BINDING OF LEAD AND COPPER(II) CATIONS TO HEMICELLULOSES OF RYE BRAN

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Several fractions of acid hemicelluloses isolated from rye bran were characterized by molar ratios of saccharides (D-Xyl, L-Ara, D-Glc, D-Gal) and 4-O-methyl-D-glucuronic acid and protein content. Binding of Pb^{2+} and Cu^{2+} ions to these acid polysaccharides was considered according to function $(M)_b = f([M^{2+}]_f)$, expressing the relationship between the amount of metal $(M)_b$ bound to 1 g of the substance and the concentration of free ions $[M^{2+}]_f$ in the equilibrium solution and according to the association degree β of these cations with carboxyl groups of uronic acid at a stoichiometric ratio of both components in the system under investigation. Acid hemicelluloses contained only a very small portion of uronic acid ((COOH) 0·05—0·18 mmol g^{-1}); the model polysaccharide, 4-O-methyl-D-glucurono-D-xylan of beech, was substantially richer in uronic acid content ((COOH) 0·73 mmol g^{-1}). Consequently, the amount of lead and copper bound to acid hemicelluloses is very small ($(M)_b$ 0·017—0·025 mmol g^{-1}) at $[M^{2+}]_f$ = = 0·10 mmol l^{-1}. On the other hand, much greater amount of cations ($(M)_f$ 0·09—0·10 mmol . . g^{-1}) was bound to the glucuronoxylan. The association degree β was like with the majority of samples (β = 0·31—0·38). The amount of lead and copper(II) bound to acid hemicelluloses from rye bran is several times lower than that bound to dietary fiber isolated from vegetables (cabbage, carrot), rich in pectic substances.

In studies concerning the physiological effect of dietary fiber of foodstuffs great attention has been paid to elucidation of influence of dietary fiber of various origin and composition on the absorption of mineral substances and metal cations from the gastrointestinal tract. First of all, the effect of dietary fiber from various vegetables, fruit, as well as bran and flour (wheat, rice, oats, corn), full-corn bread *etc.* was studied[1-5]. One of the main components of dietary fiber from vegetables and fruit is pectin, the free unesterified carboxyl groups of which are able to bind metal cations. Our preceding papers dealt with the binding of toxic lead cations to dietary fiber isolated from some vegetables (cabbage, carrot), and various species of apple[1] and with binding of lead and copper(II) cations to dietary fiber from crude and cooked potatoes[2]. The predominating constituents of dietary fiber of cereals, which contains only a small amount of pectin, are cellulose and hemicelluloses. The latter are polysaccharides of linear or branched structures formed by neutral saccharides and, in a small extent, also by D-glucuronic acid units and their 4-O-methyl derivative. According to some authors the hemicelluloses cause a higher excretion of cations

in faeces thereby lowering their absorption (*cf. e.g.* review articles[3-5]). Studies dealing with the effect of dietary fiber rich in hemicelluloses, as dietary fiber from bran of various cereals, on the absorption of mineral compounds report predominantly on the total content of hemicelluloses. Some papers present also their chemical composition, as *e.g.* that on interaction of neutral rice hemicelluloses with trace inorganic compounds under action of digestive enzymes[6,7]. So far, no attention has been paid to binding of toxic cations to acid hemicelluloses; reported was only the binding of various cations to D-glucuronic acid[8,9].

This study was aimed to contribute to elucidation of the role of hemicelluloses in foodstuffs as ligands of toxic metal cations. Therefore, this communication concerns the bonding of lead and copper(II) cations to several fractions of acid hemicelluloses isolated from rye bran, which is a considerable basis for foodstuff industry.

EXPERIMENTAL

Isolation and Fractionation of Hemicelluloses

Isolation and fractionation of hemicelluloses from rye bran has already been described in more detail[10]. First of all fat was extracted[11], water-soluble constituents including starch were removed by the action of enzymes[12] and the resulting material was delignified with sodium chlorite according to Klauditz[13]. A subsequent extraction with 1% NH_4OH and 4·5% NaOH (ref.[14]) of the holocellulose obtained in this way afforded hemicellulose fractions R_1 and R_2 in 5·8 and 13·9% yields per dry matter of bran, respectively. Both these fractions were further fractionated: preparations were dissolved in 3% NaOH and the polysaccharides were precipitated by acidification with acetic acid to pH = 5·0 (fractions R_1-A, R_2-A); further fractions were obtained from the filtrate by addition of a three-fold volume of ethanol (fractions R_1-B, R_2-B). Dialysis of the suspension of precipitated hemicelluloses furnished the water-soluble and water-insoluble portions. Fractionation of hemicelluloses R_1 and R_2 yielded four water-soluble (L) and four water-insoluble (NL) fractions. (These fractions are denoted as those reported in the previous paper[10]).

The content of neutral saccharides in the respective fractions in form of alditol trifluoroacetates[15] was determined by gas–liquid chromatographic method[10]. Uronic acids in the hemicellulose hydrolysates were identified by paper chromatography using the appropriate standards. Total content of uronic acids was alkalimetrically estimated by potentiometric titration of solutions or suspensions of hemicelluloses in H^+ form with KOH 0·05 mol l^{-1}. Carboxyl groups in sample R_2-A/NL (water-insoluble) (Table I) containing only a very small amount of uronic acid were determined by a back potentiometric titration of a small excess of potassium hydroxide with HCl 0·01 mol l^{-1}. The titration medium was KCl 0·5 mol l^{-1}; the alkaline solution was protected against carbonization with atmospheric CO_2 during titration by a toluene layer. The accurate point of equivalence was estimated by the appropriate blind experiment. Optical rotation of the 0·5% hemicellulose solutions was determined in 2% NaOH.

The beech glucuronoxylan was the preparation already employed in our previous paper[16]; since D-xylose forms 98% of the neutral saccharides content in the polysaccharide, it is a pure 4-O-methyl-D-glucurono-D-xylan. The mean molecular mass osmometrically determined M_n = 18 600, and uronic acid content 19·7%

All samples of the above-mentioned acid hemicelluloses were passed into H^+ form by washing with acidified 90% ethanol containing HCl 0·1 mol l^{-1}, 90 and 95% ethanols, and ether. The

samples were air-dried at room temperature (the dry matter content 87–89%). Dry matter was estimated at 105°C and atmospheric pressure. The absence of hydrochloric acid, removed from the samples by washing with ethanol, was argentometrically monitored by potentiometric titration with $AgNO_3$ 0·01 mol l^{-1}, silver electrode being used. The resulting hemicellulose samples (H^+ form) did not contain any traces of Cl^-.

Determination of Binding of Lead and Copper(II) Cations to Acid Hemicelluloses

Binding of Pb^{2+} and Cu^{2+} cations was investigated in solutions or suspensions of compounds of ionic strength $I = 0{\cdot}15$ mol l^{-1}. Concentration of free cations $[M^{2+}]_f$ in equilibrium solutions was estimated by ion-specific electrodes. The amount of lead or copper bound to 1 g of the dry sample was calculated from the amount of lead or copper salts added and the concentration of free Pb^{2+} (Cu^{2+}) cations in equilibrium solution.

Hemicelluloses from rye bran in H^+ form (100 mg) were dissolved or suspended in redistilled water (10 ml). The pH of the hemicellulose solution was adjusted to 7·0–7·2 by adding KOH 0·05 mol l^{-1}; KNO_3 1 mol l^{-1} was added in such an amount as the ionic strength I of the resulting solution filled with water to 20 ml was 0·15 mol l^{-1}. Solutions of $Pb(NO_3)_2$ 0·01 mol l^{-1}, or $Cu(NO_3)_2$ 0·01 mol l^{-1} were added to the solution (suspension) in half an hour intervals in graded portions together with the corresponding amount of KNO_3 necessary to keep the afore-mentioned ionic strength of the solution constant. Each addition of the lead of copper salts was followed by tempering the solution to 25·0 ± 0·1°C. Potential of the ion-specific electrode (*EMF*) was determined while stirring the solution (suspension) at constant rate; the concentration of free cations $[M^+]_f$ in equilibrium solution was calculated. Solutions for determination of the relationship $EMF = f(c_{M^{2+}})$ at a constant ionic strength I contained $2 \cdot 10^{-5}$ to $1{\cdot}3 \cdot 10^{-3}$ mol $Pb(NO_3)_2$ or $Cu(NO_3)_2$ in 1 l ($I = 0{\cdot}15$ mol l^{-1}, the additional electrolyte KNO_3). Determination of activity and concentration of free Pb^{2+} (Cu^{2+}) ions using this technique has been described in more detail[17,18]. The volume change of the solution under investigation due to addition of lead (copper) and potassium salts was considered when processing the measured data. The ionic strength of the solution I was identical with all equilibrium mixtures ($I = 0{\cdot}15$ mol l^{-1}).

Each equilibrium mixture involved in the examination of binding of lead and copper(II) cations to glucuronoxylan was separately prepared. The mixture contained 100 mg of glucuronoxylan

TABLE I

Acid hemicelluloses from rye bran (R_1, R_2) and 4-O-methyl-D-glucurono-D-xylan from beech (X-UA)

Sample	Yield	$[\alpha]_D^{20}$	Molar ratios of saccharides						Protein %
			L-Ara	D-Xyl	D-Glc	D-Gal	UA[a]	NS[b]/UA[a]	
R_1-B/L	1·1	−114·1	0·77	1·00	0·01	0·10	0·049	38	4·9
R_2-A/NL	8·6	−106·7	0·14	1·00	0·02	0·00	0·008	152	0·0
R_2-B/L	2·5	−96·0	0·98	1·00	0·14	0·14	0·057	40	1·1
R_2-B/NL	2·4	−20·7	0·55	1·00	0·31	0·07	0·039	49	0·3
X-UA	16·9		—	1·00	—	—	0·112	8·9	0·0

[a] Uronic acids, predominantly 4-O-methyl-D-glucuronic acid; [b] neutral saccharides.

neutralized with potassium hydroxide, graded amounts of lead or copper salts and the additional electrolyte KNO_3 ($I = 0{\cdot}15$ mol l^{-1}) in 100 ml. Solutions or suspensions were stirred for 2 h at an ambient temperature and left to stand overnight. The concentration of free Pb^{2+} or Cu^{2+} ions was determined as mentioned above.

Concentration of Pb^{2+} and Cu^{2+} ions in standard solutions of $Pb(NO_3)_2$ 0·01 mol l^{-1} and $Cu(NO_3)_2$ 0·01 mol l^{-1} was chelatometrically determined. Used were: Complexon IV 0·01 mol . l^{-1}, $MgCl_2$ 0·01 mol l^{-1}, interference filters IF 600 or 650 nm (Zeiss, Jena), indicators Murexide and Eriochrome Black T; the point of equivalence was spectrophotometrically indicated.

The association degree β of ions M^{2+} with carboxyl groups of hemicellulose was calculated according to equation $\beta = (c_{M^{2+}} - [M^{2+}]_f)/c_{M^{2+}}$, where $c_{M^{2+}}$ stands for the initial concentration of ions M^{2+} added in an equivalent amount in respect to carboxyl groups of hemicelluloses in the system under investigation. Values β were calculated by interpolation of results obtained by the above-mentioned technique.

Apparatuses and reagents: gas chromatograph Hewlett–Packard 5 700 A (England), digital potentiometer Radiometer PHM 64 (Denmark), ion-specific electrodes Crytur, Pb type 82-17 Cu type 29-17 (Monokrystaly, Czechoslovakia) and special two-chamber saturated calomel electrode Radiometer, type K-711 (outer chamber of the electrolyte bridge was filled wiht 10% KNO_3). The chemicals used were of *p.a.* grade; KOH 0·05 mol l^{-1} was carbonate-free, the redistilled water was free of atmospheric carbon dioxide.

RESULTS AND DISCUSSION

Characterization of Acid Hemicelluloses

Fractions of hemicelluloses from rye bran[10] were chosen for further experiments on the basis of preliminary analyses; only samples containing uronic acid units besides of neutral saccharides were employed (Table I). They consisted of L-arabino-D-xylans of various branching degree. The main polysaccharide chain consisted of D-xylopyranose units linked by a glycosidic $\beta(1 \rightarrow 4)$ bond. The side chains are formed by L-arabinofuranose units linked by an $\alpha(1 \rightarrow 3)$ bond, the highly branched polysaccharide included also $\alpha(1 \rightarrow 2)$, bonds, ref.[19]. The uronic acid is mostly the 4-O-methyl-D-glucuronic acid, which is bound to D-xylose unit of the main chain by an $\alpha(1 \rightarrow 2)$ bond. Some fractions were contamined in a small extent by neutral polysaccharides containing D-glucose and D-galactose units. The investigated fractions of hemicelluloses contained, excepting the sample R_1-B/L, a negligible amount of proteins. The samples of water-soluble hemicelluloses (R_1-B/L, R_2-B/L) constitute only a small portion of the total amount of hemicellulose fractions.

As shown in Table I, the hemicellulose samples from rye bran contained a very little amount of uronic acids. This was the reason why we have chosen 4-O-methyl-D-glucurono-D-xylan from beech sawdust containing several-times higher portion of uronic acid as model substance for investigating the binding of cations. The main macromolecule chain of this polysaccharide is composed of D-xylose units with a glycosidic $\beta(1 \rightarrow 4)$ bond. 4-O-Methyl-D-glucuronic acid units are bound to D-xylose units as monomeric side chains by glycosidic $\alpha(1 \rightarrow 2)$ bonds. Transformation of the

original glucuronoxylan sample into H^+ form by washing with acidified ethanol resulted in a small decrease of uronic acid content in the macromolecule to a final molar ratio D-xylose to uronic acid 8·9 : 1, Table I (for details see[16]).

Binding of Pb^{2+} *and* Cu^{2+} *Cations to Acid Hemicelluloses*

Binding of Pb^{2+} and Cu^{2+} cations to acid hemicelluloses was evaluated according to function $(M)_b = f([M^{2+}]_f)$ describing the relationship between the amount of metal bound to 1 g of dry sample (mmol $(M)_b\,g^{-1}$) and the equilibrium concentration of free $[M^{2+}]$ ions in solution (mmol $[M^{2+}]_f\,l^{-1}$) and according to the association degree β of M^{2+} ions with carboxyl groups of hemicelluloses. The amounts of lead and copper bound to 1 g of the sample at equilibrium concentrations $[Pb^{2+}, Cu^{2+}]_f$ 0·01 and 0·10 mmol l^{-1}, respectively, are listed in Table II for a better comparison of binding of these cations to various fractions of hemicelluloses.

The course of curves $(M)_b = f([M^{2+}]_f)$ in Figs 1 and 2, and the results presented in Table II show that binding of lead and copper cations to acid hemicelluloses from rye bran is very small, this being associated with a low content of uronic acid in these hemicelluloses ((COOH) 0·05–0·18 mmol g^{-1}). Lead and copper were prevalently bound to hemicellulose fractions soluble in water (R_1-B/L, R_2-B/L), whereas the

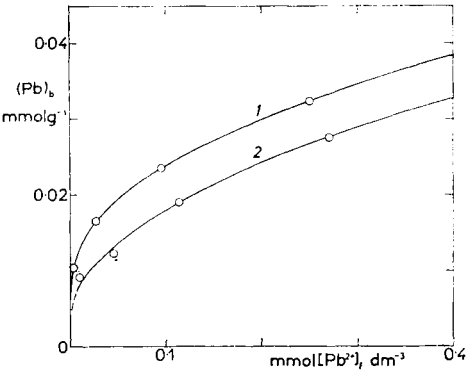

FIG. 1

Binding of lead cations to soluble fractions of acid hemicelluloses from rye bran, I = = 0·15 mol l^{-1}, $(Pb)_b$ millimols of lead bound per 1 g of dry sample, Pb^{2+} concentration of free Pb^{2+} ions in equilibrium solution. *1* R_1-B/L, *2* R_2-B/L (see Table I)

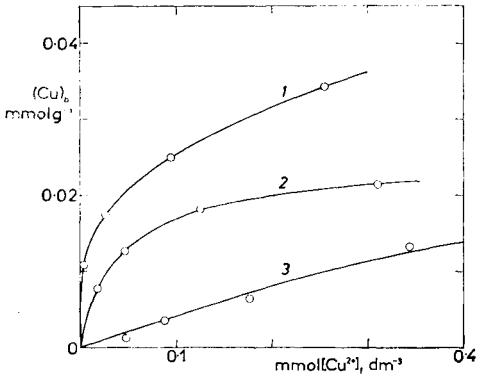

FIG. 2

Binding of copper(II) cations to fractions of acid hemicelluloses from rye bran, I = = 0·15 mol l^{-1}, $(Cu)_b$ millimols of copper-(II) bound per 1 g of dry sample, $[Cu^{2+}]_f$ concentration of free Cu^{2+} ions in equilibrium solution. *1* R_1-B/L, *2* R_2-B/L, *3* R_2-B/NL (see Table I)

binding of these cations to the majority of insoluble fractions is negligible. The insoluble fraction R_2-B/NL binds exclusively copper in a very low extent even though the content of its carboxyl groups ((COOH) 0·142 mmol g^{-1}) is comparable with that of carboxyl groups of soluble fractions of acid hemicelluloses (R_1-B/L, R_2-B/L – (COOH) 0·178 and 0·180 mmol g^{-1}, respectively). The carboxyl groups of uronic acid of insoluble fractions are probably difficultly accessible for Pb^{2+} and Cu^{2+} ions due to steric hindrance. On the other hand, the exchange of $K^+ \rightarrow H^+$ ions during neutralization of these acid hemicelluloses with potassium hydroxide proceeds easily.

Binding of lead and copper to 4-O-methyl-D-glucurono-D-xylan from beech sawdust is substantially greater in accord with the several-times greater content of carboxyl groups in preparation ((COOH) 0·728 mmol g^{-1}, Figs 3 and 4, Table II). Binding of Pb^{2+} and Cu^{2+} ions to glucuronoxylan was investigated after adjusting the pH of the sample to 7·0–7·2. Due to hydrolysis of the lead or copper salts added, the pH of the equilibrium solution decreased to 5·97–5·46 with the lead salt and to 5·94–5·18 with the copper one. Binding of Cu^{2+} was investigated also with the glucuronoxylan in H^+ form at pH 3·60 ± 0·01 (Fig. 4, curve 2). Here, due to the low pH a partial suppression of dissociation of carboxyl groups took place, this being manifested by a weaker binding of copper to glucuronoxylan in H^+ form. Binding of Pb^{2+} and Cu^{2+} ions to acid polysaccharides containing carboxyl groups considerably depends on the pH of the solution[1,2].

TABLE II

Binding of lead and copper(II) cations to acid hemicelluloses from rye bran (R_1 and R_2) and 4-O-methyl-D-glucurono-D-xylan from beech (X-UA), I = 0·15 mol l^{-1} (KNO_3)

Sample	(COOH) mmol g^{-1}	$(M)_b$, mmol g^{-1}				$\beta_{Pb^{2+}}$	$\beta_{Cu^{2+}}$
		$[Pb^{2+}]_f$ mmol l^{-1}		$[Cu^{2+}]_f$ mmol l^{-1}			
		0·01	0·10	0·01	0·10		
R_1-B/L	0·178	0·013	0·024	0·014	0·025	0·36	0·38
R_2-A/NL	0·049	*a*	*a*	*a*	*a*	*a*	*a*
R_2-B/L	0·180	0·008	0·018	0·005	0·017	0·31	0·24
R_2-B/NL	0·142	*a*	*a*	*a*	0·004	*a*	0·14
X-UA	0·728	0·031	0·097	0·049	0·086	0·34	0·32
Y[b]	—	0·32	0·37	—	—	—	—
Z[c]	—	0·28	0·33	—	—	—	—

[a] Within experimental error; Y, Z dietary fiber isolated from cabbage or carrot (ref.[1]); $(M)_b$ lead and copper bound; $[M^{2+}]_f$ concentration of free Pb^{2+} or Cu^{2+} ions in equilibrium solution; β association degree of M^{2+} ions with carboxyl groups of hemicelluloses; [b] from cabbage; [c] from carrot.

Table II is supplemented by data[1] concerning the binding of lead to dietary fiber isolated from some nutritionally important kinds of vegetables, from cabbage (Y) and carrot (Z) (substances insoluble in 80% ethanol). Binding of lead to these dietary fibers rich in pectin of a medium degree of esterification E of carboxyl groups with methanol (E 46–63%) is many-times higher than with hemicelluloses from rye bran and also with glucuronoxylan from beech sawdust. Binding of lead cannot be here correlated only with the content of free carboxyl groups of pectin in the dietary fiber ((COOH) 0·62–0·63 mmol g^{-1}), since a complex mixture of compounds is involved, which can contain also salts of some low-molecular acids insoluble in 80% ethanol in addition to macromolecular constituents (polysaccharides, proteins). It has been, however, shown that the capacity of the binding of lead to dietary fiber of cabbage and carrot was roughly twice higher than that corresponding to free carboxyl groups of pectin present in them.

Polysaccharides related to D-xylan investigated in this paper differ from each other by the content of neutral saccharides and uronic acids. Aiming to evaluate the ability of uronic acid of these compounds to bind lead and copper, the association degree β of lead and copper cations with carboxyl groups was calculated; the addition of metal cations was equivalent to the content of carboxyl groups in the system under investigation (Table II). Carboxyl groups of soluble fractions of hemicelluloses from rye bran (R_1-B/L, R_2-B/L) showed virtually the same affinity towards Pb^{2+} ions as the carboxyl groups of 4-O-methyl-D-glucurono-D-xylan from beech sawdust $\beta_{Pb^{2+}}$ = = 0·31–0·36. Mutually close were found also the β values corresponding to binding

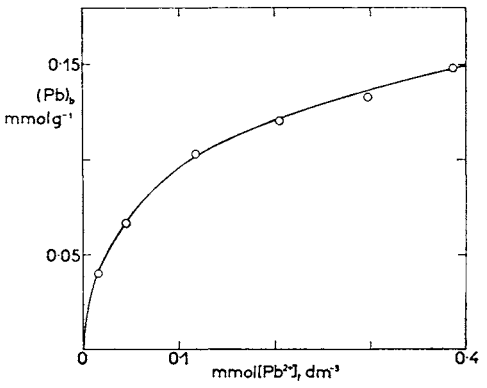

FIG. 3

Binding of lead cations to 4-O-methyl-D-glucurono-D-xylan from beech, I = 0·15 mol . l^{-1}, $(Pb)_b$, $[Pb^{2+}]_f$ *cf.* Fig. 1

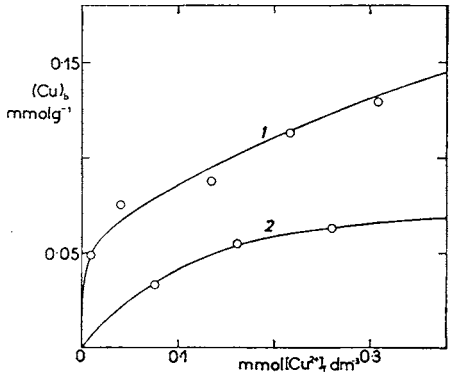

FIG. 4

Binding of copper(II) cations to 4-O-methyl-D-glucurono-D-xylan from beech, I = = 0·15 mol l^{-1}, $(Cu)_b$, $[Cu^{2+}]_f$ *cf.* Fig. 2, *1* pH 5·9–5·2, *2* pH 3·60 ± 0·01

of Cu^{2+} ions to soluble acid hemicelluloses of various origin, $\beta_{Cu^{2+}} = 0{\cdot}24-0{\cdot}38$. An exception was the value $\beta_{Cu^{2+}} = 0{\cdot}14$ found with the insoluble fraction R_2-B/NL. Binding of lead and copper(II) to other insoluble hemicellulose fractions was negligible. Obviously, the soluble acid hemicelluloses reveal very probably the same, or at least a similar mechanism of interaction of Pb^{2+} and Cu^{2+} ions with carboxyl groups irrespective of the structure of hemicelluloses. The determined values β (Table II) show that an equivalent addition of lead and copper to acid soluble hemicelluloses results in a 31- to 36%-binding of Pb^{2+} ions and 24- to 38%-binding of Cu^{2+} ions.

Finally, the β values characteristic of binding of Pb^{2+} and Cu^{2+} ions to soluble acid hemicelluloses (Table II) were compared with those already determined[20] for the binding of these cations to monomeric D-galacturonic acid at the same ionic strength of the solution $I = 0{\cdot}15$ mol l^{-1}: $\beta_{Pb^{2+}} = 0{\cdot}26$, $\beta_{Cu^{2+}} = 0{\cdot}24$. According to our earlier findings[20] the values for the glucuronic acid as the structural unit of tested hemicelluloses could be anticipated even a little lower. The hydroxyl group at $C_{(4)}$ in the D-glucuronic acid could not, however, participate in formation of the Pb- or Cu-complexes from steric reason, as was the case with D-galacturonic acid, where the axial —OH group at $C_{(4)}$ is situated at the same side of the pyran ring as the equatorial carboxyl group. Differences in stability constants of lead and copper complexes of D-galacturonic acid ($\log K_{Pb} = 2{\cdot}00$, $\log K_{Cu} = 1{\cdot}81$) and D-glucuronic acid ($\log K_{Pb} = 1{\cdot}62$, $\log K_{Cu} = 1{\cdot}48$) at the ionic streength $I = 1{\cdot}0$ mol l^{-1} determined by Makridou and coworkers[8] lead to the same conclusion.

Soluble fractions of acid hemicelluloses from rye bran displayed a very high molar ratio of neutral saccharides to uronic acid (NS/UA 38–40). At a random distribution of side chains containing uronic acid in the hemicellulose molecule mutually "isolated" uronic acid units considerably remote from each other would be involved. It could be expected that the ability of such an isolated uronic acid unit to bind cations would roughly correspond to the binding of the respective cation to the monomeric uronic acid[21]. A little higher β values found with acid hemicelluloses when compared with monomeric uronic acid could be caused by a special irregular distribution of uronic acid units in the macromolecule of hemicelluloses, as *e.g.* with acid polysaccharides from peach gum[22], 4-O-methyl-D-glucurono-D-xylan[23] from the bark of white willow (*Salix alba* L.), and 4-O-methyl-D-glucurono-D-xylan of beech[16] (*Fagus sylvatica* L.).

Hemicelluloses of rye bran contained only a very small portion of uronic acid. Due to this fact also the binding of lead and copper(II) to these substances is small in comparison with their binding to dietary fiber isolated from cabbage and carrot. The significant binding of mineral compounds to cereal bran observed *in vivo* should obviously be first of all ascribed to adsorption and not only to interaction of cations with uronic acids in hemicelluloses present.

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REFERENCES

1. Kohn R., Malovíková A., Bock W., Dongowski G.: Nahrung *25*, 853 (1981).
2. Kohn R., Dongowski G., Bock W.: Nahrung *30*, 39 (1986).
3. Kelsay J. L.: Cereal Chem. *58* (1), 2 (1981).
4. Ali R., Staub H., Coccodrilli G. jr, Schanbacher L.: Agric. Food Chem. *29*, 465 (1981).
5. James W. P. T. in the book: *Medical Aspects of Dietary Fiber* (Spiller G. A., McPherson Kay R., Eds), p. 239. Plenum Medical Book, New York 1980.
6. Mod R. R., Ory R. L., Morris N. M., Normand F. L.: J. Agric. Food Chem. *29*, 449 (1981).
7. Mod R. R., Ory R. L., Morris N. M., Normand F. L.: Cereal Chem. *59*, 538 (1982).
8. Makridou C., Cromer-Morin M., Scharff J. P.: Bull. Soc. Chim. Fr., 1, *1977*, 59.
9. Balt S., de Bolster M. W. G., Booij M., van Herk A. M., Visser-Luirink G.: J. Inorg. Biochem. *19*, 213 (1983).
10. Hromádková Z., Ebringerová A.: Nahrung, in press.
11. Theander O., Åman P. in the book: *The Analysis of Dietary Fibers in Food* (James W. P. T., Theander O., Eds), Vol. 3, pp. 51—70. Dekker, New York 1981.
12. Furda I.: Cereal Foods World *22*, 252 (1977).
13. Klauditz W.: Holzforschung *11*, 110 (1957).
14. Ebringerová A., Kramár A., Rendoš F., Domanský R.: Holzforschung *21*, 74 (1967).
15. Shapiro J.: Nature *222*, 792 (1969).
16. Kohn R., Hromádková Z., Ebringerová A., Toman R.: This Journal, in press.
17. Kohn R.: This Journal *47*, 3424 (1982).
18. Kohn R., Heinrichová K., Malovíková A.: This Journal *48*, 1922 (1983).
19. Ebringerová A., Hromádková Z., Petráková E.: Unpublished results.
20. Kohn R., Hirsch J.: This Journal, in press.
21. Kohn R., Luknár O.: This Journal *40*, 959 (1975).
22. Kohn R., Rosík J., Kubala J., Malovíková A.: This Journal *44*, 2517 (1979).
23. Toman R., Kohn R., Malovíková A., Rosík J.: This Journal *46*, 1405 (1981).

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