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Carbohydrate Research 340 (2005) 395–401
Carbohydrate RESEARCH

A novel family of glucosyl 1,5-anhydro-D-fructose derivatives synthesised by transglucosylation with dextransucrase from *Leuconostoc mesenteroides* NRRL B-512F

Gaëtan Richard,[a] Shukun Yu,[b] Pierre Monsan,[a] Magali Remaud-Simeon[a] and Sandrine Morel[a,*]

[a]*Laboratoire de Biotechnologie-Bioprocédés UMR CNRS 5504, UMR INRA 792, INSA DGBA 135 avenue de Rangueil 31077 Toulouse Cedex 04, France*
[b]*Danisco Innovation, Danisco A/S, Langebrogade 1, PO Box 17, DK 1001 Copenhagen, Denmark*

Received 29 June 2004; accepted 30 October 2004

Abstract—1,5-Anhydro-D-fructose (**AF**), a metabolite of starch/glycogen degradation, is a good antioxidant. With the prospect of increasing its applications and use as a food ingredient, **AF** glucosylation catalysed by the dextransucrase from *Leuconostoc mesenteroides* NRRL B-512F was performed in the presence of sucrose. This led to **AF** glucosylated derivatives containing α-(1→6) linkages named 1,5-anhydro-D-fructo-glucooligosaccharides (**AFGOS**). LC–MS analyses showed that **AFGOS** with a degree of polymerisation (DP) of up to 7 were synthesised. The amount of **AFGOS** produced and the average DP increased by using a high sucrose/**AF** molar ratio and high total sugar concentration. **AFGOS** were proved to present antioxidant properties quite similar to **AF**.

Keywords: 1,5-Anhydro-D-fructose; 1,5-Anhydro-D-fructoglucooligosaccharides; Dextransucrase; *Leuconostoc mesenteroides*; Acceptor reaction

1. Introduction

1,5-Anhydro-D-fructose (**AF**) was first chemically synthesised by Lichtenthaler et al. in the early 1980s.[1] **AF** was then demonstrated to be present in numerous organisms: fungi (especially *Morchella* sp.)[2] red algae[3–5] and rat liver tissues[6] from the action of α-1,4-glucan lyase (EC 4.2.2.13) on starch/glycogen. The anhydrofructose pathway[3–5] is an alternative starch/glycogen degradation route. **AF** may be further metabolised to secondary compounds with interesting properties: microthecin (an antimicrobial[7]) or ascopyrone P (an antioxidant and antimicrobial[8,9]).

AF exhibits an unusual structure with no anomeric carbon, but a prochiral carbonyl function on carbon C-2 (Fig. 1). This explains the equilibrium existing between the keto, enol, enediol and hydrated **AF** forms.[10] The hydrate form is predominant in water,[10,11] whereas in organic solvents, such as DMSO, **AF** adopts not only the keto, but also two dimeric forms, which are C-2 isomeric spiroketals.[12] **AF** has antioxidant activity[13,14] mainly due to the enol form, which is similar to ascorbic acid, and is also attractive as a chemical synthon. **AF** is thus involved in numerous patent applications as an antioxidant[15] or perfume stabilising agent.[16]

In order to enlarge its potential use and applications, especially as a food ingredient, **AF** glucosylation with glucansucrases was attempted. Glucansucrases (GS, EC 2.4.1) belong to family 70 of glycoside-hydrolases according to Henrissat's classification based on sequence similarities.[17] They are extracellular transglucosidases produced by the lactic acid bacteria *Leuconostoc mesenteroides* and *Streptococcus* sp.[18–20] They use a cheap and abundant substrate: sucrose. From this D-glucosyl unit donor, GS are able to catalyse the transfer of the

* Corresponding author. Fax: +33 561 559400; e-mail: morel@insa-toulouse.fr

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doi:10.1016/j.carres.2004.10.026

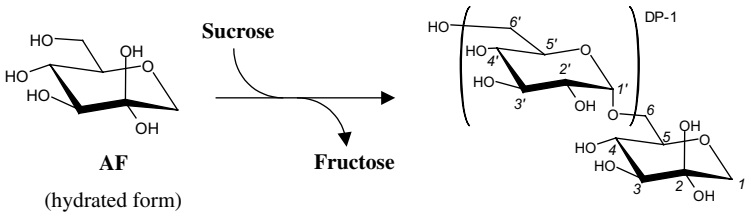

Figure 1. Glucosylation of **AF**, catalysed by dextransucrase from *Leuconostoc mesenteroides* NRRL B-512F using sucrose as D-glucosyl donor. Fructose is released in the medium, whereas the glucosyl residue is transferred to the acceptor.

glucosyl residue, with concomitant fructose release in the medium. In the presence of sucrose alone, this transfer results in the formation of high molecular weight α-glucan.[20,21] The GS used in this study is the dextransucrase produced by *L. mesenteroides* NRRL B-512F (DSR, EC 2.4.1.5). It catalyses the synthesis of a dextran with a high content of α-(1→6) glucosidic linkages.[22] GS can also transfer the glucosyl residue from sucrose to the non-reducing end of exogenous acceptors: this reaction named 'acceptor reaction'[17] is in competition with polymer synthesis and the specificity of GS is usually conserved. Numerous acceptors have already been tested: they can be grouped into two families[23] depending on their acceptor efficiency: (1) the efficient acceptors, which inhibit polymer synthesis and from which series of oligosaccharides are synthesised (e.g., maltose,[24,25] isomaltose,[26] etc.); (2) the weak acceptors to which only one or two glucosyl residues are transferred (e.g., fructose yielding leucrose: α-D-glucopyranosyl-(1→5)-D-fructopyranose[27]). Note also that water can act as an acceptor of a glucosyl residue, the reaction resulting in the formation of glucose.

Here, for the acceptor reaction in the presence of **AF**, an acceptor that has not been tested before for GS, was attempted with dextransucrase from *L. mesenteroides* B-512F. The products synthesised have been characterised, their antioxidant activity examined, and the reaction optimised in order to increase the yield reaction.

2. Results and discussion

2.1. AF glucosylation

AF glucosylation catalysed by dextransucrase (DSR) from *L. mesenteroides* NRRL B-512F was carried out in the presence of 0.29 M (52 g/L) **AF** and 0.29 M (100 g/L) sucrose. Figure 2 shows the chromatographic profile of the reaction mixture at the initial reaction phase and after total sucrose depletion: it can be seen that both sucrose and **AF** (to a lesser extent) were consumed. This led to the formation of compounds **2** to **7**, identified by reverse phase chromatography (Fig. 3).

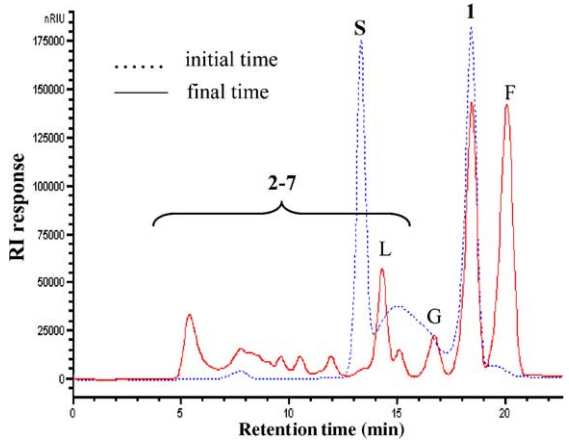

Figure 2. HPLC analysis of the **AF** acceptor reaction mixture on Ca^{2+} carbohydrate column. Identified peaks: **1**: **AF**; **2**–**7**: **AFGOS 2**–**7**; F: fructose; S: sucrose; G: glucose; L: leucrose.

Coupled to mass spectrometry, this separation method enabled the determination of the mass of compounds **2**–**7** (Table 1). The molecular mass obtained showed that each compound bears 1–6 glucosyl residues (degree of polymerisation 2–7). They were named 1,5-anhydro-D-fructo-glucooligosaccharides (**AFGOS**). Additional peaks were also observed on the chromatogram shown in Figure 3. These peaks were attributed, on the basis of their retention times, to leucrose and isomaltooligosaccharides. The latter result from the transfer to glucose formed from sucrose hydrolysis, which also seems to occur in the presence of **AF**.

2.2. Structural characterisation of AFGOS

In order to confirm the structure of the **AFGOS** and to elucidate the glucosidic linkages involved, ^{1}H, ^{13}C, HSQC and HMBC analyses were performed on purified compounds **2**, **3** and **4**. ^{1}H and ^{13}C chemical shifts are listed in Table 2.

All the AFGOS synthesised showed the same ^{1}H and ^{13}C chemical shifts as those observed on **AF** spectra, except for the chemical shift of carbon C6, moved upfield from 61 to 66 ppm. This indicates that **2**, **3** and **4**

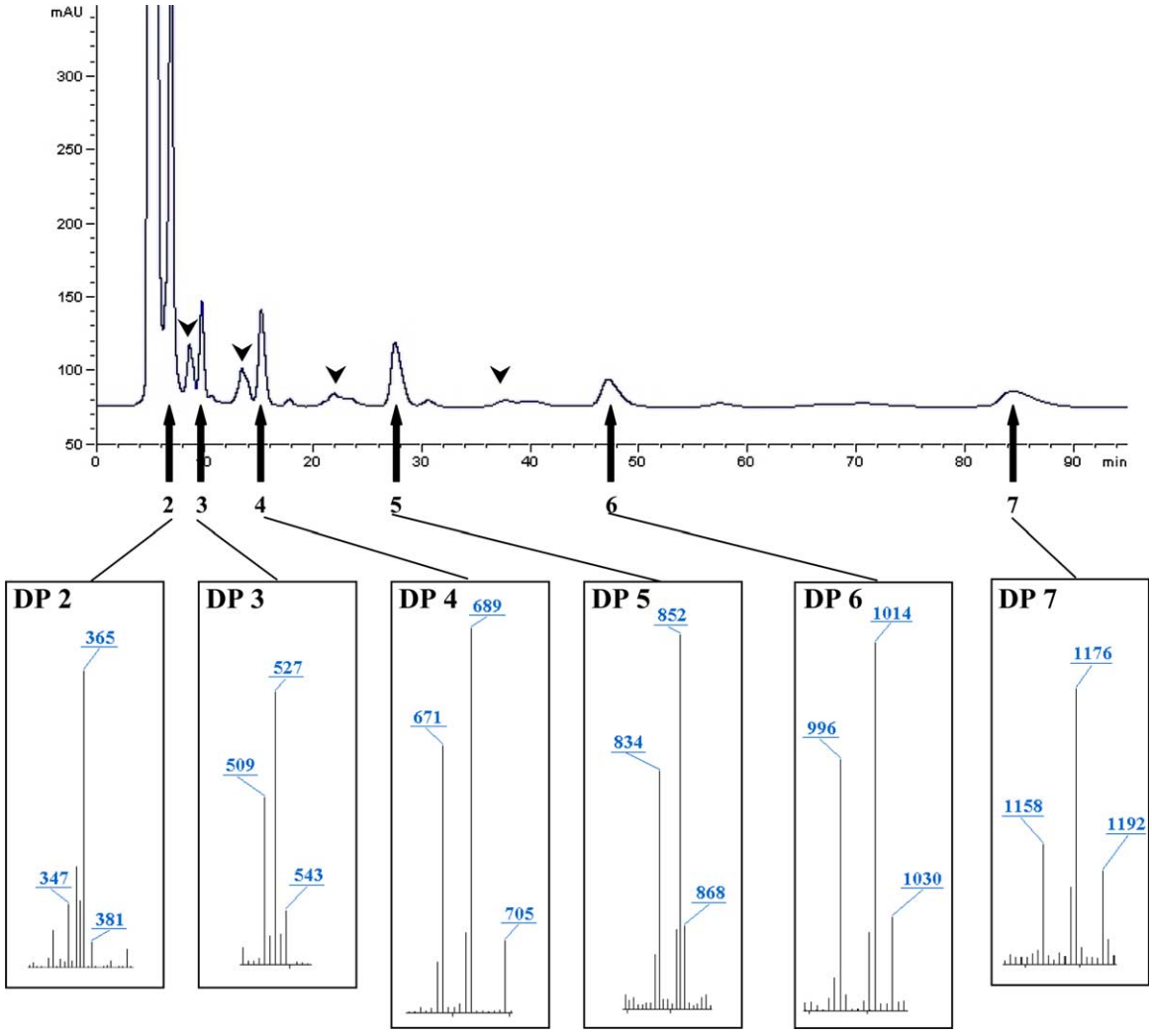

Figure 3. LC–MS analyses of the **AFGOS 2–7** on a C18 column with ultra-pure water as eluent at 0.5 mL/min; each **AFGOS** is characterised by ion peaks $[M+Na^+-H_2O]$, $[M+Na^+]$ and $[M+K^+]$ (data listed in Table 1). By-products such as isomaltooligosaccharides are indicated (⮟).

Table 1. Determination of the degree of polymerisation (DP) of **AFGOS 2–7** by LC–MS

Compound	Retention time (min)	*m/z*			Molecular mass (g/mol)	DP	**AFGOS** structure
		$[M+Na^+-H_2O]$	$[M+Na^+]$	$[M+K^+]$			
AFGOS 2	6.8	347	365	381	342	2	Glc–**AF**
AFGOS 3	9.1	509	527	543	504	3	$(Glc)_2$–**AF**
AFGOS 4	15.1	671	689	705	666	4	$(Glc)_3$–**AF**
AFGOS 5	27.4	834	852	868	828	5	$(Glc)_4$–**AF**
AFGOS 6	47.1	996	1014	1030	990	6	$(Glc)_5$–**AF**
AFGOS 7	84.2	1158	1176	1192	1152	7	$(Glc)_6$–**AF**

all possess an **AF** residue whose carbon C6 is involved in glucosidic bond. In addition, ^{13}C NMR spectra revealed no signals for C2 corresponding either to the keto form (around 200 ppm) or to the enol and enediol (130–160 ppm) **AF** forms. This demonstrates that the **AF** residue engaged in **AFGOS** is hydrated.

2.2.1. Compound 2. The molecular mass of DP2 was confirmed by HRMS analyses. The ^1H NMR spectrum showed only one anomeric proton with a coupling constant J_{1-2} of 3.7 Hz indicating that a single glucosyl residue is α-linked to **AF**. C-1 of the glucosyl residue was observed at 98 ppm and coupling occurs between proton H-1 of the glucosyl unit and C-6 of the **AF** residue. This indicates that an α-(1→6) glucosidic linkage was formed in the transglucosylation reaction. Consequently, **2** is α-D-glucopyranosyl-(1→6)-*O*-1,5-anhydro-D-fructose and was named **AFGOS 2**.

Table 2. ^{1}H and ^{13}C NMR analyses of **AFGOS 2**, **3** and **4**. Carbons and protons are numbered as indicated in Figure 1

Molecule	Residue[a]		Chemical shift (δ, ppm)							
			1		2	3	4	5	6	
			1a	1b					6a	6b
AF		^{1}H	3.36	3.66	—	3.46	3.34	3.31	3.58	3.80
		^{13}C	71.9		92.8	77.1	69.2	80.8	61.4	
AFGOS 2	AF	^{1}H	3.40	3.67	—	3.48	3.49	3.50	3.63	3.86
		^{13}C	72.2		92.8	77.3	68.7	79.2	66.2	
	Glc I	^{1}H J_{1-2} = 3.7 Hz		4.86	3.46	3.69	3.33	3.61	3.69	3.76
		^{13}C	98.2		71.7	73.3	69.7	71.9	60.7	
AFGOS 3	AF	^{1}H	3.41	3.67	—	3.47	3.48	3.48	3.64	3.89
		^{13}C	72.2		92.8	77.4	68.7	79.1	66.3	
	Glc I	^{1}H J_{1-2} = 3.0 Hz		4.88	3.48	3.64	3.42	3.81	3.67	3.88
		^{13}C	98.2		71.6	73.5	69.7	70.3	65.6	
	Glc II	^{1}H J_{1-2} = 3.1 Hz		4.87	3.48	3.63	3.35	3.64	3.68	3.77
		^{13}C	97.9		71.7	73.2	69.7	72.0	60.6	
AFGOS 4	AF	^{1}H	3.46	3.66	—	3.50	3.51	3.51	3.65	3.90
		^{13}C	72.2		92.8	77.4	68.7	79.1	66.3	
	Glc I	^{1}H		4.87	3.51	3.66	3.43	3.82	3.89	3.69
		^{13}C	98.2		71.6	73.5	69.7	70.3	65.7	
	Glc II	^{1}H		4.88	3.51	3.66	3.43	3.82	3.69	3.89
		^{13}C	97.8		71.7	73.5	69.7	70.4	65.7	
	Glc III	^{1}H		4.88	3.51	3.65	3.34	3.66	3.70	3.76
		^{13}C	7.9		71.7	73.3	69.7	72.0	60.6	

[a] Rings are numbered starting from the reducing end (AF residue).

2.2.2. Compound 3. In the ^{13}C spectrum, two anomeric carbons were present at 98 ppm, which were attributed to C-1 of two glucosyl units. DP 3 was furthermore confirmed by HRMS analyses. Both anomeric protons showed coupling constants J_{1-2} close to 3 Hz. The C-6 signal of the glucosyl residue I (the first residue transferred to **AF**) shifted from 61 ppm to 66 ppm and C-1 of glucosyl residue II (the last residue transferred) was close to 98 ppm, showing that this glucosyl unit is linked to **AFGOS 2** via an α-(1→6) linkage. Hence, **3** is α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-*O*-1,5-anhydro-D-fructose and was named **AFGOS 3**.

2.2.3. Compound 4. The signal at 98 ppm on the ^{13}C NMR spectrum represents three anomeric carbons. The molecular mass of 666 g/mol measured by HRMS was in agreement with a DP4 formed by 3 glucosyl units linked through α-(1→6) linkages. This was confirmed by the occurrence in the HMBC spectrum of coupling between anomeric protons and C-6 of the glucosyl residues. The J_{1-2} of the anomeric protons was estimated to be close to 3.5 Hz, indicative of α linkage type. Thus, **4** is α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-*O*-1,5-anhydro-D-fructose and was referred as **AFGOS 4**.

These analyses demonstrated that products **2**, **3** and **4** are 1,5-anhydro-D-fructo-glucooligosaccharides composed of glucosyl units linked through α-1,6 linkages, and an **AF** residue at the reducing end. It is reasonable to assume that compounds **5**, **6** and **7** are related structure analogues, but with a higher DP. Interestingly, when the logarithmic of **AFGOS** retention times was plotted versus their DP, a linear relationship was observed (Fig. 4), which agrees with the logarithmic law that is valid for maltose acceptor glucosylation products.[22,29] This illustrates the fact that **AFGOS 2–7** synthesised with DSR from *L. mesenteroides* NRRL B-512F belong to the same oligosaccharide family. Thus, the **AF** glucosylated derivatives obtained here differ from the disaccharide (α-D-glucopyranosyl-(1→3)-*O*-1,5-anhydro-D-fructose) recently reported by Yoshinaga et al.[30] They obtained this compound by the action of a cyclodextrin glucosyltransferase on cyclomaltoheptaose and **AF**, followed by glucoamylase digestion.

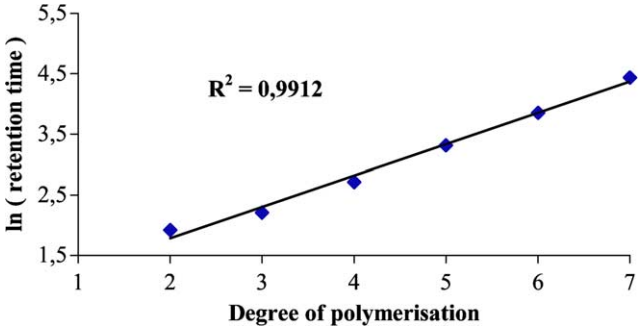

Figure 4. Correlation between the HPLC retention time (on C18 column) and the degree of polymerisation of the **AFGOS**.

2.3. Optimisation of AFGOS synthesis

In the presence of 0.29 M sucrose and **AF**, a conversion of 26% was obtained (Table 3). Among all the glucosyl residues available from sucrose, 20% were transferred to **AF**, the other were polymerised to dextran. Under such conditions, the reaction yield[34] (taking into account of **AF** conversion and the percentage of glucose transferred to **AF and AFGOS**) was 19.8%. The average DP[33,35] of the final products was 1.25, whereas it was equal to 1 at the beginning of the reaction since only **AF** was present. For comparison, in the same reaction carried out with either fructose or maltose as acceptor, 6.6% of fructose and 50.6% of maltose were glucosylated, with yields of 3.5% and 75.0%, respectively. From these observations, **AF** is a moderate acceptor: the majority of **AF** was not glucosylated, but, unlike with fructose, the reaction led to the synthesis of several compounds.

In order to improve **AF** glucosylation, the influence of the sucrose/acceptor molar ratio (S/A) and total sugar concentration (TSC) on conversion and **AFGOS** yield were studied.

By increasing the S/A ratio, more glucosyl residues were available for transglucosylation, and higher **AF** conversions were obtained. However, the percentage of glucose transferred to the acceptor decreased simultaneously: a higher proportion of glucose was incorporated to dextran. Consequently, the reaction yield decreased with S/A. In parallel, the average DP of the **AFGOS** formed increased with the S/A ratio showing that glucosylation reactions must be performed at high S/A ratios to synthesise higher DP **AFGOS** (Table 3).

At high TSC (S/A ratio being kept constant), the medium is enriched in both substrate and acceptor. This results in a more efficient glucosylation of **AF**, and hence in higher conversions, glucosylation yields and average DP of **AFGOS** (Table 4).

When the S/A ratio and TSC were increased simultaneously, **AF** conversions were further improved (Table 4). Under the optimal conditions, we were able to produce 262 g of **AFGOS**, starting with 100 g of **AF**, whereas only 72 g were obtained at 0.29 M sucrose and **AF**.

Table 3. Effect of the sucrose/acceptor molar ratio (S/A) at a total sucrose concentration of 131 g/L

	S/A ratio				
	0.6	1.2	2.3	3.5	5.8
Conversion (%)	20.1	26.1	34.8	36.0	41.2
% Glc transferred to **AF and AFGOS**	42.9	20.0	13.5	10.4	7.0
Reaction yield (%)	28.1	19.8	17.5	15.1	11.3
Average DP[a]	1.26	1.25	1.34	1.39	1.38

[a] At initial time, the average DP was set to 1.

Table 4. Influence of the total sugar concentration (TSC) at a constant sucrose/acceptor molar ratio equal to 1.2 and with a high S/A ratio for further improved glucosylation efficiency

S/A	1.2					2.5
TSC	45	89	131	169	232	575 g/L
Conversion (%)	19.2	23.5	26.1	30.5	35.9	56.4
% Glc transferred to **AF and AFGOS**	9.9	15.8	20.0	23.5	24.2	43.3
Reaction yield (%)	9.9	15.5	19.8	22.9	26.2	53.1
Average DP	1.13	1.20	1.25	1.30	1.38	1.95

2.4. Antioxidative properties of the AFGOS

In this study, the **AFGOS** synthesised reacted specifically with 3,5-dinitrosalicylic acid (DNS reagent) at ambient temperature like **AF**[31] (data not shown), indicating the high reducing power of these novel saccharides. Indeed, since no transfer occurs to the C-2 hydroxyl function of the **AF** residue, the enediol system responsible for the antioxidant activity is conserved.

Antioxidative analyses were carried out according to the 2-thiobarbituric acid (TBA) method[32] with pure **AFGOS**. It showed that **AF** exhibits an antioxidant power similar to ascorbic acid (as already observed by Fujisue et al.[32]). As for **AFGOS**, the antioxidant power tends to increase with the DP (see Fig. 5), and this is more pronounced at low concentrations. Consequently, **AF** derivatives could be used as an antioxidant in food like **AF**.[15]

In summary, it was established in this study that **AF** is a new member of the family of acceptor molecules recognised by DSR, thus leading to the synthesis of a family of **AFGOS** comprising up to 7 glucosyl residues linked though α-(1→6) glucosidic linkages. Although **AF** did not prove to be as efficiently glucosylated as maltose, we achieved **AF** conversion of 56% at high concentrations of sucrose and **AF**. Since the enediol system of the **AF** residue is not altered by glucosylation, antioxidant analyses performed with purified **AFGOS**

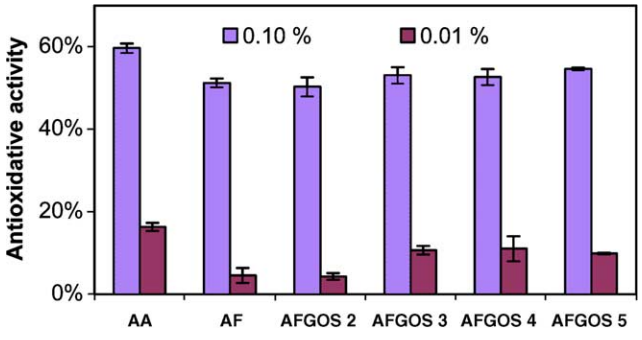

Figure 5. Evaluation of the antioxidant activity of **AF** and its glucosylated derivatives **AFGOS** in comparison to ascorbic acid (**AA**). Analyses were carried out according to the TBA method[32] at concentrations in the antioxidant tested of 0.1% and 0.01% (w/w). Antioxidant activity was evaluated as the percentage of remaining linoleic acid. The control activity was fixed at 0%. Assays were repeated five times.

confirmed that these glucosylated derivatives conserve the high antioxidant activity of **AF**.

3. Experimental

3.1. Materials

L. mesenteroides NRRL B-512F (EC 2.4.1.5) was provided by the NRRL Peoria (IL), USA. DSR was produced and purified as previously described.[28] 1,5-Anhydro-D-fructose (**AF**) was prepared from starch enzymatically by the action of α-1,4 glucan lyase (EC 4.2.2.13).[4]

3.2. Analytical methods

3.2.1. High performance liquid chromatography (HPLC). The efficiency of the glucosylation reaction was evaluated by HPLC using a Hewlett Packard 1050 series pump and autosampler, and Hewlett Packard 1100 series refractometer. With the prospect of studying **AF** consumption and the formation of **AFGOS**, HPLC analyses were performed on two columns: (1) a Ca^{2+} carbohydrate column (CHO-62O carbohydrate, Alltech column, 300 × 6.5 mm) maintained at 90 °C, using ultra-pure water as eluent with a flow rate of 0.3 mL/min; (2) an analytical C18 column (Prontosil Eurobond, 5.0 μm, 250 × 4.0 mm) kept at room temperature and ultra-pure water was pumped through at 0.5 mL/min.

3.2.2. Liquid chromatography–mass spectrometry (LC–MS). The molecular mass of **AFGOS** was determined by LC–MS using a Perkin-Elmer SCIEX model API 365 system. The separation was achieved with a C18 column (same as described in Section 3.2.1) and **AFGOS** were ionised by electron spray ionisation (ESI) at 400 °C, separated by a quadrupole and detected in positive ion mode.

3.2.3. High resolution mass spectrometry (HRMS). Accurate mass determination was carried out using an Autospec mass spectrometer arranged in an EBE geometry (Micromass, Manchester, UK). The instrument was operated at 8 kV accelerating voltage in positive mode. The caesium gun was set to 35 keV energy and 1 μL of sample was mixed in the tip of the probe with a glycerol (for **AFGOS 3**, **AFGOS 4**) or dithiothreitol/dithioerythritol (for **AFGOS 2**, **AFGOS 3**) matrix.

3.2.4. Nuclear magnetic resonance. The structure of **AFGOS** was confirmed by NMR analyses: ^{1}H (400.130 MHz), ^{13}C (100.612 MHz), HSQC and HMBC were registered on a Bruker-ARX 400 spectrometer equipped with an ultrashim system. Samples were dissolved in deuterium oxide at ca. 80 g/L.

3.3. AFGOS synthesis

The glucosylation reaction was performed in AcONa (20 mM, pH 5.2) at 30 °C with sucrose and **AF** concentrations of 100 and 52 g/L in the reference conditions (S/A = 1.2, TSC = 131 g/L). DSR was used at 1 U/mL what means that 1 μmol sucrose was hydrolysed per minute. The reaction was stopped by heating at 95 °C for 5 min and the final mixture was centrifuged at 18,000*g* for 10 min and filtered before HPLC analysis.

3.4. AFGOS purification

Dextran was eliminated by precipitation with ethanol (50% v/v) and removed by centrifugation at 18,000*g* for 10 min. Fructose was separated from **AFGOS** by gel permeation chromatography on Bio-Gel® P-2 (Stationary phase: polyacrylamide gel, fine particles: diameter = 45–90 μm, 91 × 10 cm; eluent: ultra-pure water at 14 mL/min). **AFGOS 2**, **3** and **4** were isolated by reverse phase preparative liquid chromatography on a Prontosil Eurobond C18 column (10 μm, 250 × 400 mm) with ultra-pure water used as eluent at 50 mL/min.

3.4.1. α-D-Glucopyranosyl-(1→6)-*O*-1,5-anhydro-D-fructose (AFGOS 2). Mp 121 °C; HRMS: Anal. Calcd for $C_{12}H_{22}O_{11}$: 364.10598 [MNa^{+}]. Found: 365.10570; ^{1}H and ^{13}C given in Table 2.

3.4.2. α-D-Glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-*O*-1,5-anhydro-D-fructose (AFGOS 3). Mp 152 °C; HRMS: Anal. Calcd for $C_{18}H_{32}O_{16}$: 527.15881 [MNa^{+}]. Found: 527.15873; ^{1}H and ^{13}C given in Table 2.

3.4.3. α-D-Glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-*O*-1,5-anhydro-D-fructose (AFGOS 4). Mp 166 °C; HRMS: Anal. Calcd for $C_{24}H_{42}O_{21}$: 689.21163 [MNa^{+}]. Found: 689.21229; ^{1}H and ^{13}C given in Table 2.

3.5. Assay of antioxidant activity

The antioxidant activity of the **AFGOS** was determined according to the 2-thiobarbituric acid (TBA) method used by Fujisue et al.[32] and compared to that of **AF**. It is based on linoleic acid oxidation, which is more or less prevented by the presence of an antioxidant. The test was carried out in 5 mL of an aqueous solution containing 4.5 mM linoleic acid, 0.04% (V/V) Tween 40 and either 0.1% or 0.01% (w/w) of antioxidant: **AF** or **AFGOS** (or ascorbic acid used as reference). Simultaneously, a control was run without any antioxi-

dant. The test tubes were sealed and kept at 50 °C for 14 days. After this period, the amount of malonaldehyde resulting from the linoleic acid oxidation was quantified: 200 μL of 20% trichloroacetic acid and 100 μL of 0.67% TBA were added to 200 μL of the reaction mixture. The solution was heated to 100 °C for 10 min and then cooled to room temperature. After centrifugation at 18,000*g* for 10 min, the absorbance of the supernatant was measured at 532 nm: the complex between TBA and malonaldehyde is responsible for the pink colour. The antioxidant activity was calculated as the percentage of remaining linoleic acid whereas and the control activity was set to 0%.

Acknowledgements

We are grateful to Pierre Escalier for technical assistance, the LC–MS and NMR laboratories from the University Paul Sabatier (Toulouse, France) and CESAMO (Talence, France) for HRMS analyses. This work was supported by the 5th framework programme of the European Union under cell factory with contract No QLK3-2001-02400.

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34. Equation 1: Calculation of the reaction yield

$$\text{yield } (\%) = \frac{[\text{AFGOS}]_t}{[\text{AF}]_{t=0} + 162/342 \times [\text{Sucrose}]_{t=0}}$$

35. Equation 2: Calculation of the average degree of polymerisation in the reaction mixture at the end of the reaction according to the Schulz law;[33] at the initial reaction phase, the average DP was 1 since only **AF** is present in the medium. *i* represents the DP, and *pi* the molar percentage of each DP in the medium

$$\text{average DP} = \frac{p1}{100} \times 1 + \frac{p2}{100} \times 2 + \cdots = \sum_{i=1}^{\infty} \frac{pi}{100} \times i$$