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Ferulic Acid Content and Appearance Determine the Antioxidant Capacity of Arabinoxylanoligosaccharides

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ABSTRACT: To investigate the antioxidant capacity of ferulic acid (FA) in conjunction with prebiotic arabinoxylan-oligosaccharides (AXOS), procedures for the production of FA-enriched, -depleted and cross-linked AXOS were developed, and samples were analyzed using the Trolox equivalent antioxidant capacity (TEAC) and oxygen radical absorbance capacity (ORAC) assays. Results showed that not only the level of FA but also the condition under which it appears (free, bound, or dimerized) impacts the antioxidant capacity of FA-containing AXOS samples. Although esterification of FA on AXOS and cross-linking of AXOS through dehydrodiferulic acid formation lowered the antioxidant capacity of FA by 30 and 55%, respectively, as determined with the TEAC test, the antioxidant capacity of these components still remained high compared to Trolox, a water-soluble vitamin E analog. Total antioxidant capacity of the AXOS samples determined by the ORAC assay resulted in less prominent differences between the different forms of FA than those seen with the TEAC test. Feruloylated AXOS can hence function as strong, water-soluble antioxidants.

KEYWORDS: *antioxidant, arabinoxylanoligosaccharides, ferulic acid, prebiotic, ORAC, TEAC*

1. INTRODUCTION

Western society faces new challenges because chronic diseases, totally or at least partially attributed to diet, will cause serious problems of public health as a result of increased lifetime, changed lifestyle, and dietary habits.[1] Therefore, healthier food products, such as functional foods, attract more interest. Functional foods beneficially affect one or more target functions in the body, beyond adequate nutritional effects, in a way that is relevant to either an improved state of health and well-being and/or a reduction of risk for disease.[2] Prebiotics and antioxidants are two concepts that receive a lot of attention.

Xylooligosaccharides (XOS) and arabinoxylan-oligosaccharides (AXOS), hydrolysis products of xylan and arabinoxylan (AX), exert prebiotic properties[3] and can be considered a functional food ingredient.[4] According to the definition of Gibson et al.,[5] a prebiotic is "a selectively fermented ingredient that allows specific changes, both in the composition and/or activity of the gastrointestinal microbiota and confers benefits upon host wellbeing and health". Prebiotics stimulate the growth and activity of *Bifidobacteria* and *Lactobacilli*, which are perceived as beneficial for human health.[6] However, this concept should be expanded with other genera (e.g., *Eubacterium*, *Faecalibacterium*, and *Roseburia*) in the future.[7] Prebiotics are often considered to improve overall health, reduce risk of colon cancer, obesity, and diabetes, improve mineral absorption, and reduce gut infections and other gastrointestinal disorders.[7,8]

XOS and AXOS consist of a linear backbone of β-1,4 linked D-xylopyranosyl residues with, in the case of AXOS, α-L-arabinofuranoses linked to their *C*-(*O*)-2 and/or *C*-(*O*)-3 position.[9] Some arabinose residues are esterified with ferulic acid (FA), and to a lesser extent with other hydroxycinnamic acids like coumaric acid and dehydrodiferulic acid (di-FA), to their *C*-(*O*)-5 position.[10] Besides arabinose residues, other constituents like glucuronic acids, D-glucose, D-galactose, or small oligomeric side chains consisting of two or more arabinofuranosyl residues can be bound to xylose.[10,11]

As AXOS, and to a much lesser degree XOS, often contain bound FA residues, they not only exert prebiotic but also antioxidant properties. Antioxidants are defined as any substance that when present at low concentrations compared to those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate.[12] FA and phenolic acids in general are commonly accepted as health-promoting components,[13] which is at least partially attributed to their antioxidant capacity.[14] A shortage of antioxidants in the human body causes an imbalance between oxidants and antioxidants, referred to as oxidative stress.[15] Therefore, sufficient intake of dietary antioxidants is necessary to battle oxidative stress and oxidative stress-related diseases such as Alzheimer's disease, Parkinson's disease, cancer (e.g., stomach, colon, and rectal), cardiovascular diseases, and type 2 diabetes, and antioxidants may also reduce inflammation and aging.[16]

In vitro studies have shown that feruloylated AXOS inhibit erythrocyte hemolysis and oxidative DNA damage to lymphocytes.[17−19] Diabetic rats showed lowered blood glucose levels and enhanced antioxidant capacity after being fed with feruloylated AXOS.[20] Furthermore, feruloylated AXOS inhibit lipid peroxidation, both in vitro and in vivo,[20−22] and therefore, might be able to decrease the risk of cardiovascular disease.[23] Although it is known that esterification alters the antioxidant capacity of phenolics,[21,24] the impact of the form under which FA appears (free, bound, or dimerized) on the antioxidant

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properties of feruloylated AXOS has not been studied, to our knowledge.

Furthermore, results from some studies suggest that oligosaccharide carbohydrate moieties might also possess antioxidant properties. For example, a correlation was seen between oxidative stress in plants and production of sucrosyl oligosaccharides, including raffinose-family oligosaccharides and fructans.[25] Uronyl, acetyl, phosphate, or sulfate groups contribute to the antioxidant capacity of sugars,[26,27] while their structural features like molecular weight, monosaccharide composition, and chain conformation might have an influence as well.[21,26,28]

The objective of this work was to study the influence of free, bound, or di-FA and carbohydrate structure on the antioxidant properties of feruloylated AXOS. Therefore, a set of AXOS containing both XOS and AXOS which only differed in specific structural characteristics (carbohydrate structure or FA content and form) were produced, structurally characterized, and analyzed for their antioxidant capacity using Trolox equivalent antioxidant capacity (TEAC) and oxygen radical absorbance capacity (ORAC) assays.

2. EXPERIMENTAL SECTION

2.1. Materials. All solvents, chemicals, and reagents were of at least analytical grade and purchased from Sigma-Aldrich (Bornem, Belgium), unless specified otherwise. An AXOS-rich product (Brana Vita) was provided by Fugeia (Leuven, Belgium). It was characterized by an AXOS level of 73%, an average degree of polymerization of the xylan backbone (avDPxyl) of 4.3, an average degree of arabinose substitution (avDAS) of 0.22, a protein content of 0.24%, and an ash content of 0.4%, and the product was produced by enzymic treatment of wheat bran AX using the procedure described by Swennen et al.[29] with adaptations. Low degree of polymerization (DP) XOS (XOS level of 74%, avDPxyl of 3.1, protein content of 0.01%, and ash content of 0.1%) were obtained from Shandong Longlive Biotechnology (Qingdao, China). High DP XOS (XOS level of 80%, avDPxyl of 12.2, protein content of 0.64%, and ash content of 7.7%) were from Megazyme (Bray, Ireland). Amberlite XAD-4 resin, horseradish peroxidase (EC 1.11.1.7), and bovine liver catalase (EC 1.11.1.6) were obtained from Sigma-Aldrich. A feruloyl esterase (FAE) (EC 3.1.1.73) from *Clostridium thermocellum* was purchased from Megazyme.

2.2. Methods. *2.2.1. Production of Related AXOS with Similar Carbohydrate Structure and Different Ferulic Acid Content. Ferulic Acid Enrichment of AXOS.* Production of AXOS enriched in FA was done using an adapted method based on Saulnier et al.[30] Brana Vita AXOS was dissolved in water (10% w/v) and loaded on an Amberlite XAD-4 column, a polymeric resin with affinity toward phenolic compounds, using a BioLogic DuoFlow system (Bio-Rad Laboratories, Hercules, U.S.A.). After the loading step, successive elution steps with water, ethanol–water (10/90 v/v), and ethanol–water (50/50 v/v) or higher concentrations of ethanol (ethanol–water mixtures of 70 and 80 v/v) were carried out. The adsorption and desorption process of AXOS molecules on the column was monitored through UV detection (280 nm) of the eluting samples. The qualitative UV signal was used to decide whether to proceed to the next elution step. Ethanol was vacuum evaporated out of the ethanol-containing fractions. All fractions were freeze-dried afterward. The fractionation process was executed in triplicate and the FA-enriched AXOS were obtained by combining the fractions elutedwith ethanol–water (50/50 v/v) or higher concentrations of ethanol (ethanol–water mixtures of 70 and 80 v/v) of three repetitive runs.

Ferulic Acid Depletion of AXOS. The production of AXOS depleted in FA occurred in two subsequent steps. In the first step, FA was enzymatically hydrolyzed from AXOS (Brana Vita or FA-enriched AXOS) using a FAE treatment. The FAE that was used works optimally at a pH between 4 and 7, and its optimal temperature is 50 to 60 °C.[31] As AXOS are weakly acidic and salinization of the end product needs to be avoided, no buffer was used during the enzymic hydrolysis of FA. Excess FAE (180 U on *O*-{5-*O*-[(*E*)-feruloyl]-*α*-L-arabinofuranosyl}-(1→3)-*O*-*β*-D-xylopyranosyl-(1→4)-D-xylopyranose substrate [31]) was added to an AXOS solution (5 g/100 mL deionized water), and enzymic hydrolysis was executed at 50 °C for 24 h. Afterward, the FAE was inactivated by heating for 15 min in boiling water. In the second step, free and residual bound FA were removed by use of an Amberlite XAD-4 column. The solution was brought over the column manually and the run through was collected and reloaded on the column for another two times. The nonadsorbed water and ethanol–water (10/90 v/v) eluted fractions were combined to generate FA-depleted AXOS.

Dehydrodiferulic Acid Cross-Linking of AXOS. A horseradish peroxidase–hydrogen peroxide system [32] was used to couple Brana Vita AXOS through FA dimerization to generate di-FA cross-linked AXOS. Brana Vita AXOS were dissolved in deionized water (200 mL) in a concentration that corresponds to 5 mM FA, and hydrogen peroxide was added in the same concentration. To facilitate the reaction, 11.6 units of horseradish peroxidase was added, and the sample was incubated at 25 °C for 1 h. The FA cross-linking reaction was monitored by measuring the absorbance at 325 nm, as this is the wavelength at which the absorbance of FA bound to AXOS (and dissolved in water) is maximum. Afterward, a catalase (2460 U) was added to convert excess hydrogen peroxide to oxygen and water at 25 °C. An oxygraph was used to measure the oxygen production. After 10 min, no more additional oxygen was produced. The mixture was further incubated up to 2 h to ensure total conversion of hydrogen peroxide. Finally, enzymes were heat denatured, and AXOS were freeze-dried.

2.2.2. Analysis of Total, Reducing End, and Free Monosaccharide Content. The total, reducing end, and free sugar content of all samples were analyzed with gas chromatography, as described by Courtin et al.[33] Samples were hydrolyzed using 2.0 M trifluoroacetic acid (TFA) for total sugar content determination. Resulting monosaccharides were subsequently reduced by sodium borohydride and acetylated with acetic acid anhydride to alditol acetates. Samples (1.0 μL) were separated on a Supelco SP-2380 column (30 m × 0.32 mm inside diameter (i.d.), 0.2 μm film thickness; Supelco, Bellefonte, PA, USA) with helium as the carrier gas in an Agilent 6890 series chromatograph (Agilent, Wilmington, DE) equipped with an autosampler, splitter injection port (split ratio 1:20), and a flame ionization detector. Alditol acetates were separated at 225 °C with injection and detection temperatures at 270 °C. *β*-D-Allose was used as the internal standard, and calibration samples containing the expected monosaccharides were included with each set of samples. Reducing end sugars were determined similarly as total sugars, but the order of the reduction and hydrolysis steps were switched.[33] For free sugar content determination, the hydrolysis step was omitted. AXOS levels, avDPxyl, and avDAS were calculated from the obtained arabinose and xylose levels (represented by A and X in the formulas, respectively) using the following formulas:

$$\text{AXOS level (\%)} = (\%\text{total A} - \%\text{free A}) \times \frac{132}{150} + (\%\text{total X} - \%\text{free X}) \times \frac{((\text{avDPxyl} - 1) \times 132 + 150)}{150 \times \text{avDPxyl}}$$

$$\text{avDPxyl} = \frac{\%\text{total X} - \%\text{free X}}{\%\text{reducing end X} - \%\text{free X}}$$

$$\text{avDAS} = \frac{\%\text{total A} - \%\text{free A}}{\%\text{total X} - \%\text{free X}}$$

2.2.3. Analysis of Total and Free Ferulic Acid Content. Both free and bound, monomeric, and di-FA content were analyzed according to Antoine et al.[34] with some modifications. Total FA content determination started by dissolving AXOS in 2.0 M NaOH and saponification under slow stirring for 2 h at 35 °C under nitrogen

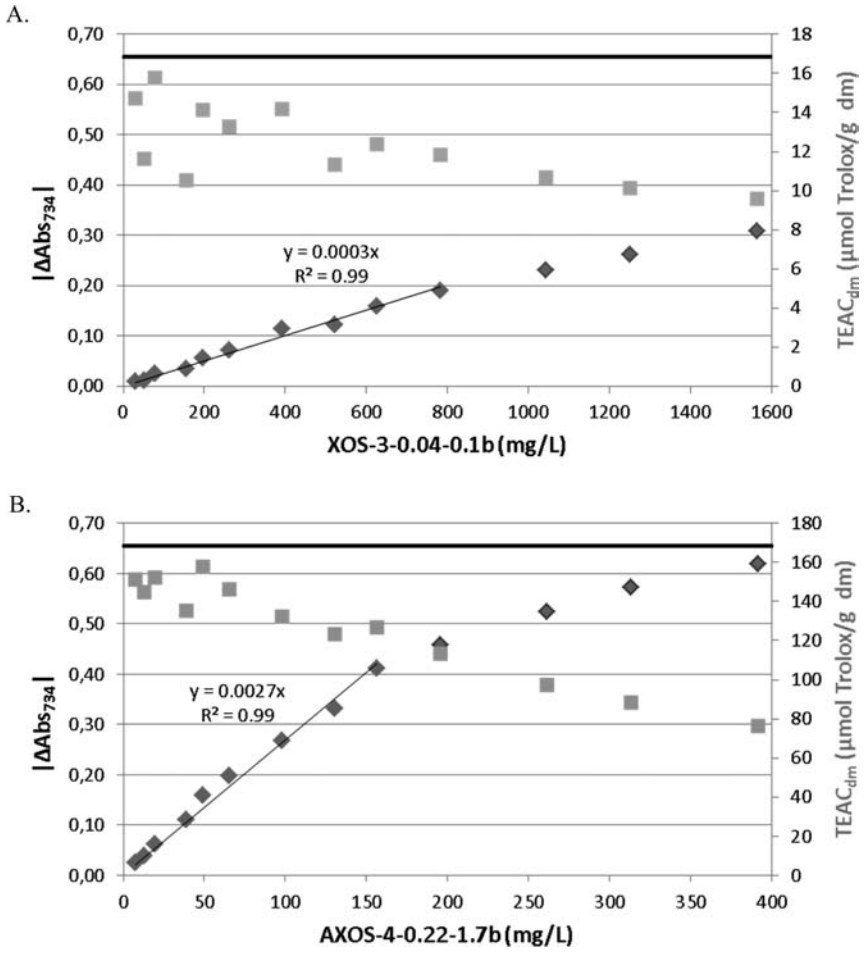

Figure 1. The decrease in absorbance, depicted as an absolute value, at 734 nm of the ABTS radical solution (dark-gray tilted square) during testing of low DP XOS (XOS-3-0.04-0.**1b**) (A) and Brana Vita AXOS (AXOS-4-0.22-1.7**b**) (B) in a different concentration range in TEAC assay. TEAC values were expressed in μmol Trolox per gram (A)XOS on dry matter base ($TEAC_{dm}$, light-gray square). A trendline was fitted for the linear part of the curve (-). The upper limit of decrease in absorbance was also shown (−).

atmosphere, protected from light. Samples were acidified to pH 2.0 with 4.0 M HCl, followed by addition of caffeic acid as the internal standard (final concentration of 50 μg/mL methanol in vial). Phenolic acids were extracted twice with diethyl ether. Ether was evaporated under a nitrogen flow, and phenolic acids were dissolved in methanol and filtered (0.45 μm). For the determination of free FA, no saponification step took place, and samples were analyzed starting from the acidification step. Monomeric phenolic acids were analyzed on a Luna Phenyl-Hexyl column (250 mm × 4.6 mm i.d., 5 μm particle size, plus 3 mm × 4.6 mm i.d. guard column; Phenomenex, Utrecht, The Netherlands) in a LC-20AT modular high-performance liquid chromatography (HPLC) system (Shimadzu, Kyoto, Japan) according to Dobberstein and Bunzel.[35] After injection of 20 μL of sample, a ternary gradient system of 1 mM aqueous TFA (eluent A), acetonitrile/1 mM aqueous TFA (90/10 v/v) (eluent B), and methanol/1 mM aqueous TFA (90/10 v/v) (eluent C) at 45 °C was used to separate phenolic acids. UV detection (Shimadzu, UV-10A detector) was carried out at 280 nm. Calibration samples containing caffeic acid, 4-hydroxybenzaldehyde, *p*-coumaric acid, vanillin, and *trans*-ferulic acid were included with each set of samples. No di-FA standards were available, but phenolic acids were also analyzed in a separate run according to Dobberstein and Bunzel[35] to determine whether different forms of di-FA were present in the samples.

2.2.4. Analysis of Acetic Acid Content. Total acetic acid content of samples (50 mg) was determined after saponification in 0.8 M sodium hydroxide (1.0 mL), as described by Van Gool et al.[36] Samples were kept for 1 h on ice and 2 h at room temperature. After acidification with hydrogen chloride to a pH below 7, succinic acid (100 μL, 5.0 mg/mL) was added as the internal standard, and samples were filtered (0.45 μm) before analysis. Acids were separated as described by Jayaram et al.[37] using a LC-20AT modular HPLC system (Shimadzu, Kyoto, Japan) with an ROA-Organic acid ion exclusion column (Phenomenex) and detected with refractive index detection (Shimadzu, RID-10A detector) using the following conditions: 60 °C column temperature, 2.50 mM sulfuric acid as eluent, and a 0.60 mL/min flow rate. Calibration samples containing succinic and acetic acid were included with each set of samples.

2.2.5. Analysis of Uronic Acid Content. The uronic acid contents in samples were determined using the *m*-hydroxydiphenyl method, as described by Blumenkrantz and Asboe-Hansen[38] with some modifications, using glucuronic acid as the standard. Samples (50 mg) were suspended in 3 mL of 13.0 M sulfuric acid and put on ice for 3 h. After hydrolysis, deionized water was added up to a total volume of 50 mL after filtering. Hydrolyzates (600 μL) were transferred to a glass test tube and placed in an ice bath to cool. After addition of 3.6 mL of a sulfuric acid/tetraborate solution (12.5 mmol/L sodium tetraborate in 96% sulfuric acid), the mixtures were shaken and placed in a water bath at 100 °C for 6 min. After the mixtures were cooled and filtered, *m*-hydroxydiphenyl reagent (60 μL, 0.15% *m*-hydroxydiphenyl in 0.5% NaOH) was added. The samples were mixed, and extinction measurements were carried out 1 min after addition of the reagent at 520 nm (Ultrospec III UV/vis spectrophotometer, GE Healthcare, Piscataway, U.S.A.).

2.2.6. TEAC Assay for Determination of the Antioxidant Capacity of AXOS. The antioxidant capacity of the samples was analyzed on the basis of their ability to scavenge 2,2′-azino-bis(3-ethylbenzothiazoline-

6-sulfonic acid) diammonium salt (ABTS) radicals according to the TEAC assay described by Fischer et al.[39] with some modifications. ABTS radicals were generated prior to the addition of antioxidants by incubation of 0.23 mM ABTS and 2.3 mM 2,2′-azobis(2-methyl-propionamidine) dihydrochloride (ABAP) in McIlvaine buffer pH 6.5, prepared by mixing appropriate amounts of 0.10 M citric acid and 0.20 M disodium phosphate at 70 °C until an absorption of 0.70 ± 0.02 at 734 nm was reached. Trolox, a water-soluble vitamin E analog, and FA were dissolved in ethanol, and AXOS in water, before 1960 μL of radical solution was added to the 40 μL of antioxidant solution. Six minutes after the addition, decolorization of the blue-green ABTS radicals due to radical scavenging of the antioxidant was determined by measuring the absorbance at 734 nm (Ultrospec III UV/vis spectrophotometer, Pharmacia Biotech, Piscataway, U.S.A.). The antioxidant capacity of the AXOS samples is expressed as μmol Trolox per gram of dry matter ($TEAC_{dm}$) by using the Trolox calibration curve. The decrease in absorbance at 734 nm was linear for Trolox concentrations ranging from 1.0 to 25.0 μM. The TEAC values are also expressed in μmol Trolox/μmol FA ($TEAC_{FA}$) present in AXOS.

To ensure that antioxidant capacity of XOS and AXOS was determined in a linear range, the concentration ranges of XOS and AXOS in which they show a linear decrease in absorbance of the radical solution was established for both XOS-3-0.04-0.1**b**, containing almost no FA (0.07%), and AXOS-4-0.22-1.7**b**, containing medium amounts of FA (1.7%). The meaning of these sample codes is explained in section 3.1. A linear decrease in absorbance was observed between 0.01 and 0.19 for concentrations of XOS-3-0.04-0.1**b** from 30 mg/L up to 800 mg/L. The absolute value of the decrease is depicted in Figure 1A. In this concentration range, the TEAC assay gave rise to more constant $TEAC_{dm}$ values ranging from 10 to 16 μmol Trolox per gram of dry matter, as $TEAC_{dm}$ values seemed to slowly decrease for higher tested concentrations. The radical scavenging capacity of AXOS-4-0.22-1.7**b** was only linear in the concentration range up to 150 mg/L, corresponding to a decrease in absorbance of up to 0.42. The absolute value of the decrease is depicted in Figure 1B. The TEAC value of AXOS-4-0.22-1.7**b** was calculated using concentrations between 30 and 150 mg/L, as too low AXOS concentrations might lead to errors during determination of antioxidant values. Within this concentration range, decreases in absorbance were measured between 0.10 and 0.42. Although the error in absorbance measurement was rather small, determination of $TEAC_{dm}$ had a higher standard deviation due to low dry matter concentration upon testing. Therefore, the TEAC for other AXOS samples was calculated by averaging the TEAC values determined at several (at least three) concentrations within this range of decrease in absorbance.

2.2.7. ORAC for Determination of the Antioxidant Capacity of AXOS. The ORAC assay, using fluorescein as a fluorescent probe, was performed according to the method described by Ou et al.[40] with some modifications. The reaction was carried out in potassium phosphate buffer (75 mM; pH 7.4) and a final reaction volume of 200 μL. A calibration curve for Trolox was made between 2.0−7.0 μM (final concentration in phosphate buffer). On the basis of TEAC values of FA, XOS, and AXOS, samples were dissolved in two different concentrations, which led to six different test concentrations within the Trolox calibration range after 8, 11.7, and 20 times (overall dilution) dilution by an automatic sampler (Multiprobe probeII plus, Perkin-Elmer, Zaventem, Belgium) before analysis. The automatic sampler filled each well of a microtiter plate with sample (50 μL) or Trolox (50 μL) and fluorescein (100 μL; 61 nM, final concentration in phosphate buffer), and the plate was preincubated for 30 min at 37 °C. After preincubation, ABAP solution (50 μL; 19 mM final concentration in phosphate buffer) was rapidly added using an 8-channel pipet, and then the microtiter plate was immediately placed in the fluorescence plate reader (Spectramax M2^e, Molecular devices, Wokingham, U.K.) for measurement (excitation 485 nm, emission 520 nm, 37 °C). Upon incubation, peroxyl radicals were generated which would react with fluorescein, leading to loss of fluorescence if not scavenged by present antioxidants. The fluoresence was measured every minute for a period of 60 min. As the reaction goes to completion within an ORAC assay, information on the total antioxidant capacity and also the inhibition degree and inhibition time is provided.[40] Phosphate buffer was used instead of sample or Trolox as a blank. The difference in the area under the curve between the fluoresescence curve of the sample and the blank is a measure for the total antioxidant capacity of the sample. Antioxidant capacities were again expressed relative to the antioxidant capacity of Trolox. The calibration curve of the ORAC assay was linear between 2.0 and 7.0 μM Trolox. On the basis of differences in concentrations of Trolox for the calibration curve of TEAC and ORAC, concentrations for testing XOS and AXOS with the ORAC assay were calculated. Within the tested range, final measurements were done on six concentrations, and antioxidant capacities proved constant and reproducible for the tested concentrations. ORAC values of the samples were expressed as μmol Trolox per gram of dry matter ($ORAC_{dm}$) and also based on their FA content (μmol Trolox/μmol FA, $ORAC_{FA}$).

2.2.8. Statistical Analysis. Statistical analysis of the results was performed using SAS software 9.3 (SAS Institute, Inc., Cary, NC). One-way analysis of variation (ANOVA) was performed to compare $TEAC_{FA}$ and $ORAC_{FA}$ of different AXOS samples. A Tukey multiple comparison procedure was used with a 5% family significance level.

3. RESULTS AND DISCUSSION

3.1. Production and Structural Characterization of AXOS. A schematic overview of the production of the structurally different AXOS samples used in this study is depicted in Figure 2. Two series of AXOS were produced. In

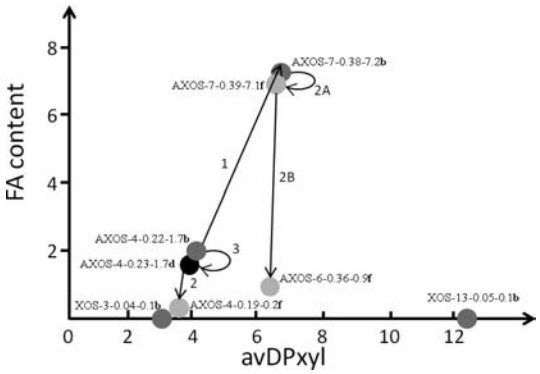

Figure 2. Overview of eight AXOS samples produced or used in this study. AXOS samples are produced starting from Brana Vita AXOS (AXOS-4-0.22-1.7**b**). Method 1 is based on FA enrichment of AXOS using Amberlite XAD-4. Method 2 consists of both enzymic hydrolysis (2A) and removal (2B) of FA. Using method 3, AXOS are coupled due to cross-linking of their FA moieties by use of a horseradish peroxidise−hydrogen peroxide system. The low (XOS-3-0.04-0.1**b**) and high DP XOS (XOS-13-0.05-0.1**b**) are depicted as well. Samples with FA mostly present as bound are depicted in dark gray, samples with FA mostly present in a free form in light gray and samples with FA mostly present in a dimeric form in black.

the first series, FA-enriched AXOS produced from Brana Vita AXOS were treated with FAE with or without subsequent FA removal to create free FA-depleted and free FA-rich AXOS with similar carbohydrate structure. For the second series, Brana Vita AXOS were used to produce FA-depleted AXOS and di-FA cross-linked AXOS with similar carbohydrate structure.

On the basis of their structural characteristics (see below), AXOS samples were given a code. The code consists of XOS or AXOS followed by the avDP, avDAS, and FA content (w/w %). After the FA content, a letter **b**, **f**, or **d** is mentioned, which indicates that most FA was present in a bound, free, or dimerized form.

Table 1. Distribution of AXOS over the Different Fractions, in Terms of Percentage of Initial AXOS and FA Weight (w/w), after Fractionation of Brana Vita Using the Amberlite XAD-4 Resin, and Characteristics of These Fractions

	Brana Vita AXOS	run through	water fraction	ethanol−water (10/90 v/v) fraction	ethanol−water (50/50 v/v) or higher fraction
FA distribution (w/w%)	100	20	1	<1	78
AXOS distribution (w/w%)	100	52	28	2	18
AXOS (% dm[a])	73 (±1)	73 (±1)	64 (±1)	53 (±1)	70 (±1)
free ara (% dm)	0.1 (±0.1)	0.1 (±0.1)	0.1 (±0.1)	0.0 (±0.1)	0.0 (±0.1)
free xyl (% dm)	0.7 (±0.1)	1.3 (±0.1)	0.9 (±0.1)	0.0 (±0.1)	0.0 (±0.1)
avDPxyl[b]	3.9 (±0.2)	3.2 (±0.4)	3.4 (±0.1)	7.4 (±0.1)	7.4 (±0.3)
avDAS[c]	0.22 (±0.01)	0.21 (±0.01)	0.15 (±0.01)	0.41 (±0.01)	0.40 (±0.03)
oligomeric glucose (% dm)	16 (±1)	14 (±1)	17 (±1)	37 (±1)	15 (±1)
total FA (% dm)	1.68 (±0.06)	0.61 (±0.06)	0.04 (±0.01)	0.05 (±0.01)	7.21 (±0.02)

[a]dm, dry matter. [b]avDPxyl, average degree of polymeriztion of the xylan backbone. [c]avDAS, average degree of arabinose substitution.

*3.1.1. First Series of AXOS (AXOS-7-0.38-7.2**b**, AXOS-7-0.39-7.1**f**, AXOS-6-0.36-0.9**f**). Production of FA-Enriched AXOS (AXOS-7-0.38-7.2**b**).* FA-enriched AXOS were produced starting from Brana Vita AXOS (AXOS-4-0.22-1.7**b**) using an Amberlite XAD-4-based fractionation process. During this fractionation process, AXOS with different sugar and FA composition were separated into different fractions. Mass balances were made, both for AXOS as well as for FA, to characterize the fractionation process. The distribution of AXOS and FA over the different fractions is summarized in Table 1. Half of AXOS did not bind to the column and ended up in the run through, but FA in this run through fraction only accounted for 20% of the total FA amount. Eluting with water and ethanol−water (10/90 v/v) resulted in desorption of another 30% of AXOS in total, while only 1% of the initial present FA was eluted. Only 18% of initial AXOS was found in the fraction eluted with ethanol−water (50/50 v/v) and higher ethanol concentrations. Nonetheless, 78% of the initially present FA was collected in this fraction.

The AXOS present in the run through were smaller (avDPxyl 3.2) and had a 3 times lower FA level (0.61%) than Brana Vita AXOS. The avDAS was the same as that found in the starting material. The AXOS eluting with water and ethanol−water (10/90 v/v) only contained 0.04 and 0.05% FA, respectively. AXOS present in the fraction eluting with water had both a lower avDPxyl (3.4) and avDAS (0.15), compared to Brana Vita AXOS (avDPxyl 3.9 and avDAS 0.22). The AXOS that were eluted with ethanol−water (10/90 v/v) were considerably bigger (avDPxyl 7.4) and more substituted (avDAS 0.41). It was also noticed that a large amount of oligomeric glucose (37%) was present in the ethanol−water 10/90 v/v eluted fraction. Finally, AXOS eluted with ethanol−water (50/50 v/v) and higher ethanol concentrations were approximately 4 times richer in FA (7.2%) in comparison to the starting sample. The FA enrichment of Brana Vita resulted not only in a higher FA content but AXOS eluting with ethanol−water (50/50 v/v) and higher ethanol concentrations also had a higher avDPxyl and avDAS (0.40), similar to the ethanol−water (10/90 v/v) eluting fraction. The above observation is logical as FA is esterified to arabinose. The selection of FA-containing AXOS hence results in AXOS samples with bigger and more arabinose-substituted AXOS. Unlike in the other fractions, no free monomeric sugars were present in these samples. The AXOS preparation of this type, further used in the article, is a combination of FA-enriched fractions of three repetitive fractionation processes (results not shown). The characteristics of this sample are summarized in Table 2. It is further referred to as AXOS-7-0.38-7.2**b**.

*Production of Free FA-Rich (AXOS-7-0.39-7.1**f**) and Free FA-Depleted AXOS (AXOS-6-0.36-0.9**f**).* AXOS-7-0.39-7.1**f** and AXOS-6-0.36-0.9**f** were produced consecutively, starting from AXOS-7-0.38-7.2**b**. First, AXOS-7-0.39-7.1**f** was produced by incubation of AXOS-7-0.38-7.2**b** with FAE, which led to hydrolysis of about 85% of the present FA. Both samples had similar sugar structure and only differed in the form in which FA was present. Subsequently, AXOS-7-0.39-7.1**f** was loaded on an Amberlite XAD-4 column as described in section2.2.1. The nonadsorbed, water and ethanol−water (10/90 v/v) eluted fractions were combined to generate AXOS-6-0.36-0.9**f**. FA removal was not absolute and some FA was recovered in the FA-depleted fractions while some nonferuloylated AXOS were lost in the FA-enriched fraction. AXOS-6-0.36-0.9**f** only contained 0.86% FA, with 0.50% FA present in a free form (Table 2). AXOS-6-0.36-0.9**f** had a similar avDPxyl (6.3) and avDAS (0.36) than AXOS-7-0.38-7.2**b** (Table 2).

3.1.2. Second Series of AXOS (AXOS-4-0.22-1.7b, AXOS-4-0.19-0.2f, AXOS-4-0.23-1.7d). Production of FA-Depleted AXOS (AXOS-4-0.19-0.2f). FA-depleted AXOS were produced from Brana Vita AXOS (AXOS-4-0.22-1.7**b**). During enzymic hydrolysis of AXOS-4-0.22-1.7**b** with FAE, 90% of the FA present was hydrolyzed (results not shown). Further processing by removal of FA using an Amberlite XAD-4 column, resulted in AXOS-4-0.19-0.2**f**. Their avDPxyl (3.5) and avDAS (0.19) were slightly smaller but similar to AXOS-4-0.22-1.7**b** (3.9 and 0.22, respectively), but they only contained 0.23% FA, with 0.16% FA present in a free form (Table 2).

Production of di-FA Cross-Linked AXOS. During the production of di-FA cross-linked AXOS, FA were rapidly cross-linked after the addition of the horseradish peroxidase. Spectrophotometric follow-up of the reaction showed that within the first minutes, the absorbance at 325 nm reached a constant level that was almost half that of the starting point. The di-FA cross-linked AXOS only contained 0.07% monomeric FA, indicating that 96% of the present FA had dimerized. The HPLC chromatogram showed the presence of a set of new peaks on run times where several forms of di-FA are expected to elute according to Dobberstein and Bunzel [35] (results not shown). As expected, the sugar composition remained unchanged during the process (Table 2). Based on the stoichiometry, only one mole of hydrogen peroxide is needed to cross-link two moles FA. Inevitably, the peroxidase - hydrogen peroxide system also oxidizes other substrates, in this case most probably the already dimerized FA.[41] Therefore, the

Table 2. Overview of the Content and Structural Characteristics of the (Produced) AXOS Determined with GC (Carbohydrates) and RP-HPLC (FA). Sugar and FA Content Are Expressed as Mass Percentage on a Dry Matter Basis

	XOS-3-0.04-0.1**b**	XOS-13-0.05-0.1**b**	AXOS-7-0.38-7.2**b**	AXOS-7-0.39-7.1**f**	AXOS-6-0.36-0.9**f**	AXOS-4-0.22-1.7**b**	AXOS-4-0.19-0.2**f**	AXOS-4-0.23-1.7**d**
AXOS (% dm[a])	80 (±1)	74 (±1)	73 (±5)	73 (±1)	76 (±2)	73 (±1)	75 (±3)	76 (±3)
free ara (% dm)	0.3 (±0.1)	0.7 (±0.1)	<0.1	0.7 (±0.1)	1.4 (±0.1)	0.1 (±0.1)	0.2 (±0.1)	0.1 (±0.1)
free xyl (% dm)	0.6 (±0.1)	0.1 (±0.1)	<0.1	<0.1	0.1 (±0.1)	0.7 (±0.1)	1.1 (±0.1)	0.9 (±0.1)
avDPxyl[b]	3.1 (±0.1)	12.2 (±0.2)	6.6 (±0.6)	6.6 (±0.1)	6.3 (±0.3)	3.9 (±0.2)	3.5 (±0.2)	3.8 (±0.2)
avDAS[c]	0.04 (±0.01)	0.05 (±0.01)	0.38 (±0.05)	0.39 (±0.01)	0.36 (±0.02)	0.22 (±0.01)	0.19 (±0.01)	0.23 (±0.01)
oligomeric glucose (% dm)	16 (±1)	2 (±1)	12 (±2)	12 (±1)	12 (±1)	12 (±1)	18 (±1)	17 (±2)
free FA (% dm)	<0.01	<0.01	0.07 (±0.01)	6.00 (±0.21)	0.56 (±0.01)	0.01 (±0.01)	0.16 (±0.02)	<0.01[d]
bound FA (% dm)	0.07 (±0.01)	<0.01	7.17 (±0.11)	1.10 (±0.29)	0.30 (±0.02)	1.67 (±0.06)	0.07 (±0.03)	0.07 (±0.01)[d]
total FA (% dm)	0.07 (±0.01)	<0.01	7.24 (±0.11)	7.11 (±0.12)	0.86 (±0.01)	1.68 (±0.06)	0.23 (±0.03)	0.07 (±0.01)[d]

[a]dm, dry matter. [b]avDPxyl, average degree of polymerization of the xylan backbone. [c]avDAS, average degree of arabinose substitution. [d]Total FA content is the same as for AXOS-4-0.22-1.7**b**, but FA moieties were mainly cross-linked.

stoichiometry will change and relatively more hydrogen peroxide was needed. Preliminary tests showed that use of hydrogen peroxide and FA (bound to AXOS) in a 1/2 molar ratio resulted in less than 90% FA dimerization. The presence of the reactants in equal concentrations increased the degree of cross-linked AXOS.

3.1.3. Comparison of the Structural Characteristics of the (A)XOS Samples. The production of these AXOS brought the total of XOS and AXOS samples to eight. The structural characteristics of all samples are summarized in Table 2.

The two XOS samples (XOS-3-0.04-0.1**b** and XOS-13-0.05-0.1**b**) differed in their DP and only contained traces of FA.

Furthermore, two sets of three AXOS samples with a similar sugar composition and content but different FA content were produced, which is ideal for the study of the impact of FA on the antioxidant properties of AXOS.

Samples in series 1 (AXOS-7-0.38-7.2**b**, AXOS-7-0.39-7.1**f**, and AXOS-6-0.36-0.9**f**) differed mainly in their FA content, while only showing subtle differences in avDPxyl (approximately 6.5) and avDAS (approximately 0.38). AXOS-7-0.38-7.2**b** and AXOS-7-0.39-7.1**f** were rich in FA (7%), which is mainly present in the bound form in AXOS-7-0.38-7.2**b** and in the free form in AXOS-7-0.39-7.1**f**. AXOS-6-0.36-0.9**f** contained only 16% of the FA present in AXOS-7-0.38-7.2**b** or AXOS-7-0.39-7.1**f**, mainly in the free form.

Samples in series 2 (AXOS-4-0.22-1.7**b**, AXOS-4-0.19-0.2**f**, and AXOS-4-0.23-1.7**d**) also differed in their FA content and form only. AXOS-4-0.23-1.7**d** had the same FA level as AXOS-4-0.22-1.7**b**, but FA was present in a dimeric or oligomeric form. AXOS-4-0.19-0.2**f** contained substantially less FA (0.23%) compared to AXOS-4-0.22-1.7**b** and AXOS-4-0.23-1.7**d** (1.7%), while the present FA was mainly in a free form. Furthermore, XOS-3-0.04-0.1**b** had a comparable but a bit smaller xylan backbone as these samples and differed from the other samples by their low avDAS and FA content.

These sets of samples were further used in antioxidant capacity measurements to study the impact of FA on the antioxidant properties of AXOS.

3.2. Determination of the Antioxidant Capacity of AXOS. The ORAC assay measures the inhibition of the action of in situ generated peroxyl radicals against fluorescein, while during the TEAC assay, relatively stable ABTS radicals are generated prior to addition of antioxidant. Furthermore, the ORAC assay measures a complete reaction of the antioxidant with generated radicals and provides information on the total antioxidant capacity and also on the inhibition degree and inhibition time,[40] while TEAC is based on a fast reaction (6 min time frame) between antioxidant and radicals.

In the following sections, first the results on the antioxidant capacity of the AXOS samples are presented and discussed on a dry matter base, followed by a discussion on the influence of FA and its form and other structural characteristics on the antioxidant capacity of AXOS.

3.2.1. Antioxidant Capacity of AXOS: $TEAC_{dm}$ and $ORAC_{dm}$. The TEAC and ORAC values of the different XOS and AXOS samples are summarized in Table 3. The $ORAC_{dm}$ values of the samples were 1.3 to 2.8 times higher than the $TEAC_{dm}$ values. This was also seen in a previous study of the antioxidant capacity of phenolic acids and their microbial metabolites,[42] due to measurement of the total antioxidant capacity by using ORAC. Antioxidants can exert both a fast reaction (in the first 10 s) and a slow reaction (over a longer period), of which the main part, but not the entire part, is

Table 3. Antioxidant Capacity of FA and AXOS Samples Determined with TEAC and ORAC Assays. $TEAC_{dm}$ and $ORAC_{dm}$ Represent the Antioxidant Capacities of a Sample Expressed on g dm Basis (μmol Trolox/g dm). $TEAC_{FA}$ and $ORAC_{FA}$ Values Are Expressed on Total FA Content of the Sample (μmol Trolox/μmol FA)

	$TEAC_{dm}$	$TEAC_{FA}$[a]	$ORAC_{dm}$	$ORAC_{FA}$[a]
	μmol Trolox/g dm[b]	μmol Trolox/ μmol FA	μmol Trolox/g dm	μmol Trolox/ μmol FA
free FA	11867 (±926)^{A}	2.3 (±0.2)^{B}	23429 (±3548)^{A}	4.5 (±0.7)^{BC}
XOS-3-0.04-0.1**b**	10.3 (±0.4)^{H}	-	27 (±4)^{F}	-
XOS-13-0.05-0.1**b**	<1^{I}	-	15 (±2)^{G}	-
AXOS-7-0.38-7.2**b**	655 (±44)^{C}	1.8 (±0.1)^{BC}	1837 (±246) ^{B}	5.0 (±0.7)^{B}
AXOS-7-0.39-7.1**f**	1339 (±111)^{B}	3.7 (±0.3)^{A}	2064 (±145)^{B}	5.6 (±0.4)^{AB}
AXOS-6-0.36-0.9**f**	184 (±11)^{D}	4.2 (±0.2)^{A}	300 (±28)^{C}	6.8 (±0.6)^{A}
AXOS-4-0.22-1.7**b**	132 (±5)^{E}	1.5 (±0.1)^{CD}	316 (±10)^{C}	3.7 (±0.5)^{C}
AXOS-4-0.19-0.2**f**	50 (±4)^{G}	4.3 (±0.3)^{A}	67 (±8)^{E}	5.6 (±0.7)^{AB}
AXOS-4-0.23-1.7**d**	82 (±5)^{F}	1.0 (±0.1)^{D}	133 (±16)^{D}	1.5 (±0.2)^{D}

[a]Values are averages with standard deviations on at least triplicate measurements. Values within columns with the same capital letter are not statistically different ($p < 0.05$) . [b]dm, dry matter.

included in the 6 min time span of the TEAC assay.[43] In both assays, XOS had the lowest antioxidant capacity (Table 3). From the AXOS samples, AXOS-4-0.19-0.2**f** had the lowest and AXOS-7-0.39-7.1**f** the highest antioxidant capacity (Table 3).

As already mentioned, AXOS-4-0.22-1.7**b**, AXOS-4-0.19-0.2**f**, and AXOS-4-0.23-1.7**d** had a similar carbohydrate structure but differed in their FA content and form, having 1.68% FA in bound form, 0.23% FA (mostly) in free form, and 1.68% FA in dimerized form, respectively. AXOS-4-0.19-0.2**f**, having an almost 7-fold lower FA content than AXOS-4-0.22-1.7**b**, also had a considerably lower antioxidant capacity. This decrease in antioxidant capacity when determined with TEAC was, however, not proportional to the decrease in FA. Cross-linking of AXOS-4-0.22-1.7**b** to produce AXOS-4-0.23-1.7**d** resulted in a decreased antioxidant capacity, which was more outspoken using the ORAC assay (decrease from 316 to 133 μmol Trolox/g dry matter) compared to the TEAC assay results (decrease from 132 to 82 μmol Trolox/g dry matter).

Also AXOS-7-0.38-7.2**b**, AXOS-7-0.39-7.1**f**, and AXOS-6-0.36-0.9**f** had similar sugar structures and only differed in their FA content and form. AXOS-7-0.38-7.2**b** and AXOS-7-0.39-7.1**f** specifically differed in the form in which FA was present as they mainly contained bound and free FA, respectively. The conversion of 85% of the FA from bound to free form doubled the antioxidant capacity from 655 to 1338 μmol Trolox/g dry matter. After removal of approximately 85% of the FA present in AXOS-7-0.39-7.1**f**, the antioxidant capacity decreased to 184 μmol Trolox/g dry matter in AXOS-6-0.36-0.9**f**.

3.2.2. Influence of FA and Its Form on Antioxidant Capacity: $TEAC_{FA}$ and $ORAC_{FA}$. To focus more on the specific impact of FA on the antioxidant capacity, TEAC and ORAC values were also expressed on sample FA content, as $TEAC_{FA}$ and $ORAC_{FA}$, respectively, and also the antioxidant capacity of free FA was determined (Table 3). $TEAC_{FA}$ and $ORAC_{FA}$ values were not calculated for XOS samples because of their very low FA content.

Free FA had a $TEAC_{FA}$ value of 2.3 and $ORAC_{FA}$ value of 4.5, which is similar to values reported before.[42,44]

Both AXOS-4-0.22-1.7**b** and AXOS-7-0.38-7.2**b** consisted of AXOS with mainly bound FA. As $TEAC_{dm}$ values were for both samples proportional to their FA content, this results in similar $TEAC_{FA}$ values of 1.6 and 1.8. Esterification of FA to AXOS led to a decrease in its antioxidant capacity of about 30%. However, FA bound to AXOS still had considerable antioxidant properties compared to Trolox. Based on $ORAC_{FA}$ values, almost no decrease in antioxidant capacity was noticed at all for nonesterified and esterified FA. It should indeed be noted that these observations can differ depending on the type of antioxidant test. In an antioxidant activity test based on scavenging of free 2,2-diphenyl-1-picrylhydrazyl radicals, free FA gave better results than when it was present in feruloylated AXOS.[22] However, when the same compounds were tested using an antioxidant capacity test based on the inhibition of copper-mediated oxidation of low density lipoprotein, the feruloylated AXOS exerted higher antioxidant activities presumably due to the presence of both a hydrophilic (carbohydrate structure of AXOS) as lipophilic part (FA), while free FA is not well-soluble in water. In the latter system, the partition coefficient between the aqueous and lipophilic phase of an antioxidant was said to influence its antioxidant capacity.[22]

As esterified FA showed a lower antioxidant capacity, it was seen that $TEAC_{FA}$ values of AXOS which contained mostly free FA (AXOS-4-0.19-0.2**f**, AXOS-7-0.39-7.1**f**, and AXOS-6-0.36-0.9**f**) were higher than $TEAC_{FA}$ of AXOS containing mostly bound FA (AXOS-4-0.22-1.7**b** and AXOS-7-0.38-7.2**b**). Also $ORAC_{FA}$ values were higher for these samples, although the effect was less outspoken. Remarkably, free FA in the presence of AXOS had higher antioxidant capacity than free FA in absence of AXOS. It might be that antioxidant capacity of free FA is influenced by its water solubility, which can be affected by the surrounding matrix.

Dimerization of the FA moieties led to a decrease of the antioxidant capacity as seen by their lower $TEAC_{FA}$ and $ORAC_{FA}$ values (1.0 and 1.5, respectively). During cross-linking, a diversity of di-FA and FA trimers or tetramers can be formed, which are cross-linked at their 4-, 5- or 8-position, varying in number of free hydroxyl groups and resonance system.[45] Antioxidant capacity studies on free FA and di-FA showed that the antioxidant capacity of the dimers is structure dependent as this number of free hydroxyl groups and the conjugates system for resonance stabilization influence the antioxidant capacity of di-FA and hydroxycinnamic acids in general. According to their TEAC values, one mole of di-FA, except for 8,8-di-FA (noncyclic form), has lower antioxidant capacity than two moles (and some even less than one mole) of FA,[24,46] explaining the observed decrease in antioxidant capacity after diFA-cross-linking. Funk et al. [47] showed that moderate cross-linking of cell wall polysaccharides by FA does not influence bacterial metabolism. However, it remains to be

demonstrated in the specific case of dimerized AXOS whether or not cross-linking leads to enhanced resistance of the molecule against enzymic hydrolysis of the ester bonds and hence to protection against metabolism of the antioxidant by micro-organisms in the gut and activity loss.

As XOS contained only very low levels of FA, expressing the antioxidant capacity in μmol Trolox/μmol FA is irrelevant and could lead to high errors.

3.2.3. Influence of Other Structural Characteristics on Antioxidant Capacity: $TEAC_{FA}$ and $ORAC_{FA}$. Due to the low FA content of XOS samples, contribution of substituents with less antioxidant capacity, like uronic and acetic acid groups, might be more important. However, the antioxidant capacity of XOS remained very low. Acetic and uronic acid contents were determined for all AXOS samples, but concentration differences were rather small and no correlation could be found between their content and the antioxidant capacity of the AXOS and XOS (results not shown). Also, proteins which were present in low concentrations (<0.7%) did not contribute to the antioxidant capacity of the samples.

Similar to the presence of other non-FA substituents, the difference in carbohydrate structure did not influence the antioxidant capacity of AXOS within the DP and DAS range tested. Therefore, it can be concluded that the contribution of the carbohydrate backbone of XOS and AXOS to the antioxidant capacity is small or nonexistent. A previous study found that the antioxidant capacity of rice or ragi AX was higher than expected based on the FA content, and the authors stated that other structural features (molecular weight and uronic acid) played an important role,[26] although this might be less important for low molecular weight FA esters.[48] Also the matrix can influence the antioxidant capacity of FA. With regard to wheat bran, for example, an increase in antioxidant capacity was seen after ultrafine grinding due to a better exposition of the phenolic moieties.[49]

In conclusion, FA content was shown to be the major factor determining the antioxidant capacity of feruloylated AXOS. The form in which FA is present also had a strong influence on the antioxidant capacity. FA esterified to AXOS had lower antioxidant capacity, as determined with TEAC, than its free form. Nevertheless, antioxidant capacity of esterified FA was still considerable in comparison with the antioxidant capacity of Trolox. However, total antioxidant capacities of free or bound FA, as determined with ORAC, were rather similar. Cross-linking AXOS through dimerization of FA led to a decrease in antioxidant capacity of this sample. Other structural characteristics (avDPxyl, avDAS, or other substituents) did not contribute to the antioxidant capacity of AXOS. Feruloylated AXOS are hence interesting water-soluble food additives as they have strong antioxidant properties.

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ABBREVIATIONS USED

ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt; ABAP, 2,2′-azobis(2-methylpropionamidine) dihydrochloride; avDAS, average degree of arabinose substitution; avDPxyl, average degree of polymerization of the xylan backbone; AX, arabinoxylan; AXOS, arabinoxylanoligosaccharides; di-FA, dehydrodiferulic acid; DP, degree of polymerization; FA, ferulic acid; FAE, feruloyl esterase; HPLC, high-performance liquid chromatography; ORAC, oxygen radical absorbance capacity; $ORAC_{dm}$, ORAC values expressed in μmol Trolox per gram of dry matter; $ORAC_{FA}$, ORAC values expressed in μmol Trolox/μmol FA; TEAC, Trolox equivalent antioxidant capacity; $TEAC_{dm}$, TEAC values expressed in μmol Trolox per gram of dry matter; $TEAC_{FA}$, TEAC values expressed in μmol Trolox/μmol FA; TFA, trifluoroacetic acid; XOS, xylooligosaccharides

REFERENCES

(1) Cordain, L.; Eaton, S. B.; Sebastian, A.; Mann, N.; Lindeberg, S.; Watkins, B. A.; O'Keefe, J. H.; Brand-Miller, J. Origins and evolution of the Western diet: Health implications for the 21st century. *Am. J. Clin. Nutr.* **2005**, *81*, 341−354.

(2) Diplock, A. T.; Aggett, P. J.; Ashwell, M.; Bornet, F.; Fern, E. B.; Roberfroid, M. Scientific concepts of functional foods in Europe. Consensus document. *Br. J. Nutr.* **1999**, *81*, S1−S27.

(3) Broekaert, W. F.; Courtin, C. M.; Verbeke, K.; Van de Wiele, T.; Verstraete, W.; Delcour, J. A. Prebiotic and other health-related effects of cereal-derived arabinoxylans, arabinoxylan-oligosaccharides, and xylooligosaccharides. *Crit. Rev. Food Sci. Nutr.* **2011**, *51*, 178−194.

(4) Grajek, W.; Olejnik, A.; Sip, A. Probiotics, prebiotics and antioxidants as functional foods. *Acta Biochim. Pol.* **2005**, *52*, 665−671.

(5) Gibson, G. R.; Probert, H. M.; Van Loo, J.; Rastall, R. A.; Roberfroid, M. B. Dietary modulation of the human colonic microbiota: Updating the concept of prebiotics. *Nutr. Res. Rev.* **2004**, *17*, 259−275.

(6) Gibson, G. R.; Roberfroid, M. B. Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. *J. Nutr.* **1995**, *125*, 1401−1412.

(7) Roberfroid, M.; Gibson, G. R.; Hoyles, L.; McCartney, A. L.; Rastall, R.; Rowland, I.; Wolvers, D.; Watzl, B.; Szajewska, H.; Stahl, B.; Guarner, F.; Respondek, F.; Whelan, K.; Coxam, V.; Davicco, M. J.; Leotoing, L.; Wittrant, Y.; Delzenne, N. M.; Cani, P. D.; Neyrinck, A. M.; Meheust, A. Prebiotic effects: Metabolic and health benefits. *Br. J. Nutr.* **2010**, *104*, S1−S63.

(8) Swennen, K.; Courtin, C. M.; Delcour, J. A. Non-digestible oligosaccharides with prebiotic properties. *Crit. Rev. Food Sci. Nutr.* **2006**, *46*, 459−471.

(9) Cleemput, G.; Roels, S. P.; Van Oort, M.; Grobet, P. J.; Delcour, J. A. Heterogeneity in the structure of water-soluble arabinoxylans in

european wheat flours of variable bread-making quality. *Cereal Chem.* **1993**, *70*, 324−329.
(10) Izydorczyk, M. S.; Biliaderis, C. G. Cereal arabinoxylans: Advances in structure and physicochemical properties. *Carbohydr. Polym.* **1995**, *28*, 33−48.
(11) Schooneveld-Bergmans, M. E. F.; Beldman, G.; Voragen, A. G. J. Structural features of (glucurono)arabinoxylans extracted from wheat bran by barium hydroxide. *J. Cereal Sci.* **1999**, *29*, 63−75.
(12) MacDonald-Wicks, L. K.; Wood, L. G.; Garg, M. L. Methodology for the determination of biological antioxidant capacity in vitro: A review. *J. Sci. Food Agric.* **2006**, *86*, 2046−2056.
(13) Andreasen, M. F.; Christensen, L. P.; Meyer, A. S.; Hansen, Å. Ferulic acid dehydrodimers in rye (*Secale cereale* L.). *J. Cereal Sci.* **2000**, *31*, 303−307.
(14) Gallardo, C.; Jiménez, L.; García-Conesa, M. T. Hydroxycinnamic acid composition and in vitro antioxidant activity of selected grain fractions. *Food Chem.* **2006**, *99*, 455−463.
(15) Sies, H. Oxidative stress: Oxidants and antioxidants. *Exp. Physiol.* **1997**, *82*, 291−295.
(16) Valko, M.; Leibfritz, D.; Moncol, J.; Cronin, M. T. D.; Mazur, M.; Telser, J. Free radicals and antioxidants in normal physiological functions and human disease. *Int. J. Biochem. Cell Biol.* **2007**, *39*, 44−84.
(17) Wang, J.; Sun, B.; Cao, Y.; Song, H.; Tian, Y. Inhibitory effect of wheat bran feruloyl oligosaccharides on oxidative DNA damage in human lymphocytes. *Food Chem.* **2008**, *109*, 129−136.
(18) Wang, J.; Sun, B.; Cao, Y.; Tian, Y. Protection of wheat bran feruloyl oligosaccharides against free radical-induced oxidative damage in normal human erythrocytes. *Food Chem. Toxicol.* **2009**, *47*, 1591−1599.
(19) Yuan, X.; Wang, J.; Yao, H.; Chen, F. Free radical-scavenging capacity and inhibitory activity on rat erythrocyte hemolysis of feruloyl oligosaccharides from wheat bran insoluble dietary fiber. *LWT—Food Sci. Technol.* **2005**, *38*, 877−883.
(20) Ou, S. Y.; Jackson, G. M.; Jiao, X.; Chen, J.; Wu, J. Z.; Huang, X. S. Protection against oxidative stress in diabetic rats by wheat bran feruloyl oligosaccharides. *J. Agric. Food Chem.* **2007**, *55*, 3191−3195.
(21) Ohta, T.; Semboku, N.; Kuchii, A.; Egashira, Y.; Sanada, H. Antioxidant activity of corn bran cell-wall fragments in the LDL oxidation system. *J. Agric. Food Chem.* **1997**, *45*, 1644−1648.
(22) Katapodis, P.; Vardakou, M.; Kalogeris, E.; Kekos, D.; Macris, B. J.; Christakopoulos, P. Enzymic production of a feruloylated oligosaccharide with antioxidant activity from wheat flour arabinoxylan. *Eur. J. Nutr.* **2003**, *42*, 55−60.
(23) Holvoet, P.; Jenny, N. S.; Schreiner, P. J.; Tracy, R. P.; Jacobs, D. R. The relationship between oxidized LDL and other cardiovascular risk factors and subclinical CVD in different ethnic groups: The Multi-Ethnic Study of Atherosclerosis (MESA). *Atherosclerosis* **2007**, *194*, 245−252.
(24) Williamson, G.; Plumb, G. W.; Garcia-Conesa, M. T. Glycosylation, esterification and polymerization of flavonoids and hydroxycinnamates: effects on antioxidant properties. *Basic Life Sci.* **1999**, *66*, 483−494.
(25) Van den Ende, W.; Valluru, R. Sucrose, sucrosyl oligosaccharides, and oxidative stress: Scavenging and salvaging? *J. Exp. Bot.* **2009**, *60*, 9−18.
(26) Rao, R. S. P.; Muralikrishna, G. Water soluble feruloyl arabinoxylans from rice and ragi: Changes upon malting and their consequence on antioxidant activity. *Phytochemistry* **2006**, *67*, 91−99.
(27) Yuan, H. M.; Song, J. M.; Zhang, W. W.; Li, X. G.; Li, N.; Gao, X. L. Antioxidant activity and cytoprotective effect of κ-carrageenan oligosaccharides and their different derivatives. *Biorg. Med. Chem. Lett.* **2006**, *16*, 1329−1334.
(28) Pristov, J. B.; Mitrovic, A.; Spasojevic, I. A comparative study of antioxidative activities of cell-wall polysaccharides. *Carbohydr. Res.* **2011**, *346*, 2255−2259.
(29) Swennen, K.; Courtin, C. M.; Lindemans, G.; Delcour, J. A. Large-scale production and characterisation of wheat bran arabinoxylooligosaccharides. *J. Sci. Food Agric.* **2006**, *86*, 1722−1731.
(30) Saulnier, L.; Vigouroux, J.; Thibault, J.-F. Isolation and partial characterization of feruloylated oligosaccharides from maize bran. *Carbohydr. Res.* **1995**, *272*, 241−253.
(31) Blum, D. L.; Kataeva, I. A.; Li, X.-L.; Ljungdahl, L. G. Feruloyl esterase activity of the *Clostridium thermocellum* cellulosome can be attributed to previously unknown domains of XynY and XynZ. *J. Bacteriol.* **2000**, *182*, 1346−1351.
(32) Zaidel, D. N. A.; Arnous, A.; Holck, J.; Meyer, A. S. Kinetics of enzyme-catalyzed cross-linking of feruloylated arabinan from sugar beet. *J. Agric. Food Chem.* **2011**, *59*, 11598−11607.
(33) Courtin, C. M.; Van den Broeck, H.; Delcour, J. A. Determination of reducing end sugar residues in oligo- and polysaccharides by gas-liquid chromatography. *J. Chromatogr. A* **2000**, *866*, 97−104.
(34) Antoine, C.; Peyron, S.; Mabille, F.; Lapierre, C.; Bouchet, B.; Abecassis, J.; Rouau, X. Individual contribution of grain outer layers and their cell wall structure to the mechanical properties of wheat bran. *J. Agric. Food Chem.* **2003**, *51*, 2026−2033.
(35) Dobberstein, D.; Bunzel, M. Separation and detection of cell wall-bound ferulic acid dehydrodimers and dehydrotrimers in cereals and other plant materials by reversed phase high-performance liquid chromatography with ultraviolet detection. *J. Agric. Food Chem.* **2010**, *58*, 8927−8935.
(36) Van Gool, M. P.; Vancsó, I.; Schols, H. A.; Toth, K.; Szakacs, G.; Gruppen, H. Screening for distinct xylan degrading enzymes in complex shake flask fermentation supernatants. *Bioresour. Technol.* **2011**, *102*, 6039−6047.
(37) Jayaram, V. B.; Cuyvers, S.; Lagrain, B.; Verstrepen, K. J.; Delcour, J. A.; Courtin, C. M. Mapping of *Saccharomyces cerevisiae* metabolites in fermenting wheat straight-dough reveals succinic acid as pH-determining factor. *Food Chem.* **2013**, *136*, 301−308.
(38) Blumenkrantz, N.; Asboe-Hansen, G. New method for quantitative determination of uronic acids. *Anal. Biochem.* **1973**, *54*, 484−489.
(39) Fischer, M. A. J. G.; Gransier, T. J. M.; Beckers, L. M. G.; Bekers, O.; Bast, A.; Haenen, G. R. M. M. Determination of the antioxidant capacity in blood. *Clin. Chem. Lab. Med.* **2005**, *43*, 735−740.
(40) Ou, B.; Hampsch-Woodill, M.; Prior, R. L. Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *J. Agric. Food Chem.* **2001**, *49*, 4619−4626.
(41) Garcia, R.; Rakotozafy, L.; Nicolas, J. Analysis and modeling of the ferulic acid oxidation by a glucose oxidase-peroxidase association. Comparison with a hexose oxidase-peroxidase association. *J. Agric. Food Chem.* **2004**, *52*, 3946−3953.
(42) Gómez-Ruiz, J. A.; Leake, D. S.; Ames, J. M. In vitro antioxidant activity of coffee compounds and their metabolites. *J. Agric. Food Chem.* **2007**, *55*, 6962−6969.
(43) van den Berg, R.; Haenen, G. R. M. M.; van den Berg, H.; Bast, A. Applicability of an improved Trolox equivalent antioxidant capacity (TEAC) assay for evaluation of antioxidant capacity measurements of mixtures. *Food Chem.* **1999**, *66*, 511−517.
(44) Anson, N. M.; van den Berg, R.; Havenaar, R.; Bast, A.; Haenen, G. R. M. M. Ferulic acid from aleurone determines the antioxidant potency of wheat grain (*Triticum aestivum* L.). *J. Agric. Food Chem.* **2008**, *56*, 5589−5594.
(45) Bunzel, M. Chemistry and occurrence of hydroxycinnamate oligomers. *Phytochem. Rev.* **2010**, *9*, 47−64.
(46) Garcia-Conesa, M. T.; Wilson, P. D.; Plumb, G. W.; Ralph, J.; Williamson, G. Antioxidant properties of 4,4′-dihydroxy-3,3′-dimethoxy-β,β′-bicinnamic acid (8−8-diferulic acid, non-cyclic form). *J. Sci. Food Agric.* **1999**, *79*, 379−384.
(47) Funk, C.; Braune, A.; Grabber, J. H.; Steinhart, H.; Bunzel, M. Moderate ferulate and diferulate levels do not impede maize cell wall degradation by human intestinal microbiota. *J. Agric. Food Chem.* **2007**, *55*, 2418−2423.

(48) Ohta, T.; Yamasaki, S.; Egashira, Y.; Sanada, H. Antioxidative activity of corn bran hemicellulose fragments. *J. Agric. Food Chem.* **1994**, *42*, 653−656.
(49) Rosa, N. N.; Barron, C.; Gaiani, C.; Dufour, C.; Micard, V. Ultra-fine grinding increases the antioxidant capacity of wheat bran. *J. Cereal Sci.* **2013**, *57*, 84−90.