Immobilization of flavan-3-ols onto sensor chips to study their interactions with proteins and pectins by SPR

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A B S T R A C T

Interactions between plant polyphenols and biomacromolecules such as proteins and pectins have been studied by several methods in solution (e.g. isothermal titration calorimetry, dynamic light scattering, nuclear magnetic resonance and spectrophotometry). Herein, these interactions were investigated in real time by Surface Plasmon Resonance (SPR) analysis after immobilization of flavan-3-ols onto a sensor chip surface. (–)-epicatechin, (+)-catechin and flavan-3-ol oligomers with an average degree of polymerization of 2 and 8 were chemically modified using *N*-(2-(tritylthio)ethyl)propiolamide in order to introduce a spacer unit onto the catecholic B ring. Modified flavan-3-ols were then immobilized onto a carboxymethylated dextran surface (CM5). Immobilization was validated and further verified by evaluating flavan-3-ol interaction with bovine serum albumin (BSA), poly-L-proline or commercial pectins. BSA was found to have a stronger association with monomeric flavan-3-ols than oligomers. SPR analysis of selected flavan-3-ols immobilized onto CM5 sensor chips showed a stronger association for citrus pectins than apple pectins, regardless of flavan-3-ol degree of polymerization.

Keywords:

Polyphenol
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1. Introduction

Flavan-3-ols belong to a large and diverse family of plant secondary metabolites, the polyphenolic flavonoids. This class of plant phenolics has widespread interest to food processors and is of particular interest for their potential health benefits. Flavan-3-ol oligomers and polymers, called proanthocyanidins, form the so-called “condensed tannins”. Tannins have long been recognized for their capacity to interact with and precipitate proteins or polysaccharides. This ability to interact with macromolecules has

implications in food chemistry (e.g. red wine fining and astringency) [1]. Insight into the mechanism of interaction between tannins and macromolecules has applications in food and beverage production and potentially in human health.

Flavan-3-ols are abundant in beverages (e.g. wine, tea, cider) and fruits (e.g. apple, grape, nuts) [2]. The term “procyanidins” specifically designates oligo/polymers of (+)-catechin and (–)-epicatechin. Procyanidins are characterized by the type of linkages between those flavan-3-ol building blocks and the number of such constitutive units or degree of polymerization (DP) [3]. Procyanidins from apple are an interesting model due to their structural homogeneity combined with their variation in size. They are composed of (–)-epicatechin units that are linked together through their C4 and C8 positions with a DP of up to 50 [4], where in some instances (+)-catechin can be found as a terminal unit. Such flavonoid oligo/polymers are able to bind to biomacromolecules such as proteins [2] or polysaccharides, including notably pectins [5].

Pectins are plant polysaccharides, which are mainly found in the middle lamella and primary cell wall. Their backbone chains are characterized by partially methyl esterified and acetylated

Abbreviations: SPR, surface plasmon resonance; CM5, carboxymethyl dextran; BSA, bovine serum albumin; PLP, poly-L-proline; DP, degree of polymerization; ITC, isothermal titration calorimetry; EPI, (–)-epicatechin; CAT, (+)-catechin; DP2, flavan-3-ols dimer; DP8, flavan-3-ols oligomer with a degree of polymerization of 8; STTrt, linker with trityl group; SH, linker with thiol group; ATR, attenuated total reflection; RU, resonance units.

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galacturonic acid units (homogalacturonan), and rhamnose units to which are linked neutral sugar side chains (rhamnogalacturonans) [6].

Interactions between polyphenols and biomacromolecules, mostly proteins, have been studied in solution by various analytical techniques such as isothermal titration calorimetry (ITC), dynamic light scattering or nuclear magnetic resonance spectroscopy, for understanding the strength and equilibrium/energy parameters of interactions [5,7–12]. Only a few studies have been performed by immobilizing one of the two compounds on a sensor surface for determining the affinity strength by Surface Plasmon Resonance (SPR). SPR is an optical-based real-time detection method which allows for the analysis of biomolecular recognition at surfaces between an immobilized ligand and an analyte. For example, salivary proteins have been immobilized onto a dextran SPR sensor chip for characterizing the interaction between protein and β -pentagalloylglucose, a common precursor of gallotannins [13–15]. Conversely, as polyphenols are known to interact in a nonspecific manner with proteins, other studies have chosen to immobilize polyphenols such as chemically modified vescaline and biotinylated vescalagin onto modified dextran and streptavidin-coated sensor chips, respectively, for examining their interaction with proteins such as topoisomerase II α and filamentous actin [16]. The literature is very scarce concerning the SPR analysis of protein and polysaccharide interaction with polyphenols. Joergensen et al. [17] have studied associations between pectins and protein immobilized onto a gold sensor chip, while Seo et al. [18] have worked on interactions between carbohydrates immobilized onto a gold surface and lectins. Moreover, Hayashi et al. [19] have shown higher affinity between β -cyclodextrin immobilized onto a carboxymethylated dextran sensor chip and (–)-epigallocatechin-3-O-gallate and (–)-epicatechin-3-O-gallate than non-galloylated (–)-epigallocatechin and (–)-epicatechin. In our study, the flavan-3-ol monomer/oligomers were immobilized onto carboxymethylated dextran sensor chips in order to minimize non-specific interactions between polyphenols and pectins or proteins, for the first time.

The aim of this study was: first, to define the appropriate chemical method for the immobilization of flavan-3-ol monomers, dimers and oligomers onto a SPR sensor chip; second, to analyze qualitatively the parameters of interactions of these flavonoids and proteins or pectins by SPR and third, to compare with interactions already observed in solution.

2. Material and methods

2.1. Chemicals

Unless specified, all chemicals used for chemical modification and (+)-catechin, (–)-epicatechin, apple and citrus pectins, bovine serum albumin (BSA) and poly-L-proline (PLP) were purchased from Sigma-Aldrich (Germany). Propiolic acid was purchased from Fluka Chemica (Germany). Sodium hydroxide, magnesium sulfate, sodium bicarbonate and hydrochloric acid were purchased from VWR (France). *tert*-butanol was purchased from Acros Organics (Geel, Belgium), CDCl₃, DMSO-*d*₆ and acetone-*d*₆ used for NMR analysis were purchased from Eurisotop (France).

2.2. Procyanidin preparation and characterization

Procyanidins of DP2 and DP8 were extracted from ‘Kermes-rien’ and ‘Golden delicious’ apple parenchyma, respectively, and were characterized as described by Watrelot et al. (Table 1) [20]. The DP2 contained 91% of (–)-epicatechin and 9% of (+)-catechin. This fraction contained >90% polyphenols and included

the dimers B2 ((–)-epicatechin-(4 β →8)-(–)-epicatechin, 85%) and B1 ((–)-epicatechin-(4 β →8)-(+)-catechin, 15%). The DP8 fraction was determined to be 83% polyphenolic and was composed of (–)-epicatechin (86%) and (+)-catechin (14%) only as terminal units [20].

2.3. Pectin characterization

Commercially available apple and citrus pectins were characterized by their galacturonic acid and neutral sugar content determined by gas chromatography as well as their partition coefficient evaluated by high performance size exclusion chromatography, as described by Watrelot et al. [5]. The pectin compositions are shown in Table 2. Both pectins differed in neutral sugars content. Citrus pectins were characterized by a higher rhamnose (2.6% w/w) and galactose (18.3% w/w) content than in apple pectins (1.6% and 11.6% w/w, respectively), while glucose content was higher in apple pectins (8.3% w/w) than in citrus pectins (4.4% w/w).

2.4. Modification of flavan-3-ols

2-[(Triphenylmethyl) thio]ethanamine (CAS number: 1095-85-8) was prepared by an adaption of the procedure reported by Liu et al. [21] To a stirred solution of 2-mercaptoethylamine hydrochloride (1.1 eq.) in anhydrous dichloromethane (4 mL) at 0 °C under argon atmosphere was added dropwise trifluoroacetic acid (TFA, 1.6 mL), followed by chlorotriphenylmethane (1 eq.). The reaction mixture was stirred for 2 h at 0 °C, then concentrated and diluted with CHCl₃ (5 mL). The solution was stirred vigorously, mixed with 3 M NaOH (8 mL) at room temperature for 1 h. The resulting suspension was then extracted with CHCl₃ (3 × 60 mL), and the combined organic layers were washed with brine (3 × 30 mL), dried over MgSO₄, filtered and evaporated to dryness to afford the desired product as a yellowish solid in quantitative yield without any purification (spectroscopic data in supporting information).

N-(2-[(triphenylmethylthio)ethyl] propiolamide was prepared by an adaptation of the procedure reported by Shiu et al. [22] To a stirred solution of propiolic acid (4 eq.) and 4-(dimethylamino) pyridine (DMAP, 0.4 eq.) in anhydrous CH₂Cl₂ (3 mL) at 0 °C under argon atmosphere was added dropwise *N,N'*-diisopropylcarbodiimide (DIC, 5 eq.). To the resulting solution was added a solution of 2-[(triphenylmethyl) thio]ethanamine (1 eq.) in anhydrous CH₂Cl₂ (2 mL). The resulting suspension was allowed to warm up slowly and was then stirred at room temperature for 20 h under argon. After removing the white precipitate by filtration and diluting with CHCl₃ (90 mL), the resulting solution was washed with 0.5 M HCl (10 mL), aqueous saturated NaHCO₃ (10 mL) and brine (20 mL), dried over MgSO₄, filtered and evaporated. The residue was purified by column chromatography, eluting with cyclohexane/EtOAc (8:2; v/v) to furnish the desired product as a yellowish solid in 43% yield (spectroscopic data in supporting information).

According to the procedure described by Ariza et al. [23], to a stirred solution of (+)-catechin, (–)-epicatechin, dimer DP2 or oligomers DP8 (1 eq.) in a mixture of anhydrous DMF (1 mL) and *tert*-butanol (1 mL) were added *N*-(2-(tritylthio) ethyl) propiolamide (1.1 eq. for monomer and dimer and 3 eq. for oligomers) and *N,N*-dimethylaminopyridine (1.5 eq. for monomer and dimer and 4.5 eq. for oligomers). The reaction mixture of monomers was heated at 40 °C under an argon atmosphere for 24 h and 52 h for dimer; while reaction mixture of oligomers was heated at 60 °C under an argon atmosphere for 5 days. The solvents and volatiles were then evaporated at 50 °C. The crude mixture of monomers was purified by column chromatography, eluting with CH₂Cl₂ to remove impurities, and then with CH₂Cl₂/acetone (8:2; v/v),

Table 1

Characterization of procyanidins DP2 and DP8 (mg/g of dry matter) (data from Watrelot et al. [5]).

	DPn	Procyanidins			Dihydrochalcones		Hydroxycinnamic acids	
		Extension	Terminal		PLZ	XPL	CQA	PCQ
			EPI	CAT				
DP2	2	464	333	79	0	0	8	0
DP8	8	562	64	21	1	0	25	1
SD	0.5	43.4	1.1	0.4	0.1	nd	7.1	nd

DPn: number average degree of polymerization; EPI: (–)-epicatechin; CAT: catechin; PLZ: phloridzin; XPL: phloretin xyloglucoside; CQA: caffeoylquinic acid; PCQ: *para*-coumaroylquinic acid; SD: pooled standard deviation.

Table 2

Characterization of commercial pectins from apple and citrus: sugar composition (mg/g) and partition coefficient (K_{av}) (data from Watrelot et al., [5]).

	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	GalA	MeOH (DM%)	K_{av}
Apple pectins	12	–	20	8	1	88	63	564	74 (73)	0.02
Citrus pectins	20	1	21	4	–	138	33	535	77 (79)	0.04
SD	1.4	0.5	0.4	0.2	0.4	1.5	0.8	10.3	1.8 (0.02)	–

Rha: rhamnose; Fuc: fucose; Ara: arabinose; Xyl: xylose; Gal: galactose; Man: mannose; Glc: glucose; GalA: galacturonic acid; MeOH: methanol; DM: degree of methylation; SD: pooled standard deviation.

to furnish a diastereoisomeric mixture of the expected products, named CAT-STrt and EPI-STrt in 48% and 53% yield respectively (spectroscopic data in supporting information). The crude mixtures of dimer and oligomer were triturated in CH_2Cl_2 to obtain the expected products named DP2-STrt (55% yield) and DP8-STrt (spectroscopic data in supporting information).

Detritylation was carried out by an adaption of the procedure reported by Basit et al. [24]. To solutions of the flavan-3-ols-STrt (1 eq.) in anhydrous CH_2Cl_2 (1 mL) were added trifluoroacetic acid (5 eq.) and triethylsilane (5 eq.), and the resulting solutions were stirred for 4 h at room temperature. The solvent and volatiles were then evaporated and the crude products were successively triturated in CHCl_3 (2 mL) and petroleum ether (10×1 mL) and further dried under vacuum to furnish the expected compounds as yellowish plastics, named CAT-SH (92% yield), EPI-SH (75% yield), B2-SH (78% yield) and DP8-SH (spectroscopic data in supporting information). These compounds reacted positively with Ellman's reagent (5,5'-dithio-bis-(2-nitrobenzoic acid)), thus confirming the presence of free thiols. All modification steps were monitored by NMR spectroscopy (300 MHz) using samples in acetone- d_6 or DMSO- d_6 .

2.5. Surface plasmon resonance experiments

All surface plasmon resonance (SPR) experiments were performed using a Biacore™ 3000 biosensor (Biacore, GE Healthcare, Uppsala, Sweden). The sensor possessed a microfluidic cartridge that provides four separate channels for different assays simultaneously. All measurements were performed at 23 °C in HBS-EP running buffer from GE Healthcare (0.01 M HEPES pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.005% surfactant P20). 1 nM flavan-3-ol-SH was dissolved in HBS-EP buffer with 5% v/v acetic acid and then filtered (0.45 μm) before injection.

2.5.1. Immobilization onto CM5 surface

Carboxymethyl dextran sensor chips (CMD 200m, Xantec, Dueseldorf, Germany) were used for immobilization of flavan-3-ol-SH according to the thiol coupling protocol from GE Healthcare. The baseline was stabilized for 300 s with HBS-EP buffer at 5 $\mu\text{L}/\text{min}$. Then 10 μL of EDC (0.2 M)/NHS (0.05 M) were injected, followed by 20 μL of 80 mM 2-(2-pyridinyldithio)-ethanamine (PDEA) prepared in a 0.1 M borate buffer pH 8.5. Two times of 10 μL of 1 nM flavan-3-ol-SH was injected. Then, 20 μL of a mixture of 50 mM cysteine and 1 M sodium chloride prepared in a 0.1 M sodium acetate

buffer pH 4.3 was injected. The reference track was prepared under the same conditions as above but without flavan-3-ol-SH.

2.5.2. Interaction analysis by SPR

Apple and citrus pectins, BSA and PLP were solubilized at 3, 30 and 300 nM in HBS-EP buffer with 5% v/v acetic acid. Interaction analyses were carried out at a flow rate of 20 $\mu\text{L}/\text{min}$. After interaction analysis with pectins or proteins, sensor chips were regenerated by injection of SDS 0.1% w/v solution.

2.6. ATR experiments

The ATR spectra of CAT, CAT-SH, DP8, and DP8-SH compounds were recorded with a Thermo Nicolet Nexus 670 FTIR spectrometer equipped with a liquid nitrogen cooled narrow-band mercury cadmium telluride (MCT) detector using a Silver-Gate (germanium crystal) ATR accessory (Specac). The electric field of the infrared beam was polarized either perpendicular (s-polarized) or parallel (p-polarized) to the plane of incidence with a BaF₂ wire grid polarizer (Specac). Each spectrum was obtained after evaporation of a drop (20 μL) of a solution (2 mg in 100 μL of EtOH), at a resolution of 4 cm^{-1} , by co-adding 500 scans.

2.6.1. Determination of the optical constants of CAT, CAT-SH, DP8, and DP8-SH compounds

The optical constants (refractive index $n(\tilde{\nu})$ and extinction coefficient $k(\tilde{\nu})$) of CAT, CAT-SH, DP8, and DP8-SH compounds have been determined from polarized attenuated total reflectance (ATR) spectra, using the interdependence of $n(\tilde{\nu})$ and $k(\tilde{\nu})$ by the Kramers-Kronig relations. Dignam et al. have shown how the Kramers-Kronig relations can be applied to polarized ATR spectra [25]. The in-plane optical constants (n_{xy} and k_{xy}) were calculated from the s-polarized ATR spectrum, whereas the out-of-plane optical constants (n_z and k_z) were obtained from the p-polarized ATR spectrum and the before determined n_{xy} and k_{xy} . Then, the isotropic optical constants of CAT, CAT-SH, DP8, and DP8-SH compounds have been calculated from the in-plane and out-of-plane optical constants using the relations: $n_{iso} = (2n_{xy} + n_z)/3$ and $k_{iso} = (2k_{xy} + k_z)/3$.

3. Results

3.1. Flavan-3-ol derivatization

In order to immobilize flavan-3-ol monomers/oligomers onto sensor chips, a linker bearing a protected thiol group at one of its extremities was synthesized. The other extremity of the linker was bound to the flavan-3-ol catecholic B-ring through a Vilarrasa reaction [23,26]. In order to optimize the mobility of flavan-3-ols immobilized onto surfaces, flavan-3-ol catechols were modified by using only one equivalent of the linker per equivalent of flavan-3-ol. Modified flavan-3-ols were then detritylated to release their thiol group(s) (Scheme 1). These reactions performed on monomers and dimers were monitored by ^1H and ^{13}C NMR analyses, whereas only ^1H NMR analysis was used to follow the appearance and disappearance of trityl groups (between 7.5 and 7 ppm) on DP8 oligomers. Mid-infrared extinction coefficient $k(\bar{\nu})$ spectra of a monomer (CAT) and flavan-3-ol oligomers (DP8) were also determined from ATR spectra before and after derivatization in order to confirm the presence of thiolate linkers (Fig. 1). The covalent linkage of flavan-3-ols to the linker via an amide bond was confirmed by the presence of amide I ($\nu_{\text{C=O}}$) and amide II ($\delta_{\text{NH}} + \nu_{\text{C-N}}$) modes of the linker at 1655 and 1500 cm^{-1} , respectively were observed for both derivatized molecules (CAT-SH and DP8-SH) whereas these bands were not present in the initial CAT and DP8 spectra. The 1630 and 1470 cm^{-1} bands, assigned to the $\nu_{\text{C=C}}$ stretching modes of the A ring of flavan-3-ols, were not observable on the DP8 spectrum, likely because the intensity of these two modes occurs from the unlinked rings.

3.2. CM5 immobilization and interaction analysis

To obtain a good flexibility and subsequent binding site availability of immobilized flavanols, flavan-3-ols-SH were immobilized onto carboxymethyl dextran (CM5) sensor chips using a routine

coupling method from GE Healthcare Life Sciences (Ligand thiol coupling, Laboratory guidelines BR-2001-21 AB). For this method, reactive disulfide groups were introduced into the dextran matrix then, the thiol coupling occurred through a thiol-disulfide exchange with the thiol groups on the ligand. EPI-SH, DP2-SH and DP8-SH were immobilized onto CM5 sensor chip with the following resonance units (312 RU, 417 RU and 305 RU respectively), then the excess reactive groups were deactivated with a 50 mM cysteine-containing 0.1 M sodium acetate solution.

The modified sensor chips were first tested with a protein (BSA) and a polypeptide (PLP) well known to interact in solution with these flavan-3-ols [9,11]. Interactions between BSA and flavan-3-ols depended on BSA concentrations. At 3 nM BSA, the association step was higher with (–)-epicatechin (85 RU) than with dimer (34 RU) or oligomer DP8 (17 RU) (Fig. 2). For BSA concentration higher than 30 nM, the association step showed two stages that is an increase of association after the beginning of injection of BSA followed by a decrease of SPR signal after 1 min before the end of injection (data not shown). With poly-L-proline no interaction was detected at 3 nM of peptide, while similar levels were obtained for all flavan-3-ols at 30 nM of peptide (Table 3).

Only weak resonance units were obtained for apple pectins with either EPI or DP8 (around 15 RU), a slightly higher level being reached for DP2 (22 RU) whatever the concentrations of apple pectins (Fig. 3 and Table 3). Higher association was found with citrus pectin up to 120 RU for all flavan-3-ols at the highest pectin concentration (Table 3).

4. Discussion

The binding affinity differences between flavan-3-ol derivatives immobilized onto CM5 surface and biomacromolecules might be explained either by the biomacromolecules or flavan-3-ol structure and conformation. On the one hand, BSA is composed of three domains and its tertiary structure (like an equilateral triangle)

Scheme 1. Reaction scheme of chemical synthesis of N-(2-[(Triphenylmethylthio)ethyl] propiolamide linker and flavan-3-ols.

Fig. 1. Extinction coefficient spectra of (+)-catechin (CAT), (+)-catechin modified by linker with thiol group (CAT-SH), flavan-3-ol oligomer (DP8) and flavan-3-ol oligomer modified by linker with thiol group (DP8-SH). Assignment of the major bands in the 1700–1300 cm^{-1} spectral range.

is similar to Human Serum Albumin. Domains II and III form a positively charged hydrophobic cavity which allows for associations with ligands [27]. On the other hand, proline-rich proteins (PRPs), more precisely PLP, form an open extended structure which presents a maximum surface area for interaction per residue [28]. Therefore, we expected that the smaller polypeptide would bind more, as the steric hindrance should be limited (smaller molecule). The opposite was observed, perhaps due to limited flexibility (i.e. no possibility to adjust its conformation to the bound flavan-3-ols) [29] or due to involvement of the B-ring of the flavan-3-ols here modified and/or bound to the sensor chip, in the interaction.

Different response levels were observed between flavan-3-ols and the two pectins. Apple pectins showed higher resonance units with the dimer DP2 while citrus pectins interacted with more intensity but indifferently to the monomer, dimer and oligomer. Apple and citrus pectins differ by their neutral sugar composition, in particular by higher rhamnose and galactose in citrus pectins, i.e. presumably more type I rhamnogalacturonans. The high rhamnose content in apple pectins could induce an increase of the molecule flexibility and lead to a change of conformation [30]. Apart from rhamnose, neutral sugars tend to adopt various conformations in solution. Watrelot et al. [31] showed that β -1 $\rightarrow$ 4 galactan chains bind more strongly to procyanidins than arabinan chains, and it was demonstrated that pectins with short neutral sugar side chains

Table 3

Resonance units corresponding to interactions between flavan-3-ols (–)-epicatechin (EPI), dimer (DP2) and oligomer (DP8) and analytes (apple and citrus pectins, poly-L-proline and bovine serum albumin) at 3, 30 and 300 nM in HBS-EP buffer at 25 °C.

Analytes/Ligands	EPI (312 RU)	DP2 (417 RU)	DP8 (305 RU)
Apple pectins			
3 nM	16	22	14
30 nM	16	21	14
300 nM	11	15	10
Citrus pectins			
3 nM	61	60	61
30 nM	118	119	120
300 nM	112	121	119
Bovine serum albumin			
3 nM	85	34	17
Poly-L-proline			
3 nM	–	–	–
30 nM	58	64	58
300 nM	61	58	65

associate with flavan-3-ol polymers. This interaction would be due to a cooperative interaction between pectins and procyanidins.

Polyphenols were immobilized by the catechol group, which is the B-ring of flavan-3-ols. Consequently, for the monomer (i.e. (–)-epicatechin), the B-ring was not available to bind to biomacromolecules. Therefore, sensor chips prepared here could only detect interactions due either to hydrophobic interactions between A- or pyran C-ring and methyl groups of pectins or amino acids of proteins, or to hydrogen bonds between hydroxyl groups of A- and/or C-ring and hydroxyl groups of pectins or proteins, or both. The dimer DP2 was immobilized by one B-ring, which left the other free to interact. Therefore, the higher association of apple pectins with DP2 could be due to both parameters (conformation of apple pectins and a higher availability of binding sites of dimer). Regarding flavan-3-ol oligomers, the number of catechol groups immobilized was unknown and the conformation is fully different from that of monomer and dimer, notably with increased rigidity [32], while orientation with the respect to the surface remains unknown. Associations, both with pectins or proteins, were not correlated to the number of hydroxyl groups of polyphenols immobilized contrary to the work of Jean-Gilles et al. [33] but rather in the conformation adopted by polyphenols.

Associations between the same polyphenols and macromolecules (pectins and proteins) were recently measured in solution by Isothermal Titration Calorimetry (ITC) [5,20]. ITC is based upon heat exchange and measures the energy released upon (or necessary for) ligand-macromolecule interactions. Weak energies of interactions were found between pectins and flavan-3-ol monomers and dimers, so that method was not an appropriate method for measuring interactions parameters between those two molecules [5]. In contrast, SPR measurements showed that, depending on pectin concentrations, both pectins tend to interact more with the dimer than with the monomer. Therefore, provided or generated heat by interactions between pectins and flavan-3-ols monomer or dimer might be too low for detection, while the injection of pectins on a surface of (–)-epicatechin or dimer leads to formation of layers of pectins which induced a change of refracted light and also resonance units.

Associations between model proteins or polypeptides and flavan-3-ols were already measured by ITC [7,10–12,20]. ITC evidenced higher affinity of flavan-3-ol for BSA than for poly-L-proline as found here by SPR. This affinity increased with the increase of the degree of polymerization of flavan-3-ols, as previously observed [34]. However, in the case of immobilized flavan-3-ols (using SPR), affinity with BSA decreased with the increase of the DP. Moreover, degree of polymerization did not influence RU levels observed with poly-L-proline. As mentioned for analysis with pectins, impact of

Fig. 2. Sensorgram of interaction double corrected between bovine serum albumin (BSA) at 3 nM and (–)-epicatechin (89 RU, red line), dimer DP2 (72 RU, green line) and oligomer DP8 (81 RU, blue line), in HBS-EP buffer at 25 °C. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 3. Sensorgram of interaction double corrected between apple pectins at 3, 30 and 300 nM and (–)-epicatechin (red line), dimer DP2 (green line) and oligomer DP8 (blue line) in HBS-EP buffer at 25 °C. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

immobilization on flavan-3-ols conformation probably modified the interactions at the molecular level.

5. Conclusions

In this work, flavan-3-ol monomer, dimer and oligomer were chemically modified through their B-ring and for the first time grafted onto CM5 surface with success. The immobilization of flavan-3-ol derivatives onto a CM5 sensor chips, confirmed using SPR, allowed comparison of interactions of the same flavan-3-ol-bearing sensor chips with varied macromolecules, which brought new information on interaction characteristics. For the first time molecules of proteic and polysaccharidic nature were directly compared in the same system: though, as expected, higher affinities were obtained with BSA, citrus pectin in particular bound remarkably to the sensor chips. Therefore (i) interactions of flavan-3-ols with polysaccharides should not be neglected, particularly in plant food systems, and (ii) further work on the impact of fine structure

of polysaccharides on the interactions with tannins is still needed. SPR analysis should be used in addition to ITC to compare the behavior of macromolecules when one is immobilized on a solid surface or both are in solution. This method is useful for a better understanding of the effect of the macromolecule conformation on associations. Because in this study the flavan-3-ols were immobilized onto a surface through B-rings and affinities were lower than obtained in solution, the results suggest that the flavan-3-ol B-ring is involved in macromolecular interactions. This method gave new information about the interaction mechanisms occurring between macromolecules (condensed tannin, protein, polysaccharide), which are involved in astringency mouthfeel perception and colloids formation.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.apsusc.2016.03.002>.

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