penetrating and co-rotating twin-screw extruder (Clextral BC45) (Fig. 2).

The extruder was composed of a 1.4 m-long barrel containing seven dedicated profile modules for alkaline treatment of plant matter. Modules C, D, E and G were heated using induction belts (5 kW). There were two reverse-pitch screw elements with grooved peripheral slots in the screw for leakage flow, and a series of 1 cm-long neutral pitch bilobal elements splayed at 90 degrees to each other, were used to knead the plant matter. The 10 cm-long filter element mounted on the last section, was perforated with conical holes (1 mm inlet, 2 mm outlet) and was used to optimize solid/liquid separation (Zeitoun et al., 2010). One hour before extrusion, wheat bran and sodium hydroxide were blended at room temperature, giving a liquid/solid ratio of 7, adjusted to 10 just before the extrusion. The mixture was injected into the extruder in section B, using an exocentric-screw pump (Nemo, 2NE40A). Straw was also introduced in section A using a screw feed, and mixed with the alkaline dough in the first zone of the barrel through the neutral pitch element and the reverse-pitch screw element successively. Wash water was injected downstream of this zone (E) by piston pump (Clextral, DKM K20-2-P32), and the mixture was carried through the second reverse pitch element located just downstream of the filtration module. Screw rotation speed and barrel temperature were monitored from a control panel. The extract was collected and kept in a cold room before further processing, while the refined cellulosic fibres were collected at the barrel outlet.

Optimal conditions for extrusion concerning hemicelluloses yields and purity, have been defined in previous studies (Maréchal, Jorda, Pontalier, & Rigal, 2004) and are summed up in Table 1.

2.2.3. Liquid–solid separation

Clarification of the extract from suspended matter that could hinder the subsequent concentration step, was performed using a continuous centrifuge separator (Alpha-Laval, CLARA20 LAPX)

equipped with 400 μm spaced discs. The extract was introduced continuously at a flow rate of 100 L/h via an impeller pump (Schneider, Reform B-FU). Clarification was at $11,130 \times g$, and separated sludge was drawn off every 4 min for trials 1 and 3 and every 5 min for trial 2.

2.3. Purification process

2.3.1. Ultrafiltration

The ultrafiltration (UF) membrane used had a molecular weight cut-off (MWCO) of 30 kDa and a surface area of 1.15 m². The 52 cm long, 5.4 cm external diameter module was composed of 1300, 0.5 mm-diameter polyethersulfone hollow fibres, (GE Healthcare, UFP-30-C9A). This membrane was selected in the light of previous results (Zeitoun et al., 2010) as discussed in Section 3. The filtration apparatus used a 36 L capacity feed reservoir, a rheostat controlled bilobal-pump, a filtration cartridge and a pressure gauge allowing manual membrane pressure adjustment. Transmembrane pressure (TMP), the average of the inlet and outlet membrane module pressures, was measured using manometers.

In order to determine the influence of transmembrane pressure and circulation flow rate on the permeate flux and define optimal operating conditions for extract concentration, ultrafiltration was first carried out by recycling both permeate and retentate flux in the feed tank, to maintain a constant concentration.

The extract was then concentrated by extracting the permeate flux, while still recycling the retentate flux in the feed tank. The permeate flow rate was measured gravimetrically using an electronic balance. The volume reduction factor (VRF) was evaluated by taking the extract density as equivalent to that of water.

Membrane cleaning used the following procedure:

– demineralized water rinse for 30 min;
– cleaning with 1 N sodium hydroxide solution for 1 h at 40 °C; and
– demineralized water rinsing until pH 7 obtained.

The concentration of the whole extract was made with the same membrane, in 20 L batches, and there were 5 batches for each trial. A new membrane was used for each of these trials.

2.3.2. Anion-exchange chromatography

Chromatography was performed with a strong anion-exchange resin (Rohm and Haas, Amberlite IRA958-Cl) on 12.5 L batches. 2 L of resin was stirred with the extract for 1 h and separation between extract and resin performed by Büchner-filtration. Before use, the resin was degassed for 24 h in 6 L demineralized water, and after use it was reconditioned for 30 min using deionized water, and regenerated with 5 L of 2% sodium hydroxide solution.

2.4. Powder recovery

2.4.1. Alcoholic precipitation

Hemicelluloses were recovered at different stages of the process by precipitating the alkaline extracts with acid and alcohol. Acetic acid was added to the extract up to pH 5.5, followed by 3 volumes of ethanol for 1 volume of solution. To improve precipitation, the solution was stored at 4 °C overnight, and aggregated hemicelluloses were then recovered, by wringing out the simple filtration cloth, and drying to obtain powders.

2.4.2. Freeze-drying

Hemicellulosic precipitates were freeze-dried in all cases (Cryo Rivoire, PILOT27). Samples were placed in aluminium trays and cooled to −40 °C. Decreasing the pressure to −300 mbar caused sublimation of moisture content which then froze in a cold-trap.
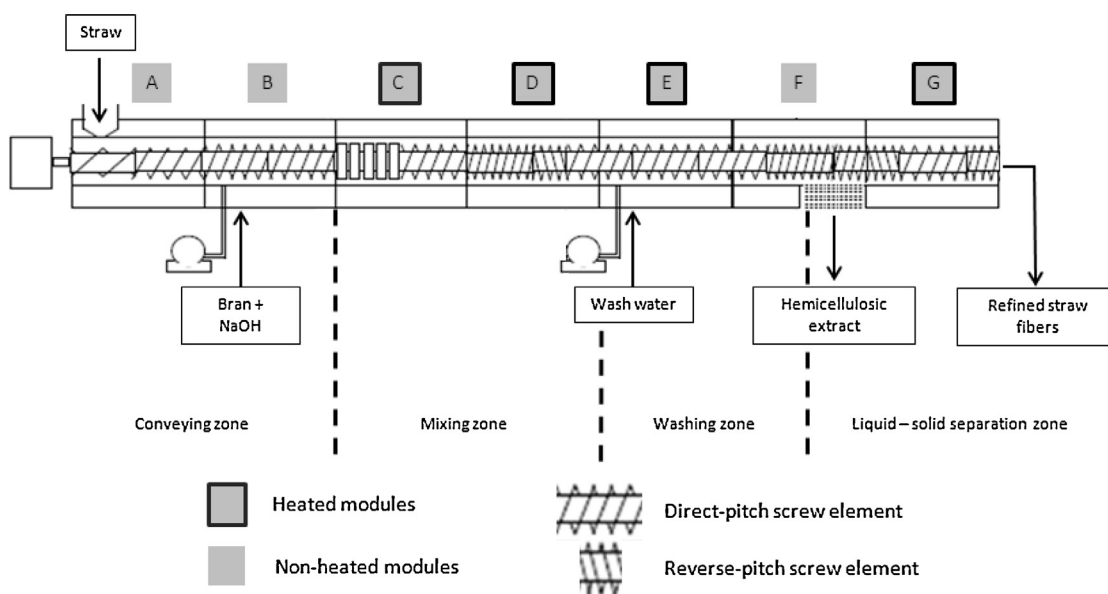
Fig. 2. Twin-screw extruder configurations for the alkaline treatment of plant matter.

Finally, the temperature was gradually increased to 20 °C and the dried samples recovered.

2.5. Analytical

Starch in bran quantification before and after destarching was made with a total starch assay kit using thermostable α-amylase and amyloglucosidase (Megazyme, Ireland). Dry matter was gravimetrically determined at 105 °C over a period of 24 h, and ash content was determined by thermogravimetric analysis after incineration at 550 °C for 5 h. An estimation of the three cell-wall components contained in the solid fraction (cellulose, hemicelluloses, and lignins), was made using the ADF–NDF method of Van Soest and Wine (1967, 1968). Lignin composition in powders was measured using the Tapi norm T222 om 88 for acid-insoluble lignin. Matter treated with sulfuric acid was filtered on Wattman pre-weighed glass-fibre filters, washed with deionized water, and dried at 105 °C overnight before being weighed. Proteins were measured on a Kjeldahl automated device. The specific factor (5.7) used for the conversion of nitrogen content to protein content, uses the average molecular weight of amino acids in the bran endosperm compared to the molar mass of nitrogen (Sosulski & Imafidon, 1990). Individual neutral sugars were analyzed by gas-liquid chromatography (GLC) after hydrolysis (2 h in 1 M H_2SO_4 at 100 °C), and conversion of individual sugars into aldidol acetates (Englyst & Cummings, 1988). A prehydrolysis step in concentrated sulfuric acid (72% w/v) was carried out for 30 min at 25 °C on the fresh bran and straw. All determinations were carried out in triplicate.

3. Results and discussion

3.1. Raw material

For the first two trials, starch was removed by water washing. For trial 1, filtration was done from the top of the tank by pumping the starch milk through an external loop, while for trial 2, water was removed by filtration through the bottom of the tank. The bran used for trial 3 was destarched using enzymes. The starch composition and amount of destarching for each batch of bran are shown in Table 2. The decrease in level of destarching from 70.7% to 46.5% between trial 1 and 2 indicates that during filtration through

the bottom of the tank, the bran cake has retained a part of the starch molecules in suspension in the wash water. Moreover, the high level of destarching observed in trial 3 shows that enzymatic hydrolysis is much more efficient, leading to a starch content of 2.5% of the dry matter weight.

Analysis of the main fractions of destarched bran allows characterization of at least 90% of the dry matter.

Bran 1 is slightly different from bran 2 and 3, especially for mineral content, which is lower (around 3% for bran 1 and around 5% for bran 2 and 3) and proteins (around 14% for bran 1 and around 20% for bran 2 and 3). This is due to the fact that bran 1 came from a different batch of the same production. Cellulose and lignin contents obtained using Van Soest's characterization are equivalent, whereas a significant difference between bran 2 and bran 3 can be seen for hemicelluloses content. However, this fraction seems to be overvalued for bran 3 compared to the gas chromatography analysis, making the total carbohydrate obtained by gas chromatography appear to be lower than the hemicellulose content evaluated

Table 2
Detailed composition of destarched bran.

	Bran, trial 1	Bran, trial 2	Bran, trial 3
Dry matter (%)	93.4	96.1	95.6
Ash (wt% dry matter)	3.1	4.9	5.2
Organic matter (wt% dry matter)	96.9	95.1	94.8
Starch (wt% dry matter)	10.9	9.9	2.5
Destarching percentage (%)	70.7	46.5	86.5
Protein (wt% dry matter)	14.1	20.0	23.0
Cellulose (wt% dry matter)	15	15	14
Hemicelluloses (wt% dry matter)	51	52	57
Lignin (wt% dry matter)	6	7	5
Total carbohydrates (wt% dry matter)	44.2	51.6	45.6
Arabinose	10.4	12.6	10.9
Xylose	18.7	22	17.4
Mannose	0.5	0.6	0.3
Galactose	1.0	1.3	1.0
Glucose	13.6	15.1	16.0
X/A[a]	1.8	1.7	1.6
X/G[a]	1.4	1.5	1.1

[a] X/A is the ratio between xylose and arabinose contents, X/G is the ratio between xylose and glucose contents.

Table 3
Comparison between the refined straw fibres and the initial straw.

	Straw	Refined straw fibres 1	Refined straw fibres 2	Refined straw fibres 3
Dry matter (%)	91.5	34.5	21.5	21.4
Ash (wt% dry matter)	8.1	3.3	8.3	8.5
Organic matter (wt% dry matter)	91.9	96.7	91.7	91.5
Protein (wt% dry matter)	2.1	2.2	3.7	6.2
Cellulose (wt% dry matter)	39	29.8	37.8	33.8
Hemicelluloses (wt% dry matter)	32	27.6	24.4	36.1
Lignin (wt% dry matter)	9	19.8	7.8	6.9

gravimetrically. Therefore, as bran contains a low proportion of cellulose and because Van Soest's method is more accurate for raw and fibrous materials, only the gas chromatography analysis results will be used for the rest of this article.

Starch has been analyzed using a total starch assay procedure with megazyne on bran, before and after the destarching treatment. In Table 2, it appears that the destarching yield is better under condition 1 than condition 2, and even though the two brans were not from the same batch and did not contain the same amount of starch initially, the amount of starch remaining in the bran is very similar (10%). Nevertheless, it can be assumed that in the second case, starch has been solubilized and then entrapped in the bran during the bottom filtration. Thus its recovery was higher during extrusion, and the X/G ratio confirms this hypothesis since it is far higher in condition 1 (0.8) than in condition 2 (0.2). The X/G ratio can thus be used as an indicator of starch contamination in the extract. Further analyses (data from another partner not shown) on the final powder also confirm this hypothesis.

3.2. Extraction

3.2.1. Composition of refined straw fibres

It has been shown in previous studies that straw is mainly added to improve liquid–solid separation, and so the source of extracted molecules is mainly the bran, pre-soaked in soda 1 h before extrusion, (Zeitoun et al., 2010; Jacquemin, Zeitoun, Sablayrolles, Pontalier, & Rigal, 2012). However, after extraction, slight differences can be observed between the composition of the straw and the refined straw fibres produced during extrusion (Table 3). These can be explained by the thermo-mechanical and chemical treatments applied simultaneously to the straw and bran, which lead to the extraction of a few compounds from straw (hemicelluloses) whereas some bran compounds remain trapped in the refined straw fibres (proteins).

Conditions used for trial 1 involved a high straw/bran ratio, a high sodium hydroxide concentration and a low liquid/solid ratio, whereas those for trials 2 and 3 had a lower straw/bran ratio, a lower sodium hydroxide concentration and a higher

liquid/solid ratio. All the trials were performed with low mechanical action (slow screw rotation speed, 150 rpm). For trial 1, the high straw/bran ratio combined with a low liquid/solid ratio increases the mechanical action on the raw material, despite the slow screw rotation speed. This results in better liquid–solid separation at the outlet of the extruder and gives a higher dry matter content of refined straw fibres (34.5% versus 21.5%). The use of a lower straw/bran ratio combined with a lower sodium hydroxide concentration for trials 2 and 3, means that more bran compounds remain entrapped in the refined straw fibres implying higher proteins and hemicelluloses in these. The less effective liquid–solid separation also means that the refined straw fibres have a higher moisture and a higher mineral content. Thus, despite using less sodium hydroxide for extraction in trials 2 and 3, a higher mineral content is found in the residue, compared to trial 1.

3.2.2. Extracts and filtration cake compositions

Table 4 shows the composition of extracts obtained just after extrusion, and the extract and centrifugation cake obtained after centrifugation.

Two extraction conditions were evaluated, the first with a low liquid/solid ratio and a high straw/bran ratio and the second, with a higher liquid/solid ratio and a lower straw/bran ratio favouring chemical action, and thus higher solubilization of the hemicelluloses. There is only a slight difference in the appearance of the crude extracts, but this difference increases after centrifugation which removes the non-solubilized molecules but not the solubilized ones. Mechanical action dominates compared to chemical action when extraction is carried out under extraction condition 1, and part of the extracted xylan is in the form of bran particles. This explains why there is a greater reduction in dry matter after the centrifugation step for condition 1.

The dry matter concentration of the crude extract obtained in trial 1 reached 3.1% versus 2.6% for trial 2 and 3.2% for trial 3. The lower value obtained in trial 2 compared to trial 3 may be the result of the less effective destarching step. However, under alkaline conditions at ambient temperature, starch can form a gel that could hinder the water washing of the molecules released at the

Table 4
Composition of extracts after extrusion and after centrifugation.

	Trial 1			Trial 2			Trial 3		
	Crude extract[a]	Centrifuged extract[b]	Cake[c]	Crude extract[a]	Centrifuged extract[b]	Cake[c]	Crude extract[a]	Centrifuged extract[b]	Cake[c]
Dry matter (%)	3.1	2.4	9.8	2.6	2.1	14.7	3.2	2.7	9.2
Ash (wt% dry matter)	34.6	42.9	16.4	20.3	22.9	13.6	20.9	23.8	13.7
Organic matter (wt% dry matter)	65.4	57.1	83.6	79.7	77.1	86.4	79.1	76.2	86.3
Protein (wt% dry matter)	8.5	10.3	3.9	15.4	18.7	6.4	22.9	27.5	12.1
Carbohydrate (wt% dry matter)	22.7	20.0	n.d.	31.2	32.9	n.d.	n.d.	n.d.	n.d.
X + A recovery yield[d] (%)	12.9	9.9	n.d.	12.4	9.8	n.d.	n.d	n.d.	n.d.

n.d. not determined.
[a] "Crude extract" is the extract obtained at the outlet of the extruder.
[b] "Centrifuged extract" is the extract obtained after centrifugation.
[c] "Cake" is the matter in suspension removed from the crude extract during centrifugation.
[d] Refers to X + A introduced by bran and straw.

Table 5

Composition of powders obtained after alcoholic precipitation and freeze-drying of the centrifuged extracts (C+P+FD), of the centrifuged and ultrafiltered extracts (C+UF+P+FD) and of the extracts percolated through the chromatographic resin (C+UF+CH+P+FD).

	C+P+FD			C+UF+P+FD			C+UF+CH+P+FD		
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
Weight (kg of powder/100 kg of extract)[a]	1.2	1.1	1.1	1.7	2.5	3.6	1.8	1.7	2.3
Dry matter (%)	94.1	97.2	96.5	95.5	97.4	87.2	95.4	95.6	94.9
Ash (wt% dry matter)	9.4	3.5	5.9	5.5	3.6	6.3	5.2	4.3	7.8
Organic matter (wt% dry matter)	90.6	96.5	94.1	94.5	96.4	93.7	94.8	95.7	92.2
Protein (wt% dry matter)	12.9	18.8	37.0	15.6	18.8	39.3	13.5	17.9	38.2
Lignin (wt% dry matter)	–	–	–	24.7	18.3	22.6	23.3	11.0	11.9
Total carbohydrates (wt% dry matter)	48.4	58.4	31.7	46.7	57	26.6	50.3	61.8	29.8
Arabinose	6.4	4.8	6.3	6.6	4.4	4.9	7.1	5.1	5.7
Xylose	17.8	10.1	12.5	17.6	9.9	10.4	18.8	11.0	11.5
Mannose	0.3	0.4	0.2	0.3	0.4	0.0	0.3	0.4	0.2
Galactose	1.1	0.8	1.0	1.2	0.7	0.9	1.2	0.8	1.0
Glucose	22.5	42.1	11.7	20.7	41.8	10.4	22.9	44.5	11.4
X/A[b]	2.8	2.1	2.0	2.7	2.2	2.1	2.6	2.2	2.0
X/G[b]	0.8	0.2	1.1	0.8	0.2	1.0	0.8	0.2	1.0
Dry matter yield of precipitation step (%)	38.0	38.9	38.6	53.1	61.9	87.3	56.5	57.6	53.4

[a] Mass of powder recovered from 100 kg of extract.

[b] X/A is the ratio between xylose and arabinose contents, X/G is the ratio between xylose and glucose contents.

extrusion outlet (Ring, 1985; Morris, 1990). The combined use of a low straw/bran ratio and low sodium hydroxide concentration for trials 2 and 3, gives greater protein and carbohydrate fractions recovered in the extract, and a lower proportion of ash compared to trial 1.

After centrifugation, the reduction in dry matter is 22.6% for trial 1 whereas it is only 15.6% for trial 3. This difference can be explained by the higher mechanical and chemical action applied to the plant matter during trial 1, due to the high straw content and sodium hydroxide concentration. Hence, a greater amount of suspended matter is recovered in this extract 1, and removed during centrifugation, whereas for trials 2 and 3 extracted molecules are mainly in solution. For all the trials, the proportion of proteins and ash in the extract has increased, and this is because a major part of them are solubilized and cannot be evacuated with the suspended matter in the cake. However, cake also contains some ash and proteins from the part of the extract evacuated with it during flushing. The variations in the proportion of carbohydrates in the extract are not significant, implying that part of them are probably in a non-solubilized form and evacuated with the cake.

The Xylose+Arabinose (X+A) extraction yield can be calculated by using only the X+A coming from bran, or all the X+A introduced, from both bran and straw. Taking all the X+A introduced, there is almost no difference between the conditions tested. Assuming that carbohydrates only come from bran, condition 1 gives the best results, with an extraction yield of 50% while this is only 22% for condition 2. For both conditions 1 and 2 the centrifugation recovery yield is only 50% of the X+A and this result indicates that the mechanical action of the extruder is producing particles containing xylans, which are not dissolved and so are recovered in the centrifugation cake.

The centrifugation cakes show differences in dry matter content (9.8% for trial 1, 14.7% for trial 2 and 9.2% for trial 3), that can be explained by a slight difference in flushing frequency used (every 4 min for trials 1 and 3 and every 5 min for trial 2). During centrifugation, the flush interval should be short enough to be able to push the matter out of the pipes, but not so short as to cause large losses in the extract. Optimization of this frequency is one of the main opportunities for improving the process.

3.2.3. Powder compositions

A fraction of each centrifuged extract was treated using alcoholic precipitation, and hemicellulosic powders were recovered after freeze-drying, used because it was assumed it would allow 100% recovery yield of the precipitated molecules. The composition of all these fractions was evaluated and the results are shown in Table 5.

The dry matter yields of the powder recovery steps (precipitation+freeze drying) after centrifugation are around 38.5% for all powders, which indicates that not all the solubilized compounds are precipitated and confirms that precipitation also has a purifying action (Jacquemin et al., 2012). The ash content is higher for the powder produced during trial 1 (as well as for its corresponding extract; Table 4), once again because of the larger amount of sodium hydroxide used here. The carbohydrate content, reached 58.4% in trial 2. This is a very high value, mainly due to the high glucose content (42.1%). And the origin of this glucose is starch, because the content from the powder produced using better-destarched bran under the same conditions (trial 3), is only 11.7%. Therefore, the total carbohydrate content of the powder from trial 3 is only 31.7% whereas powder from trial 1 reached 48.4% (but still with a substantial fraction of glucose, due to the less efficient destarching procedure). The X/G ratio can therefore be used as an indicator of starch contamination in the extract. Since it is low in extract 2, it can be supposed that this contains a larger amount of starch than extract 1 with a higher X/G ratio. Further analysis (data from another partner not shown) of the final powder also confirms this hypothesis.

The proportion of proteins in powders is consistent with previous results. Nonetheless, because less bran was used in trial 1, less protein was recovered in the extract and thus their content in the powders is lower (12.9%). There are two reasons for the smaller proportion of proteins in trial 2 compared with trial 1 (respectively, 37.0% and 18.0%). The first is that the gel formed by starch during extrusion had probably hindered their extraction. The second is that because the proportion of carbohydrate is higher, the relative proportion of proteins is lower.

The content in arabinoxylans (X+A) of trial 1 powder is 24.2% while it is only 14.9% and 18.8% for those from trials 2 and 3, respectively. This can be explained by the presence of more glucose for trial 2 and more proteins for trial 3. The X/A ratio is slightly higher for trial 1 (2.8) which confirms that a part of the arabinoxylans from the straw (that have an X/A ratio around 9; Jacquemin et al., 2012) are also extracted due to the higher mechanical action applied during this experiment.
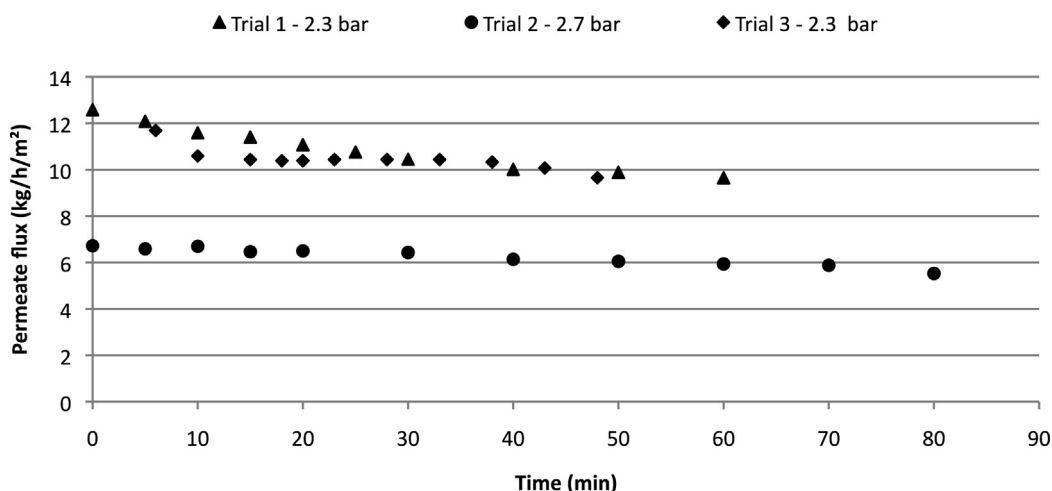
▲ Trial 1 - 2.3 bar ● Trial 2 - 2.7 bar ◆ Trial 3 - 2.3 bar

Fig. 3. Evolution of the permeate flux during concentration of the extract by a volume reduction factor of 2. All trials were conducted at a tangential flow rate of 0.23 m/s (210 kg/h) at a temperature of 20 °C.

3.3. Concentration and purification

3.3.1. Ultrafiltration

Alcoholic precipitation is the most widely used technique for recovery of hemicelluloses contained in alkaline extracts on a laboratory scale. It is a selective technique, which separates large molecules (proteins, polysaccharides) from small molecules (salts, monosaccharides). However, it requires the addition of at least 3 volumes of ethanol per volume of extract, meaning that it is expensive and has been singled out as the main restriction to the development of hemicellulose production on an industrial scale. Thus, the combination of ultrafiltration and anion-exchange chromatography has been investigated in order to define how it could be introduced into the process to replace precipitation (Jacquemin et al., 2012). Optimal conditions for filtration, defined in a previous work, showed that the best results were obtained with a 30 kDa membrane. The polarization layer that appears during ultrafiltration, changes the filtering properties of the membrane and leads to a high rejection rate of the hemicelluloses. (Zeitoun et al., 2010). Concentration of the different extracts was performed with a transmembrane pressure of 2.3 bar and a circulation flow rate of 0.23 m/s (210 kg/h). During ultrafiltration of the trial 2 extract, it was impossible to apply the same filtration conditions as for the other extracts, because the permeate flux was too low. Therefore filtration was performed with a higher transmembrane pressure. Despite this increase, the permeate flux for trial 2 extract remained below those obtained with the two other conditions (Fig. 3). For trials 1 and 3, the flux decreases from 13 to 9 kg/h/m² while for trial 2, it decreases from 7 to 5 kg/h/m². This difference can be explained by the gelling properties of the starch contained in the extract, which increased its viscosity, assuming that the high value of carbohydrate in the extract of trial 2 (Table 4) is linked to high starch content.

After ultrafiltration, a part of the extract was precipitated in order to analyse the effect of the sequences of operations on the powder composition. Results are presented in Table 5.

For powder content, comparing data from Table 5 shows a slight decrease in carbohydrate while proteins increase. The amount of arabinoxylans (X+A) and proteins recovered from 100 kg of extract after centrifugation is 0.16 kg and 0.20 kg, respectively, for trial 2. This 100 kg of extract produced 50 kg of retentate, leading to the production of 0.16 kg of arabinoxylans and 0.23 kg of proteins. It can be concluded from this result

that ultrafiltration of extract from trial 2 implies a very low loss in protein and arabinoxylans. The results are very similar for trial 3 but the recovery yields are lower than for trial 1.

It has been shown in previous studies that ultrafiltration is an efficient tool for polymer concentration and for the elimination of ash and small molecules (Jacquemin et al., 2012). However, the demineralization effect is not visible in these results because alcoholic precipitation also eliminates ash. One interesting result is the yield in dry matter of the precipitation step after ultrafiltration. This increased from 38.0% to 53.1% for trial 1, from 38.9% to 61.9% for trial 2 and from 38.6% to 87.3% for trial 3. Two reasons can be put forward to explain this result. The first is that purification by ultrafiltration removes molecules that hinder the precipitation. The second is that higher solute concentrations increase the efficiency of the precipitation step. Which could explain why for trial 3, more proteins are recovered by precipitation of retentate than by precipitation of the extract before ultrafiltration.

It can be assumed that the decrease in carbohydrates after ultrafiltration is mainly due to elimination of small molecules and monosaccharides, while the polymers are retained.

3.3.2. Ion exchange chromatography

Anion exchange chromatography has been evaluated for the removal of the coloured molecules, lignin and proteins, in order to obtain purer hemicelluloses. This step obviously decreases the lignin content (from 24.7% to 23.3% for trial 1, from 18.3% to 11.0% for trial 2 and from 22.6% to 11.9% for trial 3), leading to a change in carbohydrate composition. In fact, compared with powders from extracts that have not been percolated on resin, powders here show an increase in the proportion of carbohydrates while that of proteins remains stable or slightly lower (Table 5). Although this step is efficient for lignin removal, the result indicates that the resin also retains a fraction of proteins, maybe due to aromatic amino acids. The X/A ratio also decreases in all cases proving that some arabinoxylans are similarly retained on the resin beads. The ash content remains stable meaning that the mechanism responsible for the purification does not involve ion exchange from resin. Furthermore, the global composition of the powder produced does not change, trial 2 still contains a large part of glucose (44.5%) whereas

Table 6
Purification yields for the main fractions obtained during the three trials.

	C + P + FD			C + UF + P + FD			C + UF + CH + P + FD		
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
Dry matter yield (%)[a]	4.7	6.2	8.2	4.9	5	11.7	4.3	4.6	7.6
Organic matter yield (%)[a]	4.6	5.0	8.2	5.0	5.4	11.7	4.4	4.7	7.6
(X + A) yield (%)[a,b]	4.5	3.3	5.8	4.3	2.7	6.9	4.4	2.7	5.1
Protein yield (%)[a]	14.8	12.5	29.7	19.1	10.5	45	14.3	10	28.8
(X + A) purity (wt% dry matter)[c]	24.2	14.9	18.8	24.2	14.3	15.3	25.9	16.1	17.2

[a] Calculation refers to (bran + straw), expressed as the dry weight of recovered matter in the powder/weight of this matter in (bran + straw).
[b] (X + A) is the sum of xylose and arabinose.
[c] Content of (X + A) in the powder (wt% dry matter).

glucose content is lower for trial 3 (11.4%), and the protein content for trial 3 is still very high.

3.4. Recovery yields

Production yield of each fraction produced under the three conditions has been evaluated for dry matter, organic matter, xylose + arabinose and protein (Table 6).

For all process routes, and for all trials, the yields in (X + A) are below 10%, which is partly explained by the brevity of the residence time in the extruder. The small differences observed between the different process pathways for the same trial, can be explained by the fact that a precipitation step is always involved, which masks the effect of ultrafiltration and chromatography. Nevertheless, it can be seen that ultrafiltration is efficient at concentrating all polymers, including protein and lignin, and that chromatography seems to be an effective procedure to purify the resulting extract (Trial 3) even if it still requires optimization.

Hence, trial 3 is much more efficient (twice) than trial 2, because remaining starch created a gel during extrusion that entrapped a part of the released molecules. Trial 1 gives lower recovery yields than trial 2 because the calculation includes the mass derived from straw while in fact only a small part of the molecules in the extract come from this, as shown by the X/A difference between trial 1 extracts and the others. However, if the calculations were made using only bran-derived (X + A), the (X + A) yields would increase above 20%.

4. Conclusions

Two different large scale feed conditions (considered optimal for small scale xylan recovery; Maréchal et al., 2004), have been investigated for the extraction of arabinoxylans by straw–bran co-extrusion in a twin-screw extruder. These large-scale results confirm those found on a smaller scale, with very similar arabinoxylan recovery yields of between 9% and 10% taking into account all the arabinoxylan introduced (from both bran and straw). For the xylans coming only from bran, condition 1 gives the best result with an extraction yield of 28% after centrifugation compared to 12% with condition 2. The centrifugation step had a carbohydrate recovery yield of approximately 50% under all the conditions tested, which is because of the large amount of small particles generated during the extrusion.

The 30 kDa ultrafiltration membrane is adapted to hemicellulose concentration, and to solution demineralization, but it also concentrated large molecules such as proteins. As the loss of arabinoxylans remains low, it should be possible to wash the extract by diafiltration to ensure complete demineralization. The results also indicate that pre-extraction starch removal is necessary, because starch reduces extraction efficiency and leads to fouling of the ultrafiltration membrane. Chromatography is efficient for the elimination of coloured molecules, but it also retains

proteins, mainly aromatic compounds. Thus, the combination of ultrafiltration and chromatography enables partial purification of the extract with a final arabinoxylan purity ranging from 16% to 26%. The carbohydrate content of the final powder may exceed 50%, and the other main components are protein and lignin.

This large-scale study almost defines the industrial performance of a continuous process including extrusion, ultrafiltration and industrial chromatography. On a large scale, extraction must be made with a large straw/bran ratio, and the extract can be efficiently concentrated by ultrafiltration instead of evaporation. Nevertheless, under these conditions, extraction of the proteins is more efficient than for polysaccharides, with protein recovery yield ranging from 10% to 30%. Twin-screw extrusion is an efficient tool for the extraction of the main soluble fractions of plant matter and allows polysaccharides, proteins and lignin to be washed from the cellulose fibres. Nonetheless, the separation of these fractions remains difficult because a part of them is extracted in non-soluble particle forms, subsequently lost during the centrifugation step. Production of a refined hemicellulose powder would require an extra step to separate the large molecules (proteins and lignins) and xylans. However, the purity required for the final powder depends on its future application. For biofuel production, requirements are as yet undefined, but for material applications (data not shown) starch, protein and lignin bring properties that could be interesting, with no further purification required.

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