PRELIMINARY COMMUNICATION

An Immunomodulatory Xylan-Phenolic Complex from the Seed Hulls of Buckwheat (*Fagopyrum esculentum* Moench)

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The seeds of buckwheat, classified as a pseudocereal, are at present recognized as a suitable component of food products due to their high nutritional value as well as antioxidant activity [1, 2]. The hulls removed before utilization of the seeds represent a new source of hemicelluloses, which nowadays are considered potential biopolymers for food and nonfood applications [3, 4]. Buckwheat hulls are also rich in antioxidants comprising tocopherols, rutin, quercetin derivatives, and other phenolic substances [5, 6].

Our previous investigations on buckwheat hulls extraction with and without application of ultrasound [7] revealed that the isolated hemicelluloses are significantly contaminated with starch, pectic polysaccharides, protein, and phenolic substances. In view of this and in recognition of the reported [8] immunomodulatory activity of some xylans, a multistep fractional extraction procedure has been elaborated. From the air-dried ground hulls (BH) the mechanically nonseparated starch was removed by repeated decantation with cold water, and extractives by successive extraction with benzene/ethanol (φ_r = 2:1), ethanol, and isopropanol. The hull residue was further treated with acetate buffer of pH 6.1 at 70 °C for 10 min to release residual starch. The aqueous extract gave upon dialysis fraction E1 (1.2 % of BH). Subsequent treatment of the wet solid with α- and γ-amylases in acetate buffer yielded after dialysis of the extract the polymeric fraction E2 (0.5 % of BH), which was composed of arabinose, mannose, galactose, xylose, and free of glucose. In the next step, the separated air-dried solid residue was extracted with EDTA in acetate buffer of pH 6.8 at 70 °C for 2 h to yield pectic fraction E3. In the following alkaline steps, extraction with 1 %, 5 %, and 10 % NaOH at 60 °C for 1 h released hemicellulose fractions H1, H5, and H10, respectively. They were recovered, after adjusting the pH to 7.5 of the respective extracts, by precipitation with ethanol (φ_r = 1:4) in the yields of 9.9, 5.6, and 7.7 % (related to BH). As shown in Table 1, proteins (calculated as % N × 6.25) were gradually removed in the alkaline extractions steps and the content of phenolics, expressed in gallic acid equivalents, decreased. Neutral sugar composition of the hydrolyzates revealed the prevalence of D-xylose in the alkali-extracted fractions indicating the presence of xylan-type polysaccharides, whereas the bulk of residual starch and pectic polysaccharides was released during the previous steps. The FTIR spectrum of both H5 and H10 fractions showed the spectral pattern in the $\tilde{\nu}$ = 900—1200 cm^{-1} region typical of low-substituted glucuronoxylans [9]. The very weak ν_{as}(CO) absorption band at 1605 cm^{-1} indicated a low content of 4-*O*-methyl-D-glucuronic acid (MGA), in accord with paper chromatography of the hydrolyzates. The band at 1505 cm^{-1} confirmed the presence of phenolic substances.

Table 1. Analytical Data of Buckwheat Hull Fractions

Sample	E3	H1	H5	H10
Protein/%	19.2	6.7	0.9	0
Phenolics/%	14	62	29	22
Neutral sugars, x_i/mole %				
L-Rha	4.9	5.0	2.0	0.5
L-Ara	36.6	6.6	3.4	5.9
D-Xyl	21.3	57.4	89.7	84.8
D-Man	12.2	13.7	0	0.6
D-Glc	8.6	7.0	3.4	3.9
D-Gal	16.4	10.3	1.5	4.3

The ^{13}C NMR spectra of H5 in D_2O and DMSO-d_6 showed considerable noise, particularly in the first solvent (Fig. 1*A*), explained by the partial water solubility of the sample. However, in the HSQC-NMR spectrum of H5 in D_2O (Fig. 1*B*), the $^{13}C/^{1}H$ cross-peaks of the nonsubstituted (X) and O-2 substituted (X′)

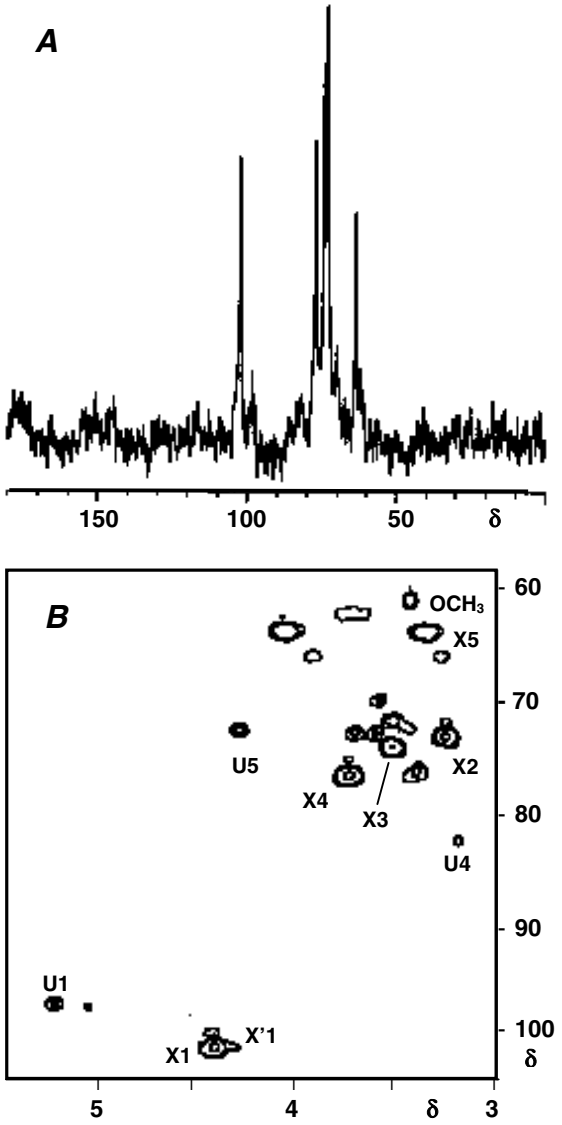

Fig. 1. ^{13}C NMR (*A*) and HSQC-NMR (*B*) spectra (in D_2O) of hemicellulose fraction H5.

Xyl*p* residues, and of the MGA units (U) were well resolved. The resulting chemical shifts are in accord with previously reported data [10, 11]. The presence of phenolic components was confirmed by the complex of resonances in the low field region of the ^{13}C NMR at $\delta \approx 113$—165.

The HPGPC elution pattern of H5 showed a main peak ($\approx$ 90 % of the curve area) with $M_{app} = 60\,000$ and a minor one with $M_{app} = 3000$. All molecular populations showed UV absorption at $\lambda = 245$ nm, indicating that phenolics are closely associated (physically and/or covalently linked) to the glucuronoxylan chains. However, the bulk of UV-absorbing material was eluted in the low-molecular-mass region.

The *in vitro* comitogenic thymocyte test [8] revealed that H5 enhanced the proliferation of thymocytes in the absence and presence of the mitogen – phytohaemagglutinin. This immunostimulatory effect was comparable to that of other mitogenic xylans and the commercial immunomodulator Zymosan [8].

The results suggested that the main hemicellulose component of buckwheat hulls comprises 4-*O*-methylglucuronoxylan, which is a typical hemicellulose component of dicotyledonous angiosperm plants [3] to which buckwheat belongs. Studies of the effect of phenolic components on the biological and other functional properties of the hemicellulose fractions will be the subject of further work.

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