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Molecular Interactions between Barley and Oat β-Glucans and Phenolic Derivatives

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Equilibrium dialysis, molecular modeling, and multivariate data analysis were used to investigate the nature of the molecular interactions between 21 vanillin-inspired phenolic derivatives, 4 bile salts, and 2 commercially available β-glucan preparations, Glucagel and PromOat, from barley and oats. The two β-glucan products showed very similar binding properties. It was demonstrated that the two β-glucan products are able to absorb most phenolic derivatives at a level corresponding to the absorption of bile salts. Glucosides of the phenolic compounds showed poor or no absorption. The four phenolic derivatives that showed strongest retention in the dialysis assay shared the presence of a hydroxyl group in *para*-position to a CHO group. However, other compounds with the same structural feature but possessing a different set of additional functional groups showed less retention. Principal component analysis (PCA) and partial least-squares regression (PLS) calculations using a multitude of diverse descriptors related to electronic, geometrical, constitutional, hybrid, and topological features of the phenolic compounds showed a marked distinction between aglycon, glucosides, and bile salt retention. These analyses did not offer additional information with respect to the mode of interaction of the individual phenolics with the β-glucans. When the barley β-glucan was subjected to enzyme degradation, the ability to bind some but not all of the phenolic derivatives was lost. It is concluded that the binding must be dependent on multiple characteristics that are not captured by a single molecular descriptor.

KEYWORDS: β-Glucan; barley; oat; bile salts; phenolic derivatives; β-glucosides

INTRODUCTION

The first publication on the relationship between dietary fibers and small molecules, namely, bile salts, was published by Cooksoon et al. in 1967 (*1*). Since then, the health-promoting effect of dietary fibers and the influence of dietary fibers in food mixtures has been investigated in a large number of studies. The health-promoting effects of dietary fibers are now well documented (*2*−*5*). Knowledge obtained in these studies has inspired this study and has served as a platform in the investigation of the interaction of a different set of small molecules with β-glucans. It has been observed in animal as well as human models that an increase in soluble, viscous nonstarch polysaccharides (e.g., β-glucan) in the diet is accompanied by an increase in fecal sterols, suggesting that these fibers interact with bile salts and cholesterol in the gastrointestinal (GI) tract (*6, 7*). Cellulose was shown not to possess any of these effects, but it was not possible to define the properties responsible for binding or retention (*8*). The adsorption capacities of different fiber types were shown to vary, and the drug colestyramine, a bile acid sequestrant, has been adopted as a standard for these measurements (*8*). Eastwood et al. (*9*) suggested a simple method to establish strong and reversible adsorption. A linear relationship between the percentage of bile acid adsorbed and the amount of fiber used regardless of the bile acid concentration was observed. However, differences between the adsorption of different bile acids made final conclusions difficult. Dietary fibers from different sources have been tested and shown to adsorb bile salts, but the adsorption was not correlated with the ability of the fiber to alter the cholesterol level in vivo. This suggests that several factors influence the properties that account for the adsorption of the bile acids and the lowering of blood cholesterol levels (*10, 11*).

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Table 1. Percentage Dialysate Retention Based on the Asymptotic UV Absorbance (Δ*A*) and Dialysis Rate Constants (k_d) from Mixed Solutions of 2.5% (w/v) Glucagel and the 21 Selected Phenolic Derivatives (Means of Minimum Two Replicates)

	dialysate retention[a] (Δ*A*, %)			dialysis rate constant[b] (k_d)		
compd (name/no.)	day 1	day 2	Δ (2 − 1)	day 1	day 2	Δ (2 − 1)
4′-hydroxy-3′-methoxyacetophenone (**1**)	23	34	11	0.008	0.009	0.001
2-hydroxy-4-methoxybenzaldehyde (**2**)	10	8	−2	0.008	0.007	−0.001
2,6-di-*tert*-butyl-4-methoxyphenol (**3**)	6	3	−3	0.013	0.011	−0.002
2,6-di-*tert*-butyl-4-methylphenol (**4**)	12	18	6	0.012	0.011	−0.001
4-hydroxy-3-methoxybenzyl alcohol (**5**)	12	18	6	0.016	0.012	−0.004
3-hydroxy-4-methoxybenzyl alcohol (**6**)	13	17	4	0.015	0.011	−0.004
3-ethoxy-4-hydroxybenzaldehyde (**7**)	14	25	11	0.008	0.010	0.002
3,5-dimethoxyphenol (**8**)	14	21	7	0.011	0.012	0.001
2,3-dimethylphenol (**9**)	5	−2	−7	0.011	0.009	−0.002
2,5-dimethylphenol (**10**)	13	18	5	0.008	0.010	0.002
2′-hydroxy-4′-methoxyacetophenone (**11**)	19	13	−6	0.009	0.008	−0.001
ethyl 4-ethoxy-2-hydroxybenzoate (**12**)	5	10	5	0.013	0.011	−0.002
3,5-dimethoxy-4-hydroxybenzaldehyde (**13**)	35	38	3	0.009	0.010	0.001
3,5-dimethoxy-4-hydroxybenzoic acid (**14**)	40	34	−6	0.010	0.006	−0.004
2,3,5-trimethylphenol (**15**)	2	−4	−6	0.011	0.008	−0.003
2,3,6-trimethylphenol (**16**)	6	5	−1	0.012	0.010	−0.002
4-hydroxy-3-methoxybenzaldehyde (**17**)	12	4	−8	0.011	0.009	−0.002
4-hydroxy-3-methoxybenzoic acid (**18**)	2	15	13	0.010	0.011	0.001
methyl 4-hydroxy-3-methoxybenzoate (**19**)	5	0	−5	0.009	0.008	−0.001
ethyl 4-hydroxy-3-methoxybenzoate (**20**)	34	32	−2	0.010	0.008	−0.002
4-hydroxybenzyl alcohol (**21**)	17	20	3	0.012	0.009	−0.003

[a] Compounds **1**, **2**, **7**, **11**, **13**, **14**, **17**−**27** were measured at 280 nm and compounds **3**−**6**, **8**−**10**, **12**, **15**, and **16** were measured at 220 nm. [b] Values represent k_d values from mixed solutions of vanillin derivatives and Glucagel.

Fiber-induced changes in fecal bile salt concentrations or composition may not be the sole mechanism involved in the lowering of serum cholesterol. No direct correlation between the viscosity of the matrix and the adsorption has been observed, whereas adjustments of pH and salt strength have been observed to alter the adsorption properties of the fibers (*12*). Multiple adsorption mechanisms are possible, mediated by the same, partly overlapping, or different molecular parameters contributing to viscosity. One major mechanism could be the formation of micelles by the bile acid and the adsorption of these within the fiber (*13, 14*).

β-Glucans are known as hydrocolloid-forming glucose polymers and are used as texture-enhancing additives in the food industry. Several studies have shown that hydrocolloids influence the rate and intensity of flavor release in foods (*15*−*17*). It is recognized that viscosity affects overall flavor perception (*15, 18*). Thickened solutions of similar viscosity do not necessarily offer the same flavor perception. This demonstrates that viscosity as well as adsorption affects flavor release and perception (*19*). The molecular mechanisms that govern the functionality of β-glucans in human health and in food matrices thus remain elusive. Knowledge of the physicochemical interactions that occur between aroma compounds and food constituents is required to be able to describe the behavior of aroma and flavor compounds in food products.

To study interactions between β-glucans and small molecules such as aroma compounds, phenolic derivatives, and bile salts, several different methods have been used, including static headspace, NMR, dynamic exponential dilution, and size exclusion chromatography (*9, 19*−*22*). Thus, thermodynamic and other dynamic approaches have been used to study the behavior of aroma compounds in model complex media that possess different microstructure.

In equilibrium dialysis, a liquid is partitioned through a semipermeable membrane that separates a cell into two compartments, a sample and an assay chamber, of which one contains the dietary fiber. If the interactions that occur between two components (e.g., small molecules such as aroma compounds and macromolecules such as dietary fiber) are strong enough, only the nonretained small molecules will participate in the equilibrium. If the total concentration of a small compound in the two compartments at equilibrium is known, it is possible to calculate the quantity that is adsorbed or retained by the macromolecule matrix (*23*). This enables quantitative assessment of molecular interactions between small compounds and macromolecular food constituents (e.g., β-glucan). Molecular affinities and mechanisms by which β-glucans function may be elucidated by combined studies of small compound retention in β-glucan matrices, molecular modeling, and multivariate data analysis. This knowledge can be correlated to the interactions of other small molecules and β-glucans and provide ideas on how β-glucans affect the aroma of foods and function as health promoters in the intestine.

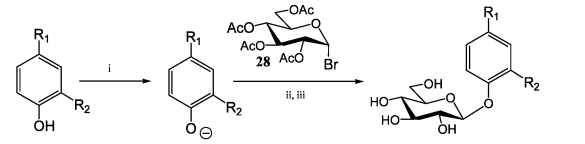
The objective of the present study was to determine the possible interaction between 21 different vanillin-inspired phenolic derivatives, 6 glucosides of these derivatives, and 4 bile salts and specific barley β-glucan (Glucagel) and oat β-glucan (PromOat) preparations using equilibrium dialysis. Differences in the β-glucan-induced retention of the small molecules were related to the specific physicochemical properties of these molecules using molecular modeling and multivariate data analysis.

MATERIALS AND METHODS

Phenolic Derivatives and Bile Acids. Vanillin and 20 different related phenolic structures (**1**−**21**), tryptophan, and the 4 bile acids deoxycholic acid, glycocholic acid, taurocholic acid, and cholic acid were obtained from Sigma-Aldrich (Copenhagen, Denmark) and selected for screening.

Glucosylation of Phenolic Compounds. The investigated phenolic glucosides **22**−**27** (**Table 2**) used in this study were chemically synthesized as shown in **Figure 1**.

Glucosylation of the aglycons **1**, **7**, **17**, and **19**−**21** with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**28**) was performed in aqueous organic basic media using homogeneous reaction conditions and aqueous NaOH with acetone as the organic cosolvent (*24*) to provide the related aryl O-protected-glucosides, which by Zémplen deacetylation afforded the phenolic compounds **22**−**27**. The purity and structural conformation was verified by NMR spectroscopy.



Comp. No.	R_1	R_2
22	CHO	OCH_3
23	$COOC_2H_3$	OCH_3
24	COCH3	OCH_3
25	$COOCH_3$	OCH_3
26	CH2OH	H
27	CHO	OCH3

Figure 1. Chemical synthesis of the phenolic glucosides **22**−**27**: (i) NaOH, H_2O, <10 °C, 15 min; (ii) acetone, room temperature, 24 h; (iii) MeOH, MeONa/MeOH, room temperature, 1−2 h.

Barley *β*-Glucan. Glucagel, a commercial soluble β-glucan extracted from hull-less barley, was obtained from GraceLinc Ltd. (Christchurch, New Zealand). Glucagel has a declared content of ≥75% β-glucan, <18% starch, <10% moisture, <5% protein, <2% ash, and <2% fat. The β-glucan is of moderate molecular weight, $(0.12-0.18) \times 10^6$.

Oat *β*-Glucan. PromOat, a commercial soluble β-glucan extracted from oat, was obtained from Biovelop (Kimstad, Sweden). PromOat has a declared content of 30−40% β-glucan, 6% pentosans, 49% carbohydrates (described as dextrins by the supplier), 4.5% moisture, <2.5% protein, 3.5% ash, and 0.5% fat. According to the supplier, molecular weights of 1.0×10^6 are routinely achieved, which characterizes PromOat as a high molecular weight β-glucan.

Equilibrium Dialysis Assays with Glucagel. The phenolic compounds and β-glucan were dialyzed in sterile 0.5% DMSO and 10 mM Tricine buffer (pH ~7) using 1 mL in-line equilibrium dialysis cells (Bel-Art Products, Pequannock, NJ) and dialysis membranes with size exclusion of 6−8 kDa for globular molecules (Spectrum Laboratories Inc., Breda, The Netherlands). According to the manufacturer, this cutoff is calculated for proteins, and for dextrans the cutoff is calculated to be 1−1.5 kDa. Glucagel 5% (w/v) was dispersed in distilled water in a 50 mL conical flask covered with aluminum foil and then heated for 30 min at 80−82 °C using a hot plate stirrer controlled by a thermostat. A sufficient quantity of phenolic derivatives or bile salts was dissolved in 1 mL of DMSO. Two 5 mL samples of 2.5% (w/v) β-glucan were prepared in the described buffer. To one sample were added phenolic derivatives or bile salts to achieve 2 and 10 mM final concentrations, respectively. Additional 5 mL samples of 2 mM phenolic derivative or 10 mM bile salt were prepared in buffer. The concentrations of the phenolic derivative relate to the maximum possible concentration to be kept in solution. The concentrations of the bile salts were chosen as those previously used in similar experiments (*14, 20*). All samples were stored at 4 °C prior to performance of the dialysis experiment. To minimize the differences in the gel-setting samples, the dialysis experiments were initiated 12 and 36 h (1 and 2 days) after sample preparation. All samples were then heated to 25 °C and thoroughly mixed using a vortex mixer, again to keep the gel setting to a minimum. One milliliter of each sample was applied into two individual sample chambers of the dialysis cells under aseptic conditions and subsequently dialyzed against 1 mL of sterile buffer in the assay chambers. The time of application of the first sample was denoted time zero, and a time gap of 30 s between applications of samples was maintained, thus keeping track of the exact dialysis time for each sample. After application, the dialysis cells were quickly transferred to a thermostat-regulated rotating water bath at 37 °C. Preliminary kinetic studies showed that dialysis equilibrium was reached in <5 h. Aliquots (10 μL) were withdrawn from the assay chamber after 15, 30, 45, 60, 75, 90, 120, 150, 180, 240, and 300 min (5 h), transferred to a 96-well plate (Nunc A/S, Roskilde, Denmark), and diluted with distilled water in the ratio 1:9. Absorbance of the diluted dialysate sample and of a reference of distilled water was measured at 210, 220, and 280 nm on a Spectra Max 190 plate reader (Molecular Devices Corp., Sunnyvale, CA) depending on the wavelength providing maximum absorbance for the phenolic derivative used in the individual experiments. All compounds were tested four times; that is, two replicates were dialyzed 12 h after mixing and two replicates of the same sample were dialyzed 36 h after mixing. The 12 h (day 1) and 36 h (day 2) dialysis experiments were treated as two separate experiments.

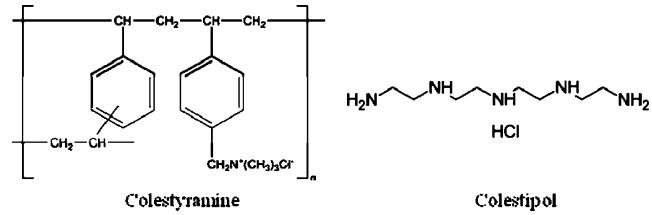


Figure 2. Chemical structures of colestyramine and colestipol.

Enzymatic Breakdown Studies. The studies were performed using Glucagel and the methodology reported above. After aliquot sampling at 240 min, 2 μL of 50 mU/mL Lichenase enzyme (Megazyme, Ireland) or 2 μL of a 1:100 dilution of the original product solution of Viscozyme L (batch KTN02140, Novozymes, Denmark) was added to the β-glucan-containing compartment of the dialysis instrument. Aliquots (10 μL) were withdrawn from the assay chamber after 255, 270, 285, 300, 315, 330, 360, 390, 420, and 450 min from the start of the experiment. The aliquots were analyzed as described above.

Comparison of Glucagel and the Bile Salt Sequestrant Drugs Colestyramine and Colestipol Hydrochloride. The studies were performed using the methodology reported above and a 2.5% (w/v) assay concentration of Glucagel. Using the commercial drug formulations, 1% (w/v) solutions of colestyramine (Questran, Bristol-Myers Squibb) and colestipol hydrochloride (Lestid, Pfizer) were prepared. Chemical structures are shown in **Figure 2**. The drugs were obtained directly from the manufacturer through the pharmacy at the University of Copenhagen, and the 1% (w/v) concentration used reflects the recommend dose of the drugs.

Comparative Equilibrium Dialysis Assays with Glucagel and PromOat. This series of dialysis experiments was carried out as above except that the concentrations of both β-glucan preparations were reduced to 1% (w/v) (the glucan concentration in the assay) to circumvent handling problems related to the high specific viscosity of PromOat.

Exponential Curve Fitting of Dialysis Data. Initial data handling was conducted in Excel (Microsoft Office 2003) where measured absorbencies were corrected for the background absorbance, mean values were calculated for the two replicates, and moving averages were calculated over five continuous measurements. Regression analysis procedures were employed to explore the relationship between dialysate absorbance and dialysis time. Analysis of moving averages reduces the impact of nonrelevant dialysis information, leads to simpler and more robust data sets for regression models, and improves interpretation of the dialysis data. Generally, linearity of the absorbance to concentration relationship over the concentration range of 0−2 mM for the phenolic compounds was observed when measured in the 210−280 nm range. Subsequent mathematical modeling was conducted in SigmaPlot (version 4.01). A curve derived from the exponential equation

$$\text{absorbance} = A(1 - e^{-kt}) \quad (1)$$

was fitted to data from each individual dialysis experiment, where A is the asymptotic or equilibrium UV absorbance value, t is the dialysis time, and k is the dialysis rate constant. All model fits were evaluated using correlation coefficients (r^2). Asymptotic values were compared between dialyses of each of the pure phenolic derivatives and for the phenolics mixed with β-glucan to quantitatively determine the level of dialysate retention (ΔA) by β-glucan. Asymptotic values derived from the pure β-glucan samples were subtracted from all of the β-glucan/aroma compound mixtures to account for the dialysate from β-glucan alone.

Multivariate Data Analysis. Dialysate retention data from day 1 and day 2 dialysis experiments were subjected to principal component analysis (PCA) and partial least-squares (PLS) regression.

In PCA, a data matrix is decomposed by consecutive orthogonal extraction of the largest variation (principal components, PCs) in data

Table 2. Percentage Dialysate Retention Based on the Asymptotic UV Absorbance (Δ*A*) from Mixed Solutions of 2.5% (w/v) Glucagel and Six Selected Glucosides of Phenolic Compounds (Means of Minimum Two Replicates, All Meseaured at 280 nm)

compd (name/no.)	aglycon no.	dialysate retention (Δ*A*, %)		
		day 1	day 2	Δ (2 − 1)
4-β-D-glucopyranosyloxy-3-methoxybenzaldehyde (**22**)	17	7	9	2
ethyl 4-β-D-glucopyranosyloxy-3-methoxybenzoate (**23**)	20	−8	−5	3
4′-β-D-glucopyranosyloxy-3′-methoxyacetophenone (**24**)	1	−3	2	5
methyl 4-β-D-glucopyranosyloxy-3-methoxybenzoate (**25**)	19	−12	−10	2
4-β-D-glucopyranosyloxybenzyl alcohol (**26**)	21	−10	−9	1
3-ethoxy-4-β-D-glucopyranosyloxybenzaldehyde (**27**)	7	−9	−7	2

until the variation left is unsystematic. The loading vectors can be considered as pure hidden profiles that are common to all measurements. Two-dimensional scatter plots of the score vectors show the covariance between samples, providing a characterization of data. The purpose of PLS regression is to build a linear model enabling prediction of a desired chemical/physical characteristic (*Y*) from measured data (*X*). During the regression, *X* is decomposed as in PCA, but the PCs are found as the underlying structures that covary best with the *Y* variable (*25*).

All molecular structures in this study were optimized with MM3* in MacroModel (*26*). A total of 234 molecular descriptors were calculated for the phenolic derivatives and glucosides with CDK (*27*) and QikProp (*28*). CDK descriptors are divided into five major classes: electronic (atomic polarizabilities, bond polarizabilities, charged partial surface areas, hydrogen bond acceptors, and hydrogen bond donors), geometrical (geometrical index, length over breadth, moments of inertia, and Petitjean shape indices), constitutional (*A*log*P*, bond, element, and atom type counts, largest chain, Lipinski's rule of five, rotable bonds count, *X*log*P*, molecular weight), hybrid (BCUT and WHIM), and topological (carbon types, Chi indices, eccentric connectivity index, fragment complexity, Kier and Hall molecular shape indices, topological polar surface area, Wiener numbers, Zagreb index, and Moreau-Broto autocorrelation descriptors). QikProp provides approximately 40 descriptors, of which several (e.g., predicted brain/blood partition coefficient, QPLogBB) are of pharmaceutical relevance, whereas others [e.g., PM3 calculated ionization potential, IP(eV)] are of a more general nature. Without any a priori knowledge of the mechanisms involved, we anticipate that this diverse set of molecular descriptors captures information relevant to the dialysis characteristics. To reduce the amount of noise in the descriptor matrix and improve the subsequent interpretation of PCA plots and PLS regressions, a simple two-step variable selection scheme was employed. First, descriptors were deleted unless they assumed distinct values for at least 12 samples. Second, the Pearson product moment correlation coefficient, *r*, between response (Δ*A* or k_d) and the descriptors was evaluated. In cases when *r* fell below a certain threshold (0.5 for Δ*A*, 0.2 for k_d), the corresponding descriptor was deleted. The lower threshold for k_d was required, because response−descriptor correlations were very low in this case. The number of descriptors produced by the variable selection was 62 for day 1 Δ*A*, 16 for day 2 Δ*A*, 82 for day 1 k_d, and 91 for day 2 k_d. The variables were autoscaled prior to data analysis, and full (leave-one-out) cross-validation was used. The PLS models were evaluated on the basis of the root-mean-square error of cross-validation (RMSECV), which is the estimation of the error of the predicted values. Multivariate data analysis was performed using Unscrambler (*29*), Matlab (*30*), and Latentix (*31*).

Molecular Mass Determination. The molecular mass of the β-glucans was estimated by size exclusion chromatography (SEC) using a Superdex 200 column (16 mm × 60 cm, GE Healthcare Bio-Sciences AB, Uppsala, Sweden) fitted with a refractive index detector. Superdex 200 is a cross-linked agarose and dextran material with a nominal bead size of 13 μm, a pore size of 100−120 Å, and an optimal separation range of 10,000−600,000 Da. The mobile phase consisted of 50 mM ammonium formate and 200 mM NaCl, and the column was eluted at room temperature at 1.6 mL/h. Dextrose standards of 5, 12, 25, 50, 80, and 150 kDa (Fluka, Buchs, Switzerland) were used for calibration.

RESULTS

Vanillin and 20 other phenolic derivatives (**1**−**21**), 6 phenolic glucosides (**22**−**27**), and 4 bile salts were tested with respect to their ability to bind to Glucagel using the dialysis setup. The phenolic derivatives can be divided into different chemical classes according to the functional groups present in the different compounds. All of them possess a central benzene ring as the core structure. The benzene ring is substituted with a minimum of two groups, of which one would be an oxy group. The aglycon structures may be grouped into 2 ketones (**1**, **11**), 4 aldehydes (**2**, **7**, **13**, **17**), of which 2 were hydroxylated on the benzene ring (**2**, **17**), 10 phenols (**3**−**6**, **8**−**10**, **15**, **16**, **21**), of which 2 had one additional hydroxyl group (**5**, **6**), 3 esters of either ethyl or methyl character (**12**, **19**, **20**), and 2 aromatic acids (**14**, **18**) (**Tables 1** and **2**). The molecules cover a broad range of log *P* values ranging from −0.5 for the glucosides to 5.1 for compound **4**. With respect to their physical dimensions, the phenolic derivatives and bile salts also span a significant range from the large bile salts (18−19 Å in diameter) to the smallest, being compound **21** (7 Å in diameter).

The ability of all of the compounds **1**−**27** to bind to Glucagel was analyzed in dialysis experiments. Exponential curve fitting of all dialysis data was performed to fit eq 1. Dialysis curves of **8** and **10** in the presence of 2.5% (w/v) Glucagel as well as the exponential curve fit are presented in **Figure 3** as typical examples. Dialysate retentions (Δ*A*) and dialysis rate constants (k_d) from the screening of all 21 phenolic derivatives are presented in **Table 1**. The values represent the mean of two replicates. Values obtained by dialysis of β-glucan in the absence of any added compound were subtracted from the values obtained with added compounds. All dialysis experiments exhibited patterns similar to those presented in **Figure 3**. The patterns are composed of an initial steep slope during the first ∼15−90 min and an asymptotic convergence toward a maximum after 90−300 min. Generally, there was good agreement between the dialysis curve and the exponential fit. Correlation coefficients from the curve fits were >0.95 in all dialysis experiments. At equilibrium/asymptotic level, the absorbance of **8** reaches a value of 0.96, whereas the asymptotic value of **8** + 2.5% (w/v) Glucagel is 0.76. This gives a relative difference of 22%, which corresponds to the retention of dialysate (**8**) by the β-glucan, as seen in **Table 1**. The different classes of chemical compounds within the 21 phenolic derivatives such as ketones, aldehydes, phenols, esters, and acids did not differentiate significantly from each other and within the groups with respect to being retained by the barley β-glucan. Only four compounds (**1**, **13**, **14**, **20**) gave rise to a Δ*A* > 30. All four compounds shared the presence of a hydroxyl group at position 4 and a CHO group at position 1. However, other compounds such as **18**, **5**, and **19** also possess these features and give rise to much lower Δ*A* values. The difference between day 1 and day 2 retentions (Δ 2 − 1) showed a weak tendency of increased

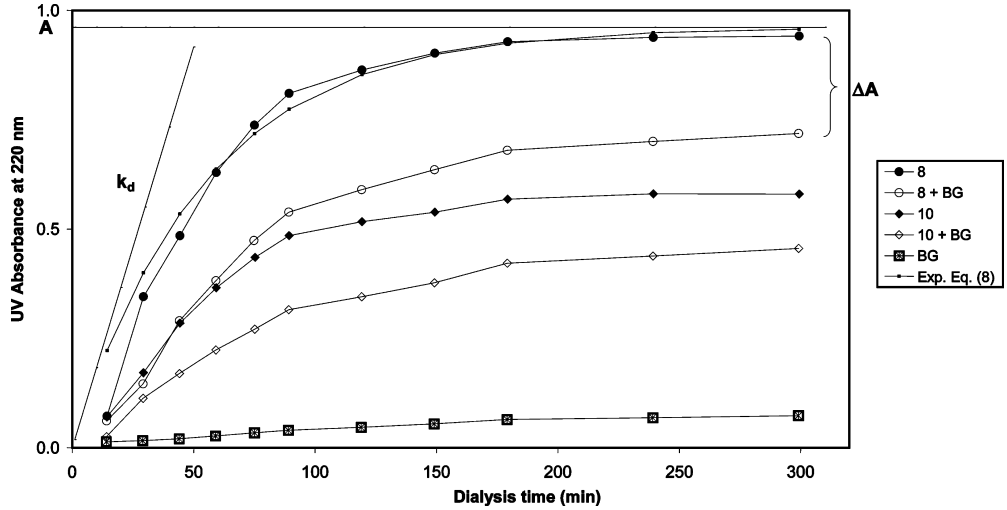


Figure 3. Dialysis curve of compounds **8** and **10** in the presence of 2.5% (w/v) Glucagel (BG). The line of k_d is shown, and the horizontal line illustrates the maximum absorbance to be obtained at equilibrium of the dialysis of the exponential data curve.

Table 3. Percentage Dialysate Retention Based on the Asymptotic UV Absorbance (ΔA) and Dialysis Rate Constants (k_d) from Mixed Solutions of 2.5% (w/v) Glucagel and Four Different Bile Salts and Tryptophan (Means of Minimum Two Replicates)

	dialysate retention (ΔA, %)			dialysis rate constant (k_d)		
compd	day 1	day 2	Δ (2 − 1)	day 1	day 2	Δ (2 − 1)
deoxycholic acid	69	76	7	0.008	0.009	0.001
glycocholic acid	78	86	8	0.008	0.007	−0.001
taurocholic acid	73	81	8	0.013	0.011	−0.002
cholic acid	38	42	4	0.012	0.011	−0.001
tryptophan	23	28	5	0.005	0.004	−0.001

dialysate retention on day 2, that is, positive Δ values. Comparison of the k_d values from days 1 and 2 (Δ 2 − 1) indicated lower dialysis rates at day 2. For the more hydrophilic phenolic derivative glucosides (**Table 2**) no or only very weak retention was observed. Compared with the values obtained for the aglycons, the glucosides are significantly less adsorbed by the fiber.

Four bile salts were tested in the dialysis equilibrium system (**Table 3**). All four bile salts showed good retention in the system with ΔA from 42 to 86. This confirmed the published in vivo evidence for the ability of dietary fibers to adsorb bile salts (*4, 5*). Tryptophan has previously been used as a reference molecule in dialysis tests (*14*) and was also tested in this study and showed a retention of 23 with is compliance with previous studies (*14*).

Comparison of Glucagel and PromOat. To evaluate the ability of a different commercial β-glucan to bind phenolic derivatives, the oat β-glucan product PromOat was investigated and compared to the barley β-glucan product Glucagel. The results (**Table 4**) show no significant differences between the two products with respect to retention of phenolic derivatives under the conditions used in our dialysis equilibrium system. The secondary structure, product composition, and viscosity of these two fiber preparations are not the same (*32*), which may be important for the adsorption. However, this is not captured by our dialysis assay.

Comparison with Commercial Bile Salt Sequestrant Drugs. To evaluate the efficacy of the fiber to adsorb bile salts and other small compounds, a comparative study with the commercial drugs colestyramine and colestipol hydrochloride was carried out. The results presented in **Table 5** show that for

Table 4. Comparison of Glucagel and PromOat (Means of Minimum Two Replicates)[a]

	dialysate retention	
compd (name/no.)	Glucagel	PromOat
3-ethoxy-4-hydroxybenzaldehyde (**7**)	23	26
ethyl 4-hydroxy-3-methoxybenzoate (**20**)	15	19
4-hydroxy-3-methoxybenzaldehyde (**17**)	14	17
4′-hydroxy-3′-methoxyacetophenone (**1**)	10	14

[a] Percentage dialysate retention based on the asymptotic UV absorbance (ΔA) from mixed solutions of 1% (w/v) β-glucan solutions and four different phenolic compounds.

Table 5. Percentage Dialysate Retention Based on the Asymptotic UV Absorbance (ΔA) of Four Phenolic Compounds and Four Bile Salts by Glucagel, Colestyramine, and Colestipol Hydrochloride

	dialysate retention		
compd (name/no.)	Glucagel	colestyramine	colestipol hydrochloride
3-ethoxy-4-hydroxybenzaldehyde (**7**)	23	2	4
ethyl 4-hydroxy-3-methoxybenzoate (**20**)	15	3	6
3,5-dimethoxy-4-hydroxybenzoic acid (**14**)	40	95	93
4-hydroxy-3-methoxybenzoic acid (**18**)	10	96	92
deoxycholic acid	69	89	85
glycocholic acid	78	95	97
taurocholic acid	73	94	93
cholic acid	38	96	95

nonacid compounds (**7**, **20**), the fibers have higher retention capability than the drugs. The drugs are characterized as having an anionic exchange nature. In agreement, the two drugs were able to retain the acidic compounds (**14**, **18**) up to 9 times better than the fiber. This demonstrates that ionic forces are not the main property responsible for the adsorption of small molecules to the fiber. This also confirms that the dialysis assay works with matrices other than the fibers.

Enzymatic Breakdown of the Fibers. To investigate the importance of fiber molecular mass for the adsorption ability, the rerelease of the small compounds from the fiber matrix was measured after partial enzymatic breakdown of the fibers (**Figure 4**). Some, but not all, of the compounds retained in the experiments with intact fibers were released as a result of enzyme breakdown of the fibers and moved freely between the two dialysis compartments. This shows that different parameters contribute to retention of the compounds. The fibers were broken

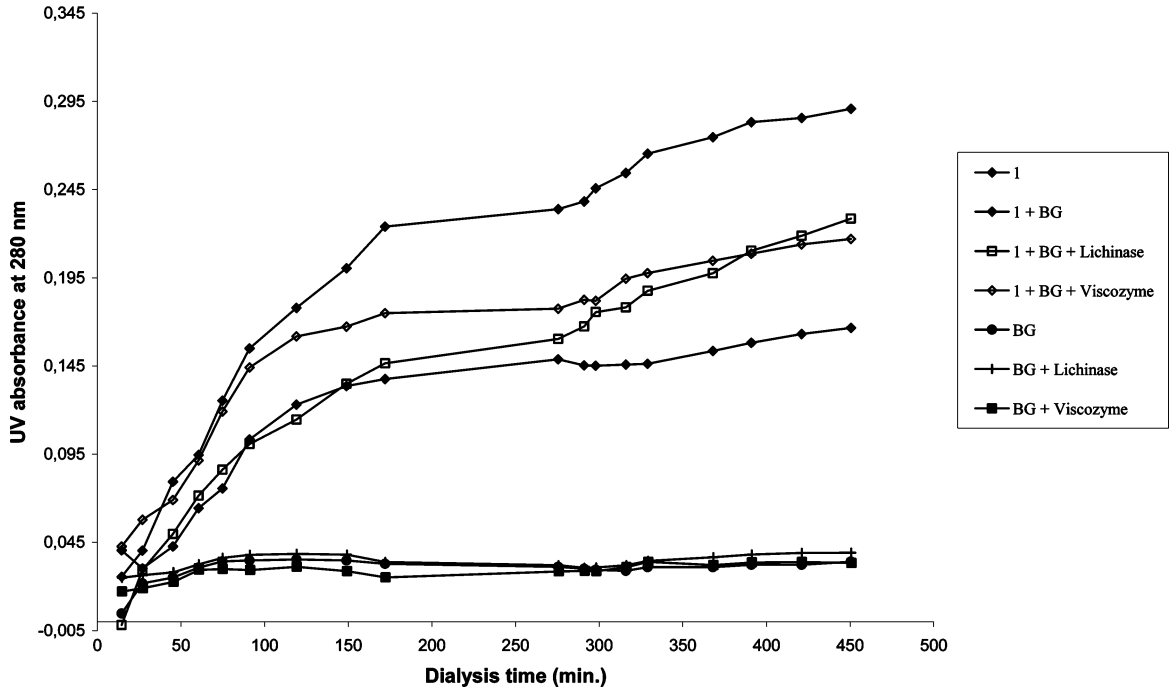


Figure 4. Effect of enzymatic breakdown of the barley β-glucan on its ability to bind phenolic derivatives as determined by dialysis assays. The experiment was carried out using 2.5% (w/v) barley β-glucan Glucagel (BG) as fiber matrix and 4′-hydroxy-3′-methoxyacetophenone (**1**) in the 2.5% (w/v) Lichenase and Viscozyme enzymatic breakdown of the fibers.

down to masses of <5000 Da, which supposedly disrupt the majority of the tertiary structures and some of the secondary structures of glucans. **Figure 4** also shows that even though the fibers were broken down, they were retained in the sample champers, indicating that they could not move freely as the small molecules can.

Multivariate Data Analysis. PCA was conducted using the dialysate retention data sets obtained at day 1 as well as day 2 using the dialysate retentions (ΔA) and rates (k_d). The correlation plot of the free aglycone, glycoside, and of the bile salt data (ΔA and k_d) (**Figure 5**) showed a marked distinction between these three groups of compounds according to ΔA. Additionally, the plot shows a tendency of decreasing k_d values between the data obtained at days 1 and 2 (arrows directed downward) and a weak trend of increasing ΔA values from day 1 to day 2 (arrows directed to the right). No obvious groupings within the 21 tested structures were observed. **Figure 6** shows the PCA score plot for the 21 phenolic derivatives and 6 glucosides represented by 62 descriptors selected using the value of day 1 ΔA as explained under Materials and Methods. The samples have been colored according to the value of ΔA. Although phenolic derivatives with high and low values of ΔA have scores on the extreme left and extreme right of PC1, respectively, the change in ΔA along PC1 is not systematic. Inspection of the remaining PCs did not reveal any improvements in describing the variation of ΔA. The PCA plots based on descriptors selected for the explanation of differences between day 1 and day 2 k_d values did not reveal any structure. The fact that a lowered threshold for correlation with the dependent variable had to be employed in the descriptor selection step supports the notion that a proper explanation of k_d cannot be achieved with the current set of descriptors. PLS regression for the prediction of the day 1 retention value seemed initially to hold some promise, but permutation testing of the model revealed that it was based on a chance correlation. The low quality of the model was also reflected in similar regression coefficients, indicating that no important descriptors could be singled out. The situation did not improve with respect to the remaining independent variables for days 1 and 2. In conclusion, the physical mechanisms involved in fiber retention of the compounds as monitored in the dialysis experiments cannot be explained with our current multivariate data analytical approach. Data analytical exploration of fiber-binding properties has to await the development of a more suitable set of descriptors. At this point the nature of such descriptors is unknown.

DISCUSSION

The metabolic health benefits and viscous properties of β-glucans have been reported by several investigators (*2*−*8*). However, their potential uses, mechanisms of action, and means of incorporation into foods and diets require further exploration. The aim of the present study was to investigate the molecular interactions between β-glucans and selected classes of small molecules. The approach of equilibrium dialysis was chosen because it has been proven useful in interaction studies on bile salts and barley β-glucan (*14*) and because the approach offers a relatively fast method for the analysis of large series of small samples. Dialysis conditions were set to mimic physiological conditions in the sense of continuous movement, a temperature of 37 °C, and pH of 7, which could provide evidence for β-glucan behavior in solutions in the GI tract independent of enzymatic degradation. However, this very simple in vitro study cannot provide an exact description of the physiological actions of β-glucans but rather affords knowledge on their molecular affinities toward small molecules. The effect of hydrocolloids on aroma release from food may be due to numerous mechanisms; one is the physical entrapment of aroma within the food matrix (*15*). Another mechanism involves chemical interaction between the aroma compound and the hydrocolloid components, for example, β-glucan (*16*). In the current study, both types of interactions were studied experimentally.

Along with the test of the 21 phenolic compounds, 6 β-glucosides of these compounds were tested. This would

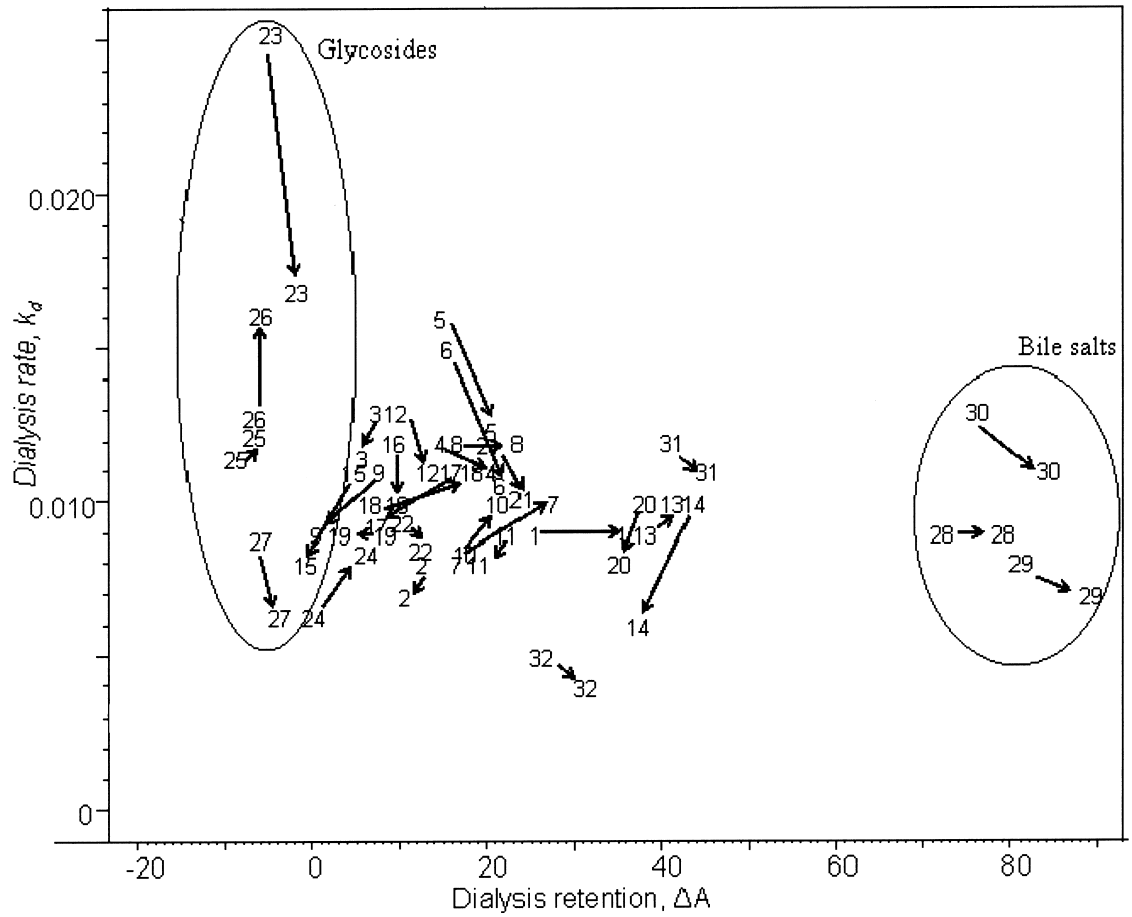


Figure 5. Correlation plot of day 1 dialysate retentions (ΔA) and dialysis rates (k_d). Numbers refer to the compounds listed in **Tables 1**−**3**, and arrows show the direction of movement in the plot between day 1 and day 2 data.

represent the native form in which the aroma compounds are present in the plants, and it was therefore of interest to test whether they are retained in the same fashion as the aglycons. As seen in **Table 2**, the glucosides are not retained or are retained at only a very low degree compared to the retention of the aglycons. This indicates that hydrophobic properties of the small molecules are of importance. Boland et al. (*15*) observed that of 11 flavor compounds tested, the hydrophobic compounds had the significantly lowest partition coefficients, that is, aroma release in a gelatin gel. In this study, an observed increased percentage of retention as a result of increased log *P* values for compounds **3** and **4** would suggest a similar contribution of hydrophobic properties to fiber retention. No such easily envisioned trends were observed in the present study using either simple curve fitting of retention versus log *P* or multivariate data analysis. Story and Kritchevsky (*8*) stated that the hydrophobic properties of the bile salts tested in their publication did not correlate directly with the binding to the tested fibers. This is in good agreement with the observations in our study both for the bile salts and for the phenolic compounds.

The differences observed in percentage of fiber retention of the different phenolic derivatives tested in the day 1 experiment (**Table 1**) do indeed indicate that the ability of the fibers to retain the different derivatives could be of a different nature depending on the physicochemical properties of each individual phenolic derivative. The corresponding glucosides have a significantly lower log *P* value than the aglycons, whereas at the same time containing a sugar moiety that might be expected to be able to interact with the β-glucan via hydrogen bond formation. As seen in **Table 2**, the glucosides are not retained by the fiber. High water solubility could therefore be one of the properties of the small molecule that would reduce possible interactions with the fiber. Among the tested phenolics the four compounds **1**, **13**, **14**, and **20** had significantly higher retentions than the others. The four molecules possess different functional groups at position 1, but all share a hydroxyl group at position 4. The log *P* values for the four molecules range from 0.83 to 1.68 and cannot be correlated to the significantly higher retention of these molecules compared to the remaining 17 compounds. The four molecules do not possess different physical dimensions, nor do they contain other functional groups compared to the rest of compounds tested. This indicates that multiple different binding properties are of importance for the retention of the small molecules to the β-glucan fiber.

The differences between the results obtained at day 1 and day 2 are most likely due to time-dependent changes in the β-glucan matrix, for example, network formation and increased rigidity in the β-glucan solution induced by the increased incubation time prior to dialysis of the day 2 samples (*32*). In a preliminary study on the viscous properties of a 2.5% (w/v) Glucagel solution, the viscosity (at 37 °C and 30 s^{-1}) of the sample increased approximately 6-fold from day 1 to day 2, which would agree with the slower dialysis rates observed for the day 2 samples. The matrix-dependent changes in dialysate retention indicate that some of the retention is due to the hygroscopic and tertiary structure of the β-glucan. The current findings are in agreement with those of Boland et al. (*15*), who found that flavor release was significantly affected by the texture of gelatin, starch, and pectin gels. The most rigid gel showed the lowest flavor release.

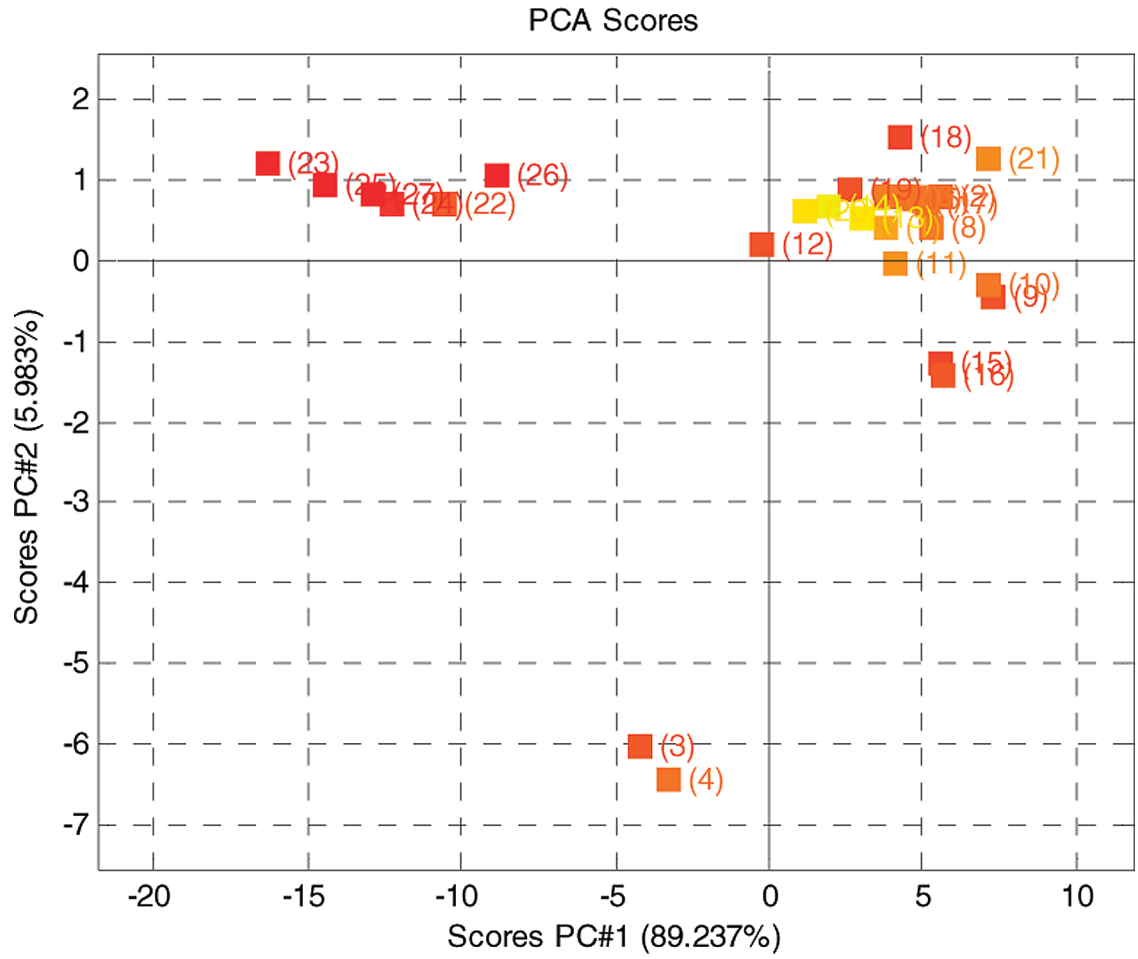


Figure 6. PCA score plot. The value of ΔA on day 1 has been used to color the samples (21 phenolic derivatives and 6 phenolic glucosides). The PCA is based on 62 calculated physicochemical descriptors, selected as described under Materials and Methods. Numbers refer to the compounds listed in **Tables 1** and **2**.

Because the term β-glucan is not a uniform definition, this study included a comparative study of the two commercial products Glucagel (barley) and Promoat (oat). The two matrices of these products are chemically quite different, for example, with respect to purity and molecular masses (*32*). Despite these differences, the two β-glucans show nearly identical properties with respect to their ability to retain phenolic derivatives in the dialysis equilibrium assays. This suggests that the retention of the different phenolics is due to several and interacting properties of the fiber matrix and not a few single parameters.

The efficacy of the β-glucan fibers to adsorb bile salts and other small compounds was also evaluated in comparison with the commercial drugs colestyramine and colestipolhydrochloride. This study indicated that ionic interactions are not the main property responsible for the adsorption of small molecules to the fiber, again confirming that the retention is composed of multiple properties. This also confirms that the dialysis assay works with matrices other than the fibers.

The importance of the fiber molecular mass and thereby the physical size of the molecules for the adsorption ability was also evaluated. A clear rerelease of some, but not all, of the retained compounds was observed upon enzymatic degradation of the barley β-glucan-based matrix. Partial rerelease of the small compounds again indicates that various factors contribute to retention of the different phenolic derivatives. The fibers were broken down to an extent that would disrupt the tertiary structure along with some parts of the secondary, which can explain why some phenolic derivatives were retained.

PCA and PLS regression analyses are powerful tools for extraction of important variances in a data matrix consisting of many variables. In the present study, comparison of β-glucan binding of different phenolic derivatives was conducted using molecular modeling and multivariable data analysis. No strong tendency of sample grouping was found by PCA either for day 1 or for day 2. This implicates that the employed set of descriptors is not well suited for explaining the variation in ΔA and k_d. Additionally, no reliable model fit was found using PLS regression to dialysis data. This could be due to the use of insufficient or ineffective descriptors, the need for more data (a larger number of phenolics screened in the dialysis assay) to strengthen the robustness of prediction, or measurement errors within the present data set from the dialysis assay. Even though no reliable prediction model was found, indications of a correlation between the binding data and some of the molecular descriptors were evident. This indicates that multiple parameters are involved in determining the binding of small molecules to fibers and that the multiplicity of parameters involved obscures the correlations of the observed binding to specific descriptors.

The dialysis data presented provide some information on the complex mechanisms controlling the ability of β-glucans to bind low mass compounds. To more accurately determine the nature of the interactions between β-glucans and low mass

compounds, a range of advanced spectroscopy and molecular modeling methodologies will need to be introduced.

In conclusion, the retention of aroma compounds by β-glucan is of great interest from a food composition point of view and from a health perspective. In recent years, the increased health consciousness among consumers has led to extended additional use of hydrocolloids as replacements for fats. The food industry would benefit greatly from an improved understanding of the mechanisms involved in the flavor retention and release from β-glucan matrices along with the nature of the interactions occurring in the GI tract. Knowledge of the action of model compounds in β-glucan matrices can provide general information on the β-glucan affinity toward small molecules, which would be applicable in the studies of, for example, the bioavailability of natural compounds found in association with β-glucans and for health claims on β-glucan.

It was confirmed that β-glucans from barley and oat are able to adsorb bile salts, and for the first time β-glucans' ability to absorb vanillin and other phenolic compounds was demonstrated. The retention in our newly developed dialysis assay depends on numerous and interacting physicochemical properties of the small molecules. The interaction could not be explained by simple correlation to any of the descriptors included in the multivariable data analysis, and the results could not confirm or disprove the previously described hydrophobic binding or micelle capture of the small molecules to the β-glucan.

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