The Structural, Molecular and Functional Properties of Lignin-Containing Beechwood Glucuronoxylan

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Summary: The samples of 4-O-methyl-D-glucurono-D-xylan (GX) with different lignin and uronic acid (MeUA) contents were prepared from beechwood by various procedures. The re-dissolution process of the partially water-soluble GX-2 lyophilizate in aqueous medium depended on the content and distribution of MeUA. In contrast to the supernatant after ultracentrifugation, the sediment had a significantly lower MeUA content and showed mainly high-molecular mass components. A part of lignin degradation products is separable by repeated dissolution and precipitation of GX in acidic and alkaline 80% ethanolic media with assistance of ultrasound. Irradiation in acidic ethanol (GX-3/ac) showed no effect on the distribution of the UV_{254}-absorbing component. GX-3 sonicated in neutral and alkaline media (GX-3/ne, GX-3/alk) lost water solubility. The lignin-rich GX samples showed remarkable emulsifying efficiency and protein foam stabilization effect. In addition, the presence of phenolics provided antioxidant properties to these xylan preparations indicating their potential as polymeric radical scavengers.

Keywords: antioxidants; gel permeation chromatography (GPC); irradiation; lignin; 4-O-methylglucuronoxylan

Introduction

Because of the continuous exhaustion of the ready sources of feedstocks, such as coal and petroleum-based compounds, there is a need for developing alternate renewable feedstocks. The 4-O-methyl-D-glucurono-D-xylan (GX) type of hemicelluloses, the most abundant in cell walls of hardwoods (20-28%)[1,2] represents one such readily available natural feedstock. In the last decade, many useful applications of xylans as crude mixtures or relatively pure polymers in papermaking, textile printing, food and pharmaceutical industries have been reviewed.[3–6] Recently, the preparation of hydrogels from aspenwood GX in combination with chitosan has been reported.[7] However, the potential of this biopolymer has not yet been completely realized.

The structural studies on the GX component of various hardwood species revealed[1,8] that it comprises a family of closely related polymers composed of a $(1 \rightarrow 4)$-β-D-xylopyranan backbone which have single 4-*O*-methyl-D-glucopyranosyl uronic acid (MeUA) residues attached at O-2 of some of the xylose monomers. The polymers show heterogeneity in the content and distribution of the MeUA side chains as well as in molecular mass distribution, dependent on the wood source and extraction method used. The isolation of GX usually needs a previous or simultaneous partial delignification of the wood.[1,4,6,9–14] During this process, also the chemical and physicochemical properties of the polysaccharide might be affected to various extent resulting in chemical modification and/or degradation of the xylan molecule.

A disadvantage of many GX preparations, that limits the investigation of their

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physicochemical properties and application possibilities as well, is that they are only in part soluble in aqueous medium, whereupon the low-branched samples are even insoluble in solvents applied for cellulose. In earlier studies[1] the molecular properties of GX from hardwoods have been investigated by osmometry, ultracentrifugation as well as LS methods using various solvents such as aqueous sodium hydroxide solutions, DMSO, and complexing agents applied for cellulose. Later a few studies dealing with gel chromatography of GX have been reported as well.[15]

For a better understanding of the overall properties of GX polymers, further work is needed to ascertain the effect of various factors which govern the behavior of this biopolymer in solution. This can help to find new applications for hardwood xylans in many industrial fields. In our study we investigated the solubility, structural, molecular and functional properties of beechwood GX.

Material and Methods

All GX samples were isolated from beechwood on semitechnical scale in the pilot plant of our Institute. GX-1 was extracted from beechwood holocellulose, obtained by acidic chlorite delignification, with 5% NaOH in the second step of the two-step extraction method.[16] The water-soluble fraction of GX-1 was separated and lyophilized yielding sample GX-2. Sample GX-3 was isolated from beechwood sawdust according to the two-step extraction method[17] using 5% NaOH/3% H_2O_2 in the first step. GX-4 was separated from GX-3 as the water-soluble part.

The sugar composition of the GX samples was determined by acid hydrolysis followed by quantitative analysis of the released neutral sugars by gas chromatography of their alditol trifluoroacetates (Hewlett-Packard instrument, Model HP 5890) as described in a previous paper.[18] The uronic acid content was determined by potentiometric titration of the protonated samples.[19] The content of phenolics was determined by the Klason method as the resistant residue from the two-stage acid hydrolysis.[20]

Fourier-transform infrared (FT-IR) spectra of samples (2 mg) in KBr pellets (200 mg) were obtained on the NICOLET Magna 750 spectrometer with DTGS detector and OMNIC 3.2 software using 128 scans at a resolution of 4 cm^{-1}. The ^{13}C-NMR spectra (in D_2O) were recorded in the inverse gated decoupling mode at 25 °C on a Bruker DPX AVANCE-300 spectrometer operating at 75.46 MHz for ^{13}C. UV spectra were recorded with a Pye-Unicam 1700 UV spectrophotometer. High performance gel permeation chromatography (HPGPC) was performed using a commercial instrument (Laboratorni pristroje, Prague, Czechoslovakia) equipped with Separon HEMA-BIO 100 and 1000 exclusion columns (Tessek Ltd., Prague) using 0.1 M aqueous $NaNO_3$ as the solvent and eluent. The eluate was monitored by refractometry and UV absorption at 254 nm. The columns were calibrated with a set of Pullulan standards P5 – P800 (Shodex Standard P-82, Macherey-Nagel, Germany).

Fractionation and Purification of GX

The freeze-dried polysaccharide GX-2 (50 mg) was solubilized in water (5 ml) and the solution was ultracentrifuged (170000 g) for 1h to separate the soluble (GX-2/Spt-1) and insoluble (GX-2/Sed-1) portions of GX-2. Then, the centrifugation was prolonged at the same conditions for further 1 h yielding fractions GX-2/Spt-2 and GX-2/Sed-2.

For purification, the GX-3 sample (0.5 g) was immersed into 80% EtOH (50 ml) and the pH of the dispersion was adjusted to 3.0 and 9.0 with 5% HCl and 5% NaOH, respectively. The ultrasonic irradiation of the polysaccharide dispersion (1%, w/v) was carried out with the aid of the Ultragen system PERSON (Slovakia, 20 kHz) at sonic power 100 W using a cylindric sonotrode. The dispersion was poured into a glass vessel surrounded by ice. After irradiation at pH 3.0, 7.0 and 9.0 for 5 min at 30 ± 5 °C, the treated dispersions were

filtered yielding the purified samples GX-3/ac, GX-3/ne, and GX-3/alk as well as the corresponding filtrates Fil-ac, Fil-ne, and Fil-alk, respectively.

Functional Properties

The emulsifying efficiency was tested on emulsions of the oil/water (O/W) type. They were prepared by mixing 9 ml of distilled water containing 0.05 g of the sample and 1 ml of paraffin oil dyed with SUDAN IV in the laboratory mixer (Heidolph DIAX 600) at 20500 rpm for 1 min. The stability of the emulsion was determined at three different time intervals after the emulsions had been prepared, i.e. after 5 min (h_1), 1 h (h_2) and 24 h (h_3), and expressed in terms of the height (h, mm) of oil and cream layers formed on the surface of the emulsion.

The protein foam stabilization effect was estimated according to Izydorczyk[21] by adding 0.25 ml of an aqueous solution (1 %, w/v) of the sample to 1 ml of bovine serum albumin (BSA) solution (2 %, w/v), followed by vigorous shaking for 30 s. The foam volume (ml) attained was measured immediately after the foam was generated (V1) and after heat treatment in a water bath at 95 °C for 3 min (V2).

Antioxidant activity was determined by measurement of chemiluminiscence (CL) using Luminometer BioOrbit 1251 as described in detail.[22] The chemiluminescence signal is driven by the production of luminol derived radicals. The antioxidant activity was expressed as total radical trapping potential (TRAP) and calculated from the duration of the period of time (t) during which the serum sample resists lipid peroxidation. The $TRAP_{exp}$ value for the sample was obtained by equation:

$$TRAP_{exp} = 2.0 \times t_{sample}/c \times t_{trolox},$$

where c is the concentration of the sample, and 2.0 is the stoichiometric factor of trolox, used as antioxidant standard.

Results and Discusion

Analysis of GX samples isolated from beechwood by different extraction procedures (Table 1) revealed that the content of xylose varied from 87.1 mole% to 93.0 mole % related to the neutral sugar constituents. Uronic acids were found in all samples and the mole ratios of Xyl:MeUA varied from 8.6:1 to 11.2:1. All samples contained small amounts of phenolics determined as Klason lignin.

Table 1.
GX samples prepared from beechwood by various alkaline extraction procedures.

Xylan	Source	Xylose	Xyl: MeUA	Klason lignin
		Mole %[a]	Mole ratio	%
GX-1	Holocellulose	93.0	8.6:1	1.9
GX-2	Holocellulose	91.8	10.1:1	2.8
GX-3	Sawdust	88.9	10.9:1	4.3
GX-4	Sawdust	87.1	11.2:1	6.5

[a] Based on neutral sugars.

The ^{13}C-NMR spectra of all GX samples, shown for GX-4 in Figure 1, exhibited the same pattern typical of the 4-O-methyl-D-glucurono-D-xylan type.[8] The presence of lignin compounds is indicated by signals of the aromatic components in the region of δ 110–165.[23]

As seen in Figure 2, the FT-IR spectra of the samples from the chlorite holocellulose (GX-1, GX-2) and sawdust under alkaline-oxidative conditions (GX-3, GX-4) show similar patterns in accord with their similar sugar composition. The bands at 1047, 985 and 898 cm^{-1} are typical of glucuronoxylan-type polysaccharides.[24]

The presence of lignin and its degradation products in all samples was indicated by absorptions bands at 1500–1520 cm^{-1} in the FT-IR spectra.[24] Delignification of the wood prior to xylan extraction resulted in a significantly smaller band at 1505 cm^{-1} in the FT-IR spectrum of GX-1 and GX-2.

Fractionation and Purification of GX

Usually the GX isolated from hardwoods by alkali extraction are only partially soluble in water. This is because the GX component is chemically heterogeneous, composed of fractions differing in the proportion of the MeUA side chains, as reported for beechwood xylans.[25] Moreover, the distribution

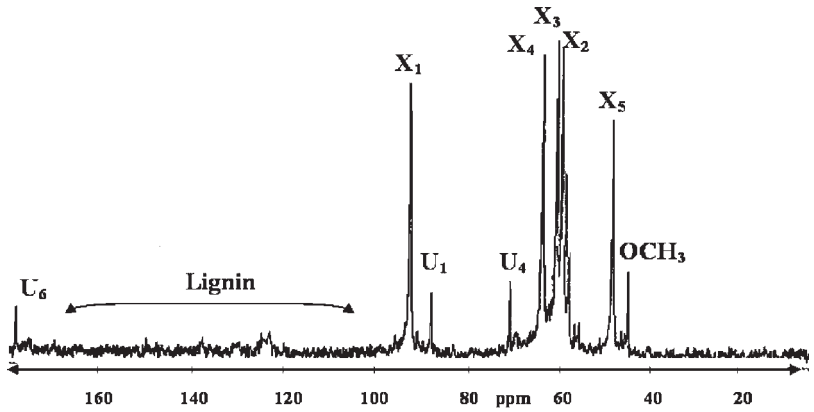

Figure 1.
^{13}C-NMR spectrum of GX-4 (in D_2O). X: 4-linked β-xylopyranosyl residues; U: 4-O-methyl-α-glucuronic acid.

of the side chains is irregular.[8] The sample GX-2, separated from GX-1 as its water-soluble portion, became after recovery from the solution by lyophilization only partially soluble.

HPGPC analysis of GX-2 (Figure 3) using UV- and RI- monitoring revealed a bimodal distribution of polymer chains. The chromatogram shows two components (peak I) and (peak II) in the region of high molecular mass (HMM) and medium molecular mass (MMM), respectively. In further experiment, GX-2 was subjected to ultracentrifugation for 1 and 2 h yielding water-soluble and water-insoluble subfractions (Table 2). Chemical analyses of GX-2 and their subfractions showed that most of the MeUA residues were present in the supernatant fraction obtained after 2h (GX-2/Spt-2). In contrast, the corresponding sediment GX-2/Sed-2 had a significantly lower MeUA content. HPGPC analysis of the subfractions revealed that the HMM population was accumulated mainly in the sediment GX-2/Sed-2. After 1 h centrifugation the proportion of HMM and MMM

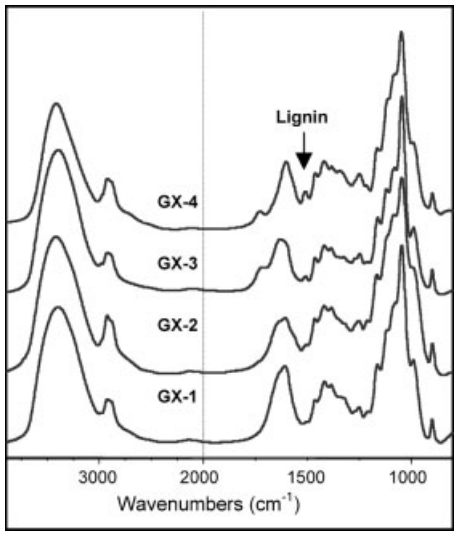

Figure 2.
FT-IR spectra of xylan samples isolated by different extraction procedures from beechwood holocellulose (GX-1, GX-2) and beechwood sawdust (GX-3, GX-4).

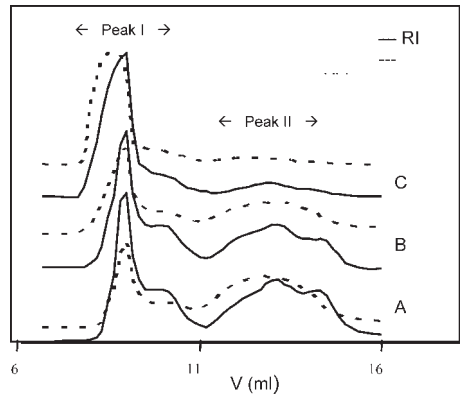

Figure 3.
HPGPC chromatograms of GX-2 measured on pullulan-calibrated Separon HEMA-BIO 1000 columns. A: GX-2/Spt-2; B: GX-2; C: GX-2/Sed-2.

Table 2.
Characteristics of GX-2 and its fractions separated by ultracentrifugation.

Xylan	Yield	MeUA[a)]	$[\eta]$	Molecular mass distribution[b)]			
				Peak I		Peak II	
	Wt-%	mmol/g	ml/g	$M_w \times 10^{-3}$	Area %[c)]	$M_w \times 10^{-3}$	Area %[c)]
GX-2	100	0.634	88.0	479	51.6	22.9	48.4
GX-2/Spt-1	—	—	—	489	56.2	19.7	43.8
GX-2/Spt-2	66	0.753	50.5	424	50.6	24.0	49.4
GX-2/Sed-1	—	—	—	477	78.8	20.0	21.2
GX-2/Sed-2	34	0.296	89.0	568	71.1	25.0	18.9

[a)] Content of 4-O-methylglucuronic acid; [b)]Determined by HPGPC on pullulan-calibrated Separon HEMA-BIO columns; [c)]Area % calculated from the elution curve.

components in the subfractions (GX-2/Spt-1, GX-2/Sed-1) was similar. The polydispersity ($P = M_w/M_n$), calculated from the RI-detected elution curves ranged for peak I from 1.1 to 1.25 and for peak II from 1.4 to 1.6 for all subfractions.

The HPGPC record of all fractions (Figure 3) obtained by RI- as well as UV_{254}-detection showed the same pattern, what indicated the distribution of UV-absorbing components over all the xylan molecules. The results suggested a close association of the contaminating lignin and/or lignin degradation products.

Due to the presence of longer unsubstituted regions, the GX chains tend to form strong intermolecular hydrogen bonds during water removal by the drying process.[8] The re-dissolution of GX particles in aqueous medium depends on their swellability and scission of the hydrogen bond system. Macroscopic gel particles can be disintegrated by intense mechanical stirring. More intense disintegration of the gel particles was achieved by ultrasonication, as reported for the corn cob xylan[26] and corn hull xylan.[27]

However, in dependence on the ultrasonication conditions, polymer chain degradation occurred. Lignin closely associated to the xylan chains seems to contribute to formation of microgels as well.[8]

It was of interest to find out the capability of ultrasound treatment to remove the lignin component from the GX samples. The effects of irradiation on GX-3 was studied by treatment in neutral, acidic, and alkaline 80 % ethanol and by characterizing the treated xylan samples isolated from the suspensions by filtration (GX-3/ac, GX-3/alk and GX-3/ne) using RI- and UV_{254}-detected HPGPC chromatograms (Figure 4) as well as by UV-spectroscopy of the filtrates (Figure 5).

HPGPC analysis of GX-3 before and after irradiation indicated the presence of different proportions of two molecular populations, one with molecular mass in the range of $20–50 \times 10^3$ (peak II) and the second one at $M_w > 10^5$ (peak I). The UV-absorbing material was associated mainly to the HMM component. As seen in

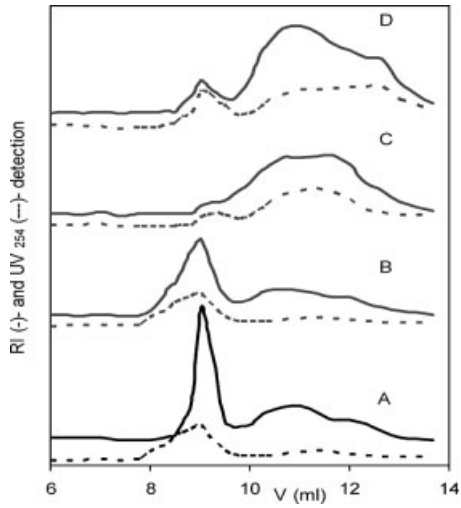

Figure 4.
HPGPC chromatograms of the water-soluble part of GX-3 before and after purification by ultrasound. A: GX-3; B: GX-3/ac; C: GX-3/alk; D: GX-3/ne.

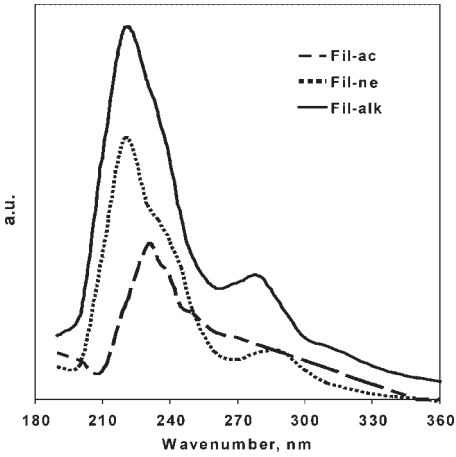

Figure 5.
UV-spectra of the GX-3 ethanolic filtrates after irradiation at various pH.

Figure 4, irradiation in acidic ethanol (GX-3/ac) had no effect on the distribution of the UV_{254}-absorbing material. However, the proportions of the HMM component changed. GX-3 sonicated in neutral and alkaline media (GX-3/ne, GX-3/alk) lost solubility and the HPGPC records of the water-soluble parts show very small peaks in the HMM region, particularly in the case of GX-3/alk. Similar changes were observed also in the corresponding UV_{254}-detected records. However, in both cases the UV-absorbing material increased in the MMM region. Due to the sonomechanical effect, disintegration of the particles and solubilization of GX-3 was achieved, however, some lignin remained associated to the xylan.

The removal of the UV-absorbing components during irradiation was followed by UV-spectral changes of the corresponding aqueous ethanolic filtrates. The UV-spectrum of Fil-alk (Figure 5) as well as that after acidification of the filtrate to pH 3 (not shown) were similar and had a main maximum at 220 nm with a shoulder at 230–240 nm, and a minor maximum at 280 nm. The UV-spectrum (Figure 5) of the filtrate from the irradiation in acidic ethanol (Fil-ac) showed no maximum at 220 nm, but one at 230 and shoulders at 240 and 250 nm, also after changing the pH of the filtrate to 9 (not shown). The results correspond with the presence of solubilized lignin degradation products.[28] After irradiation, FT-IR analyses indicated nor significant structural differences of the GX-3 polymer, nor significant changes of the vibration band at $\sim$1500 cm^{-1}, typical of lignin structures.

From these results it can be suggested that a part of lignin and lignin degradation products are in part separable by repeated dissolution and precipitation of GX with assistance of ultrasound using different pH of the dispersion medium. This indicates that a part of lignin is very strongly associated to the xylan molecules, probably by covalent bonds supposed by various authors.[13,23]

Functional Properties of GX

Lignin, physically and/or covalently bound, is suggested to create hydrophobic sites on the hydrophilic xylan chains. In contrast to GX-1, the samples with a higher content of lignin showed remarkable surface active properties. They form emulsions of the O/W type (Table 3). The emulsifying efficiency is affected by the lignin content. The GX became surface-active, when a certain thydrophilic/hydrophobic balance was achieved, what depends not only on the content of lignin, but also of the glucuronic acid side chains. Therefore, in contrast to GX-1, xylans GX-2, GX-3 and GX-4 formed emulsions with stabilities comparable to that of the commercial emulgator Tween 20, used as standard. The cream layer formed with the lignin-richest GX-4

Table 3.
Emulsifying efficiency[a] of various GX samples.

Xylan	Oil (mm)/Cream (mm)layers		
	h_1	h_2	h_3
GX-1	0/7	0.5/10	1/9
GX-2	0/0	0/14	0/15
GX-3	0/0	0/16	0/15
GX-4	0/0	0/5	0/11
Tween 20	0/0	0/0	0/4

[a] Expresed as the oil and cream layers, formed after (h_1) 5 min, (h_2) 60 min and (h_3) 24 h. Tween 20 (oxyethylated monolauransorbitol) - standard.

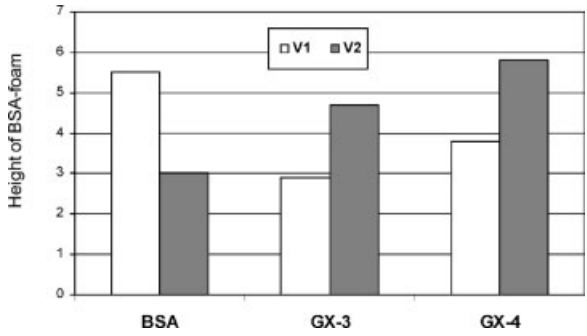

Figure 6.
The protein foam stabilization effect of GX-3 and GX-4. Foam volume (V1) before heating and (V2) after heating at 95 °C for 3 min. BSA: Bovine serum albumine.

was stable even after 5 days of rest, as no oil was separated after this time.

The stabilizing effect of the lignin-containing GX on the foam generated by the protein BSA is illustrated in Figure 6. The high foam volume generated by the control solution (BSA) diminished after heating. The samples GX-2 (not shown), GX-3 and GX-4 depressed the formation of the BSA foam due to the increased viscosity of the liquid medium. However, after heating the foam volume increased and was in presence of GX-4 even higher than the BSA foam volume before heating. This behavior is similar to that reported also for other xylan types,[6,8] which were assumed to protect gas cells against thermal disruption due to their viscosity-enhancing and film-forming properties,[29] both depending on the chemical structure and molecular mass.[8] As seen, the stabilizing effect of the GX with higher lignin content was high. Very probably, both the film-forming property of the GX chains and their hydrophobization due to the associated lignin component might contribute to the observed stabilizing effect. The results indicated that lignin-rich GX-type hemicelluloses represent potential polysaccharide-based polymeric surfactants.

Due to the presence of lignin, which in the case of alkali-lignin isolated from cereal fibers has been reported[30] to exhibit significant free radical scavenging effects, the antioxidant activity of all tested GX samples to peroxyl radicals was determined using the chemiluminiscence method.[22] The results are shown in Table 4. The xylans extracted from holocellulose (GX-1, GX-2), which had a relatively low content of lignin (Table 1), exhibited a lower antioxidant activity in comparison to that of GX-3, GX-4 isolated from the sawdust. Evidently, the higher antioxidant activity to peroxyl radicals correlated with the higher lignin content. It is of importance that the range of TRAP values for samples GX-3 and GX-4 at polymer concentrations >0.5 mg/ml and 1.0 mg/ml, respectively, even exceeded the limit of the method.

The presence of phenolics provides antioxidant properties to the GX indicating the potential of lignin-rich samples as polymeric radical scavengers. It can be concluded that such GX preparations are useful as additives with tensioactive as well as antioxidant properties, what makes them attractive for application in many industrial branches.

Table 4.
Total radical trapping antioxidant potential (TRAP) of various GX samples.

Xylan	Antioxidative activity (μmol/l peroxyl radical)			
	3 mg/ml	1 mg/ml	0.5 mg/ml	0.1 mg/ml
GX-1	490	335	132	<100
GX-2	6740	2023	306	39
GX-3	>limit	7029	2474	169
GX-4	>limit	>limit	396	188

1 mmol TROLOX (standard) deactivates 2 mmol peroxyl radicals.

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