An overview on the application of ultrasound in extraction, separation and purification of plant polysaccharides

Anna Ebringerová*, Zdenka Hromádková
Institute of Chemistry, Center for Glycomics, Slovak Academy of Sciences, SK-845 38, Bratislava, Slovakia

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Abstract: In view of the recent emphasis on non-conventional chemistry, application of ultrasound in isolation of plant polysaccharides represents a viable alternative to traditional extraction processes. This review presents an extensive literature survey of ultrasound-assisted extraction of polysaccharides from different plant materials, particularly herbal plants and secondary agricultural plant sources. Targeted, multistep methods were applied with respect to differences in the types of polysaccharides and their location in plant cell walls. The effectiveness of the methods was evaluated according to yield and properties of the isolated polysaccharides in comparison to classical extraction methods. Substantial shortening of extraction time, reduction of reagent consumption and/or extraction temperature are the most important advantages of the ultrasonic treatment. In combination with sequential extraction steps using different solvents, sonication was shown to be effective in separation and/or purification of polysaccharides. The disadvantages of the sonication treatment, such as degradation and compositional changes of the polysaccharide preparations are discussed as well.

Keywords: Ultrasound • Hemicellulose • Pectic polysaccharides • Separation • Degradation

1. Introduction

In connection with the emerging concept of ‘Green Chemistry’, recent emphasis has been given to non-conventional chemistry based on a combination of chemical and physical treatments. Power ultrasound has great potential in a wide variety of technological processes. The technique has been used in commercial applications for many years, such as in cleaning, diagnostic analysis, cosmetics and preparation of nanoparticles. Over the past two decades, application of ultrasound in chemistry as well as food and pharmaceutical industries became an exciting new field of research [1-3]. Very early, ultrasound has been recognized as an alternative approach to traditional extraction methods [4-6].

Ultrasound-assisted extraction is a process that uses acoustic energy and solvents to extract target compounds from various plant matrices. The generally accepted explanation for solvent extraction enhancement by using ultrasound is the propagation of ultrasound pressure waves and resulting cavitation phenomena, whereby the collapse of cavitation bubbles and highly localized temperature breaks cell walls and releases the contents of the cell into the extraction medium [2,4,6]. In accord with recently published reports [7,8], the solvent extraction of polysaccharides from plant tissues, used usually in dried form, is based on (i) steeping the material in solvent to facilitate swelling and hydration processes and (ii) mass transfer of the soluble constituents from the material to the solvent by diffusion and osmotic processes. The entire extraction process is significantly improved by the use of power ultrasound. The mechanical effect of ultrasound enhances the process of softening via hydration, provides a greater penetration of solvent into the material, improves mass transfer and facilitates the release of soluble polysaccharides due to break-up of vegetative tissues.

However, the preponderance of published studies concerned low molar mass substances. Intense research on the ultrasound-assisted extraction of...
Plant polysaccharides started in 1995 as a part of the European community COPERNICUS project ERB-CIPA-CT/94-0227 and the COST D10/0016-“SAFE” project. In the subsequent years, an increasing number of papers dealing with the application of ultrasound in extraction of bioactive principles and various functional polymers from primary and secondary plant sources have been registered and were included in part in recent reviews [4,9].

This review presents a complete literature survey of ultrasound-assisted extraction of polysaccharides from various higher plants, such as herbs containing bioactive polysaccharides, and other plant sources. Of particular interest are secondary agricultural plant products, which are rich in hitherto underutilized non-cellulosic polysaccharides (hemicelluloses and pectic polysaccharides) and represent renewable and carbon-neutral resources for sustainable society. A goal of this review was to judge the potential of ultrasonic-assisted extraction of various plant polysaccharides in comparison to conventional extraction processes and evaluate the applicability of ultrasound in separation and purification of polysaccharides. The future perspectives of ultrasound applications in polysaccharide research will be discussed as well.

2. Range of plant materials subjected to ultrasound-assisted extraction of polysaccharides

The type and physical nature of plant tissues included in this review, as well as brief information about the ultrasonic conditions used in the corresponding papers (sonic power, time, temperature) and the device type, solvents and application in the extraction, are presented in Table 1. Although most of the studies were performed on a laboratory scale using direct (probe system) or indirect sonication (water bath), larger-scale ultrasonic devices and reactors have been developed as well and applied in pilot plants of several drug factories [7,9].

2.1. Herbal plants

The extraction of biologically active compounds is the most important stage in the production of medicines from herbs. Usually, this process is performed using methods of percolation and maceration, characterized by low efficiency, long processing times (up to 21 days), high-energy consumption, and decomposition of some of the isolated compounds [10]. As reported earlier [1-3], the sonomechanical effect of ultrasound causes disruption of biological cell walls, thus, facilitating better penetration of solvent into the cellular materials and release of cell contents. A further benefit is the improvement of mass transfer. Remarkable increases of efficiency and consequent reductions of time were observed with ultrasound-assisted extraction of low molar mass bioactive principles from plant materials [4-8]. No attention has been paid to the polysaccharides present in herbal plants, although many of them were reported to exhibit various biological activities [11,12].

Within the framework of the abovementioned multilateral EU projects, the Slovak group initiated isolation of polysaccharides from the plant residues obtained after production of medicinal tinctures from the aerial part of sage (Salvia officinalis L.) and roots of valerian (Valeriana officinalis L) in the pilot plant of the drug factory Mediplant (Modra, Slovakia). The tinctures were prepared from both plants without and with application of ultrasound using as solvents 65% and 60% aqueous ethanol, respectively [13]. The ethanol-insoluble drug residue (EIR) obtained by these treatments from the aerial part of sage were subsequently treated with hot water and cold water (washing) in the pilot plant. After drying in air, they were sequentially extracted with hot water, 1% NaOH and 5% NaOH in the laboratory to isolate non-cellulosic polysaccharides [14]. As shown in Table 2, the yields of polysaccharides obtained, either by washing of EIR in the pilot plant or in the first step of its fractional extraction in the laboratory, are slightly higher in case of the ultrasound-assisted experiment.

Similarly, the effect of ultrasound on the extractability of polysaccharides from EIR of valerian roots was studied [15] and the extraction of valerian EIR by hot water is illustrated in Table 3. The yield of polysaccharide (P1) isolated from EIR was lower in the case of the ultrasound-assisted experiment. However, dialysis of the ethanolic tincture provided a further polysaccharide fraction (TP). The dry mass content of the ethanolic tincture from the ultrasound experiment was higher as was the net amount of recovered polysaccharides. The water-extracted polysaccharides from the EIR of both sage [14] and valerian [15] revealed a predominance of pectic polysaccharides that exhibited pronounced immunomodulatory activities.

The results indicated that the sonomechanical effect on herbal plant particles in aqueous ethanol was efficient enough to enhance the extractability of polysaccharides in subsequent neutral and alkaline extraction steps. Of importance is the fact that the ultrasound treatment during extraction had no negative effect on the immunological activity of the polysaccharides rich in pectic arabinogalactans isolated from sage (Salvia officinalis L.) [14,16].
Table 1. Overview of plant sources and conditions of ultrasound-assisted extraction of polysaccharides.

| Plant source                              | Ultrasonic processing conditions | Solvent for US | Extract target | Ref. |
|-------------------------------------------|----------------------------------|----------------|---------------|------|
| Salvia officinalis - aerial part           | Pilot plant; Ultrasound USD 600 (Slovakia), horn type, 20 KHz, 600 W; US: 2h at beginning of extraction, L/S 1.67 (v/w) | 65% EtOH | Tincture | [13] |
|                                           |                                  | Water | Polysaccharides* | [14] |
| Valeriana officinalis - roots             | Pilot plant; Ultrasound USD 600 (Slovakia), horn type, 20 KHz, 600 W; US: 2h at beginning of extraction, L/S: 1.67 (v/w) | 60% EtOH | Tincture | [13] |
| Zizyphus jujube - seeded fruit            | Laboratory; J96-ultrasonic generator (Shanghai, China), horn type, 20 KHz, 7.2–40.3 W; 40–80°C, 10–30 min, L/S 10–30 (v/w) | Water | Polysaccharides* | [17] |
| Birch wood                               | Laboratory; Optimum: 200 W, 60°C, 40 min, L/S: 25 (v/w) | 1.8M NaOH | Xylan | [29] |
| Corn cobs (1-2 mm)                       | Laboratory; Ultrasound PERSON (Slovakia), horn type, 20 KHz, 100 W, 8W cm²; 10–30 min, 50–70°C, L/S 10–25 (v/w) | Water, 1% NaOH | 5% NaOH | Xylan | [30] |
| Corn hulls (~0.25 mm)                    | Laboratory; Ultrasound PERSON (Slovakia), horn type, 20 KHz, 100 W, 8W cm²; 5–30 min, 30–70°C, L/S 10–25 (v/w) | Water, 1% NaOH | 5% NaOH | Xylan | [32] |
| Buckwheat hulls (1-2 mm)                  | Laboratory; Ultrasound PERSON (Slovakia), horn type, 20 KHz, 100 W, 8W cm²; 5–10 min, 40 and 60°C, L/S 10 (v/w) | 3% NaOH | 5% NaOH | Xylan/phenolics complex | [33] |
| Almond shells (<1 mm)                     | Laboratory; Ultrasound PERSON (Slovakia), horn type, 20 KHz, 100 W, 8W cm²; 10 min, 60°C, L/S 10 (v/w) | 0.5% NaOH | 5% NaOH | Xylan/phenolics complex | [36] |
| Wheat bran (~0.25 mm)                     | Laboratory; Ultrasound PERSON (Slovakia), horn type, 20 KHz, 100 W, 8W cm²; 5–10 min, 40–60°C, L/S 7.5–15 (v/w) | Water | 0.5–5% NaOH | Xylan/phenolics complex | [37] |
| Wheat straw (~0.7 mm)                     | Laboratory; Sonic system SOMERSET (England), horn type, 20 KHz, 100 W; 5–30 min, 35°C, L/S 10 (v/w) | 0.5M KOH | Hemicellulose/Cellulose | [38] |
| Wheat straw (~0.8 mm)                     | Laboratory; Sonic system ELMA (Beijing, China), horn type, 20 KHz, 100 W; 5–30 min, 60°C, L/S 30 (v/w) | 0.5M KOH in 60% MeOH | Hemicellulose | [39] |
| Sugar cane bagasse (~0.8 mm)              | Laboratory; Sonic system SOMERSET (England), horn type, 20 KHz, 100 W; US: 40 min, L/S 30 (v/w) | Water | Hemicellulose/Cellulose | [40] |
| Dimocarpus longan - fruit                 | Laboratory; Ultrasonic cleaner SB-5200DTD (Ningbo, China) 40 kHz, 120, 300 W; US: 5–40 min, 25–70°C, L/S 25 (v/w) | Water | Polysaccharides | [43] |
| Korean pine-kernel                        | Laboratory; Ultrasonic Cleaner Model 250DB (China) 40 kHz, US: 30 min, 30–60°C, L/S 10–40 (v/w) | Water | Polysaccharides | [42] |
| Apple pomace                              | Laboratory; Ultrasound cleaning bath SK200H (China), transducers, 59 kHz, 160 W; US: 4–4 h, L/S 20–45 | 2–4M KOH | Xyloglucan | [41] |
| Apple pomace                              | Laboratory; Ultrasound generator (Bulgaria), horn MP-1 type, 22 KHz, 1–1.2 W cm²; US: 70–80°C, 30–60 min, L/S 20 (v/w) | 0.5% HNO₃ | HM-Pectin (high-methoxy) | [46] |
| Apple pressing                            | Laboratory; Ultrasonic generator (Bulgaria) tube type GU-6, 22 KHz, 1–1.2 W cm²; US: 40–60°C, 2–6 h, L/S 20–30 (v/w) | 1.7–7% HNO₃ | LM-Pectin (low-methoxy) | [47] |
| Kenaf, Eucalyptus - woody parts (60 mesh) | Laboratory; Sonorex-Super RK 225 H, bath type, 35 kHz; US: 25°C, 1–3 h and 10 min (delignification step), L/S 200 | 0.5M CDTA | Pectin, Hemicellulose, Cellulose | [49] |
| Cassava root - pulp                       | Laboratory; Ultrasonic Processor Model SL (USA), horn type; Optimum: 80% of max. power, 5–40 s, L/S 25 (v/w) | 0.05M CH₃COONa | Starch | [52] |
| Marine sediments                          | Laboratory; Cell disruption VP-30s (Japan), 20 KHz, 150 W US: 20 min at 1:1 pulse or 10–90 min | 0.5M NaCl | 0.5M Na₂CO₃ Carbohydrates | [54] |

L/S, Liquid-to-solid ratio in mL g⁻¹; *) The polysaccharides were isolated from the ethanol-insoluble drug residues of sage and valerian [13];”) No information available concerning the sonication device.
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Very recently, ultrasound-assisted extraction was applied to Chinese Jujube (Zizyphus jujuba cv. jinsixiaozao) used as a food and pharmaceutical for over 3000 years. The aim was to isolate and characterize the polysaccharide components [17]. Optimization of the extraction conditions using water as a solvent by response surface methodology yielded the following parameters: extraction temperature 45−53°C, sonic power 31.7 W, and sonication time 20 min [18]. Using this short period of ultrasound treatment, the yield of polysaccharides, which belong to the group of pectic polysaccharides [19], was 20.2% greater than the classical extraction and the purity increased by a factor of 1.2.

2.2. Agricultural and silvicultural plants

Processing of agricultural plants (cereals, vegetables, fruits, grasses) and silvicultural plants (hardwoods, softwoods) generated by different kinds of industries can generate environmental problems. Because these by-products are immense renewable resources of hemicelluloses (xylans, mannoglycans, xyloglucans, cereal beta-glucans, arabinogalactans) [20] and pectic polysaccharides [21], intense efforts for more efficient utilization and potential industrial exploitation of these secondary sources are underway.

2.2.1. Extraction of hemicelluloses

Ultrasound can be applied to potential industrial application in the extraction of hemicelluloses, which are closely associated by physical and/or covalent bonds with other cell wall components (lignin, cellulose, pectic polysaccharides). Therefore, an essential prerequisite for their extraction is to cleave these bonds and enable the polysaccharides to dissolve in the solvent used (usually dilute alkalies). Among hemicelluloses, the most abundant are xylans [20], which comprise more than 25−35% of the dry biomass of woody tissues and occur up to 50% in some cereal grain tissues. Potential sources of xylans include many agricultural by-products such as straw, cereal bran, hulls, stalks, shells, etc. The primary xylan-types differing in branching sugars and their location in the β-1,4-D-xylan backbone are illustrated in Fig 1. For hardwood and softwood tissues, the 4-O-methylglucuronoxylan (MGX) and arabinoglucuronoxylan (AGX) types, respectively, are typical. Cereals and grasses contain the arabinoxylan (AX), AGX and (glucurono)arabinoxylan (GAX) types [20].

Aiming to perform the ultrasound-assisted extraction of hemicelluloses without substantially changing their structural and molecular properties, it was important to establish the range of sonication conditions in solvents used for hemicellulose extractions. Detrimental effects of ultrasound on carbohydrates caused by radical formation, and their recombination and termination reactions were reported [22,23], which result also in cleavage of glycosidic bonds in the main polymeric chain and in the branching units. Such effects depending on the applied power

Table 2. Yield of polysaccharides extracted from the ethanol-insoluble drug residue of the classical and ultrasound-assisted treatment of sage at the pilot plant and in laboratory experiments.

| Pilot plant          | Classical | Ultrasound |
|----------------------|-----------|------------|
| Conditions           |           |            |
| Water, 60°C, 2 h     | 1.8       | 1.9        |
| Washing (water)      | 0.6       | 0.9        |
| Laboratory           |           |            |
| Conditions           |           |            |
| Water, 90°C, 1 h     | 2.2       | 3.4        |
| 1% NaOH a            | 3.5       | 3.9        |
| 5% NaOH a            | 3.5       | 2.7        |

a Related to dry EIR.

Table 3. Effect of ultrasonication on the yield of polysaccharides extracted from the ethanol-insoluble residue (EIR) of valerian by hot water (P1) and by dialysis of the ethanolic extract (TP).

| Extraction  | Ethanolic extract | EIR |
|-------------|-------------------|-----|
| Classical   |                   |     |
| MT (g 100 mL⁻¹) | TP (%)a     |
| Classical   | 7.5               | 5.8 |
| Ultrasound  | 8.6               | 4.3 |

Figure 1. Schematic structure of some xylan-type hemicelluloses depicting the substituents and their sites of attachment to the xylan backbone. MGX: 4-O-methylglucuronoxylan; AGX: arabinoglucuronoxylan; GAX: glucuronoxylan; AX: arabinoxylan; Xylp: D-xylopyranose; Araf: L-arabinofuranose; MeGlcpA, 4-O-methylglucopyranosyluronic acid.
and operating parameters were recently reported for red algae polysaccharides [24] and tamarind xylotragan [25]. At longer ultrasonication times of the xylotragan (120 min) in distilled water, depolymerization was accompanied by changes in the sugar composition Glc:Xyl:Gal (mole ratios) from 10:7:8:4.5 to 10:8:3:3.4 due to cleavage of the more susceptible glycosidic bonds of the side chains (see Fig. 2). In addition, formation of carboxyl groups and UV-absorbing structures indicated degradation of glycosyl moieties that were attacked by radical reactions. The well known depolymerization effect of ultrasound on polysaccharides [1,2] has been utilized in targeted reduction of their molar mass and is usually performed in neutral aqueous solutions. However, this effect must be minimized during the extraction of polysaccharides to preserve structural and molecular properties.

For extraction of most of the types of hemicellulose (xylans and xylotragans), dilute aqueous solutions of various alkalies such as NaOH, KOH, Na$_2$CO$_3$, Ca(OH)$_2$, etc., sometimes in combination with oxidants (H$_2$O$_2$), have been applied [20]. Due to the known effects of alkalies on carbohydrates including decomposition by alkali-induced disproportion reactions and/or formation of novel functional groups [26], it was of cardinal interest to study the effects of ultrasonication on hemicelluloses in alkaline media. Suitable as well as undesirable changes of the functional properties of the isolated hemicelluloses can result.

### 2.2.1.1. Effect of ultrasonic irradiation on xylans

In view of the abovementioned facts, prior to investigation of the ultrasound-assisted extraction of xylan-type hemicelluloses from corn cobs and corn hulls, the effect of ultrasonication (20 kHz, 100 and 200 W) on their structural and molecular properties was studied using structurally well-defined model xylans—corn cob xylan (CCX) and corn hull xylan (CHX)—of the AGX and GAX types (see Fig. 1), respectively. The CCX was subjected to sonication in distilled water and dilute alkalies at 60°C [27]. The sonication time varied between 0 to 60 min. The Ara/Xyl ratios reflecting the degree of branching of xylan chains by arabinosyl residues showed only a minor drop from 0.18 to 0.17 indicating no structural changes. Conversely, changes were observed in the molar mass distribution determined by high performance gel permeation chromatography (HPGPC). The proportion of the very high $M_w$-fraction decreased from 8 to 2% as sonication time increased in 1% and 5% NaOH. This observation can be explained by disruption of aggregates. The $M_w$ of the primary xylan population (92–99%) decreased from 71 kDa to 51 kDa due to depolymerization of the individual xylan chains. In addition, a UV$_{247}$-absorbing component appeared in the very high $M_w$-region of the HPGPC chromatogram after sonication in 5% NaOH for a longer time (30 min) indicating formation of unsaturated and/or cross-linked structures. Formerly, that polymeric substances were reported [23] to be produced by recombination of macroradicals generated during sonication of carbohydrates.

The effect of ultrasound on the structural and molecular properties of CHX was studied in water and 5% NaOH at 30°C and sonication times of up to 60 min [28]. The degree of branching showed no changes. Changes in the molar mass distribution of CHX were similar to those observed in CCX. The proportion of the primary xylans extracted increased from 81% to 99%, and the $M_w$ decreased in water and 5% NaOH from 141 to 85 and 57 kDa, respectively. The UV-absorbing component appearing at 60 min in 5% NaOH was shifted to the very high $M_w$-region (> 460 kDa). The results indicated the suitability of 5% NaOH for ultrasound-assisted extraction of cereal xylans in the range of sonication conditions studied.

Recently, a kinetic study describing the effect of ultrasound on the physical properties of birch wood xylan was published [29]. The xylan was sonicated in aqueous H$_2$SO$_4$ or KOH solutions in order to elucidate the effect of the medium on molar mass distribution. A recycling-style ultrasonic spray system, composed of a 20 kHz frequency generator and a spraying nozzle, was used. Xylan was repeatedly sonicated at 25°C for 0–15 cycles. The results indicated that the mean molar mass of sonicated xylan determined by HPGPC decreased with as the number of sonication cycles increased in alkaline solution, whereas no significant decrease was observed in the acidic medium. In 1 and 2% KOH the mass ratio of the higher $M_w$-component decreased rapidly within the first 5 cycles of sonication. However, sonication in acid solution caused aggregation.

### 2.2.1.2. Extraction of xylans

The ultrasound-assisted extraction of xylan from corn cobs and corn hulls was further investigated based on previous results [27,28]. For both plant sources, a two-
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A step extraction procedure with and without application of ultrasound at various conditions in the first (extraction) step and constant conditions (5% NaOH at ambient temperature for 60 min) in the second (washing) step was applied. The xylan preparations were recovered from individual or combined extracts by ethanol precipitation and subsequent dialysis to remove low molar mass compounds.

The effect of sonication on the yields of xylan preparations obtained from corn cobs is illustrated in Fig. 3. In comparison to the case of the classical procedure using 5% NaOH, the total yield increased as the temperature increased. Increasing temperature is known to enhance lignin removal and, thus, the release of the xylan component. The same trend was observed upon the short application of ultrasound in both 1% and 5% NaOH. In comparison to the classical procedure performed at 60°C with 5% NaOH, approximately the same yields of total extracted xylan were achieved with 1% NaOH after 30 min of sonication at 70°C or 10 min of sonication in 5% NaOH at a lower temperature (50°C). An important advantage was that yields greater by 15–17% were achieved by 10 min of sonication in 5% NaOH at 60°C and 70°C. The sonication treatment did not change the structural properties of the xylan, but the proportion of the water-soluble component was affected. This AGX-type xylan component (Fig. 1) exhibited significant immunostimulatory activity, which was not affected by the sonication treatment during the xylan extraction [31]. However, the molecular properties of the water-soluble (ws) xylan fractions were significantly changed and lower mean \( M_w \) values were obtained after sonication at the highest temperature examined. The lower the liquid-to-solid ratio the lower the total xylan yields. The sonic power affected the xylan yield to a greater extent at the lower alkali concentration.

Various conditions in the extraction step were used for isolation of xylan from corn hulls [32] of the GAX type (Fig. 1). In the ultrasound-assisted procedures, distilled water was applied in the extraction step at various liquid-to-solid ratios in order to remove starch granules not separated from the hulls. In comparison to the classical procedure, a short sonication treatment (5 or 10 min) at 50 and 70°C at the lower alkali concentration (1% NaOH) resulted in yields of total extracted xylan that were increased by 9 and 12%, respectively, and increased in 5% NaOH by 14% (Fig. 4). Even upon sonication in water, the total yield increased by 9%. All xylan preparations were contaminated with residual starch, indicated by the relatively high glucose content of neutral sugars varying between 31–60 mol%. An advantage of the sonication step in water was that starch was primarily separated in this step, and the xylan isolated in the subsequent washing step showed a substantially lower glucose content. Therefore, the application of ultrasound in 1% NaOH might be used to lower significantly the contamination of xylan with starch.

The two-step extraction process with corn cobs and corn hulls can be significantly shortened by application of ultrasound. The consumption of chemicals can be reduced by applying 1% NaOH and short ultrasonic treatments and somewhat lower temperatures, whereby the xylan yields were similar with corn cobs and higher with corn hulls in comparison to the classical extraction with 5% NaOH. In case of corn hulls, the contaminating starch can be separated by sonication in water, and the xylan component isolated in the washing step in a higher yield.

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Figure 3. Yield (% on dry basis) of total extracted corn cob xylan X(I+II) and its water-soluble fraction ws-X(I+II) obtained by classical (CE) and ultrasound-assisted (UAE) extractions.

* Added 2% \( \text{H}_2\text{O}_2 \).

Figure 4. Yield (% on dry basis) of total extracted corn hull xylan F(I+II) and fraction (F-I) from step I obtained by classical (CE) and ultrasound-assisted extractions (UAE).

* Added 2% \( \text{H}_2\text{O}_2 \).
Using the two-step alkaline extraction procedure, polysaccharides from almond shells were investigated and phenolics. The isolation of non-cellulosic polysaccharides from almond shells was investigated using the two-step alkaline extraction procedure, thereby confirming the advantages of the two-step procedures with classicaly extracted hemiceluloses, thereby confirming that structural, molecular properties as well as the immunological activity of the ultrasonically extracted buckwheat MGX-type hemiceluloses were preserved as compared with classically extracted hemiceluloses, thereby confirming the advantages of the two-step procedures observed with the xylans from corn cobs and corn hulls.

Almond shells, which may comprise from 35 to 75% of the total fruit mass, represent a rich source of xylans and phenolics. The isolation of non-cellulosic polysaccharides from almond shells was investigated using the two-step alkaline extraction procedure.

Buckwheat (Fagopyrum esculentum Moench), a pseudocereal, has been cultivated since ancient times for food and medicinal applications. The hulls are a rich source of natural antioxidants and an immunogenic 4-O-methylglucuronoxylan (MGX) closely associated with phenolics. The two-step extraction procedures described earlier were applied with and without a short application of ultrasound at the beginning of the extraction step and the effects of sonication on the extractability, purity and functional properties of the hemicelulose component were compared. In the extraction step, either 3% or 5% NaOH were used as solvents. Sonication was applied at 40°C and 60°C for 5 to 10 min. Similarly as reported for corn cobs and corn hulls, the total yield of xylan extracted from buckwheat hulls was increased by the assistance of ultrasound. Using 3% NaOH at 60°C, recovery increased from 8.3 to 10.3% and from 11.2 to 16.5% with 5% NaOH. At 40°C, the yields were lower and approximately the same (~10.5%) in both extraction media. In one-step experiments (omitting the washing step), the suspension in 3% and 5% NaOH was sonicated for 10 min at 60°C and the extraction time was prolonged for 60 and 80 min, respectively. The yields of hemicelulose were approximately equal to those obtained in the two-step procedure with 3% NaOH, lower in comparison to the 5% NaOH experiment. Only minor differences were found in the molecular properties of the samples between the classical and ultrasonic experiments. The results indicated that the structural and molecular properties as well as the immunological activity of the ultrasonically extracted buckwheat MGX-type hemiceluloses were preserved as compared with classically extracted hemiceluloses, thereby confirming the advantages of the two-step procedures observed with the xylans from corn cobs and corn hulls.

Ultrasound was applied in 5% NaOH for 10 min without or with prolongation of the extraction time up to a total of 60 min, and in 0.5% NaOH for 10 min. The isolated polysaccharides were separated into water soluble (ws) and water-insoluble (wis) fractions. The yield of total extracted material and polysaccharides isolated in both extraction steps and their ws-fractions (ws-F) are summarized in Table 4. In comparison to classical extraction, a short sonication at the beginning of extraction step lasting 60 min (AS-US1) increased the yield of both the total extracted material and isolated polysaccharides due to a higher amount of released wis-polymers. By performing the extraction only 10 min with sonication (AS-US2), the yield of the extracted material decreased in comparison to the experiment without sonication. However, the amount of the polysaccharides isolated predominately in the extraction step increased and comprised about 67% of the extract. Using a lower alkali concentration (0.5% NaOH), the yield of the extract was approximately the same as with 5% NaOH, but the yield of hemiceluloses was low. In spite of the sonomechanical attack on the cell wall matrix, only a part of the polysaccharides released became solubilized due to the low swelling effect of 0.5% NaOH. This is indicated by the high proportion of the ws-fraction in sample AS-US3.

Chemical and spectral analysis of the isolated polysaccharides revealed that they are primarily composed of the MGX-type but are slightly contaminated with pectic polysaccharides and contain 8–13% of phenolics. The radical scavenging activity of the xylan/phenolic complexes was tested by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical assay. The derived EC50 values represented the amount of a sample needed to decrease the initial radical concentration by 50%. The tested xylan/phenolic complexes exhibited antioxidant activity, expressed by the inverse EC50 values (1/EC50), which varied between 48 and 80%. The activities were higher with the ultrasonically extracted polysaccharide fractions.

Wheat bran, an important by-product of the cereal industry is recognized as a good source of dietary fiber...
An overview on the application of ultrasound in extraction, separation and purification of plant polysaccharides possessing a wide range of beneficial physiological effects. It contains hemicelluloses, mainly of the arabinose-rich heteroxylan types, GAX and AX (Fig. 1), reported to exhibit immunostimulating and antioxidant activities [20]. The extractability of heteroxylan from industrial wheat bran was investigated using the two-step extraction method without and with ultrasonication (5 or 10 min) in the first (extraction) step [37]. The yield of isolated polysaccharides depended strongly on the extraction conditions of the first step (solvent type, solvent/solid ratio, extraction and sonication time, and temperature). Using water and 0.5% NaOH, ws-polysaccharides were obtained by the classical extraction as well as by the short extraction during the sonication treatment, whereas, with increasing the NaOH concentration, extraction temperature and/or prolonged extraction after sonication, the polysaccharides contained a higher proportion of the wis-fraction. Similar yields of total isolated polysaccharides were obtained using 2% NaOH by prolonged extraction after sonication as with 5% NaOH in the classical extraction.

The effects of the short sonication treatment in water (A) and solutions of 0.5% NaOH (B), 2% NaOH (C) and 5% NaOH (D) on the yield and properties of isolated ws-polysaccharides are best illustrated for experiments performed at otherwise constant conditions: liquid/solid ratio of 10 mL g⁻¹, at 40°C, and 5 min sonication (Fig. 5). As illustrated, the short ultrasound-assisted extractions in the same solvents produced similar yields of totally isolated ws-polysaccharide fractions as obtained by the classical extraction lasting 60 min. Moreover, nearly identical yields were achieved with 2% NaOH by 5 min extraction/sonication and as were determined classically with 5% NaOH. The results suggested that under certain extraction conditions the total extraction time can be shortened and/or the consumption of NaOH decreased by about 60%.

The antioxidant activity (AOA) of ws-polysaccharide fractions from both classical and ultrasound-assisted extractions was expressed by the EC₅₀ values from the DPPH⁺ test. The lower the EC₅₀ values, the higher the antioxidant activity. Fig. 5 shows that, with the exception of fraction A1, the EC₅₀ values ranged between 3.3 and 5.9 (step I) and between 4.2 and 7.3 (step II) indicating somewhat higher activities of the polysaccharides from step I. The total phenolics content of all tested samples varied between 1.6 to 4%. With increasing TP content, the EC₅₀ values showed a decreasing tendency indicative of increasing AOA in both the classical and ultrasound-assisted experiments. The polysaccharides obtained comprised a complex of phenolics-rich heteroxylans contaminated to various extents with co-extracted amylose-rich starch as well as protein. The levels of the contaminants can be governed by the extraction conditions.

Intense research of polysaccharide extraction by means of ultrasound began at the University of Wales (Bangor, UK) and at Universities in Guangzhou and Yangling (People’s Republic of China). Attention has been paid to agricultural sources, such as wheat straw and sugar cane bagasse. The aim was to develop techniques for fractionation of lignocellulosic materials into their primary polymer components. One-step alkaline extractions were used to study the effect of ultrasound on the extractability of hemicelluloses from de-waxed wheat straw [38]. The extractions were performed in 0.5 M KOH at 35°C without and with application of ultrasound for the first 5 to 35 min, followed by continuation of the alkaline extraction for a
Table 5. Yield, composition and molecular properties of hemicelluloses isolated from wheat straw by alkaline and alkali/methanol extractions with and without sonication.

| US min | 0.5 M KOH | 0.5 M NaOH in methanol−H₂O |
|--------|-----------|---------------------------|
|        | Yield %a  | Lignin %b | X+A Mw Rel.% | Yield %a | Lignin %b | X+A Mw Rel.% |
| 0      | 24.8      | 6.4       | 76.7 10.7     | 12.3      | 5.3       | 75.1 22.3    |
| 10     | 24.8      | 5.7       | 80.7 18.4     | 13.8      | 4.9       | 76.1 21.6    |
| 20     | 25.1      | 5.4       | 80.8 22.4     | 15.6      | 3.3       | 77.8 21.5    |
| 35     | 25.5      | 4.5       | 82.1 19.1     | 15.8      | 3.9       | 77.7 19.1    |

Abbrevations: X, Xylose; A, Arabinose; a Related to dry starting material; b Related to hemicellulose;

Total period of 2.5 h. Hemicelluloses were isolated from the extract after acidification by ethanol precipitation. The results demonstrated that ultrasonication produced a slightly higher yield of hemicelluloses. In comparison to the classical procedure, yield increased from 24.8% to 25.5% (Table 5). Sugar composition analysis revealed that the sum of xylose and arabinose increased from 76.7 mol% without sonication to 82.1 mol% with sonication indicating the predominance of AGX reported to be the main hemicellulose component of straw [20]. Although in the extraction process lignin was separated in amounts ranging from 7.5 to 8.4%, the xylan preparations still contained lignin including phenolic acids, and aldehydes in amounts that decreased from 6.4% to 4.5% as the sonication time increased. The relative molar mass, $M_w$, of the isolated hemicelluloses increased from 10.7 to 22.4 kDa at 20 min of sonication due to the release of polymers with higher molar mass.

Based on the knowledge that the alkali-methanol (organosolv) pulping process can produce pulps in higher yields and with lower content of residual lignin, the extractability of hemicelluloses from de-waxed wheat straw using as a solvent 0.5 M NaOH in methanol−H₂O (60:40, v/v) was investigated [39]. The extraction was performed similarly to that published by Sun and Tomkinson [38]. As documented in Table 5, as the sonication time increased from 5 to 35 min, the yield of hemicelluloses increased from 12.3% (obtained without sonication) to 15.8%. In comparison to the methanol-free alkaline extraction method [38], the $M_w$-values of the ultrasound-extracted xylans were similar. Although the presence of methanol supported the release and solubilization of lignin, the xylan preparations contained approximately 1% less amount of lignin. This can be explained by the simultaneously inhibited solubilization of hemicelluloses by the methanol present.

Sugar cane bagasse, containing 25–35% hemicelluloses, is a residue from the refining process of sugarcane, which is of low economic value and constitutes an environmental problem to sugar mills and surrounding districts. The extractability of bagasse was investigated [40] using a two-step extraction procedure in which the de-waxed bagasse was treated in water with ultrasound for 40 min at 55°C, followed by stirring for 80 min at the same temperature. Water-soluble hemicelluloses (WS₁) were isolated from the extract. In the second step, the residue was treated by sequential extractions with NaOH and alkaline peroxide solutions at 55°C for 2 h, and from the extracts, hemicelluloses were separated by ethanol precipitation. The results showed that under these conditions the ultrasonic treatment and sequential extractions released greater than 90% of the hemicelluloses originally present. The increase of the NaOH concentration from 0.5 to 2 M and of the alkaline peroxide content from 0.5% to 3.0% resulted in degradation of the hemicellulosic polymers as shown by a decrease in their $M_w$-values from 43.6 to 14.5 and 30.2 to 18.1 kDa, respectively. However, no significant differences existed in the structural features of the alkali- or alkaline peroxide-soluble hemicellulosic fractions, which contained primarily AGX. All xylan fractions contained lower amounts of associated lignin (0.41–2.90%) than those obtained under the corresponding conditions without sonication (0.46–5.12%). In contrast, the WS₁ fraction exhibited the highest content (7.36%) of phenolics, including ferulic and p-coumaric acids.

2.2.1.3. Extraction of xylloglucan

Ultrasound-assisted extraction was evaluated as an alternative to the conventional extraction method for the isolation of xylloglucan (XG, Fig. 2) from apple pomace, a by-product from the food industry [41]. Apple pomace samples were extracted under indirect sonication in an ultrasound cleaning bath. Using response surface methodology, the effects of three variables, i.e. the liquid:solid ratio, the KOH concentration and the ultrasound-assisted extraction time, on the yield of isolated xylloglucan, was studied. The ultrasound-assisted extraction was found to be approximately three times faster than the extraction without ultrasound which required 16 h. Regression analysis of the data
revealed that the optimum combination for achieving the highest yield was a liquid:solid ratio of 34.4:1 (v/w), a 3.3 M concentration of KOH and an ultrasonication time of 2.5 h. The extraction efficiency was estimated by absorbance values of XG in the iodine test. The authors presented no information about the actual yields of the xylolucan isolated.

2.2.2. Extraction of other polysaccharides
The extractability of ws-polysaccharides from defatted kernels of Korean pine (Pinus koraiensis) was investigated using hot water as a solvent, with and without application of ultrasound [42]. The factors affecting the extraction yield and polysaccharide content, such as extracting temperature and time, ratio of water to defatted kernel and concentration of ethanol for precipitation of polysaccharides, were compared. The optimal extracting parameters of ultrasound-associated extraction were: ultrasonic temperature, 70°C; liquid/solid ratio, 20:1 (v/w); extraction time, 40 min; and 80% ethanol. Under such extraction conditions, the yield of the crude ws-polysaccharides was 3.65% and the average polysaccharide content was 45.38%. Both the extraction yield and the polysaccharide contents of preparations extracted with assistance of ultrasound were greater than in case of the classical hot water extraction. The study demonstrated that ultrasound is a reliable and extremely effective tool for the rapid extraction of ws-polysaccharides from kernels of the Korean pine.

Longan (Dimocarpus longan Lour.) is an exotic fruit in Southeast Asia. The pericarp of longan—rich in polysaccharides—was subjected to ultrasound-assisted extraction [43]. Effects of ultrasonic power, sonication time and temperature on the ultrasound-assisted extraction of the polysaccharides of longan fruit pericarp (PLFP) were compared with the classical hot water extraction using distilled water at temperatures ranging from 25 to 70°C and extraction times ranging from 5 to 40 min. The sonication was performed at the same temperature and time intervals. A greater recovery rate of PLFP was obtained at an ultrasonic power of 300 W when compared to 120 W. The recovery rate of PLFP was slightly increased by elevating the ultrasonic temperature to 60°C. The highest recovery rate of PLFP was achieved at 120 W and 70°C for 20 min. Scanning electron micrographs revealed that ultrasonic power was an effective factor for modifying the physical properties of PLFP, and may have contributed to the modification in the molar mass and other physical and chemical properties. The UV-VIS spectra exhibited no apparent differences between the classically and ultrasonically extracted polysaccharides in the range from 195 to 550 nm, with a predominant peak at 199–200 nm. The results indicated that sonication might not cause significant chemical modification of functional groups of the polysaccharide chains.

In an additional study on longan fruit pericarp [44], the effects of ultrasonic extraction conditions on the radical scavenging activity of the isolated PLFP, determined by the DPPH• test, were investigated. Using response surface methodology, the optimum conditions to obtain the greatest recovery and the strongest DPPH radical scavenging activity of PLFP were determined: 120 W, 22 min and 60°C as well as 241 W, 18 min and 51°C, respectively. No further information about purity and structural types of the isolated polysaccharides and origin of the radical scavenging activity were presented.

2.2.3. Extraction of commercial-type polysaccharides
2.2.3.1. Pectin
As an improvement of the extraction technology of pectin (Fig. 6) from apple pomace, a pulsating hydrodynamic action performed at approximately 80°C was reported [45]. An increase of the pectin yield (30–60%) was achieved, whereby the extraction was 2–5 times faster, but still required several hours. A significantly greater improvement was achieved using ultrasound-assisted extraction [46]. The pectin was extracted by continuous or pulsed sonication at 40–80°C for 30 to 60 min. Optimum conditions were: intermittent sonication (2 min on, 3 min off) for 45 min at 80°C. The yield increased by 23–28% compared to the previously mentioned technique [45] with no loss of gel strength. Results obtained at 70°C were also acceptable. The degree of esterification (DE) ranged between 66.4 and 68.8%, indicative of high-methoxy (HM) pectin. By intermittent sonication, the separation of the liquid and solid phases was approximately 100-fold faster than with the pulsating hydrodynamic extraction. Several years later, the same authors applied ultrasound treatment successfully in the production of low-methoxy (LM) pectin from apple pressings [47]. By this process, the subsequent technological step for partial removal of methyl esters from the HM-pectin can be omitted. This improvement was achieved by combining acid maceration of the pectic raw materials with ultrasonication. An additional

\[
\text{HG} \quad \text{AG} \\
(\rightarrow 4)\alpha\text{G} \text{a}(1 \rightarrow 4)\alpha\text{G} \text{a}(1 \rightarrow 3)\alpha\text{G} \text{a}(1 \rightarrow 2)\beta\text{R} \text{h}(1 \rightarrow 4)\beta\text{G} \text{a}(1 \rightarrow 3)\beta\text{G} \text{a}(1 \rightarrow 2)\beta\text{R}
\]

Figure 6. Schematic structure of pectic polysaccharides depicting the homogalacturonan (HG) and rhamnogalacturonan (RG) sequences in the pectin backbone with (R) the attached neutral side chains (arabinan, galactan, arabinogalactan).
decrease of DE (approximately 6%) and an important increase of the pectin yield (approximately 18%) as compared to the reference sample were established at the optimum sonication time of 4−30 min. However, by prolonging the sonication time, the pectins showed lower gel strength. Reportedly [48], the rheological and optical properties of HM-pectin can be regulated by high-intensity ultrasound treatment. The results were attributed to an overall reduction in the average molar mass of pectin due to cavitational effects.

### 2.2.3.2. Cellulose

Cellulose is a widely used potential feedstock for a number of technologies, such as the production of pulp and paper, liquid fuels, chemicals and pharmaceuticals. Depending on plant sources, various isolation methods have been elaborated, and recently, classical and ultrasound-assisted isolation procedures were compared according to the yield and technical parameters of the isolated cellulose. Cellulose was isolated from kenaf (Hibiscus cannabinus L.) and eucalyptus wood (Eucalyptus rodustrus Sm.) [49], using a multistep procedure without and with assistance of ultrasound in order to remove successively protein, lipids, pigments, salts, pectic substances, hemicelluloses, and lignin (key step). Ultrasound was applied in each step. Although the type and amounts of solvents and chemicals were the same in both procedures, the temperature and time conditions differed. In comparison to the classical procedure using ultrasound at ambient temperature in the delignification step (vs. 70°C in the classical one), the total time of treatment decreased from 170 h to 1.5 h, the yield of kenaf and eucalyptus cellulose increased by 0.6% and 0.5%, respectively, and the purity of the cellulose preparations obtained was high.

In continuation of the ultrasound-assisted extraction of hemicellulose from sugarcane bagasse [40], three different multistep procedures (A, B, C) were compared for isolation of the cellulose component [50]. In the first step of procedure (A), water was used at 55°C for 2 h without or with sonication for the first 40 min followed by prolonged extraction for 80 min. The procedures B and C were performed classically. As illustrated in Table 6, the yield of cellulose ranged between 45.9% and 43.0%. Due to the severe delignification conditions of method C, the yields of cellulose as well as its intrinsic viscosities were the lowest. Interestingly, the short ultrasound treatment in water at elevated temperature in the first extraction step of method A protected the cellulose against depolymerization as indicated by the viscosity, and the yield was in the range of cellulose yields from method B.

| Method                  | Cellulose (%) | Hemicellulose % | Lignin % | Mw kDa |
|-------------------------|---------------|-----------------|----------|--------|
| (A) Cla: 120 min        | 45.9          | 7.21            | 3.86     | 192.0  |
| US: 40 min              | 44.7          | 5.97            | 3.35     | 218.9  |
| (B) 10% KOH             | 44.7          | 4.67            | 1.62     | 227.9  |
| 10% NaOH                | 44.2          | 3.67            | 1.45     | 226.2  |
| (C) Acidc, 110°C        | 43.6          | 4.88            | 0.58     | 160.6  |
| Acidc, 120°C            | 43.0          | 3.23            | 0.18     | 133.3  |

* Related to dry bagasse; † Related to dry cellulose;  ‡ 80% CH$_3$COOH/70% HNO$_3$.

The separation and characterization of cellulose from de-waxed wheat straw was performed by three-step sequential treatments using 0.5 M KOH at 35°C for 2.5 h without and with ultrasonication up to 35 min in the first step [38] and classical treatment with an mixture of 80% acetic acid/70% nitric acid at 120°C for 15 min in the third (delignification) step [51]. The yield of crude cellulose preparations obtained by the first two steps ranged between 45.3% and 46.9% of the dry straw, and contained 7.3 to 7.9% residual hemicelluloses and 3.3 to 3.7% residual lignin. The purified cellulose samples, obtained after the final (acid) treatment, were relatively free of bound lignin (0.1 to 0.2%) and contained only minor amounts of associated hemicelluloses (approximately 3%); however, the molar masses significantly decreased. The results indicated a noticeable degradation of the cellulose by the 80% acetic acid/70% nitric acid treatment, similarly to that described with sugar cane bagasse.

### 2.2.2.3. Starch

As a consequence of starch production from the roots of Cassava (Manihot esculenta Crantz), the solid waste (pulp) produced in large amounts is still rich in starch (50-60% dry basis). In order to recover this residual starch, ultrasound-assisted extraction was compared
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with treatments using a multi-enzyme mixture of amylases and pectinase [52]. Both treatments improved the efficiency of starch extraction resulting in about 40% recovery of the residual starch. The authors concluded that both methods had the ability to disrupt the complex structure of polysaccharides associated with and entrapped in starch granules. As earlier reported by Mason et al. [3], the mechanical and physical effects of ultrasonication of rice grain particles in water caused surface erosion and/or particle size reduction leading to a faster release of starch during cooking and a shorter period required to form a gel.

2.3. Marine sediments
Carbohydrates are ubiquitous compounds in marine sediments, representing a significant fraction of total sediment organic carbon [53]. Carbohydrates adsorbed on sediment particles were extracted by various combinations of heating, ultrasonic radiation and alkali treatment, and the extraction efficiencies of these treatments were compared [54]. Heating (121°C, 60 min) in the presence of alkali (20 mM Na₂CO₃) followed by ultrasonication (20 min on ice) yielded maximal recovery. By repeating this extraction three times, 50% of total carbohydrate in the sediment could be extracted. The major portion of extracted carbohydrates (68–84%) was found in the macromolecular fractions (Mₘ ~10 kDa).

2.4. A Two-step ultrasound-assisted extraction of immunogenic xylan from corn cobs
Irradiation was carried out using the Ultragen system PERSON (Ultragen Nitra, Slovakia) at 100 W sonic power and sonication time intervals of 10 min. The dispersion was sonicated for the given time in a glass beaker of standard geometry in order to preserve the same ultrasonic intensity of 8 W cm² in all experiments.

In the first extraction step, 5 g of ground cobs (particle size 1–2 mm) were suspended in 75 ml of 5% NaOH and sonicated under stirring for 10 min at 60°C. After rapid cooling to room temperature, an insoluble residue, I, was separated by centrifugation (3000 g, 15 min), and subsequently treated in the second (washing) step with 50 ml of 5% NaOH at ambient temperature for 1 h. A second extract, II, was separated by centrifugation (3000 g, 15 min). Both extracts were combined and poured into four volumes of ethanol. The precipitate was decanted twice with 80% ethanol acidified with acetic acid to pH 6, and subsequently combined twice with 80% ethanol and filtered. The remaining solids were suspended in distilled water and subjected to exhaustive dialysis against distilled water in a cellophane bag. The non-dialysable retentate was subjected to centrifugation and the water-soluble portion (ws-X) was dried by lyophilisation and represented the immunogenic corn cob xylan. The water-insoluble portion (wis-X) was recovered by lyophilization. The yield of the ws-X and wis-X fractions was 29% and 8%, respectively.

3. Application of ultrasound in polysaccharide purification
As stated in most of the studies examined for this review, polysaccharides isolated by extraction are usually contaminated with non-carbohydrate components (protein, lignin and other phenolics) and colored degradation products. They are physically associated or chemically bound to polysaccharides, and might adversely affect their solubility and functional properties. To address the solubilization problems, ultrasound was used to purify beechwood glucuronoxylan (GX-3) with a lignin content of 4.3% [55]. GX-3 suspensions in 80% ethanol (1%, w/v) at pH 7.0 and 9.0 were sonicated for 5 min at 30±5°C with the aid of the Ultragen system PERSON (20 kHz) at sonic power 100 W using a cylindric sonotrode. The sonication effects were characterized by HPGPC analysis of the treated xylan sample isolated from the suspension by

![Figure 7. HPGPC chromatograms of beech wood xylan GX-3 suspended in 80% aqueous ethanol (a) before and after purification by sonication for 5 min at (b) pH 9 and (c) pH 7. Inserted are UV-spectra of the filtrates; the arrows indicate the absorption bands of unsaturated and phenolic structures.](image-url)
filtration, and by UV-spectra of the ethanolic filtrates. As demonstrated in Fig. 7a, the UV-absorbing material of GX-3 was associated predominately with the high-$M_w$ component and comprised a large proportion of the sample. The xylan treated in neutral (Fig. 7c) and alkaline-ethanolic (Fig. 7b) media showed substantial changes in the distribution of the UV$_{254}$-absorbing material. Its proportion increased in the medium-$M_w$ and low-$M_w$ regions. Similar changes were also observed in the molar mass distribution indicating accumulation of the medium-$M_w$ and low-$M_w$ populations. These results suggested that the xylan-lignin complexes, forming micelles or aggregates [56], were disintegrated by sonication. A portion of the resulting products became soluble in the neutral and, primarily, in the alkaline 80% ethanolic medium. The result was confirmed by UV spectra of the filtrates (inserted in Figs. 7b and 7c), which showed a shoulder at 230–240 nm and a peak at 280 nm which are typical absorptions of unsaturated and phenolic functional groups, respectively. Although solubilization of GX-3 was achieved, a portion of the phenolics remained associated to the xylan molecules.

Effective degrees of extraction and purification were reported with polysaccharides isolated from Chlorella pyrenoidosa by disruption of seaweed cells in distilled water with ultrasonic waves [57]. The greatest yield (44.8 g kg$^{-1}$) and purity were obtained by ultrasonication for 800 s at 400 W, followed by incubation of the dispersion in a water bath at 100°C for 4 h, and recovery of the polysaccharide using 80% ethanol.

The results indicated that the effects of ultrasonic treatments (mass transfer intensification, cell disruption, improved penetration and capillary effects) [4] are influenced not only by the solvent but also by type of plant tissues, their size, hardness and swellability in the solvent used [7], and therefore, also by the liquid/solid ratio. However, this has been tested only in a few studies.

## 4. Future opportunities

One of the various opportunities suggested for ultrasound processing in the food industry [9] comprises the simultaneous extraction of plants and encapsulation of the extracted substances with proteins, through hydroxyl-radical-initiated covalent bonding and microsphere formation. As indicated in this review, polysaccharides extracted by the assistance of ultrasound might serve simultaneously as encapsulating agents for co-extracted phenolics and other extractives. Such processes can explain the isolation of xylan/phenolics complexes [33,36,37] and difficulties to remove the phenolics from beechwood xylan [55]. For xylan utilization, a novel hybrid process has been reported [58], which involves the ultrasound-assisted extraction of xylan from corn cobs followed by enzymic hydrolysis of the isolated xylan to xylose. Analytical procedures combining acid hydrolysis with ultrasound treatments have been proposed for the simultaneous extraction and total carbohydrate analysis of environmental and food samples [59]. The methods were faster and exhibited significantly improved analytical accuracy and increased recoveries of carbohydrates in comparison to conventional analysis. A combination of ultrasound and microwave treatments was also presented as a new technique for digestion of solid and liquid samples suitable for chemical and food analysis [60].

## 5. Conclusions

This review summarized the importance and potential of ultrasound to enhance extraction of various polysaccharide components present in different plants and plant tissues. From this point of view, the polysaccharides that were investigated most were the major hemicellulosic components of cereals and grasses, which comprise various heteroxylan types (MGX, AGX, GAX and AX) and xylan/phenolic complexes. Attention has also focused on pectic polysaccharides from herbal plants, pectin and xyloglucan from apple pomace, cellulose from kenaf, sugar cane bagasse, eucalyptus wood and wheat straw, and further undefined hemicelluloses and polysaccharides. Ultrasound represents a new and powerful tool for acceleration of polysaccharide extraction and reduction of the consumption of chemicals. Ultrasound extraction is useful in the separation of different types of polysaccharide from plant materials by application of multistep procedures such as separation of starch from hemicelluloses or pectic polysaccharides from hemicelluloses and cellulose and removal of co-extracted phenolics from the isolated hemicelluloses. A further potential benefit is to combine ultrasound with microwave treatments and/or enzymes for isolation and targeted modification of polysaccharides as well as to provide simultaneous extraction and encapsulation of useful co-extracted components. All these advantages may attract industrial interest for ultrasound applications in the field of polysaccharide extraction and modification technologies.
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